ORIGINAL RESEARCH

KLF13 Loss-of-Function Mutations Underlying Familial Dilated Cardiomyopathy

Yu-Han Guo, MD*; Jun Wang, MD*; Xiao-Juan Guo, MD*; Ri-Feng Gao, MD; Chen-Xi Yang, MD; Li Li, PhD; Yu-Min Sun, MD; Xing-Biao Qiu, MD; Ying-Jia Xu, MD; Yi-Qing Yang, MD

BACKGROUND: Dilated cardiomyopathy (DCM), characterized by progressive left ventricular enlargement and systolic dysfunction, is the most common type of cardiomyopathy and a leading cause of heart failure and cardiac death. Accumulating evidence underscores the critical role of genetic defects in the pathogenesis of DCM, and >250 genes have been implicated in DCM to date. However, DCM is of substantial genetic heterogeneity, and the genetic basis underpinning DCM remains elusive in most cases.

METHODS AND RESULTS: By genome-wide scan with microsatellite markers and genetic linkage analysis in a 4-generation family inflicted with autosomal-dominant DCM, a new locus for DCM was mapped on chromosome 15q13.1–q13.3, a 4.77-cM (≈3.43 Mbp) interval between markers D15S1019 and D15S1010, with the largest 2-point logarithm of odds score of 5.1175 for the marker D15S165 at recombination fraction (θ)=0.00. Whole-exome sequencing analyses revealed that within the mapping chromosomal region, only the mutation in the KLF13 gene, c.430G>T (p.E144X), cosegregated with DCM in the family. In addition, sequencing analyses of KLF13 in another cohort of 266 unrelated patients with DCM and their available family members unveiled 2 new mutations, c.580G>T (p.E194X) and c.595T>C (p.C199R), which cosegregated with DCM in 2 families, respectively. The 3 mutations were absent from 418 healthy subjects. Functional assays demonstrated that the 3 mutants had no transactivation on the target genes ACTC1 and MYH7 (2 genes causally linked to DCM), alone or together with GATA4 (another gene contributing to DCM), and a diminished ability to bind the promoters of ACTC1 and MYH7. Add, the E144X-mutant KLF13 showed a defect in intracellular distribution.

CONCLUSIONS: This investigation indicates KLF13 as a new gene predisposing to DCM, which adds novel insight to the molecular pathogenesis underlying DCM, implying potential implications for prenatal prevention and precision treatment of DCM in a subset of patients.

Key Words: biological assay ■ dilated cardiomyopathy ■ functional genomics ■ KLF13 ■ molecular genetics ■ transcriptional factor

Dilated cardiomyopathy (DCM), defined by left ventricular enlargement with systolic dysfunction at the exclusion of other usual clinically detectable causes responsible for impaired global contractile function, is the most prevalent form of myocardial disease in humans, with an estimated prevalence of up to 1 in 250 individuals worldwide.1–3 Notably, there is a significant sex difference in the incidence of DCM, with an overall combined mean male:female sex ratio of 2.5:1.4 DCM confers a significantly enhanced risk for heart failure, ventricular and supraventricular dysrhythmias, cardiac transplantation, and death.5–9 Factually, DCM is the second most prevalent cause of heart failure next to coronary heart disease, accounting for 30% to 40% of all heart failures, and the most common indication for cardiac transplantation worldwide, with an average 5-year survival rate of approximately 50% after diagnosis of DCM, though a significant improvement of heart function has been achieved by evidence-based therapy for heart failure.4,10–14 It was reported that in female patients the annual incidence of...
What Is New?
• By genome-wide screening with microsatellite markers and genetic linkage analysis in a 4-generation family suffering from autosomal-dominant dilated cardiomyopathy (DCM), a new locus for DCM was mapped on chromosome 15q13.1–q13.3.
• A novel mutation in the KLF13 gene, c.430G>T (p.E144X), was identified by whole-exome sequencing analysis to cosegregate with DCM in the family within the mapping chromosomal region; sequencing analyses of KLF13 in another cohort of 266 unrelated patients with DCM and their available family members revealed 2 novel mutations, c.580G>T (p.E194X) and c.595T>C (p.C199R), which cosegregated with DCM in 2 families, respectively.
• The 3 mutants had no transactivation on the target genes ACTC1 and MYH7, alone or together with GATA4, and the 3 mutations showed a diminished ability to bind the promoters of ACTC1 and MYH7; the E144X-mutant KLF13 showed a defect in intracellular distribution.

What Are the Clinical Implications?
• The discovery of a new DCM-causative gene has potential implications for antenatal prophylaxis and precise management of DCM.
• The DCM-associated genetic data may be used for prognostic risk stratification of patients with DCM.

Nonstandard Abbreviations and Acronyms

DCM  dilated cardiomyopathy
WES  whole-exome sequencing

DCM-related death was about 2%, whereas in male patients, the annual incidence of DCM-causative death was nearly 4%, and in the United States alone, DCM contributed to ~46,000 hospitalizations and ~10,000 deaths annually.3,15 Obviously, DCM causes substantial morbidity and mortality as well as medical care cost, imposing an enormous socioeconomic burden on humans. Despite the clinical significance, the molecular pathogenesis underlying DCM remains incompletely understood.

Epidemiological investigations demonstrate that DCM is frequently associated with miscellaneous structural heart diseases, systemic comorbidities, and other environmental predisposing factors, including valvular heart disease, coronary heart disease, tachycardia-mediated cardiomyopathy, viral myocarditis, neuromuscular disease, essential hypertension, autoimmune disorder, endocrine homeostatic imbalance, inadequate epigenetic modifier, nutritional deficiency, hemochromatosis, systemic amyloidosis, alcohol abuse, and exposure to cardiotoxic chemicals.3,4,11,16 However, there is accumulating evidence highlighting the pivotal role of genetic defects in the pathogenesis of DCM, especially for familial DCM, which is considered when ≥2 first-degree relatives have been diagnosed with DCM or have experienced sudden cardiac death with ages ≤35 years.1,3,4 Familial DCM, accounting for approximately 20% to 50% of all DCM, is predominantly inherited in an autosomal-dominant mode, although other inheritance patterns, encompassing autosomal-recessive, X-linked recessive, and mitochondrial inheritance, are also reported.17 To date, DCM-causing variations in >250 genes have been implicated with the development of DCM, of which the vast majority code for sarcomere proteins, nuclear envelope proteins, cytoskeleton proteins, Z-band proteins, intercalated disc proteins, ion channel proteins, gap junction channel proteins, RNA-binding proteins, mitochondrial proteins, and transcriptional factor proteins.1–4,18–55 Additionally, genome-wide association studies have led to the discovery of many new common variants involved in DCM, in addition to novel rare variants involved in DCM.33 Nevertheless, the genetic architecture of DCM is highly complex and diverse owing to pronounced genetic heterogeneity, and the genetic determinants underpinning DCM in a large proportion of cases remain to be identified.56

METHODS

The supporting data are available within the article, its online supplemental files, and upon reasonable request. Data analysis came from our institutional laboratories, including the cardiovascular research laboratory and central laboratory of Shanghai Fifth People’s Hospital, Fudan University, Shanghai, China.

Recruitment of Study Participants

For the current research, a 43-member Chinese family spanning 4 generations with a high incidence of DCM was identified, and the available family members were recruited. Another cohort of 266 probands with DCM and their available family members were enlisted. A total of 418 unrelated healthy individuals, who were matched with the patients for ethnicity, sex, and age, were enrolled as controls. This cohort of DCM patients with DCM and their family members was specifically included for the purpose of this study, and the same for the healthy individuals. They were recruited from the inpatient and outpatient individuals of the Shanghai Fifth People’s Hospital. All research participants underwent
a comprehensive clinical assessment, encompassing a thorough review of personal, familial, and medical histories, detailed physical examination, standard 12-lead electrocardiogram, transthoracic echocardiogram, and routine laboratory tests. All patients with DCM also experienced coronary artery angiography and exercise performance tests, but cardiovascular magnetic resonance imaging, coronary computed tomography angiography, and endomyocardial biopsy were performed only when indicated. DCM was diagnosed as described elsewhere: a left-ventricular end-diastolic diameter >117% of the predicted value corrected for body surface area and age and a left-ventricular ejection fraction <45% and/or a fractional shortening <25%, in the absence of abnormal loading conditions, coronary artery disease, toxin exposure, and other systemic diseases. 2,57 The inclusion criteria for the patients with DCM were consistent with the diagnosis of DCM. 2 The exclusion criteria included peripartum DCM and secondary DCM caused by other cardiovascular diseases, systemic diseases, or known environmental risk factors, including rheumatic heart disease, ischemic heart disease, viral myocarditis, primary hypertension, metabolic disorder, and exposure to toxic chemicals. The current investigation involving human participants complies with the tenets of the Declaration of Helsinki. All study procedures used in this research were approved by the Medical Ethics Committee of Shanghai Fifth People’s Hospital, Fudan University, Shanghai, China (approval number 2020–011). Before recruitment into the study, all study participants signed informed consent. Approximately 6 mL of whole blood sample was collected from each study subject.

**Genome-Wide Screening With Microsatellite Markers and Genetic Linkage Analysis**

Genomic DNA was extracted from blood leukocytes using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Genome-wide screening for genotyping in Family 1 with DCM was implemented with the ABI PRISM Linkage Mapping Set (Applied Biosystems, Foster City, CA), using 386 polymorphic microsatellite markers spaced at a mean resolution of ≈10 cM from chromosomes 1 to 22. Multiplex amplification of markers was conducted using the AmpTaq Gold DNA Polymerase (Applied Biosystems) by polymerase chain reaction (PCR) under the T100 Thermal Cycler (Bio-Rad, Hercules, CA). Genotyping was carried out on the ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems) as per the manufacturer’s protocol. Genetic linkage analysis was made with the software Statistical Analysis for Genetic Epidemiology (http://darwin.cwru.edu/sage/), under an autosomal-dominant mode of inheritance with the allele frequency and penetrance rate of DCM being set at 0.1% and 95%, respectively. 58 A 2-point logarithm of odds (LOD) score between each marker and the disease locus was calculated as described previously. 59 To finely map the chromosomal region determined by the marker D15S165 with an initial supportive LOD score, 5 additional markers (D15S1002, D15S1019, D15S3976, D15S1010, and D15S144) near the region were genotyped to delimit the critical chromosomal interval by refining the recombination boundaries. The distances between loci and marker order were derived from the Généthon genetic map. 58 A haplotype map of Family 1 with DCM was constructed with the software Cyrillic v2.1.3 (Cherwell Scientific, Oxford, UK) to reveal the shared genomic regions among affected members from the DCM family and confine the recombination boundaries.

