ORIGINAL ARTICLE

WILEY

# Whole-exome sequencing reveals two *de novo* variants in the *RBM20* gene in two Chinese patients with left ventricular non-compaction cardiomyopathy

Qiqing Sun[1*] | Jun Guo[2,3*] | Chanjuan Hao[2,3] | Ruolan Guo[2,3] | Xuyun Hu[2,3] | Yuanying Chen [2,3] | Weili Yang[3] | Wei Li[2,3] | Yingjun Feng[1]

[1]Department of Cardiology, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou, China
[2]Beijing Key Laboratory for Genetics of Birth Defects, Beijing Pediatric Research Institute; MOE Key Laboratory of Major Diseases in Children; Genetics and Birth Defects Control Center, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China
[3]Henan Key Laboratory of Pediatric Inherited & Metabolic Diseases, Henan Children's Hospital, Zhengzhou Hospital of Beijing Children's Hospital, Zhengzhou, China

**Correspondence**
Yingjun Feng, Department of Cardiology, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou, 450018, China.
Email: fengyingjunvip@163.com
Wei Li, Beijing Children's Hospital, Capital Medical University, Beijing 100045, China.
Email: liwei@bch.com.cn

*These authors contributed equally to this paper.

Received: 20 August, 2019
Accepted: 31 January, 2020

**ABSTRACT**

**Importance:** Pathogenic variants in the *RBM20* gene are associated with aggressive dilated cardiomyopathy (DCM). Recently, *RBM20* was found to be associated with left ventricular non-compaction cardiomyopathy (LVNC). Thus far, only five families with LVNC have been reported to carry variants in *RBM20*. It remains unknown whether the variants in *RBM20* associated with DCM can also cause LVNC.

**Objective:** To elucidate the causative *RBM20* variant in two unrelated patients with both LVNC and DCM, and to identify the clinical characteristics associated with variants in *RBM20*.

**Methods:** Trio whole-exome sequencing (WES) was performed. Variants were filtered and classified in accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG).

**Results:** We identified two distinct *de novo* variants in *RBM20* (one per patient) in these two patients with LVNC. Both variants have been reported in patients with DCM, without the LVNC phenotype. Patient 1 was an 11-year-old girl who had DCM, LVNC, and heart failure; the ratio of noncompacted-to-compacted myocardium was 2.7:1. A *de novo* heterozygous variant c.1907G>A (p.Arg636His) in exon 9 was identified in this patient. Patient 2 was a 13-year-old boy who had clinical phenotypes identical to those of Patient 1; the ratio of noncompacted-to-compacted myocardium was 3.2:1 in this patient. WES revealed a *de novo* heterozygous variant c.1909A>G (p.Ser637Gly) in exon 9. Both variants were previously characterized as pathogenic, and our study classified them as pathogenic variants based on the ACMG guidelines.

**Interpretation:** We found that two patients with LVNC had variants in *RBM20*. Our results extended the clinical spectrum of the two *RBM20* variants and illustrated that the same variant in *RBM20* can cause DCM, with or without the LVNC phenotype.

**KEYWORDS**

Left ventricular non-compaction cardiomyopathy, Dilated cardiomyopathy, RNA-binding motif protein 20, Trio whole-exome sequencing

DOI: 10.1002/ped4.12183

## INTRODUCTION

Left ventricular non-compaction cardiomyopathy (LVNC) is characterized by the presence of excessive left ventricular trabeculae, deep intratrabecular recesses, and a thin compacted myocardial layer.[1] The LVNC phenotype may occur either in isolated form or in combination with additional cardiomyopathy phenotypes including dilated, hypertrophic, and restrictive cardiomyopathies, and/or other congenital heart diseases.[2,3] LVNC may be asymptomatic or manifest as arrhythmia, thromboembolism, and/or heart failure; compared with other phenotypes, LVNC with a dilated phenotype is associated with higher mortality and more severe cardiac events.[4]

Most LVNC-associated genes have also been reported in patients with hypertrophic cardiomyopathy and dilated cardiomyopathy (DCM).[5] Variants of sarcomere genes have been identified in a majority of patients with genetic LVNC; *MYH7*, *MYBPC3*, and *TTN* were the most common mutated genes in these patients.[5,6] Variants in *RBM20*, which encodes RNA-binding protein 20, have been found in patients with clinically aggressive DCM.[7] Recently, *RBM20* was also identified as a novel causative gene in patients with LVNC[8]; to the best of our knowledge, only five variants in *RBM20* have been identified in patients with LVNC thus far.[5,6,8]

Here, we used trio whole-exome sequencing (WES) to identify two *de novo* heterozygous *RBM20* variants in two unrelated patients with LVNC and DCM; these variants had previously been found only in patients with DCM who did not have the LVNC phenotype.

