Identification and functional characterization of BICD2 as a candidate disease gene in an consanguineous family with dilated cardiomyopathy

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Abstract

Background: Familial dilated cardiomyopathy (DCM) is a genetic cardiomyopathy that is associated with reduced left ventricle function or systolic function. Fifty-one DCM-causative genes have been reported, most of which are inherited in an autosomal dominant manner. However, recessive DCM-causative gene is rarely observed.

Methods: Whole-exome sequencing (WES) was performed in a consanguineous family with DCM to identify candidate variants. Sanger sequencing was utilized to confirm the variant. We then checked the DCM candidate gene in 210 sporadic DCM cases. We next explored BICD2 function in both embryonic and adult bicd2-knockout zebrafish models. In vivo cardiac function of bicd2-knockout fish was detected by echocardiography and RNA-seq.

Results: We identified an autosomal recessive and evolutionarily conserved missense variant, NM_001003800.1:c.2429G > A, in BICD2, which segregated with the disease phenotype in a consanguineous family with DCM. Furthermore, we confirmed the presence of BICD2 variants in 3 sporadic cases. Knockout of bicd2 resulted in partial embryonic lethality in homozygotes, suggesting a vital role for bicd2 in embryogenesis. Heart dilation and decreased ejection fraction, cardiac output and stroke volume were observed in bicd2-knockout zebrafish, suggesting a phenotype similar to human DCM. Furthermore, RNA-seq confirmed a larger transcriptome shift in bicd2 homozygotes than in heterozygotes. Gene set enrichment analysis of bicd2-deficient fish showed the enrichment of altered gene expression in cardiac pathways and mitochondrial energy metabolism.

Conclusions: Our study first shows that BICD2 is a novel candidate gene associated with familial DCM, and our findings will facilitate further insights into the molecular pathological mechanisms of DCM.

Keywords: Dilated cardiomyopathy, BICD2, Zebrafish model, RNA-seq

Introduction

Dilated cardiomyopathy (DCM) is one of the major cardiomyopathy subtypes [1]. It is the third leading cause of heart failure and the most common cause of heart transplantation [2], but its aetiology and pathogenesis remain elusive.

Familial DCM is now more commonly diagnosed owing to the improved awareness of disease-causing...
genetic variants and genetic screening. Familial DCM is found in 20–35% of patients with DCM, 80% of DCM patients inherit in autosomal dominant pattern, and 10–15% of DCM patients exhibit autosomal recessive or X-linked inheritance [3]. Previous DCM studies revealed > 250 associated genes spanning > 10 gene ontologies, suggesting a complex and diverse genetic architecture [4]. A DCM gene curation expert panel curated a final set of 51 genes proposed to have a monogenic role in isolated, idiopathic DCM in humans [4]. In addition, 12 out of these 51 genes, namely, BAG3, DES, FLNC, LMNA, MYH7, PLN, RBM20, SCN5A, TNNT2, TTN, and DSP, were classified as having definitive or strong evidence [4]. Another 7 genes, ACTC1, ACTN2, JPH2, NEXN, TNNI3, TPM1, and VCL, were classified as having moderate evidence [4]. However, these 19 genes explain only a minority of cases, leaving the majority of the genetic architecture of DCM incompletely addressed [4].

Most reported DCM variants are inherited in an autosomal dominant manner [3–5]; autosomal recessive (AR) inheritance in adult DCM is less frequently observed [6] and is more common in paediatric DCM [7–9]. Consanguineous families are ideal for investigating AR DCM. However, few studies have focused on familial DCM in consanguineous families [9–11].