**Sequencing Analysis of the Candidate Genes Within the Mapped Chromosomal Region**

Whole-exome sequencing (WES) and bioinformatic analyses in 3 affected family members (II-5, III-11, and III-16) and 2 unaffected family members (II-6 and III-14) from Family 1 were completed as described elsewhere. 59–62 Briefly, for each family member selected to undergo WES, 3 μg of genomic DNA were used for construction of a whole exome library, which was captured with the SureSelect Human All Exon V6 Kit (Agilent Technologies, Santa Clara, CA) following the manufacturer’s manual. The captured exome libraries were sequenced on the Illumina HiSeq 2000 Genome Analyzer (Illumina, San Diego, CA), with the HiSeq Sequencing Kit (Illumina) according to the manufacturer’s protocol. Raw image data were processed by the Pipeline software to call bases and generate the set of sequence reads. Sequence reads were mapped to the human referential genome (hg19, GRCh37) through the Burrows-Wheeler Aligner software. Sequence variants were called via the GATK software. When a potential DCM-causative variation was identified by WES analysis within the mapped chromosomal region, Sanger sequencing analysis in the whole family with DCM was fulfilled to confirm it. Once a gene possessing an identified DCM-causing variation was discovered, Sanger sequencing analysis of the gene was carried out in another cohort of 266 index patients with DCM, the available family members of the index patients harboring an identified DCM-causing variation, and 418 unrelated healthy subjects. For a verified deleterious mutation, such population genetics databases as the UK Biobank (https://www.ukbiobank.ac.uk/), the Single Nucleotide Polymorphism database (https://www.ncbi.nlm.nih.gov/snp/), and the Genome...
Aggregation Database (http://gnomad.broadinstitute.org/) were consulted to check its novelty.

**Construction of Eukaryotic Gene Expression Plasmids**

Isolation of total mRNA from discarded human heart tissue and production of cDNA by reverse-transcription PCR were described previously. The full-length cDNA of the human KLF13 gene (GenBank accession no. NM_015995.4) was yielded by PCR amplification of the produced cDNA with the PfuUltra High-Fidelity DNA Polymerase (Stratagene, Santa Clara, CA) and a specific pair of primers (forward primer: 5′-GAGGATTCTCGGATCCGCGGCTGACGAC-3′; reverse primer: 5′-CTCTCTAGAGCGGCTGCTATGGCTGTC-3′). The yielded KLF13 cDNA was doubly digested with restriction endonucleases EcoRI (NEB, Ipswich, MA) and XbaI (NEB) and then ligated to the plasmid pcDNA3.1 (Invitrogen, Carlsbad, CA) at the EcoRI-XbaI sites to create the eukaryotic gene expression plasmid of wild-type (WT) KLF13-pcDNA3.1. Each mutant-type KLF13-pcDNA3.1 plasmid was generated by site-targeted mutagenesis of WT KLF13-pcDNA3.1 with the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA), as well as a complementary pair of primers, and was confirmed by Sanger sequencing analysis. Similarly, the entire open reading frame of the human GATA4 gene (GenBank accession no. NM_001308093.3) was yielded by PCR amplification of the produced cDNA with the PfuUltra High-Fidelity DNA Polymerase (Stratagene) and a specific pair of primers (forward primer: 5′-ACTGAAATTCGGTGACCTGCG-3′; reverse primer: 5′-AGTTCTAGACAGCGCGCTGCTATGGCTGTC-3′), cut with EcoRI (NEB) and XbaI (NEB), and then inserted to the plasmid pcDNA3.1 (Invitrogen) at the EcoRI-XbaI sites to construct WT GATA4-pcDNA3.1. A 680-bp genomic DNA fragment (from −620 to +60, with the transcriptional start site numbered as +1) of the human ACTTC1 gene (GenBank accession no. NC_000015.10) was amplified from human genomic DNA by PCR with a specific pair of primers, and was confirmed by Sanger sequencing analysis. Similarly, the entire open reading frame of the human MYH7 gene (GenBank accession no. NC_000014.9) was amplified with a specific pair of primers (forward primer: 5′-GCGAGCCCAGACCCAGCTCCTCT-3′; reverse primer: 5′-GGACTGAGCCGCGCTGCTACTCAGCG-3′), digested with NheI (NEB) and XhoI (NEB), and inserted into the pGL3-Basic vector (Promega) to construct the MYH7 promoter-driven firefly luciferase reporter (MYH7-luc).

**Cell Culture, Transfection, and Dual-Luciferase Assays**

Hela cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% fetal bovine serum (Invitrogen) in an atmosphere with 5% CO₂ at 37 °C. Twenty-four hours before transient transfection using the Lipofectamine™ 3000 Transfection Reagent (Invitrogen), cells were seeded into 24-well plates at a density of 1×10⁵ cells per well. Different amounts (ranging from 0.025 to 0.8μg) of WT KLF13-pcDNA3.1 were used to assess its dose-dependent activation of ACTC1-luc and MYH7-luc (1.0μg, respectively). For the analysis of the transactivation of ACTC1 promoter by KLF13, Hela cells were transfected with 0.4μg of empty pcDNA3.1 or 0.4μg of WT KLF13-pcDNA3.1 or 0.4μg of mutant KLF13-pcDNA3.1 or 0.2μg of WT KLF13-pcDNA3.1 plus 0.2μg of empty pcDNA3.1 or 0.2μg of WT KLF13-pcDNA3.1 plus 0.2μg of mutant KLF13-pcDNA3.1, together with 1.0μg of ACTC1-luc and 0.04μg of pRL-TK (Promega). For the analysis of the transactivation of MYH7 promoter by KLF13, the quantity of WT or mutant KLF13-pcDNA3.1 or empty pcDNA3.1 were halved whereas the amount of MYH7-luc or pRL-TK (Promega) remained the same as described previously. For measurement of the synergistic transcriptional activation between KLF13 and GATA4, the same amount (0.1μg) of empty pcDNA3.1, WT KLF13-pcDNA3.1, mutant KLF13-pcDNA3.1, or GATA4-pcDNA3.1 was used alone or together, in the presence of 1.0μg of ACTC1-luc or MYH7-luc and 0.04μg of pRL-TK (Promega). The pRL-TK plasmid expressing Renilla luciferase (Promega) was used for the normalization of transfection efficiency. Cells were harvested 48 hours after transfection and the luciferase activity was assayed by using the Dual-Luciferase Reporter Assay System (Promega). The promoter activity value was expressed as fold activation of firefly luciferase to Renilla luciferase. Experiments were conducted independently 3 times in triplicate for each sample.

**Nuclear Protein Isolation and Electrophoretic Mobility Shift Analysis**

Nuclear extracts were prepared from Hela cells transfected with 10μg of WT KLF13-pcDNA3.1 or mutant KLF13-pcDNA3.1 by using the NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific, Rockford, IL) according to the manufacturer’s instructions. Oligonucleotide probes used for electrophoretic mobility shift analysis were synthesized, purified, and 5′ end-labeled with biotin. For the binding reactions, biotinylated probes (0.2 pmol) were incubated...
with nuclear extracts (5 μg) and the binding buffer (Beyotime Biotechnology, Shanghai, China) for 20 minutes at room temperature. Of note, unlabeled cold probes (20 pmol) were preincubated with the nuclear extracts for 10 minutes. The mixture was fractionated on a 6% nondenaturing polyacrylamide gel in 0.5x Tris-borate-EDTA buffer at 100V for 1 hour, transferred to a nylon membrane (Beyotime Biotechnology) in ice-cold 0.5x Tris-borate-EDTA buffer at 380mA for 30 minutes, and crosslinked by ultraviolet irradiation for 20 minutes. The biotin-labeled probe was detected by using the streptavidin-horseradish peroxidase conjugate and the chemiluminescent substrate (Beyotime Biotechnology) according to the manufacturer’s instructions.

**Cellular Immunofluorescence Assay of the KLF13 Proteins**

Hela cells (5 × 10⁴) grown on glass coverslips in a 12-well plate were transfected with 0.4 μg of WT or mutant KLF13-pcDNA3.1. After 48 hours, cells were harvested and fixed with 4% paraformaldehyde for 15 minutes, heated in antigen retrieval buffer at 95 °C for 10 minutes, and permeabilized with 0.5% TritonX-100 for 20 minutes. Subsequently, cells were blocked with 3% bovine serum albumin at room temperature for 30 minutes and incubated with rabbit anti-KLF13 polyclonal antibody (Affinity, 1:100) overnight at 4 °C, followed by incubation with the conjugated secondary antibody, goat antirabbit Alexa-Flour 594 (Affinity, 1:100) for 1 hour at room temperature in the dark. Nuclear staining was conducted by using 4′,6-diamidino-2-phenylindole for 1 minute. Finally, coverslips were mounted with ProLong Glass Antifade Mountants (Thermo Fisher Scientific, Waltham, MA) and sealed with clear nail polish. The images were acquired by using confocal laser-scanning fluorescence microscopy (Leica, Mannheim, Germany) under an oil objective (Leica).

**Statistical Analysis**

Continuous variables were presented as mean±SD and compared between the 2 groups using the Student’s unpaired t test. Categorical variables were expressed as numbers and percentages and compared between the 2 groups using the Fisher’s exact test.

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**Figure 1.** Pedigree and haplotypes for Family 1 affected with dilated cardiomyopathy.