## METHODS

### Ethical approval

The study was approved by the Ethics Committee of Henan Children's Hospital. Written informed consents for participation and publication were obtained from the guardians of each patient.

### Patients

Patient 1 was an 11-year-old girl, who was admitted to Henan Children's Hospital because of chest tightness and hypodynamia. Patient 2 was a 13-year-old boy without a family history of cardiomyopathy; he was admitted to Henan Children's Hospital because of chest tightness, cough, and tachypnea during sports. Clinical data, pedigree information, and blood samples were collected from the two probands, as well as the probands' parents and other available family members. All participants underwent detailed physical evaluations, including blood examination, electrocardiography, chest radiography, and echocardiography. LVNC was diagnosed on the basis of a characteristic morphological appearance comprising: 1) multiple trabeculations, 2) deep intertrabecular recesses visible on color flow, and 3) a two-layered myocardial structure with a > 2:1 ratio of non-compacted to compacted myocardium in systole.[9]

### Sequencing

Trio WES of the two probands and their parents was performed as previously described.[10] In brief, DNA was isolated from 200 μL blood samples from the two probands and their family members, using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Exon capture was performed using the Agilent SureSelect Human All Exome V6 kit (Agilent Technologies Inc., Santa Clara, CA, USA). WES was performed on an Illumina Hiseq X Analyzer (Illumina, San Diego, CA, USA) in 150-bp paired-end mode, in accordance with the manufacturer's recommendations. Sequences obtained from WES were aligned to the GRCh37/hg19 human reference genome. Variants were filtered if they had a minor allele frequency > 0.5%, or if they had a minor allele frequency > 2% when the variant was homozygous or there was a second variant in the gene in three SNP databases (db-SNP, gnomAD v2, and 1000 Genomes Project Database). SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org), and CADD (http://cadd.gs.washington.edu/) were used to predict the potential impacts of the identified mutations on protein function. Variants were classified in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines.[11] A virtual cardiomyopathy-related gene panel was generated that included 409 genes based on the HPO term "cardiomyopathy" (HP:0001638; http://www.human-phenotype-ontology.org/); this panel was supplemented with 10 genes that were not included in this HPO term, but were associated with LVNC (Supplementary Table S1).[12] Putative pathogenic variants in cardiomyopathy-related genes detected by WES were confirmed by Sanger sequencing with a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) in the two probands, their parents, and available family members.

## RESULTS

### Clinical information

Chest radiography of Patient 1 revealed pneumonia and marked cardiomegaly. Echocardiography examination revealed dilation of both atria and both ventricles with prominent trabeculations, a depressed left ventricular ejection fraction of 22%, moderate to severe tricuspid regurgitation, and mild mitral regurgitation. The ratio of noncompacted-to-compacted myocardium was 2.7:1 (Figure 1A); trabeculations and deep intertrabecular recesses were observed (Figure 1B). The left and right ventricular diastolic dimensions were 66.6 mm and 16.7 mm, respectively; left and right atrial diameters were

52.8 mm and 52.4 mm, respectively. Electrocardiography revealed sinus rhythm with P-wave abnormalities, ST-T changes, transient atrial tachycardia, and atrial fibrillation (Figure 1C). Serum biochemistry tests showed high levels of N-terminal fragment of pro-B-type natriuretic peptide (8482 pg/mL; normal range, < 100 pg/mL). The patient was treated with optimal doses of an angiotensin-converting-enzyme inhibitor (captopril), diuretics (hydrochlorothiazide and spironolactone), and potassium citrate, as well as oral anticoagulant (aspirin). She was diagnosed with LVNC, DCM and heart failure, and showed no response to treatment. Her parents were nonconsanguineous and reported no family history of cardiomyopathy. The patient's parents and her younger brother had no symptoms of heart failure; their echocardiography findings were normal.