Bicaudal-D2 (BICD2) is a dynein-activating adaptor protein that plays a critical role in minus-end-directed microtubule-based transport [12, 13]. Mutations in human BICD2 have been linked to a spectrum of neuronal disorders and particularly to a dominant mild early onset form of spinal muscular atrophy [14–17]. The BICD2 protein could bind to the kinesin tail and kinesin-activating protein filaments, which then activates the kinesin and allows the kinesin complex to travel along the microtubule cytoskeleton to transport cargo [18]. Bicd2-KO mice showed significantly lower LMNA expression at the protein level, decreased MKL-1/SRF activity, and significantly downregulated expression of the downstream α-actin, α-coactin and membrane association proteins, as well as microtubulin [19]. MKL-1, a member of the actin family, plays an important role in cardiovascular system development and functions as a cofactor of serum response factor (SRF), which activates SRF-dependent transcriptional regulatory elements. In nuclear fibrillar protein A/C gene (LMNA) knockout mice, the MKL1/SRF signalling pathway is also inhibited, and the nuclear translocation of endogenous MKL-1 in cardiomyocytes is dysfunctional, leading to reduced expression of SRF, α-actinin and membrane association proteins and subsequently the development of dilated heart disease [20]. Simple blockade of SRF expression in mouse myocardial tissue can lead to the development of DCM [21].

The zebrafish model of DCM is one of the ideal systems in which to study the function of gene mutations in DCM. These model zebrafish harbour an enlarged heart, reduced shortening fraction during cardiac contraction and no cardiomyocyte proliferation, consistent with the characteristics of human DCM [22].

In this study, we performed whole-exome sequencing (WES) in a consanguineous family with DCM to identify candidate DCM-causing genes. Subsequently, functional studies of the candidate DCM gene BICD2 were conducted in zebrafish to demonstrate the relationship between BICD2 and DCM.

Materials and methods

Patients and clinical evaluation

The proband and his families from consanguineous family was recruited in 2011. In total, 210 sporadic DCM patients were enrolled for further BICD2 variant validation. Written informed consent was obtained from all subjects participating.

Genomic DNA preparation

Peripheral blood from DCM cases was collected into EDTA anticoagulant tubes, and genomic DNA was extracted using a blood DNA extraction kit according to the manufacturer’s protocol (Qiagen, Germantown, MD, USA).

Exome sequencing and bioinformatics analysis

To identify additional genes for DCM, WES was performed on four members of Family 1 (the proband, sibling of the proband, parents of the proband). The normal human population database consisted of The Thousand Genomes Project, ESP6500SI-V2, ExAC Human Exome Integration Database and the sequencing company’s internal database. All variants were annotated with ANNOVAR software (version 2014).

Quantitative RT-PCR

Total RNA was isolated from cells using the Qiagen RNeasy Mini kit. In parallel, we analysed the mRNA concentration of the housekeeping β-actin as an internal control for normalization. The real-time monitoring of the PCR reaction, the precise quantification of the products in the exponential phase of the amplification and the melting curve analysis were performed with the Bio-Rad CFX Manager software, following recommended instructions of the manufacturer.

Immunocytochemistry

Heart tissue fixed with 4% paraformaldehyde was dehydrated by an automatic dehydrator, embedded, and sectioned as follows. Firstly, the dewaxed sections were
placed in 3% methanolic hydrogen peroxide at room temperature for 10 min. Secondly, PBS wash 3 times. Secondly, immersing the sections in 0.01 M citrate buffer (PH 6.0), heating to boiling and disconnecting, after an interval of 5 min, repeated once, after cooling, washed 2 times with PBS. Then, adding normal goat serum blocking solution dropwise. Incubated with primary antibodies at 4 °C overnight. Adding biotinylated secondary antibody dropwise, 37 °C for 30 min. PBS wash 3 times. Mixing the reagents of DAB Color Development Kit, and add dropwise to the sections at room temperature, for about 2 min, then wash with distilled water. Hematoxylin lightly re-stained, dehydrated, transparent, and sealed with neutral gum.

**CRISPR/Cas9 bicd2 knock out in zebrafish**
Zebrafish fertilized eggs were collected. 200 ng/μl Cas9 protein were mixed with ~80 ng/μl sgRNAs and subsequently microinjected into zebrafish fertilized eggs (1 nl per embryo). Five embryos were taken when the injected embryos developed to 24 hpf. PCR amplification products were cloned into pGEM-T Easy plasmid (5 μl total volume of reaction). The above 5 μl ligation product was transformed into 50 μl E. coli DH5α receptor cells (pfu ≥ 108). Then coated in LB plates containing ampicillin (50 μg/ml) and incubated upside down overnight at 37 °C. Positive clones were validated by Sanger sequencing.