The 4-generation family with a high incidence of autosomal-dominant dilated cardiomyopathy was designated as Family 1, with members identified by generations-numbers (Roman-Arabic numerals) shown below member symbols. A vertical bar beneath a member denotes the chromosomal region defined by genetic linkage analysis. Polymorphic markers spanning the linkage region on chromosome 15q13.1–q13.3 were displayed to the left of the pedigree, with members’ genotypes (represented by numbers) for markers given next to the chromosome bars. The filled bars mean disease haplotype. Key recombination events occurred with marker D15S1019 in member III-16, and with marker D15S1010 in member III-3. “+” indicates a carrier of the heterozygous mutation of c.430G>T in the KLF13 gene; an “−” indicates a noncarrier.
test. A 2-tailed P value of <0.05 was considered statistically significant.

RESULTS
Demographic and Clinical Features of the Research Participants

As shown in Figure 1, a 43-member 4-generation family suffering from autosomal-dominant DCM (Family 1) was identified from the Chinese Han population, including 40 living family members (19 male members and 21 female members, with ages ranging from 21 to 69 years). The proband of Family 1 (II-5, Figure 1) was initially diagnosed with DCM at the age of 45 years and received pharmacologic therapy for heart failure. His brother (II-1, Figure 1) and father (I-1, Figure 1) had a past medical history of DCM and died of progressive congestive heart failure caused by DCM at ages 63 and 69 years, respectively. In Family 1 (Figure 1), all the affected members had echocardiogram-documented DCM, whereas the unaffected family members had neither history of DCM nor symptoms of DCM, with normal echocardiographic findings. None had structural heart diseases or other diseases contributing to DCM, such as valvular heart disease, coronary heart disease, viral myocarditis, and essential hypertension. The demographic and baseline clinical features of the affected family members alive from Family 1 are summarized in Table 1. Additionally, another cohort of 266 probands inflicted with DCM was clinically investigated in contrast to a total of 418 unrelated healthy subjects without a history of DCM and found that the control individuals (217 male people and 201 female people, with a mean age of 48 years ranging between 36 and 64 years) were matched with the DCM probands (136 male patients and 130 female patients, with an average age of 48 years varying from 36 to 64 years) for ethnicity, age, and sex, as shown in Table S1.

Mapping of a Novel Genetic Locus for DCM on Chromosome 15q13.1–q13.3

A genome-wide scan was fulfilled in the 40 living members from Family 1 with a high incidence of DCM (Figure 1) with 386 microsatellite markers with a 10 cM-resolution. Genetic linkage analysis suggested a significant linkage of DCM to marker D15S165, with a maximum 2-point LOD score of 5.117510 at recombination fraction (θ)=0.00 (Table 2). The 2-point LOD scores for D15S165 remained >3 regardless of variations in the penetrance from 60% to 99% and variations in the phenocopy prevalence from 0% to 5%. The pairwise LOD scores indicated no significant linkage to markers in other chromosomal regions (Table 2). To confine the DCM-linked chromosomal region, 5 additional markers (D15S1002, D15S1019, D15S3976, D15S1010, and D15S144) near the marker D15S165 were genotyped, and based on haplotype analysis (Figure 1), a novel genetic locus for DCM was ultimately mapped to chromosome 15q13.1–q13.3 (GRCh38, chr15:29371221-32798467), a 4.77-cM (=3.43 Mbp) interval between markers D15S1019 and D15S1010. There are 99 genes defined within the locus between markers D15S1019 and D15S1010, including 27 protein-encoding genes, 35 nonencoding RNA genes, and 37 pseudogenes (Table S2).

Identification of DCM-Causative Mutations in KLF13

WES was carried out for 3 affected members (II-5, III-11, and III-16) and 2 unaffected members (II-6 and III-14) from Family 1. Analysis of the candidate genes within the mapping locus from marker D15S1019 to D15S1010,

Table 1. Demographic and Phenotypic Characteristics of the Living Pedigree Members Inflicted With Dilated Cardiomyopathy Harboring the Heterozygous KLF13-E144X Mutation

| Individual (Family 1) | Sex | Age, y | Phenotype | LVEDD, mm | LVESD, mm | LVEF, % | LVFS, % |
|----------------------|-----|--------|-----------|-----------|-----------|--------|--------|
| II-4                 | F   | 68     | DCM       | 69        | 60        | 31     | 15     |
| II-5                 | M   | 66     | DCM       | 63        | 54        | 33     | 17     |
| II-9                 | M   | 61     | DCM, ASD  | 77        | 66        | 29     | 14     |
| III-2                | F   | 47     | DCM       | 68        | 55        | 38     | 19     |
| III-3                | M   | 45     | DCM       | 74        | 63        | 30     | 16     |
| III-9                | M   | 44     | DCM       | 56        | 48        | 30     | 14     |
| III-11               | M   | 44     | DCM       | 60        | 50        | 39     | 19     |
| III-16               | F   | 40     | DCM       | 58        | 46        | 47     | 24     |
| III-19               | M   | 36     | DCM, ASD  | 56        | 44        | 44     | 22     |
| IV-2                 | F   | 22     | DCM       | 54        | 43        | 43     | 29     |
| IV-6                 | M   | 21     | DCM       | 53        | 41        | 46     | 24     |

ASD indicates atrial septal defect; DCM, dilated cardiomyopathy; F, female; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; LVFS, left ventricular fractional shortening; and M, male.
only the mutation chr15: 31619845G>T (GRCh37.p13: NC_000015.9), equivalent to chr15:31327642G>T (GRCh38.p14: NC_000015.10) or NM_015995.4: c.430G>T in the KLF13 gene, coding for an important cardiac transcription factor, was identified and validated to be in cosegregation with DCM in Family 1 by Sanger sequencing analysis. The sequence chromatograms illustrating the heterozygous c.430G>T variation in KLF13 and its WT control sequences are given in Figure 2A. Notably, in the mapped chromosomal region, only the rare variant in the KLF13 gene was confirmed to cosegregate with DCM in the family, and no other rare variants were shared by all affected family members and were absent in the unaffected family members. Additionally, via sequencing analysis of the coding regions and splicing junctions of KLF13 in an other cohort of 266 index patients inflicted with DCM, 2 new KLF13 mutations, NM_015995.4:c.580G>T;p.E194X and NM_015995.4:c.595T>C;p.C199R, were discovered in 2 index patients, respectively. The sequence electropherograms showing the heterozygous KLF13 mutations c.580G>T and c.595T>C and their WT control sequences are given in Figure 2B and 2C, respectively. The pedigrees of the 2 index patients carrying the discovered KLF13 mutations from Family 2 and Family 3 are displayed in Figure 3. Genetic analysis of the available members from Family 2 or Family 3 showed that the discovered KLF13 mutation cosegregated with autosomal-dominant DCM in the whole family, with complete penetrance. The demographic and baseline clinical characteristics of the affected family members alive from Family 2 and Family 3 are summarized in Table 3. Notably, these families had high incidences of miscarriages, of which women members III-2 and III-16 in Family 1, member III-6 in Family 2, and member III-4 in Family 3 had a history of miscarriage. The 3 heterozygous KLF13 mutations were neither observed in 418 unrelated controls nor released in multiple population genetics databases including the UK Biobank, the Single Nucleotide Polymorphism database, and the Genome Aggregation Database, indicating novel mutations.

### Failed Transactivation Function of the Mutant KLF13 Proteins

As shown in Figure 4A and 4C, WT KLF13 transcriptionally activated the ACTC1 or MYH7 promoter in a dose-dependent pattern. Specifically, 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 μg of WT KLF13-pcDNA3.1 transcriptionally activated the ACTC1 promoter in Hela cells by ≈18 (18.1939±0.8119)-fold, ≈22 (21.8076±0.4779)-fold, ≈27 (26.6103±0.4405)-fold, ≈33 (33.4114±0.8934)-fold, and ≈44 (44.3071±1.6442)-fold, respectively (Figure 4A); while 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 μg of WT KLF13-pcDNA3.1 transcriptionally activated the MYH7 promoter in Hela cells by ≈33 (33.7500±1.6561)-fold, ≈44 (44.4307±1.6442)-fold, respectively (Figure 4A); while 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 μg of WT KLF13-pcDNA3.1 transcriptionally activated the MYH7 promoter in Hela cells by ≈49 (48.5386±2.2430)-fold whereas homozygous E144X-

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**Table 2. Two-Point Logarithm of Odds Scores for the Markers on Chromosome 15q13.1-q13.3 at Different Recombination Fractions (θ) in Family 1 With Dilated Cardiomyopathy**

| Marker       | LOD scores at θ |
|--------------|------------------|
|              | 0.00  | 0.01  | 0.05  | 0.10  | 0.20  | 0.30  | 0.40  |
| D1SS975      | ↓∞    | 0.063799 | 1.283779 | 1.619547 | 1.580413 | 1.169251 | 0.545682 |
| D1SS1002     | ↓∞    | 1.339973 | 2.415505 | 2.594912 | 2.276644 | 1.590878 | 0.691651 |
| D1SS1019     | 5.117510 | 5.033457 | 4.694259 | 4.248125 | 3.276644 | 2.180703 | 0.964652 |
| D1SS165      | 5.117510 | 5.033457 | 4.694259 | 4.248125 | 3.276644 | 2.180703 | 0.964652 |
| D1SS976      | 4.816480 | 4.737913 | 4.415505 | 3.992852 | 3.072524 | 2.034575 | 0.885471 |
| D1SS1010     | 3.913990 | 3.847918 | 3.579244 | 3.227035 | 2.460170 | 1.596620 | 0.658374 |
| D1SS144      | ↓∞    | 2.742278 | 3.136751 | 3.038610 | 2.470470 | 1.667027 | 0.719827 |
| D1SS1007     | ↓∞    | 2.320679 | 2.733177 | 2.685948 | 2.144605 | 1.408812 | 0.569412 |

