

## Molecular studies in familial dilated cardiomyopathy – A pilot study

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### ABSTRACT

**Aim:** To study genetic variants in patients of familial dilated cardiomyopathy.

**Methodology:** Patients with reduced ejection fraction of less than 45% and dilated left ventricle are considered to have dilated cardiomyopathy. Clinical history was taken and possible secondary causes of dilated cardiomyopathy were excluded. Family history of  $\geq 2$  affected relatives or sudden cardiac death in a relative with age less than 35 years were included. Such patients blood sample were sent for next generation sequencing and analysed for presence of genetic variants.

**Results:** As part of pilot study 20 patients (44% were female and 66% were male) were included. There was presence of 16 different pathogenic variants in 14 patients. Two patients had more than one variants in them. Most common of which were sarcomeric mutations constituting 32%. Titin followed by Filamin, Lamin and Desmosomal where the most commonly repeated mutations.

**Discussion:** In our patients of familial dilated cardiomyopathy, 70% were detected to have pathogenic variants in them. Most common variations were seen on Titin gene. Thus those with familial dilated cardiomyopathy should be considered for next generation sequencing. First degree relatives of those with pathogenic variants should be screened using cascade testing for earlier detection and disease monitoring in them.

### 1. Introduction

Cardiomyopathies are a heterogeneous group of myocardial diseases associated with mechanical and/or electric dysfunction that usually exhibit inappropriate hypertrophy or dilatation. These are due to various causes that can frequently be genetic [1]. When there is depressed ejection fraction of the heart along with dilatation of the left ventricle which is not caused by hypertension, congenital, valvular or ischemic heart disease it is called as dilated cardiomyopathy (DCM) [2]. These accounts for 10,000 deaths and 46,000 hospital admissions annually in the USA [3]. Unfortunately such data are lacking in India. Ushasree et al. [4] studied 107 patients of DCM and suggested that epidemiological factors like age, sex, vegetarian diet in conjunction with sarcomeric gene mutation may play role in its expression. On segregation analysis they demonstrated a deviation from autosomal dominant mode of inheritance. Dhandapandy et al. [5] described a deletion of 25 base pairs (bp) in the gene encoding cardiac myosin binding protein C (MYBPC3) is associated with heritable cardiomyopathies and increased

risk of heart failure in Indian populations, with prevalence as high as ~4%. Subsequently Indian studies were mostly limited to case reports [6–8], and study of single gene in a series of patients [9,10]. A large study on mitochondrial DNA analysis in Indian patients with DCM revealed 48 novel, 42 disease associated and 97 private variants [11].

Etiology of dilated cardiomyopathy is heterogenous, varying from viral infection to cardiotoxic drugs [12]. There is a large syndromic group listed [13], but this group is outside the scope of the present study. Many causal genes have been identified [14–15], including those encoding cytoskeletal (DES, FLN), sarcomeres (TTN, TPM1, ACTC1), mitochondrial, desmosomes (DSG, PKP), nuclear membrane, and RNA-binding proteins. Recommendation have been made by various organizations for diagnosis and evaluation of dilated cardiomyopathy [16–18]. In DCM about 40 to 50% have identifiable genetic cause with variations in Titin gene being most common around 15 to 25% in various studies [18]. Best method currently for analysing pathogenic variants in multiple genes simultaneously is of next generation sequencing (NGS).

The inheritance of the disease can be autosomal dominant (most

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commonly), autosomal recessive, x-linked recessive or mitochondrial. However there is variable expressivity and incomplete penetrance of the pathogenic variants. The penetrance is age dependent varying from fourth to seventh decade of life. Occasionally additional insult is required for manifestation of symptoms e.g cardiomyopathy in peripartum period, myocarditis or toxin [19]. If there is a pathogenic variant and no family history than such cases are termed as sporadic. The prevalence of familial DCM is around 30% to 50% based on clinical, echocardiographic and familial criteria [20].