**Zebrafish echocardiogram**
The Vevo2100® Imaging System and Vevo Imaging Station was used to perform transthoracic echocardiography on 7-month-old zebrafish to examine indicators of ventricular function and ventricular size during systole and diastole with a 22–55 MHz transducer probe. Before echocardiography, zebrafish were anesthetized with 0.02% tricaine to induce sedation without cessation of breathing. The two-dimensional B-mode was used for measurement of heart rate, ejection fraction, fractional shortening, end-diastolic and end-systolic area, end-diastolic and end-systolic volume, cardiac output, and stoke volume. The left ventricular volumes are calculated by tracing the endocardial border manually at end diastole and at end systole. B-mode imaging quality was further optimized by adjusting focal depth, gain, image width and depth.

**RNA-seq analysis**
Before further data analysis, we firstly checked raw data quality and removed reads with poor quality to get clean reads with high quality. Then all clean reads were mapped to zebrafish genome Assembly GRCz11 using HISAT (version 2.2.1) [23]. StringTie (version 2.2.0) [23] was used to quantify gene expression in different samples and FPKM values were extracted as the expression metric. EBSeq (version 1.34.0) [24] was utilized on raw reads matrix to find out differentially expressed genes. Genes with absolute fold change not less than 2, and adjusted P-value less than 0.05 were defined as differentially expressed genes.

**Statistical analysis**
Statistical significance was determined using Student’s t test or Wilcox test. Quantitative data are presented as the means ± SD, as indicated in the figure legends. Statistical analyses were performed using R 4.10.

**Results**
Identification of candidate variants in a consanguineous family with DCM
A consanguineous family with 3 DCM-affected members (Family 1) was recruited (Fig. 1A). Conventional and dynamic electrocardiograms suggested occasional first-degree atrioventricular block, paroxysmal sinus tachycardia and complete left bundle branch block (Fig. 1B). The proband was treated with cardiac resynchronization therapy to provide simultaneous electrical activation. The proband’s brother was also DCM patient, and he received a heart transplant. However, the surgery did not substantially reduce his symptom severity, and he died one year later. The proband's sister was also diagnosed with DCM and she showed frequent atrial premature contractions, and partial of the premature electrical impulse not transmitted...
Fig. 1 (See legend on previous page.)
downstream to the ventricle. All three DCM patients in the consanguineous family did not show any skeletal muscle abnormalities and their CK serum level was within the normal range. None of the parents or other antecedents showed cardiac dysfunction.

WES was performed on consanguineous family members with DCM but revealed no known DCM-causing gene mutations. We therefore sought new candidate pathogenic variants. WES data was analysed as illustrated (Additional file 1: Fig. S1). In total 14,208 candidate rare variants were obtained. Considering the possible autosomal recessive inheritance pattern, we obtained 56 candidate variants. Finally, 51 variants were excluded based on the American College of Medical Genetics and Genomics 2015 guidelines. The remaining 5 variants in 5 separate genes, BICD2, SERINC1, BVES, ADCY1 and PAPPA, were considered for further evaluation (Additional file 1: Table S1).

BICD2 is a dynein-activating adaptor protein that functions in minus-end-directed microtubule-based transport by docking dynein motor proteins to appropriate cargos. Reduced LMNA protein expression and MKL-1/DSR expression was observed in Bicd2-deficient mice [19]. LMNA is a known causative gene for DCM, accounting for 4–8% of patients with DCM [25–27]. In addition, some individuals with spinal muscular atrophy caused by the BICD2 ‘hot spot’ mutation, c.302C>T:p.Ser107Leu, presented with unexpected heart failure-associated symptoms and exertional and supine dyspnoea [17]. Considering the possible interaction between LMNA and BICD2 and the heart failure symptoms of BICD2 mutant patients, we hypothesized that the homozygous variant (NM_001003800.1:c.2429G>A) in the C-terminal region of BICD2 (Fig. 1C) would be a candidate DCM-causative variant in this family.