θ indicates recombination fraction; and LOD, logarithm of odds.
Figure 2. New KLF13 mutations responsible for dilated cardiomyopathy. Sequencing electropherograms showing 2 nonsense mutations and 1 missense mutation in the KLF13 gene as well as their corresponding wild-type sequences were exhibited. Arrows direct to the heterozygous mutations or homozygous wild-type sequences. A, Sequencing electropherograms of the affected proband and an unaffected member from Family 1, showing the heterozygous KLF13 mutation c.430G>T and its wild-type control. B, Sequencing electropherograms of the affected proband and an unaffected member from Family 2, showing the heterozygous KLF13 mutation c.580G>T and its wild-type control. C, Sequencing electropherograms of the affected proband and an unaffected member from Family 3, showing the heterozygous KLF13 mutation c.595T>C and its wild-type control.
E194X- and C199R-mutant KLF13 activated the MYH7 promoter by \( \approx 14 \) \((13.7403\pm 1.6790)-\)fold, \( \approx 13 \) \((13.0757\pm 1.1269)-\)fold, and \( \approx 15 \) \((15.2651\pm 2.0540)-\)fold, respectively (WT versus E144X: \( t =21.5118, P =0.0003 \); WT versus E194X: \( t =24.4700, P =0.0001 \); WT versus C199R: \( t =18.9490, P =0.0005 \)); whereas heterozygous E144X-, E194X-, and C199R-mutant KLF13 activated the MYH7 promoter by \( \approx 20 \) \((20.2243\pm 0.8723)-\)fold, \( \approx 22 \) \((21.9228\pm 1.0852)-\)fold, and \( \approx 22 \) \((22.0585\pm 1.2527)-\)fold, respectively (WT versus WT+E144X: \( t =20.3774, P =0.0002 \); WT versus WT+E194X: \( t =18.5013, P =0.0003 \); WT versus WT+C199R: \( t =17.8525, P =0.0004 \)). These results indicate that E144X-, E194X-, and C199R-mutant KLF13 proteins have decreased transcriptional activation in either homozygous or heterozygous status compared with their WT counterparts.

Disrupted Synergistic Transactivation Between Mutant KLF13 and GATA4

As shown in Figure 5A, the same amount (0.1 \( \mu \)g) of WT, E144X-, E194X-, and C199R-mutant KLF13-pcDNA3.1 transactivated the ACTC1 promoter by \( \approx 22 \) \((21.8553\pm 2.1319)-\)fold, \( \approx 3 \) \((3.0649\pm 0.2210)-\)fold, \( \approx 3 \) \((3.0519\pm 0.0946)-\)fold, and \( \approx 3 \) \((2.9397\pm 0.3180)-\)fold, respectively (WT versus E144X: \( t =12.4275, P =0.0006 \); WT versus E194X: \( t =12.4710, P =0.0006 \); WT versus C199R: \( t =12.4631, P =0.0006 \)); when cotransfected with 0.1 \( \mu \)g of GATA4-pcDNA3.1, the transcriptional activation effect of each group became \( \approx 43 \) \((43.0359\pm 2.7444)-\)fold, \( \approx 6 \) \((6.3061\pm 0.7807)-\)fold, \( \approx 7 \) \((6.5421\pm 0.8767)-\)fold and \( \approx 6 \) \((5.7587\pm 0.3689)-\)fold, respectively (WT+GATA4 versus E144X+GATA4: \( t =22.2965, P =0.0001 \); WT+GATA4 versus E194X+GATA4: \( t =21.9398, P =0.0001 \); WT+GATA4 versus C199R+GATA4: \( t =23.3169, P =0.0001 \)). Similarly, as shown in Figure 5B, 0.1 \( \mu \)g of the WT, E144X-, E194X-, and C199R-mutant KLF13-pcDNA3.1 transcriptionally activated the MYH7 promoter by \( \approx 36 \) \((36.3427\pm 1.3384)-\)fold, \( \approx 10 \) \((9.6471\pm 0.3500)-\)fold, \( \approx 11 \) \((10.7531\pm 0.3837)-\)fold, and \( \approx 11 \) \((10.3900\pm 0.8694)-\)fold, respectively (WT versus E144X: \( t =33.4224, P <0.0001 \); WT versus E194X: \( t =28.5768, P =0.0001 \); WT versus C199R: \( t =28.1653, P =0.0001 \)); whereas in the presence of GATA4-pcDNA3.1, the transcriptional activation effect became

### Table 3. Clinical Features and KLF13 Mutations of the Living Family Members Suffering From Dilated Cardiomyopathy

| Family | Individual | Sex | Age, y | Phenotype | LVEDD, mm | LVESD, mm | LVEF, % | LVFS, % | KLF13 mutations |
|--------|------------|-----|--------|-----------|-----------|-----------|--------|--------|-----------------|
| Family 2 | II-8 | F | 62 | DCM | 77 | 66 | 29 | 14 | c.580G>T |
| | III-3 | M | 66 | DCM | 57 | 47 | 37 | 18 | c.580G>T |
| | III-6 | M | 61 | DCM, atrial septal defect | 62 | 53 | 32 | 16 | c.580G>T |
| | III-10 | F | 47 | DCM | 57 | 46 | 40 | 20 | c.580G>T |
| | IV-2 | M | 45 | DCM | 56 | 44 | 44 | 22 | c.580G>T |
| Family 3 | III-3 | M | 65 | DCM | 63 | 55 | 26 | 13 | c.595T>C |
| | III-4 | F | 41 | DCM | 58 | 45 | 43 | 22 | c.595T>C |

DCM indicates dilated cardiomyopathy; F, female; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; LVFS, left ventricular fractional shortening; and M, male.
Diminished DNA-Binding Ability of the Mutant KLF13 Proteins

As is shown in Figure 6, nuclear extracts form Hela cells transfected with WT KLF13-pcDNA3.1 had the ability to specifically bind to biotinylated ACTC1- or MYH7-DNA probes because the binding could be competed by the corresponding excess cold probes. The ability of E144X-, E194X-, or C199R-mutant KLF13 protein to bind to the ACTC1-DNA probes diminished to an undetectable level (Figure 6A). Similarly, E144X-, E194X-, or C199R-mutant KLF13 protein showed a diminished binding ability to the MYH7-DNA probes (Figure 6B).

Distinct Nuclear Distribution of the Mutant KLF13 Proteins

As shown in Figure 7, in transfected Hela cells, WT, E144X-, E194X-, or C199R-mutant KLF13 protein was normally
localized to the cellular nuclei. However, the E144X-mutant KLF13 protein was distributed not only in the nuclei but also in the cytoplasm.

**DISCUSSION**

In the current investigation, by sequencing analysis of the candidate genes in the mapped region, human chromosome 15q13.3, 3 mutations in the **KLF13** gene, c.430G>T (p.E144X), c.580G>T (p.E194X), and c.595T>C (p.C199R), were identified to cosegregate with the DCM phenotype in 3 families, respectively, with complete penetrance. Functional assays demonstrated that the 3 mutants had no transactivation on the target genes **ACTC1** and **MYH7**, 2 genes causally linked to DCM.\(^1\)–\(^3\),\(^63\)–\(^65\) Furthermore, the 3 mutations abrogated the synergistic transactivation between KLF13 and GATA4, another gene contributing to DCM.\(^66\)–\(^68\) Besides, the 3 mutants failed to bind the promoters of **ACTC1** and **MYH7**, and the E144X-mutant KLF13 showed a defect in cellular nuclear distribution. Hence, it is very likely that genetically defective **KLF13** contributes to DCM in these mutation carriers.

In human beings, **KLF13** maps on chromosome 15q13.3, coding for a Krüppel-like transcription factor (KLF) with 288 amino acids, which belongs to a family of transcription factors containing 3 classical zinc finger DNA-binding domains and binds to GC-rich DNA sequences as well as related GT and CACCC boxes in the promoters of target genes.\(^69\) In addition to the DNA-binding domains, which function to bind the promoters of target genes and interact with other transcriptionally cooperative partners, the KLF13 protein possesses 3 other key structural domains, encompassing a transcriptional activation domain, which serves to transactivate the expression of downstream genes, a transcriptional inhibition domain, which works to transcriptionally inhibit the expression of target genes, and a nuclear localization signal responsible for cellular nuclear localization.\(^70\) Previous studies have demonstrated that KLF13 is highly expressed in the hearts of both humans and vertebrates during the whole life from embryos to adults and plays a pivotal role in cardiovascular morphogenesis and postnatal structural remodeling.\(^71\)–\(^77\) Recently, multiple experimental investigations together with our present research have substantiated that KLF13 transactivates the expression of multiple downstream genes, including **MYH7**, **ACTC1**, **CCND1**, **NPPB**, **NPPA**, and **VEGFA**, alone or synergistically with GATA4, GATA6, and TBX5.\(^72\)–\(^74\) Moreover, pathogenetic mutations in its target genes **MYH7** and **ACTC1** as well as its transcriptionally cooperative partners GATA4, GATA6, and TBX5 have been reported to cause DCM in humans.\(^63\)–\(^68,78\)–\(^81\) These findings strongly indicate that **KLF13** haploinsufficiency is an alternative molecular mechanism of DCM in a subset of patients.