In the present study patients of dilated cardiomyopathy with a positive family history were evaluated for presence of pathogenic variants by NGS. It was a pilot study carried out at single centre of North India with the objective to determine the landscape of genes involved, and examine the feasibility of providing genetic counselling to the patients and at risk relatives.

## 2. Materials and methods

This observational study was done at Cardiology department of our hospital after approval from the institutional ethical committee (EC/07/18/1371). Patients of familial dilated cardiomyopathy (FDC) were evaluated for pathogenic variants in a 154 panel of genes using NGS. Genes selected were those that have been described to cause dilated cardiomyopathy. Patients of 18 years or more in age with presence of dilated cardiomyopathy defined as left ventricular (LV) ejection fraction <45%, fractional shortening <25% and LV end diastolic diameter >117% of the predicted value corrected for body surface area and age were included in study. Family history in first degree relatives of  $\geq 2$  members with DCM, or sudden cardiac death in them before 35 years of age were considered for NGS.

For all patients a detailed clinical history, symptom onset and its aggravation, and a three generation family history was taken. Echocardiographic parameters such as ejection fraction, LV end diastolic and systolic diameter, wall motion abnormalities and valvular regurgitation were determined. Patients with possible secondary causes of dilated cardiomyopathy and refusal to consent from study were excluded. In

eligible patients coronary artery disease was ruled out by coronary angiography or CT coronary angiography. About 8 ml of blood sample was collected in EDTA vials. DNA extracted by the salt precipitation method [21]. DNA aliquotted in Eppendorf for Next Generation Sequencing to look for variations in cardiomyopathy related genes. The systematic flow chart of the study is shown in Fig. 1. List of genes tested is given in Annexure. The variant identified was then categorised to be pathogenic, uncertain significance and benign as per ACMG guidelines [17].

### 2.1. Statistical analysis

The sample size was calculated based on the report of 40% rate of variation detection in a study conducted by Ganesh et al. [22] With confidence level of 95% and precision of 10%, required sample sizing worked out to be 93. Since it was a pilot study sample size was reduced to 20 subjects.

Categorical variables are presented in number and percentage (%) and continuous variables as mean  $\pm$  SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used.

Statistical tests were applied as follows-

1. Quantitative variables were associated using Independent *t* test or Mann-Whitney Test (when the data sets were not normally distributed) between the two groups.
2. Qualitative variables were associated using Chi-Square test or Fisher's Exact test (when the data sets were not normally distributed) between the two groups.
3. A *p* value of less than 0.05 was considered statistically significant.

## 3. Results

Twenty patients of dilated cardiomyopathy with positive familial history and satisfying the inclusion and exclusion criteria were enrolled in study. The mean age of the population was 46 years ranging from 26