The missense variant of BICD2 (Fig. 1C), segregated with the disease phenotype. The Sorting Intolerant From Tolerant [28] algorithm prediction score for the variant was 0.003 (Additional file 1: Fig. S2), indicating a damaging role. Sanger sequencing confirmed that both parents were heterozygous carriers and that the two surviving DCM patients were homozygous at this locus (Fig. 1D). Furthermore, the amino acids affected by this variant are highly evolutionarily conserved in multiple species (Fig. 1E). Together, these findings indicate that the BICD2 missense variant could be harmful to protein function.

### Three BICD2 variants in 210 sporadic DCM cases
We further performed Sanger sequencing of BICD2 in 210 DCM cases and found one missense variant and two synonymous variants (Additional file 1: Fig. S3). The missense variant, located in exon 2 (NM_001003800.1:c.421C>A) (Additional file 1: Fig. S3A), contributed to an amino acid substitution from arginine to serine. Bioinformatic prediction suggested that it was a deleterious variant (Additional file 1: Table S2). The other two variants of BICD2 were synonymous (Additional file 1: Table S2). These results support the hypothesis that BICD2 may play a significant role in DCM pathogenesis.

### BICD2 expression in heart tissue
We explored the expression of BICD2 in heart tissues from humans as well as mice and zebrafish. Real-time PCR results showed that Bicd2 mRNA expressed in the hearts of mice and that its expression level increased with age (Fig. 2A). A similar expression pattern was also observed in the zebrafish heart, in which the expression of bcd2 was much higher at 7 months than at 5 months (Fig. 2B).

In addition, immunohistochemical staining of BICD2 in normal left atrial appendage supported its high expression in human (Fig. 2C). Moreover, RNA-seq of BICD2 from the GTEx database confirmed its relatively high expression in the human heart (Fig. 2D), and scRNA-seq data of BICD2 suggested that it was mainly expressed in myocytes, endothelial cells, and fibroblasts (Additional file 1: Fig. S4). Thus, we demonstrated that BICD2 expressed in the hearts of human, mice and zebrafish at both the mRNA and protein levels.

We further conducted in situ hybridization of bicd2 in zebrafish at embryonic stages (Fig. 2E). At 48 hpf, bicd2 was expressed in the retina, forebrain, midbrain and hindbrain regions, with strong expression in the pectoral fin bud base (Fig. 2E). Taken together, these data indicate that BICD2/Bicd2/bicd2 are expressed in the heart in multiple species, implying their involvement in heart development and function.

### Fig. 2 Expression of BICD2 in human, mouse and zebrafish heart tissues

A qPCR of Bicd2 in the hearts of C57 mice suggested an increasing trend in expression over the course of development (n = 3). Data are represented as the mean ± SD. B qPCR of BICD2 in the hearts of zebrafish suggested an increasing trend in expression over the course of development (n = 3). Data are represented as the mean ± SD. C Immunohistochemistry with anti-BICD2 antibody of normal human heart showing ubiquitous staining. D Bulk RNA-seq revealed the mRNA expression level (transcripts per million fragments) of BICD2 in multiple tissues from humans. E In situ hybridization of bicd2 in zebrafish revealed that bicd2 was expressed in the heart at the embryonic stage.
Fig. 2 (See legend on previous page.)
Knockout of bicd2 leads to partial embryonic lethality and altered cardiac function in homozygotes

We designed a bicd2 knockdown assay in zebrafish to unravel the associated phenotypic changes; the experimental workflow is shown in Fig. 3A. We injected Cas9/sgRNA into embryos of F0 generation zebrafish and further screened for bicd2-deficient zebrafish in F0 adult zebrafish (Fig. 3A) (CRISPR target sequence in Additional file 1: Table S3). Three fish were obtained and mated with each other to produce bicd2 heterozygous F1 offspring (Fig. 3A) (PCR primers for F1 mutant identification in Additional file 1: Table S4). Details for the generation of bicd2-KO zebrafish are provided in Additional file 1: Fig. S5. We then allowed the F1 generation fish to self-cross to obtain F2 generation fish with three segregating genotypes (Fig. 3A). We measured the survival rate of embryos with different genotypes at three embryonic time points (Fig. 3A). At the adult stage, we performed echocardiographic measurements and transcriptome sequencing to delineate possible regulatory mechanisms (Fig. 3A).