Interestingly, 2 members from Family 1 and 1 member from Family 2 also had an atrial septal defect. Notably, previous investigations have implicated **KLF13** loss-of-function mutations with distinct types of congenital heart disease, including transposition of the great arteries, double outlet of the right ventricle, tricuspid valve atresia, ventricular septal defect, patent ductus arteriosus, bicuspid aortic valve, and atrial septal defect.\(^71\),\(^82\) Li and coworkers\(^71\) sequenced **KLF13** in a cohort of 309 patients with congenital heart disease and identified 2 heterozygous variants, c.487C>T (p.L163S) and c.467G>A (p.S156N), in 2 patients with tricuspid valve atresia and transposition of the great arteries, respectively. Functional analyses demonstrated...
that the variant c.467G>A (p.S156N) had significantly increased functionality, including increased protein expression, enhanced transactivation on the BNP promoter, and increased physical and functional interactions with TBX5; whereas the other variant, c.487C>T (p.P163S), inhibited the transcriptional activation of the target gene and the physical and functional interactions with TBX5, with no abnormality in protein expression.71 Wang and colleagues82 made a whole-exosome sequencing analysis in a Chinese family with double-outlet right ventricle and ventricular septal defect and found a novel heterozygous variation in the KLF13 gene, c.370G>T (p.E124*), which cosegregated with the disease. Biological analysis revealed that the E124*–mutant KLF13 protein failed to transactivate its cardiac target genes ACTC1 and ANP and disrupted the synergistic transactivation between KLF13 and GATA4, as well as GATA6.82 Additionally, pathogenetic mutations in its target genes MYH7 and ACTC1 as well as its transcriptionally cooperative partners GATA4, GATA6, and TBX5 have also been reported to cause multiple forms of congenital heart disease in humans.83–85 Therefore, this study supports the notion that genetically compromised KLF13 predisposes to congenital heart disease. Of note, in clinical practice, we do see some patients with previous atrial septal defect surgery present years later with DCM. Two rare diseases in the same individual seem unlikely so this does make sense and would recommend that this is the subject of future research. Analyzing the databases of adult congenital heart disease would be very interesting.

It has been revealed in experimental animals that genetically compromised KLF13 contributes to heart disease. In Xenopus, ample expression of KLF13 in the heart and vessels was observed during embryogenesis, and knockdown of Klf13 in the embryos gave rise to myocardial trabecular hypoplasia and atrial septal defect, similar to those observed in mice or humans carrying hypomorphic alleles of GATA4.73 Moreover, defects in growth, proliferation and differentiation of cardiac progenitor cells were observed in Xenopus embryos with downregulated KLF13 levels.74 Additionally, in Xenopus embryos, KLF13-depletion led to an almost complete absence of GATA4, GATA5 and TBX5 gene expression, a severely reduced expression level of GATA6, NKX2.5 and CCND1.73 Expression of ANP as well as α-MLC was severely reduced at an early stage in KLF13-depleted embryos but was upregulated at a late stage, reflecting cardiac dysfunction.73 The phenotypes of the Xenopus embryos with Klf13 knocked down, small hypoplastic hearts, pointed to an important role of KLF13 in cardiac cell survival, proliferation and differentiation. Consistent with the findings that KLF13 and GATA4 are mutual cofactors, the injection of GATA4 mRNA rescued the cardiac defects of KLF13-depleted embryos in a dose-dependent manner.73 In mice, KLF13 is abundantly expressed at all stages of embryogenesis in the heart, including ventricular trabeculae, atrioventricular cushion, truncus arteriosus, and atrial myocardium.73,86 In mice with homozygous deletion of Klf13, heart enlargement, cardiac vacuolar lesion, and embryonic lethality occurred.87 Although mice with heterozygous knockout of Klf13 showed no obvious cardiac structural anomaly, compound heterozygous ablation of both Tbx5 and Klf13 remarkably decreased the postnatal viability and increased

Figure 6. Diminished DNA-binding ability of the mutant KLF13 proteins. A, The ability of WT KLF13, E144X, E194X or C199R mutant to bind to the ACTC1-DNA probe. B, The ability of wild-type KLF13 (WT), E144X, E194X, or C199R mutant to bind to the MYH7-DNA probe. Electrophoretic mobility shift assay indicated that WT bound specifically to the ACTC1-DNA probe (A) or the MYH7-DNA probe (B), whereas all the mutants showed diminished DNA-binding affinity to the ACTC1-DNA (A) or MYH7-DNA probe (B). WT indicates wild type.
the penetrance of cardiac septal abnormality resulted from *Tbx5* haploinsufficiency. In rat cardiomyocytes, down-regulating expression of KLF13 suppressed cell viability and promoted cell apoptosis; whereas overexpression of KLF13 increased cell viability and decreased cell apoptosis. In addition, as a major mediator of glucocorticoid receptor signaling key to the regulation of myocardial function, KLF13 protected adult cardiomyocytes from DNA damage and demise. Besides, as a direct target gene of KLF13, *CCND1* could suppress DCM caused by *TTN* insufficiency. Taken collectively, these results suggest that KLF13 dysfunction contributes to congenital heart disease and DCM.

During the past few years, the field is trying to make a better distinction between monogenic causes of DCM and polygenic contributors. The ClinGen consortium curated a long list of DCM-associated genes and concluded that only a handful of genes were truly monogenic associated with DCM. Other studies came to the same conclusion. In the current study, the discovery of KLF13 as a new DCM-associated gene was based on a genome-wide scan and functional ascertainment and adequately accounted for background population variation. Therefore, KLF13 is likely to be the monogenic cause of DCM, though we could not rule out the possibility that other genes might also make a minor contribution. Presently, the diagnostic gene panels that are used to test patients with DCM are being reduced in the number of genes. Only the true robust DCM-associated genes are being tested, also to reduce the number of reported variants of uncertain significance. Hence, it is better to include this gene in diagnostic gene panels for DCM after more immediate evidence is available. The prevalence of KLF13 variants in other cohorts of patients with DCM will be of extreme importance in the validation of KLF13 as a DCM-associated gene. In addition, in a knock-in animal model expressing a mutant KLF13, DCM spontaneously occurs.

Notably, 2 patients with peripartum DCM were excluded from the current investigation. Specifically, the 2 patients with peripartum DCM were prospectively excluded from the extended cohort of 268 probands with DCM, and neither was in family 1 or family 2, hence
not included in the analysis. However, peripartum cardiomyopathy has been reported as a part of familial DCM, and aggregating evidence has demonstrated the strong genetic basis of familial peripartum cardiomyopathy. Factually, in roughly 20% of patients with peripartum DCM, screening for cardiomyopathy-causative genes reveals pathogenic mutations, with TTN truncations most commonly implicated, occurring in approximately 10% of patients with peripartum DCM. Hence, it is the right time to offer genetic testing to patients with peripartum DCM, and referral for genetic testing should be considered if there is a positive family history of cardiomyopathy or sudden cardiac death.

In the current research, we used the traditional positional candidate gene analysis strategy to identify a new disease-causing gene, as we did previously. Recently, with the rapid advances in massively parallel sequencing approaches, the old approach of Sanger sequencing of a single candidate gene is being replaced by new sequencing technologies, including whole-exome and whole-genome sequencing technologies, which are applied more and more in genetic diagnostic routines because of a high ratio of efficiency to cost. Next-generation sequencing provides the opportunity to fulfill parallel analysis of a large number of genes in an unbiased approach and has been demonstrated to be successful in identifying novel pathogenic mutations responsible for Mendelian diseases. However, though it is slow and costly, traditional Sanger’s method has higher fidelity as compared to next-generation sequencing methods and there are limitations associated with next-generation sequencing methods themselves, including limited bioinformatic tools, existence of functionally-undefined genes and failure to analyze noncoding regions for WES.

CONCLUSIONS

In conclusion, this research maps a novel genetic locus of DCM on human chromosome 15q13.1-q13.3 and identifies KLF13 as a new gene underpinning DCM, which adds new insight into the molecular pathogenesis underlying DCM, implying potential implications for precision medicine of DCM in a subset of patients.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Table S1–S2

REFERENCES

1. Jordan E, Peterson L, Ai T, Asatryan B, Bronicki L, Brown E, Celeghin R, Edwards M, Fan J, Ingles J, et al. Evidence-based assessment of genes in dilated cardiomyopathy. Circulation. 2021;144:7–19. doi: 10.1161/CIRCULATIONAHA.120.053033
2. Mazzarotto F, Tayal U, Buchan RJ, Midwinter W, Wilk A, Whiffin N, Govind R, Mazaika E, de Marvao A, Dawes TJ, et al. Reevaluating the genetic contribution of monogenic dilated cardiomyopathy. Circulation. 2020;141:387–398. doi: 10.1161/CIRCULATIONAHA.119.037661
3. Chen SN, Mestroni L, Taylor MR. Genetics of dilated cardiomyopathy. Curr Opin Cardiol. 2021;36:288–294. doi: 10.1097/HCO.0000000000002082
4. Jain A, Norton N, Brown KA, Cooper LT Jr, Atwal PS, Fairweather D. Sex differences, genetic and environmental influences on dilated cardiomyopathy. J Clin Med. 2021;10:2289. doi: 10.3390/jcm1012289
5. Giri P, Mukhopadhyay A, Gupta M, Mohapatra B. Dilated cardiomyopathy: a new insight into the rare but common cause of heart failure. Heart Fail Rev. 2022;27:431–454. doi: 10.1007/s10741-021-10125-6
6. Nuzzi V, Cannatà A, Manca P, Castrichini M, Barbati G, Alessova A, Fabris E, Zecchin M, Merlo M, Boriani G, et al. Atrial fibrillation in dilated cardiomyopathy: outcome prediction from an observational registry. Int J Cardiol. 2021;333:140–147. doi: 10.1016/j.ijcard.2020.08.062
7. Piers SR, Androulakis AF, Yim KS, van Rein N, Venlet J, Kapel GF, Siebelink HM, Lamb HJ, Ciechanover SC, Man SC, et al. Nonsustained ventricular tachycardia is independently associated with sustained ventricular arrhythmias in nonischemic dilated cardiomyopathy. Circ Arrhythm Electrophysiol. 2022;15:e009979. doi: 10.1161/CIRCEP.121.009979
8. Rossano JW, Kantor PF, Shaddy RE, Asatryan B, Bronicki L, Brown E, Celeghin R, Edwards M, Petrillo M, Rizzuto MG, et al. Elevated heart rate and survival in children with dilated cardiomyopathy: a multicenter study from the pediatric cardiomyopathy registry. J Am Heart Assoc. 2020;9:e015916. doi: 10.1161/JAHA.119.015916
9. Stolfo D, Albani S, Savarese G, Barbati G, Ramani F, Gigli M, Biondi F, Dal Ferro M, Zecchin M, Merlo M, et al. Risk of sudden cardiac death in New York Heart Association class I patients with dilated cardiomyopathy: a competing risk analysis. Int J Cardiol. 2020;307:75–81. doi: 10.1016/j.ijcard.2020.02.025
10. Tabish AM, Azzinatto V, Alexiadis A, Buyandelger B, Knöll R. Genetic epidemiology of tnni-truncating variants in the etiology of dilated cardiomyopathy. Biophys Rev. 2017;9:207–223. doi: 10.1007/s12551-017-0265-7
11. Giudicessi JR, Shrivastava S, Ackerman MJ, Pereira NL. Clinical impact of secondary risk factors in TTN-mediated dilated cardiomyopathy. Circ Genom Precis Med. 2021;4:e000240. doi: 10.1161/CIRCGEN.120.032240
12. Enzan N, Matsuhashima S, Ide T, Kabu H, Toyohama T, Nishimura S, Kudo T, Tsutsui H, Beta-blocker use is associated with prevention of left ventricular remodeling in recovered dilated cardiomyopathy. J Am Heart Assoc. 2021;10:e019240. doi: 10.1161/JAHA.120.019240
13. Domae K, Miyagawa S, Yoshikawa Y, Fukushima S, Hara S, Saito S, Kawai K, Kashiwagi N, Ito H, et al. Clinical outcomes of autologous stem cell-patch implantation for patients with heart failure without nonischemic dilated cardiomyopathy. J Am Heart Assoc. 2021;10:e008649. doi: 10.1161/JAHA.117.008649
14. Raafs AG, Boscotti A, Henkens MTHM, van den Broek WWA, Verdonchot JAJ, Weerts J, Stolfi D, Nuzzi V, Manca P, Hazeboek MR, et al. Global longitudinal strain is incremental to left ventricular ejection fraction for the prediction of outcome in optimally treated dilated cardiomyopathy patients. J Am Heart Assoc. 2022;11:e024505. doi: 10.1161/JAHA.122.024505