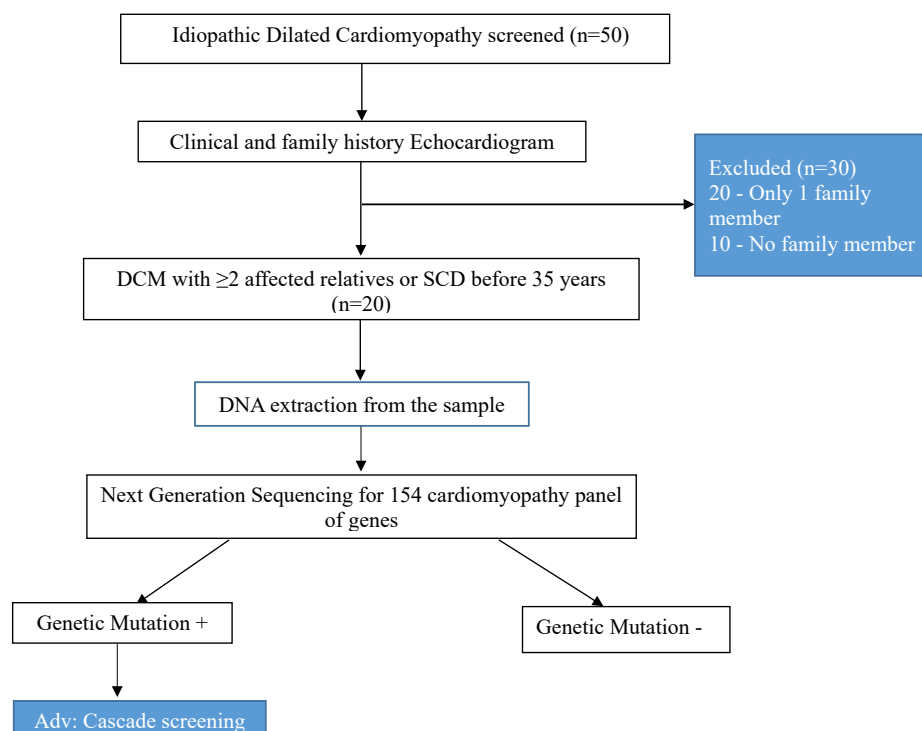


Fig. 1. Flow chart for the study.

to 60 years of which 14 patients (70%) were less than 50 years of age. There were 11(55%) male patients and 9(45%) female patients. 15 (75%) had history of more than or equal to 2 family members affected in them. Pedigree chart analysis showed that transmission of the disease was more from father to children with more of male children being affected compared to female. It suggests male predilection for the disease. Off springs also showed earlier onset of disease either symptom manifestation or sudden cardiac death compared to the parents. Most of the patients where in NYHA class 2 (90%), one had CRT (Cardiac resynchronisation therapy), 3(15%) had ICD (implantable cardioverter defibrillator) implanted for the primary prevention. Coronary angiography was done to rule out ischemic cause in 70% of the patients. Clinical features are shown in Table 1.

Basic echocardiographic features are mentioned in Table 2. Patients left ventricular end diastolic diameter for their age, sex and body surface area was calculated. The mean dilation was  $120.05 \pm 4.64\%$  (115–134). One had severe aortic stenosis and four had moderate mitral regurgitation (MR) with none having severe MR. Two had presence of left ventricular clot.

We identified pathogenic variants in 14 patients (70%) and variation of unknown significance in one of them (Table 3). Most common frequency of mutation seen was of sarcomeric (*TTN*, *MYH6* in 5 i.e 32%), followed by cytoskeletal (*FLNC* in 20%), 12% each of extracellular matrix (*LAMA4*) and desmosomal (*PKP2*, *CTNNA3*). Other mutations like *RYR2*, *RBM20*, *KCNQ* and *CHRM2* constituted 24% of variations. Most commonly reported mutation was in *TTN* gene (25%) (Table 4).

The chance of detecting pathogenic variant was greater in those with age less than 50 years compared to those more than 50 years (12 vs 2,  $p < 0.05$ ). There was no association of gender, presenting complains, family history or echocardiographic parameters with chance of detecting pathogenic variant. Out of all mutations, incidence of variants that were likely pathogenic was 88% and of variant of unknown significance (VUS) was 12%. All the detected variants were heterozygous in this study suggesting autosomal dominant mode of inheritance. All of the patients were given genetic counselling regarding the risk of disease transmission to the off-springs and the need for cascade screening in them for early detection and regular follow up.

#### 4. Discussion

In this study done in 20 patients, 14 (70%) were found to have genetic variation. Autosomal dominant was the mode of inheritance in all the patients. The incidence of presence of genetic mutation in population of familial dilated cardiomyopathy in this study is more than that reported in other studies which is around ~40% [17,23–4]. This may be due to larger coverage of genes in the panel. Most common single gene detected was Titin gene (25%), which is consistent with other studies as this is the largest gene in this group [25]. Age less than 50 years had

**Table 2**

Echocardiographic features.

Left ventricular ejection fraction (%)	28.9 ± 7
Left ventricular end systolic diameter	43 ± 2.5
Left ventricular end diastolic diameter (mm)	50 ± 3.7
Dilatation of LVDD	120.05 ± 4.6

significant chance for variation being detected. Other parameters like sex, history of affected family members or sudden cardiac death, severity of left ventricular dysfunction, left ventricular end diastolic dimension were not associated with mutation being detected.