We firstly examined bicd2 immunostaining to determine whether bicd2 was efficiently knocked out. Immunostaining images showed lower bicd2 expression in the bicd2–fish (Fig. 3B). Viable embryos’ number was much lower in bicd2– than in the other two groups at all three developmental stages (Fig. 3C, Additional file 1: Fig. S6). At 50 hpf, only one bicd2–embryo survived (Additional file 1: Fig. S6) (Additional file 1: Table S5), while the number of surviving bicd2++ and bicd2++embryos were twenty-two and thirteen (Additional file 1: Fig. S6) (Additional file 1: Table S5), respectively. Thus, the proportion of viable homozygous embryos was 2.78%, far lower than the theoretically predicted ratio of 25%. A similar trend was observed at both 76 hpf and 120 hpf (Fig. 3C, Additional file 1: Fig. S6) (Additional file 1: Table S5). The results suggests that bicd2 plays a vital role in zebrafish embryogenesis and supports the conclusion that bicd2 knockout leads to partial embryonic lethality. The heart rate of bicd2–, bicd2++ and bicd2++fish at three embryonic stages showed no significant difference (Additional file 1: Fig. S7) (Additional file 1: Table S6). In addition, no obvious phenotype was observed during the embryo development period (images not shown).

Electrocardiography was performed on bicd2– and bicd2++fish at seven months. Ventricular chamber size was assessed based on volume and area. We therefore measured the two-dimensional end-diastolic area (VAd) and end-systolic area (VAs) and three-dimensional end-diastolic volume (EDV) and end-systolic volume (ESV) in both bicd2–and bicd2++zebrafish. Compared with bicd2++zebrafish, bicd2–fish showed slightly larger values in both VAd and VAs (Fig. 3D, Additional file 1: Table S7). The EF was much lower in bicd2–than in bicd2++fish (Wilcox test, P value = 0.063) (Fig. 3D, Additional file 1: Table S7). EF < 45% is diagnostic for DCM [29]. In our studies, most bicd2–fish presented EF < 45%, supporting that bicd2 is crucial for cardiac function. Moreover, cardiac output was significantly lower in the bicd2–fish than bicd2++fish (Wilcox test, P value < 0.05) (Fig. 3D, Additional file 1: Table S7). Echocardiographic images representing zebrafish hearts with two different genotypes were extracted from echocardiograms and were compared (Fig. 3E). In summary, these results indicate that bicd2 knockout may cause abnormal contraction of the heart.

RNA-seq of bicd2-deficient zebrafish revealed a transcriptomic shift in cardiomyocytes

We conducted transcriptome sequencing of heart tissues from the bicd2–, bicd2++ and bicd2++fish to examine the transcriptional differences. Consistent with expectations, bicd2 expression was significantly lower in the knockout group than in the bicd2++fish (Additional file 1: Table S8). Gene set enrichment analysis (GSEA) [30] based on ranking of all expressed genes can provide more informative biological evidence than enrichment analysis focused solely on differentially expressed genes (DEGs). Therefore, we first utilized GSEA to identify significantly enriched gene sets. Compared to the bicd2++fish, the bicd2–group showed enrichment of cardiopathy-related signaling pathways and metabolic pathways, for example, KEGG_CARDIAC_MUSCLE_CONTRACTION, KEGG_CALCIUM_SIGNALING_PATHWAY, KEGG_OXIDATIVE_PHOSPHORYLATION, KEGG_GLYCOL.
Fig. 3 (See legend on previous page.)
YSIS_GLUCONEOGENESIS, KEGG_CITRATE_CYCLE_TCA_CYCLE and KEGG_PENTOSE_PHOSPHATE_PATHWAY (Fig. 4A), as well as pathways associated with nervous system disease (Additional file 1: Table S9). These results are consistent with functional explorations of Bicd2 in mice, which proved a causal role of Bicd2 in neuronal disorders [19]. In addition, pathways related to mitochondrial energy metabolism, such as oxidative phosphorylation (OXPH) and the tricarboxylic acid cycle (TCA), are common characteristics of distinct heart failure, as demonstrated previously [31, 32]. Interestingly, 6 out of 14 pathways (Fig. 4B) exhibited increases in expression in both the bicd2− and bicd2++ fish, including KEGG_CACLIUadox_SIGNALING_PATHWAY and KEGG_GLYCOLYSIS_GLU.