15. Marrow BA, Correa SA, Prasad SK, McCann GP. Emerging techniques for risk stratification in nonischemic dilated cardiomyopathy. JACC review topic of the week. J Am Coll Cardiol. 2022;75:1196–1207. doi: 10.1016/j.jacc.2019.12.058

16. Wada K, Misaka T, Yokokawa T, Kominishita Y, Kaneshiro T, Oikawa M, Yoshishita A, Takeishi Y. Blood-based epigenetic markers of FKB5 gene methylation in patients with dilated cardiomyopathy. J Am Heart Assoc. 2022;11:e027578. doi: 10.1161/JAHA.122.027578

17. Sveinbjornsson G, Olafsdottir EF, Thorolfsdottir RB, Davidsson OB, Helgardottir A, Jonasdottir A, Jonasondottir A, Bjornsson EJ, Jonsdottir BO, Arnadottir GA, et al. Variants in NKX2-5 and FLNC cause dilated cardiomyopathy and sudden cardiac death. Circ Genom Precis Med. 2018;11:e021251. doi: 10.1161/CIRCGEN.117.021511

18. Akhtar MM, Lorenzini M, Ciricchia M, Ochoa JP, Hey TM, Salabet Molina M, Restrepo-Cordoba MA, Dal Ferro M, Stollo D, Johnson R, et al. Clinical phenotypes and prognosis of dilated cardiomyopathy caused by truncating variants in the TTN gene. Circ Heart Fail. 2020;13:e006832. doi: 10.1161/CIRCHEARTFAILURE.119.006832

19. Hey TM, Rasmussen TB, Henkens MTHM, van den Broek WWA, Guo K, et al. KLF13 Mutations in DCM. Circ Genom Precis Med. 2020;13:e016195. doi: 10.1161/CIRCGEN.120.003031

20. Kato K, Ohno S, Sonoda K, Fukuyama M, Makiyama T, Ozawa T, Horie M. LMNA missense mutation causes nonsense-mediated mRNA decay and severe dilated cardiomyopathy. Circ Res. 2020;126:435–443. doi: 10.1161/CIRCRESAHA.119.313006

21. Nielsen SK, HansenF, Schroder HD, Wibrand F, Gustafsson F, Mogensen J. Recessive inheritance of a rare variant in the nuclear mitochondrial gene for AARS2 in late-onset dilated cardiomyopathy. Clin Genom. 2020;2020:1356–582. doi: 10.1161/CIRCRESAHA.119.316195

22. Levitas A, Muhamad E, Zhang Y, Perea Gil I, Serrano R, Diaz N, Arafat M, Gavidia AA, Kapiloff MS, Mercola M, et al. A novel recessive mutation in SPEG causes early onset dilated cardiomyopathy. PLoS Genet. 2020;16:e1009000. doi: 10.1371/journal.pgen.1009000

23. Oual D, Witkowski L, Zouk H, Daly KP, Roberts AE. Retrospective analysis of clinical genetic testing in pediatric primary dilated cardiomyopathy. J Am Heart Assoc. 2022;11:e024505. doi: 10.1161/JAHA.122.024505

24. Quiat D, Witkowski L, Zouk H, Daly KP, Roberts AE. Retrospective analysis of clinical genetic testing in pediatric primary dilated cardiomyopathy. J Am Heart Assoc. 2022;11:e027578. doi: 10.1161/JAHA.122.027578

25. Al- Yacoub N, Colak D, Mahmoud S, Morlot S, Yigit G, Lee TM, Al- Yacoub N, Colak D, Mahmoud S, Morlot S, Yigit G, Lee TM. Identification and functional characterization of KLF5 as a novel cardiac disease gene responsible for familial dilated cardiomyopathy. J Am Heart Assoc. 2021;10:e021101. doi: 10.1161/JAHA.121.021101

26.land-Alemaí M, Dunér P, Kähre A, Hagen P, et al. Implications of genetic testing in dilated cardiomyopathy. Cardiovasc Res. 2021;109:1118–1131. doi: 10.1093/cvr/cva158

27. Rico Y, Ramis MF, Massot M, Torres- Juan L, Pons J, Fortuny E, Ripoll-Puigventura R, Vidal JF, et al. Implications of genetic testing in dilated cardiomyopathy. J Am Heart Assoc. 2022;11:e024505. doi: 10.1161/JAHA.122.024505

28. Di RM, Yuan F, Guo J, Xu YJ, Yang YQ. Identification and functional characterization of KLF5 as a disease associated familial dilated cardiomyopathy. Eur J Med Genet. 2020;63:e103827. doi: 10.1016/j.ejmg.2019.103827

29. Miura A, Kondo H, Yamamoto T, Okumura Y, Nishio H. Sudden unexpected death of infantile dilated cardiomyopathy with JPH2 and PKD1 gene variants. Int Heart J. 2020;61:1079–1083. doi: 10.15363/ihj.2010-2015

30. Garrnier S, Harakalova M, Weiss S, Mokry M, Regitz-Zagrosek V, Heiligenstein G, Cappolla TP, Lisa A, Artbustiniet E, Cook SA, et al. Genome-wide association analysis in dilated cardiomyopathy reveals two new players inystolic heart failure on chromosomes 3p25.1 and 22q11.23. Eur Heart J. 2021;42:2020–2011. doi: 10.1093/eurheartj/ehab030

31. Almammari M, Luo S, Faeqeh E, Almutari F, Li Q, Agrawal PB. Homozygous SPEG mutation is associated with isolated dilated cardiomyopathy. Circ Genom Precis Med. 2021;14:e003310. doi: 10.1161/CIRCGEN.120.003310

32. Ware SM, Wilkinson JD, Tarig M, Schubart JA, Colan SD, Shi L, Canter CE, Hsu DT, Webber SA, et al. Genetic causes of cardiomyopathy in children: first results from the pediatric cardiomyopathy genes study. J Am Heart Assoc. 2021;10:e017731. doi: 10.1161/JAHA.121.020840

33. Sejadghad-Hamedani F, Rebs S, El- Battrawy I, Chasan S, Krause T, Haas JS, Heymansk SRB, et al. Clinical impact of re-evaluating genes and variants implicated in dilated cardiomyopathy. Genet Med. 2021;23:2186–2193. doi: 10.1038/s41436-021-01255-1

34. Fischer B, Dittmann S, Broedel A, Unger A, Stallmeyer B, Paul M, Seebohm G, Kayser A, Peischard S, Linke WA, et al. Functional characterization of a novel alpha-helical rod domain desmin (DES) pathogenic variant associated with dilated cardiomyopathy. Int Heart J. 2021;62:167–174. doi: 10.1553/ijcard2120650

35. Li M, Xia S, Xu L, Tan H, Yang J, Wu Z, He X, Li L. Genetic analysis using targeted next-generation sequencing of sporadic Chinese patients with idiopathic dilated cardiomyopathy. J Transl Med. 2021;19:185. doi: 10.1186/s12967-021-02832-3

36. Guenter A, Bloebaum L, Broedel A, Klaube K, Silemann K, Kassner A, Fox H, Morshuis H, Tiesmeyer J, Schutz U, et al. The combined human genotype of truncating TTN and RBM20 mutations is associated with severe and early onset of dilated cardiomyopathy. Genes (Basel). 2021;12:883. doi: 10.3390/genes12060883

37. Rico Y, Ramiès MF, Massot M, Torres-Juárez L, Pons J, Fortuny E, Ripoll-Torres J, González R, Peral V, Rossiello X, et al. Familial dilated cardiomyopathy and sudden cardiac arrest: new association with a SCNSA5 mutation. Genes (Basel). 2021;12:1899. doi: 10.3390/genes12121899

38. Heliló K, Mäyränpää MI, Saarinen I, Ahonen S, Junnila H, Tommiska T, Weckström S, Holmström M, Toivonen M, Nikus K, et al. GRINL1A complex transcription unit containing GC0M1, MYZAP, and POLR2M genes associates with fully penetrant recessive dilated cardiomyopathy. Front Genet. 2021;12:786705. doi: 10.3389/fgene.2021.786705
46. Robles-Mezcua A, Rodríguez-Miranda L, Morcillo-Hidalgo L, Jiménez-Navarro M, García-Pinilla JM. Phenotype and progression among patients with dilated cardiomyopathy and RBM20 mutations. *J Am Heart Assoc.* 2021;64:104278. doi: 10.1161/JAHA.121.104278