One patient had two disease causing mutations observed in same gene. In them segregation analysis is indicated in parents/siblings to determine whether these two variants are on same chromosome or different chromosome (i.e one inherited from each parent). One patient (6%) had variation of unknown significance (VUS). VUS means that the causal relation of this gene causing DCM is not established, lack of their reported frequencies in the literature or not studied adequately. It is very commonly reported with next generation sequencing due to a broader cardiomyopathy panel of genes being tested. This has to be established further with either sanger sequencing in the families or other studies to be called as pathogenic. Patients with VUS or pathogenic has to be followed up over years as classification of the variant pathogenicity keeps changing from current status to either benign or pathogenic. In patients with no variant detected can again be considered for testing if the test sensitivity rises by 10% [17].

Phenotypic-genotypic association was done for genes that affected more than two patients. Titin variants manifests in younger (<50 years) population (75%), has male predilection (75%), more severely depressed left ventricular function and family history of 2 or more affected members. *LAMA* and *FLNC* mostly reported in younger population (60%) and female predilection (90%). They had severely depressed left ventricular function with more dilatation of left ventricle. Patients more often had family history of sudden cardiac death (30%) suggesting increased susceptibility of arrhythmias in them.

In our study frequency of mutations was *TTN* (25%), *FLNC* (18%), *LAMA* 4 (12%) and 6% each for *MYH6*, *PKP2*, *CTNNA3*, *KCNQ1*, *RBM* 20, *RYR* 2, *CHRM* 2. A study of 766 patients of DCM, sporadic and familial with gene testing ranging from limited sanger analysis to NGS over 10 years the frequency of detecting mutation was 27–37%, with most reported frequency of Titin (19%) [23]. The frequency of other mutations compared to our study was *LAMA* 4 (7%), *TNNT2* (3%), *RBM* 20 (3%) [23]. Similarly in a study of 639 DCM patients incidence of detecting mutation was 48% with frequency of various genes being Titin (13%), *RYR* 2 (7%), *TNNT2* (4%), *RBM* 20 (3%), *MYH* 6 (3%), *PKP* 2 (2%), *KCNQ* 1 (2%) and others with frequencies less than 1% [24]. Rai et al screened 130 patients DCM and HCM for mutations in *MYH7* gene, and reported 14 mutations in 6 probands (5 proband in HCM 1 proband in DCM) [10]. Jadhav et al reported a case of familial DCM with novel LMNA mutation presenting as Emery Dreifuss myopathy [6]. Rani et al reported a familial DCM with mutation in R144w in troponin T gene [26]. Thus Indian studies mostly based on case reports and single gene analysis. This is first such study to detect prevalence to the best of our knowledge.

Patient with *MYH6* was also having severe aortic stenosis suggesting some link of this with increased risk for degeneration in aortic valve. Our patient with *RBM20* mutation was 26 years old female and had similar location compared to reports in Western and Indian case reports [9]. Such patients have early onset of disease and needs to be monitored.

*PKP2* which is known for causing arrhythmogenic right ventricular cardiomyopathy but can also cause isolated left ventricular dysfunction. *RYR2*, *CHRM2*, *KCNQ1* and *CTNNA3* were also seen in the study population. They are not frequently reported in the literature but case reports have shown association with DCM [27–9] which requires further validation in large scale studies.

**Table 1**

Demographics of the study population.

Age (years)	46.8 ± 9
Male : Female ratio	1.22:1
<b>CLINICAL FEATURES</b>	
Dyspnea	95%
Fatigue	15%
NYHA Class II	90%
NYHA Class III	10%
SCD in family	30%
≥2 family members affected	75%
Hypertension	15%
Diabetes	20%
H/O smoking	5%
QRS duration > 120 msec	15%
ICD	15%
CRT	5%
Coronary Angiography	70%