-CONEOGENESIS. Only 3 common pathways exhibited decreased expression in those two groups (Fig. 4C), implying the transcriptome differences between the bicd2− and bicd2++ fish.

We then extracted DEGs to further delineate biologically meaningful pathways involved in the bicd2 gene regulatory network. As expected, the bicd2− fish displayed the largest change among all three groups, with a total of 1708 DEGs (483 upregulated genes, 1225 downregulated genes, Fig. 4D, Additional file 2: Table S10). Surprisingly, the difference between the two groups of bicd2-KO genotypes was much larger than that between the bicd2− and bicd2++ fish. In detail, there were 1645 DEGs (417 upregulated genes, 1228 downregulated genes, Fig. 4D, Additional file 2: Table S10) between the bicd2− and the bicd2++ fish. And 535 DEGs (334 upregulated genes, 201 downregulated genes, Fig. 4D, Additional file 4: Table S12) between the bicd2++ fish and the bicd2++ fish. The top 15 enriched GO pathways based on 1708 DEGs discovered between the bicd2− and the bicd2++ fish are displayed (Additional file 1: Fig. S8-9). Genes with increased expression in the bicd2++ fish were mainly related to blood vessel development, extracellular matrix organization, cell–cell adhesion, carbohydrate metabolic process and circulatory system process (Additional file 1: Fig. S8). However, the genes with decreased expression were mainly related to the immune system, including regulation of immune system processes, response to biotic stimulus, and leukocyte activation (Additional file 1: Fig. S9). Notably, an increased expression of genes encoding extracellular matrix proteins in DCM patients was previously demonstrated by microarray [31]. In our study, extracellular matrix genes (Fig. 4E) (Additional file 1: Fig. S10), such as col4a2, col4a4, col5a1, col8a1b, col18a1b, and genes involved in circulatory system processes showed higher expression in the homozygous group than in the wild-type group (Fig. 4F) (Additional file 1: Fig. 11). Genes related to the regulation of immune system processes (Fig. 4G) (Additional file 1: Fig. 12), such as cd74b and cd79a, exhibited decreased expression in the two bicd2-KO groups, implying activation of the immune system after bicd2 knockout in zebrafish.

A set of 51 genes with evidence of association with susceptibility to inheritable DCM in humans was established in a previous study [4]. Orthologue retrieval in the zfin data report and Ensembl identified zebrafish homologues for 48 of these genes (Additional file 5: Table S13). Among these 48 genes, 28 had a single orthologue in zebrafish (Additional file 1: Fig. S13, Additional file 5: Table S13). 8 out of these 28 orthologues, including bag3, nebi, pse2, jph2, dtta, pse1, gata1 and pleklm2, showed the lowest expression in the homozygous group (Additional file 1: Fig. S13). In contrast, 10 out of 28 orthologues, including mybpc3, lama4, nppa, tnii3k, cmlc1, nexn, lrrc10, csrp3, abcc9, and tcap, showed the highest expression in the homozygous group (Additional file 1: Fig. S13). Multiple zebrafish orthologues were identified for 20 human DCM candidate genes (Additional file 1: Fig. S14).