47. Hirayama-Yamada K, Inagaki N, Hayashi T, Kimura A. A novel titin truncation variant linked to familial dilated cardiomyopathy found in a Japanese family and its functional analysis in genomically edited cells. *Int Heart J.* 2021;62:353–365. doi: 10.1016/j.ijhj.2021.05.066

48. Hakki H, Kioka H, Miyashita Y, Nishimura S, Matsuoka K, Kato H, Tsuchimoto O, Kuramoto Y, Takawa A, Takahashi Y, et al. Loss-of-function mutations in the co-chaperone protein BAG5 cause dilated cardiomyopathy requiring heart transplantation. *Sci Transl Med.* 2022;14:eabd2374. doi: 10.1126/scitranslmed.abd2374

49. Xiao L, Wu D, Chen Y, Hu D, Dai J, Chen W. Whole-exome sequencing reveals genetic risks of early-onset sporadic dilated cardiomyopathy in the Chinese Han population. *Sci China Life Sci.* 2022;65:770–780. doi: 10.1007/s11427-021-10194-4

50. Vihoreva PG, Vihoreva NN, Yeung W, Li A, Lal S, Dos Remedios CG, Bernt A, Schulze-Bahr E, Borlepawar A, Zimmermann WH, et al. Heart failure and diverse genetic architecture of dilated cardiomyopathy. *Circ Res.* 2022;121:1752–1761. doi: 10.1161/CIRCRESAHA.121.042356

51. Ramchand J, Wallis M, Macciocca I, Lynch E, Farouque O, Martyn M, Malakootian M, Bagheri Moghaddam M, Kalayinia S, Farrashi M, Yuen M, Worgan L, Iwanski J, Pappas CT, Joshi H, Churko JM, Arbuckle A, et al. A novel locus for material hypertension. *J Am Heart Assoc.* 2022;11:e027578. doi: 10.1161/JAHA.122.027578

52. Khan RS, Pahl E, Dellefave-Castillo L, Rychlik K, Ing A, Yap KL, Brew P, Bernt A, Schulze-Bahr E, Borlepawar A, Zimmermann WH, et al. Association of titin variations with outcomes in pediatric dilated cardiomyopathy. *J Pediatr Cardiol.* 2022;15:106. doi: 10.1016/j.jpcardio.2021.05.003

53. Kurebayashi Y, Endo J, Sano M, Fukuda K, et al. TNFRSF13B c.226G>A (p.Gly76Ser) as a novel causative mutation for pulmonary artery hypertension. *J Am Heart Assoc.* 2022;11:e022854. doi: 10.1161/JAHA.121.022854

54. Malakootian M, Bagheri Moghaddam M, Kalayinia S, Farrashi M, Maleki M, Sadeghpour P, Amin A. Dilated cardiomyopathy caused by a novel mutation in the CARD9 gene in a Chinese patient. *Int J Mol Med.* 2022;48:357–362. doi: 10.3892/ijmm.2022.7042

55. Guo ET, Soltani M, Firoozabadi AD, Namayandeh SM, Crockford J, Maroofian R, Jamshidi Y. Digenic inheritance of mutations in the cardiac troponin (TNNT2) and cardiac beta myosin heavy chain (MYH7) as the cause of severe dilated cardiomyopathy. *J Eur Heart J.* 2017;60:485–488. doi: 10.1093/jeuhj/hwx208

56. Robles-Mezcua A, Rodríguez-Miranda L, Morcillo-Hidalgo L, Jiménez-Guó et al. KLF13 Mutations in DCM patients with dilated cardiomyopathy. *Biochem Biophys Res Commun.* 2013;439:591–596. doi: 10.1016/j.bbrc.2013.09.023

57. Zhao L, Xu JH, Xu WJ, Hu Y, Wang Q, Zheng HZ, Jiang WF, Jiang JF, Yang YQ. A novel KAT4 loss-of-function mutation responsible for familial dilated cardiomyopathy. *Int J Mol Med.* 2014;33:654–660. doi: 10.3892/ijmm.2013.1600

58. Li J, Liu WD, Yang ZL, Yuan F, Xu L, Li RG, Yang YQ. Prevalence and spectrum of KAT4 mutations associated with sporadic dilated cardiomyopathy. *Gene.* 2014;548:174–181. doi: 10.1016/j.gene.2014.07.022

59. Scroby S, Gabant P, Van Reeth T, Hertvelt V, Derezé PL, Van Vooren P, Rivière M, Spierer J, Spierer C. Identification of KLF13 and KLF14 (SP6) novel members of the SP/XKLF transcription factor family. *Genomics.* 2018;110:93–101. doi: 10.1016/j.ygeno.2018.01.010

60. Song A, Patel A, Thamatrakoln K, Liu C, Feng D, Claberger C, Krensky AM. Functional domains and DNA-binding sequences of RFLAT-1/KLF13, a Krüppel-like transcription factor activated by T lymphocytes. *J Biol Chem.* 2002;277:30055–30065. doi: 10.1074/jbc.M202782200

61. Li J, Li B, Li T, Zhang E, Wang Q, Chen S, Sun K. Identification and analysis of KLF13 variants in patients with congenital heart disease. *BMJ Med Genet.* 2020;21:78. doi: 10.1136/bmjgenetics-2019-100099

62. Darwich R, Li W, Yamak A, Komati H, Andelfinger G, Sun K, Nemer M. KLF13 is a genetic modifier of the Holt-Oram syndrome gene TBX5. *Hum Mol Genet.* 2017;26:942–954. doi: 10.1093/hmg/ddx009

63. Lavallée G, Andelfinger G, Nadeau M, Lefebvre C, Nemer G, Horb ME, Nemer M. The Kruppel-like transcription factor KLF13 is a novel regulator of heart development. *EMBO J.* 2006;25:5201–5213. doi: 10.1038/sj.emboj.7601379

64. Nemer M, Horb ME. The KLF family of transcriptional regulators in cardiomyocyte proliferation and differentiation. *Cell Cycle.* 2007;6:117–121. doi: 10.4161/cc.6.2.3718

65. Cruz-Topete D, He B, Xu X, Cibulskis JA. Krüppel-like factor-13 is a macromolecular mediator in dilated cardiomyopathy and protects these cells from DNA damage and death. *J Biol Chem.* 2016;291:1937–19386. doi: 10.1074/jbc.M116.725903

66. Bayoumi AS, Park KM, Wang Y, Teoh JP, Aonuma T, Tang Y, Su H, Weintraub NL, Kim IM. A cardedvillo-responsive microRNA, miR-125b-5p protects the heart from acute myocardial infarction by repressing pro-apoptotic bak1 and kitl3 in cardiomyocytes. *J Mol Cell Cardiol.* 2018;114:72–82. doi: 10.1016/j.yjmcc.2017.11.003

67. Gu M, Wang J, Wang Y, Yu Z, Yang Y, Wu W, Liao S. mir-147b inhibits its cell viability and promotes apoptosis of rat H9c2 cardiomyocytes via down-regulating KLF13 expression. *Acta Biochim Biophys Sin.* (Shanghai). 2018;50:288–297. doi: 10.1093/abbs/gmx144

68. Xu L, Zhao L, Yuan F, Jiang WF, Liu H, Li RG, Xu YJ, Zhang M, Feng YW, Qu XK, et al. GATA6 loss-of-function mutations contribute to familial dilated cardiomyopathy. *J Mol Med.* 2014;92:1516–1522. doi: 10.3892/ijmm.2014.1896

69. Zhang XL, Qiu XB, Yuan F, Wang J, Zhao CM, Li RG, Xu L, Xu YJ, Shi HY, Hou XM, et al. TBX5 loss-of-function mutations contribute to familial dilated cardiomyopathy. *Biochem Biophys Res Commun.* 2015;459:166–171. doi: 10.1016/j.bbrc.2015.02.094

70. Zhou W, Qiu XB, Yuan F, Wang J, Zhao CM, Li RG, Xu L, Xu YJ, Shi HY, Hou XM, et al. TBX5 loss-of-function mutation associated with sporadic dilated cardiomyopathy. *Int J Mol Med.* 2015;36:282–288. doi: 10.3892/ijmm.2015.2206

71. Patterson J, Coats C, McCowan R. Familial dilated cardiomyopathy associated with pathogenic TBX5 variants: expanding the cardiac phenotype associated with Holt-Oram syndrome. *Am J Med Genet A.* 2020;182:1725–1734. doi: 10.1002/ajmg.a.61835

Cardiac a-actin (ACT1) gene mutation causes atrial-septal defects associated with late-onset dilated cardiomyopathy. *Circ Genom Precis Med.* 2019;12:e002491. doi: 10.1161/CIRCGENE.119.002491
82. Wang SS, Wang TM, Qiao XH, Huang RT, Xue S, Dong BB, Xu YJ, Liu XY, Yang YQ. KLF13 loss-of-function variation contributes to familial congenital heart defects. *Eur Rev Med Pharmacol Sci*. 2020;24:11273–11285. doi: 10.26355/eurrev_202011_23617

83. van der Linde IHM, Hiemstra YL, Bökenkamp R, van Mil AM, Breuning MH, Ruivenkamp RF, van Waning JI, van Slegtenhorst MA, et al. A Dutch MYH7 founder mutation, p.(Asn1918Lys), is associated with early onset cardiomyopathy and congenital heart defects. *Neth Heart J*. 2017;25:675–681. doi: 10.1007/s12471-017-1037-5

84. Augière C, Mégy S, El Malti R, Boland A, El Zein L, Verrier B, Mégarbané A, Deleuze JF, Bouvagnet P. A novel alpha cardiac actin (ACTC1) mutation mapping to a domain in close contact with myosin heavy chain leads to a variety of congenital heart defects, arrhythmia and possibly midline defects. *PLoS One*. 2015;10:e0127903. doi: 10.1371/journal.pone.0127903