**Table 3**  
Mutation detected in study population.

Patient No	Gene	Variant	Pathogenicity	Zygosity	
1.	<i>FLNC</i>	Exon 30	c.4991C>T (p.Thr1664Met)	Pathogenic	Heterozygous
2.	<i>RYR2</i>	Exon 17	c.1640A>G (p.Asn547Ser)	Pathogenic	Heterozygous
3.	<i>CTNNA3</i>	Exon 18	c.2407A>G (p.Ser803Gly)	Pathogenic	Heterozygous
4.	<i>RBM20</i>	Exon 9	c.1900C > T (p.Arg634Trp)	Pathogenic	Heterozygous
5.	<i>TTN</i>	Intron 319	c.67637-2A > G (3' splice site)	Pathogenic	Heterozygous
6.	<i>FLNC</i>	Exon 36	c.5944C > T (p.Arg1982Cys)	Pathogenic	Heterozygous
	<i>LAMA4</i>	Exon 35	c.5029C > T (p.Arg1677Cys)	VUS	Heterozygous
7.	<i>LAMA4</i>	Exon 10	c.1176_1177insT (p.Asp393Ter)	Pathogenic	Heterozygous
8.	<i>KCNQ1</i>	Exon 3	c.514G > A (p.Val172Met)	Pathogenic	Heterozygous
9.	<i>PKP2</i>	Intron 7	c.1688+1G>A (5' splice site)	Pathogenic	Heterozygous
10.	<i>MYH6</i>	Exon 38	c.5688_5689insTC (p.Lys1897SerfsTer?)	Pathogenic	Heterozygous
11.	<i>FLNC</i>	Exon 40	c.6518G>A (p.Arg2173His)	Pathogenic	Heterozygous
12.	<i>CHRM2</i>	Exon 3	c.169G>A (p.Val57Ile)	VUS	Heterozygous
13.	<i>TTN</i>	Exon 343	c.95122delT (p.Ser30067ProfsTer13)	Pathogenic	Heterozygous
14.	<i>TTN</i>	Exon 325	c.69420delT (p.Gly23141AlafsTer36)	Pathogenic	Heterozygous
	<i>TTN</i>	Exon 70	c.20324delA (p.Asn6775MetfsTer74)	Pathogenic	Heterozygous

**Table 4**  
Variant frequency in the study population.

<b>TTN (4), MYH 6</b>	<b>Sarcomere mutations (32%)</b>
<b>FLNC (3)</b>	Cytoskeletal protein mutations (20%)
<b>LAMA 4 (2)</b>	Extracellular matrix mutations (12%)
<b>PKP2, CTNNA 3</b>	Desmosomal mutations (12%)
<b>KCNQ 1</b>	Ion channel mutations (6%)
<b>RBM 20</b>	Nuclear mutations (6%)
<b>RYR 2</b>	Sarcoplasmic reticulum and cytoplasm mutations (6%)
<b>CHRM 2</b>	Cholinergic receptor mutations (6%)

The number of study subjects was small to draw much inference from the study. Moreover the population was in selected area of India (North India) so can not be generalised to all over India. The family history was available in the form of verbal form in few patients, the reason being lack of computerised data recording in the country. Authors assume if testing done after echocardiographic confirmation in first degree relatives might increase the yield of the study. In those where VUS was

reported we did not do Sanger sequencing to confirm it, and also we did not do Segregation studies.

## 5. Conclusion

Authors conclude that in those with familial dilated cardiomyopathy, Next generation sequencing should be considered to detect any genetic variation in them. If any known mutation found cascade screening be done in their first degree relatives so that they can be followed up for earliest detection of disease manifestation and disease modifying therapy be started. Authors also call for a large scale multi-centre study for detecting and reporting genetic variants in Indian population.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix. List of analysed genes