**Discussion**

Most previously reported familial DCM cases are caused by variants inherited in an autosomal dominant pattern [3]. Currently, more than 250 variant genes spanning >10 gene ontologies have been suggested to contribute to inherited DCM [4]. However, the heritability of DCM cannot be completely explained by the variants discovered thus far. Here, we searched for the pathological variant causing DCM in a consanguineous family and discovered BICD2 as a novel DCM candidate disease gene. Notably, the inheritance mode of DCM in the

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**Fig. 4** bicd2-deficient hearts exhibited cardiac transcriptome shift. A, GSEA plots of six KEGG pathways showing higher expression in bicd2 zebrafish. B, Venn diagrams showing enriched KEGG pathways among genes with increased expression in the bicd2-deficient groups. C, Venn diagrams showing enriched KEGG pathways among genes with decreased expression in the bicd2-deficient groups. D, Differentially expressed genes in the two bicd2-deficient groups. Cells are filled according to z score: red indicates higher (activated), blue indicates lower (inhibited). Differentially expressed genes involved in extracellular matrix organization. Cells are filled according to z score: red indicates higher (activated), blue indicates lower (inhibited). Differentially expressed genes involved in circulatory system processes. Cells are filled according to z score: red indicates higher (activated), blue indicates lower (inhibited).
Fig. 4 (See legend on previous page.)
consanguineous family was autosomal recessive, suggesting that loss of function contributes to the disease. 

*BICD2* is a dynein-activating adaptor protein that was previously implicated as a causative gene in autosomal dominant spinal muscular atrophy. Individuals carrying heterozygous missense variants in *BICD2* exhibit muscle weakness and atrophy predominantly of the proximal lower limbs. However, the inheritance mode of *BICD2* in our DCM family was autosomal recessive. None of the previous DCM genetic researches have reported functional role of *BICD2* or the association of *BICD2* to DCM. Our functional characterization of *BICD2* in DCM may advance our understanding of the genetic underpinnings of DCM, facilitating early genetic screening of familial DCM.

However, a limitation of our work is that we have not yet proven a direct relationship between the *BICD2* variant identified and phenotypes associated with an enlarged heart. We will continue to explore the cardiac dysfunction caused by this *BICD2* variant. We will continue our work in elucidating molecular mechanisms underlying the pathogenesis of familial DCM.

**Conclusions**

Our study shows that *BICD2*, an adapter protein linking the dynein motor complex to various cargos, is a novel DCM candidate gene and conceptually expands our horizons regarding pathogenesis of DCM.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12920-022-01349-y.

**Acknowledgements**

The authors would like to thank all patients who provided samples used in this study.

**Author contributions**

KL: Conception and design, Collection and assembly of data, Data analysis and interpretation, Manuscript writing; CZ: RL: Provision of study material, Data analysis and interpretation; XC: HS: Provision of study material, Assembly of data; HM, JH, YX: Responsible for manuscript writing and revision of the manuscript; XW: Collection and assembly of data, Data analysis and interpretation, Final approval of manuscript; XL: Conception and design, Manuscript writing, Financial support, Final approval of manuscript. XL is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

**Funding**

This work was supported by grants from Chinese National Natural Science Foundation (No. 81770379, 32171182, 81470521, and 81670290), the Foundation of Chengdu Medical College (CYZZD21-04, 2021LHPJ-02) and Health and Family Planning Commission of Sichuan Province (18PJ373).

**Availability of data and materials**

The datasets are available on China National GeneBank DataBase(CNGBdb) under project accession CNP0003349 (https://db.cnbg.org/cnsa/variant/CNP0003349_2f12e5dB/reviewlink/).

**Declarations**

**Ethical Approval and consent to participate**

All experiments and methods were performed in accordance with relevant guidelines and regulations. Animal experiments of the study is reported in accordance with ARRIVE guidelines. Human research of this study was approved by the Institutional Research Committee of Sichuan Provincial People's Hospital. Written informed consent was obtained from all subjects participating.

**Consent for publication**

Written informed consent was obtained from the patient for publication of clinical details.

**Competing interest**

The authors declare that they have no competing interests.

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**Received:** 25 May 2022   **Accepted:** 23 August 2022
**Published online:** 06 September 2022

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