85. Yasuhara J, Garg V. Genetics of congenital heart disease: a narrative review of recent advances and clinical implications. *Transl Pediatr*. 2021;10:2366–2386. doi: 10.21037/tp-21-297

86. Martin KM, Metcalfe JC, Kemp PR. Expression of Klf9 and Klf13 in mouse development. *Mech Dev*. 2001;103:149–151. doi: 10.1016/s0925-4773(01)00343-4

87. Gordon AR, Outram SV, Keramatipour M, Goddard CA, Colledge WH, Metcalfe JC, Hager-Theodorides AL, Crompton T, Kemp PR. Splenomegaly and modified erythropoiesis in KLF13−/− mice. *J Biol Chem*. 2008;283:11897–11904. doi: 10.1074/jbc.M709569200

88. Liao D, Chen W, Tan CY, Wong JX, Chan PS, Tan LW, Foo R, Jiang J. Upregulation of Yy1 suppresses dilated cardiomyopathy caused by Ttn insufficiency. *Sci Rep*. 2019;9:16330. doi: 10.1038/s41598-019-52796-0

89. van Spaendonck-Zwarts KY, van Tintelen JP, van Veldhuisen DJ, van der Werf R, Jongbloed JD, Paulus WJ, Doijes D, van den Berg MP. Peripartum cardiomyopathy as a part of familial dilated cardiomyopathy. *Circulation*. 2010;121:2169–2175. doi: 10.1161/CIRCULATIONAHA.109.929646

90. Slwa K, Bauersachs J, Arany Z, Spracklen TF, Hiltiker-Kleiner D. Peripartum cardiomyopathy: from genetics to management. *Eur Heart J*. 2021;42:3094–3102. doi: 10.1093/eurheartj/ehab458

91. Spracklen TF, Chakafana G, Schwartz PJ, Kotta MC, Shaboodien G, Ntusi NAB, Slwa K. Genetics of peripartum cardiomyopathy: current knowledge, future directions and clinical implications. *Genes (Basel)*. 2021;12:103. doi: 10.3390/genes12010103

92. Goll R, Li J, Brandimarto J, Levine LD, Riis V, McAfee Q, DePalm A, Haghhighi A, Seidman JG, Seidman CE, et al. Genetic and phenotypic landscape of peripartum cardiomyopathy. *Circulation*. 2021;143:1852–1862. doi: 10.1161/CIRCULATIONAHA.120.052395

93. Arany Z. It is time to offer genetic testing to women with peripartum cardiomyopathy. *Circulation*. 2022;146:4–5. doi: 10.1161/CIRCULATIONAHA.122.059177

94. Chen YH, Xu SJ, Bendahhou S, Wang XL, Wang Y, Xu WY, Jin HW, Sun H, Su XY, Zhuang QN, et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science*. 2003;299:251–254. doi: 10.1126/science.1077771

95. Yang Y, Yang Y, Liang B, Liu J, Li J, Grunnet M, Olsen SP, Rasmussen HB, Ellinor PT, Gao L, et al. Identification of a Kir3.4 mutation in congenital long QT syndrome. *Am J Hum Genet*. 2010;86:872–880. doi: 10.1016/j.ajhg.2010.04.017

96. Li RG, Xu YJ, Ye WG, Li YJ, Chen H, Qiu XH, Yang YG, Bai D. Connexin43 (GJC1) loss-of-function mutation contributes to familial atrial fibrillation and conduction disease. *Heart Rhythm*. 2021;18:684–693. doi: 10.1016/j.hrthm.2020.12.033

97. Kalayinia S, Goodarzynejad H, Maleki M, Mahdieh N. Next generation sequencing applications for cardiovascular disease. *Ann Med*. 2018;50:90–109. doi: 10.1080/07853890.2017.1392595

98. Al-Mursshed F, Meltah D, Scott P. Underdiagnoses resulting from variant misinterpretation: time for systematic reanalysis of whole exome data? *Eur J Med Genet*. 2019;62:39–43. doi: 10.1016/j.ejmg.2018.04.016
**Table S1. Clinical Characteristics of Another Cohort of 266 Unrelated Patients Affected with Dilated Cardiomyopathy.**

| Parameters                           | Patients (n = 266) | Controls (n = 418) | P-value |
|--------------------------------------|-------------------|--------------------|---------|
| Age (years)                          | 48 ± 9            | 48 ± 8             | >0.9999 |
| Male, n (%)                          | 136 (51)          | 217 (52)           | 0.9027  |
| Positive family history of DCM, n (%)| 93 (35)           | 0 (0)              | <0.0001 |
| Body mass index (kg/m²)              | 23 ± 3            | 23 ± 4             | >0.9999 |
| Systolic blood pressure (mmHg)       | 117 ± 10          | 118 ± 9            | 0.1755  |
| Diastolic blood pressure (mmHg)      | 77 ± 7            | 77 ± 6             | >0.9999 |
| Rest heart rate (bpm)                | 78 ± 11           | 77 ± 10            | 0.2206  |
| LVEDD (mm)                           | 67 ± 8            | 46 ± 6             | <0.0001 |
| LVESD (mm)                           | 55 ± 6            | 35 ± 5             | <0.0001 |
| LVEF (%)                             | 35 ± 7            | 62 ± 6             | <0.0001 |
| LVFS (%)                             | 20 ± 4            | 35 ± 3             | <0.0001 |

**NYHA classification of cardiac function**

| NYHA classification | Patients (n = 266) | Controls (n = 418) | P-value |
|---------------------|--------------------|--------------------|---------|
| I, n (%)            | 53 (20)            | NA                 | NA      |
| II, n (%)           | 93 (35)            | NA                 | NA      |
| III, n (%)          | 88 (33)            | NA                 | NA      |
| IV, n (%)           | 32 (12)            | NA                 | NA      |

Data were given as mean ± standard deviation or n (%).

DCM, dilated cardiomyopathy; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; NA, not applicable; and NYHA, New York Heart Association.
Table S2. A List of All the 99 Genes Mapped to the Defined Chromosomal Locus, between the two Boundaries of Markers D15S1019 and D15S1010.

| Gene          | Gene          | Gene          | Gene          | Gene          |
|---------------|---------------|---------------|---------------|---------------|
| FAM189A1      | TUBBP8        | LOC100130111  | LOC105370743  | TJP1          |
| (pseudo)      | (pseudo)      | (uncharacterized) | (uncharacterized) |               |
| HMGN2P5       | NCAPGP2       | SYNGR2P1      | GOLGA8J       | RN7SL673P     |
| (pseudo)      | (pseudo)      | (pseudo)      | (pseudo)      | (pseudo)      |
| DNM1P28       | ULK4P3 (pseudo) | GOLGA8T      | RN7SL469P     | DNM1P30       |
| (pseudo)      | (pseudo)      | (pseudo)      | (pseudo)      | (pseudo)      |
| LOC105370747  | LINC02249     | LOC728424     | RNU6-17P      | LOC102724055  |
| (uncharacterized) | (intron)    | (pseudo)      | (pseudo)      | (uncharacterized) |
| CHRFA07A      | LOC105370751  | DNM1P29       | GOLGA8R       | LOC101927788  |
| (uncharacterized) | (pseudo)      | (pseudo)      | (pseudo)      | (uncharacterized) |
| RN7SL196P     | LOC100288482  | LOC105376704  | LOC100288203  | LOC100652840  |
| (pseudo)      | (uncharacterized) | (uncharacterized) | (uncharacterized) |               |
| LOC100996413  | GOLGA8Q       | RN7SL796P     | DNM1P50       | ULK4P2 (pseudo) |
| LOC102725021  | GOLGA8H       | RN7SL628P     | ARHGAP11B     | LOC100288637  |
| (uncharacterized) | (pseudo)      | (pseudo)      | (pseudo)      | (pseudo)      |
| LOC107984025  | LOC101930434  | RN7SL82P      | HERC2P10      | LOC100629121  |
| LOC388104     | FAN1          | MTMR10        | RNU6-466P     | TRPM1         |
| (pseudo)      |               |               | (pseudo)      |               |
| LOC105370752  | MIR211 (intron) | LINC02352     | LOC238710     | KLF13         |
| (uncharacterized) | (intron)      | (intron)      | (pseudo)      |               |
| LOC105376707  | LOC105370939  | UBE2CP4       | LOC400347     | LOC107987220  |
| (uncharacterized) | (uncharacterized) | (pseudo)      | (pseudo)      | (uncharacterized) |
| OTUD7A        | LOC105370753  | DEPDC1P1      | LOC105370940  | CHRNA7        |
| (uncharacterized) | (pseudo)      | (pseudo)      | (uncharacterized) |               |
| LOC105370754  | LOC102724078  | RNU6-18P      | LOC644110     | LOC105376709  |
| (uncharacterized) | (uncharacterized) | (pseudo)      | (pseudo)      | (uncharacterized) |
| LOC112268159 (uncharacterized) | DNM1P31 (pseudo) | **GOLGA8K** | LOC107987221 (uncharacterized) | RN7SL185P (pseudo) |
|--------------------------------|-----------------|-------------|-------------------------------|-------------------|
| ULK4P1 (pseudo) | DNM1P32 (pseudo) | **GOLGA8O** | RN7SL539P (pseudo) | LOC100289543 (pseudo) |
| LOC105376710 (uncharacterized) | LOC101060588 (uncharacterized) | **LOC100653133** | LOC107987215 (pseudo) | LOC107987222 (uncharacterized) |
| WHAMMP1 (pseudo) | LINC02256 (intron) | LOC101928042 (uncharacterized) | **GOLGA8N** | RN7SL286P (pseudo) |
| **ARHGAP11A** | **ARHGAP11A-SCG5** | **SCG5** | LOC105370757 (uncharacterized) | LOC105370756 (uncharacterized) |
| **GREM1** | LOC100131315 (uncharacterized) | **FMN1** | LOC107984089 (uncharacterized) |