Gene	Gene	Gene	Gene
<i>AARS2</i>	<i>ABCC9</i>	<i>ACAD9</i>	<i>PSEN2</i>
<i>ACADVL</i>	<i>ACTA1</i>	<i>ACTC1</i>	<i>RAF1</i>
<i>ACTN2</i>	<i>AGK</i>	<i>AGL</i>	<i>RIT1</i>
<i>ALMS1</i>	<i>ANK2</i>	<i>ANKRD1</i>	<i>SCN10A</i>
<i>ANOS</i>	<i>BAG3</i>	<i>BIN1</i>	<i>SCNN1G</i>
<i>BRAF</i>	<i>CALR3</i>	<i>CASQ2</i>	<i>SEPN1</i>
<i>CAV3</i>	<i>CFL2</i>	<i>CHKB</i>	<i>SHOC2</i>
<i>CHRM2</i>	<i>COX15</i>	<i>CPT1A</i>	<i>SLC25A4</i>
<i>CPT2</i>	<i>CRYAB</i>	<i>CSR3P</i>	<i>TAZ</i>
<i>CTNNA3</i>	<i>DES</i>	<i>DMD</i>	<i>TCAP</i>

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Gene	Gene	Gene	Gene
<i>DNAJC19</i>	<i>DNM2</i>	<i>DOLK</i>	<i>TMEM70</i>
<i>DPP6</i>	<i>DSC2</i>	<i>DSG2</i>	<i>TNNI3</i>
<i>DSG3</i>	<i>DSP</i>	<i>DTNA</i>	<i>TPM1</i>
<i>DYSF</i>	<i>EMD</i>	<i>EYA4</i>	<i>TTR</i>
<i>FHL1</i>	<i>FHL2</i>	<i>FHOD3</i>	<i>XK</i>
<i>FKTN</i>	<i>FLNC</i>	<i>FOXRED1</i>	<i>SLC22A5</i>
<i>FXN</i>	<i>GAA</i>	<i>GATA4</i>	<i>SOS1</i>
<i>GATAD1</i>	<i>GJA1</i>	<i>GJA5</i>	<i>TBX20</i>
<i>GLA</i>	<i>GLB1</i>	<i>GNE</i>	<i>TGFB3</i>
<i>GUSB</i>	<i>HCN4</i>	<i>HFE</i>	<i>TMPO</i>
<i>HRAS</i>	<i>ILK</i>	<i>ISCU</i>	<i>TNNI3K</i>
<i>JPH2</i>	<i>JUP</i>	<i>KBTBD13</i>	<i>TSFM</i>
<i>KCNA5</i>	<i>KCND3</i>	<i>KCNJ8</i>	<i>VCL</i>
<i>KLHL9</i>	<i>KRAS</i>	<i>LAMA4</i>	<i>SRL</i>
<i>LAMP2</i>	<i>LDB3</i>	<i>LMNA</i>	<i>TBX5</i>
<i>LZTR1</i>	<i>MAP2K1</i>	<i>MAP2K2</i>	<i>TMEM43</i>
<i>MEGF10</i>	<i>MIB1</i>	<i>MSTN</i>	<i>TNNC1</i>
<i>MTM1</i>	<i>MTO1</i>	<i>MYBPC1</i>	<i>TNNT2</i>
<i>MYBPC3</i>	<i>MYF6</i>	<i>MYH2</i>	<i>TTN</i>
<i>MYH6</i>	<i>MYH7</i>	<i>MYL2</i>	<i>VCP</i>
<i>MYL3</i>	<i>MYLK2</i>	<i>MYOT</i>	<i>PTPN11</i>
<i>MYOZ2</i>	<i>MYPN</i>	<i>NEB</i>	<i>RANGRF</i>
<i>NEBL</i>	<i>NEXN</i>	<i>NF1</i>	<i>RRAGC</i>
<i>NOS1AP</i>	<i>NPPA</i>	<i>NRAS</i>	<i>SCN5A</i>
<i>OBSCN</i>	<i>PABPN1</i>	<i>PDLIM3</i>	<i>SCO2</i>
<i>PKP2</i>	<i>PLEC</i>	<i>PLN</i>	<i>SGCD</i>
<i>PRDM16</i>	<i>PRKAG2</i>	<i>PSEN1</i>	<i>SLC25A20</i>
<i>PTRF</i>	<i>RBM20</i>	<i>RYR2</i>	<i>SCNN1B</i>
<i>SDHA</i>	<i>SGCG</i>		

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