Echocardiography examination of Patient 2 revealed marked ventricular dilation (left and right ventricular diastolic dimensions of 71.3 mm and 23.7 mm, respectively) and atrial dilation (left and right atrial diameters of 58.4 mm and 42.4 mm, respectively). Diffuse hypokinesis was present with depressed systolic function (left ventricular ejection fraction of 25%). The ratio of noncompacted-to-compacted myocardium was 3.2:1 (Figure 2A); trabeculations and deep intertrabecular recesses were observed (Figure 2B). The patient was diagnosed with LVNC, DCM and heart failure. Electrocardiography revealed sinus rhythm with ST-T changes and occasional premature ventricular contraction (Figure 2C). The patient was treated with an angiotensin-converting-enzyme inhibitor (captopril), diuretics (hydrochlorothiazide and spironolactone), and potassium citrate, as well as an oral anticoagulant (aspirin). The treatment was ineffective; subsequently, the patient died due to heart failure and respiratory infection. Echocardiography analyses of the patient's other family members (his parents and his elder sister) did not demonstrate any clinical evidence of DCM or LVNC.

**Genetic findings**

WES data were filtered by using db-SNP, gnomAD v2, and the 1000 Genomes Project Database to exclude common variants. In total, 227 unique variants were detected in Patient 1, while 270 unique variants were detected in Patient 2. After cardiomyopathy-related gene filtering, six variants in five genes were identified in Patient 1 (Supplementary Table S2); seven variants in five genes were identified in Patient 2 (Supplementary Table S3). All filtered data were predicted by four separate bioinformatics programs (SIFT, Polyphen-2, MutationTaster, and CADD).

A *de novo* missense variant, c.1907G>A (p.Arg636His, rs267607004), was identified in *RBM20* in Patient 1 (Figure 3). Sanger sequencing of this variant was performed in all four family members; the results showed that the proband's unaffected parents and younger brother did not carry this variant. c.1907G>A was located in a highly conserved genomic region in exon 9; it was not present in gnomAD v2 or the 1000 Genomes Project Database. This variant was predicted to have a deleterious effect on the gene or gene product by SIFT, PolyPhen-2, MutationTaster, and CADD. In accordance with the ACMG guidelines, c.1907G>A was classified as a pathogenic variant. Furthermore, no other pathogenic or likely pathogenic variants in cardiomyopathy disease genes were identified in Patient 1.

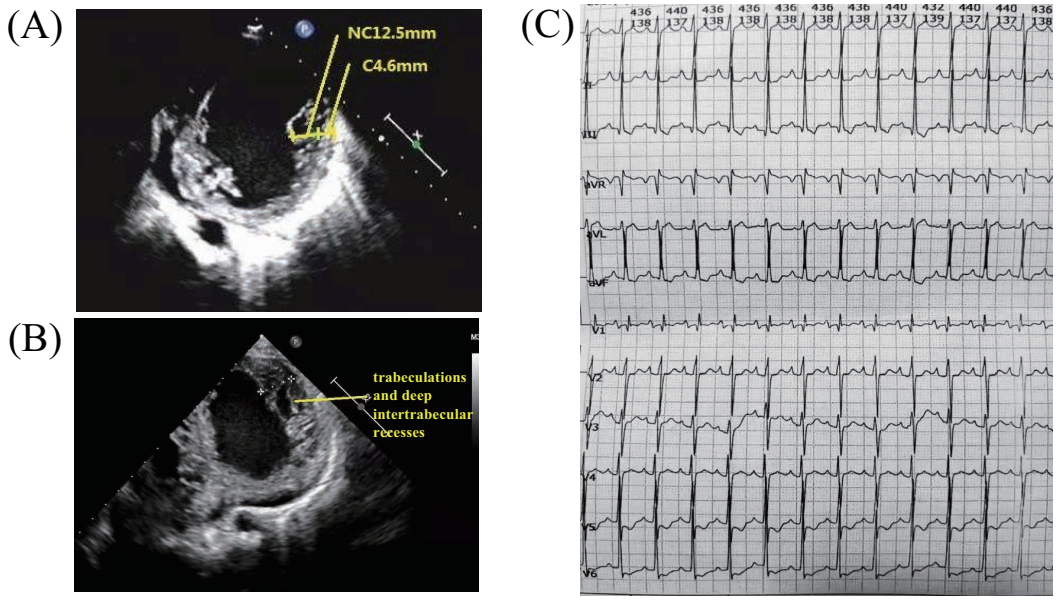


**FIGURE 1** Echocardiography and electrocardiography findings in Patient 1. (A) Echocardiography examination revealed a 2.7:1 ratio of noncompacted-to-compacted myocardium. (B) Trabeculations and deep intertrabecular recesses were observed in echocardiography examination. (C) Electrocardiography revealed sinus rhythm with P-wave abnormalities and ST-T changes.

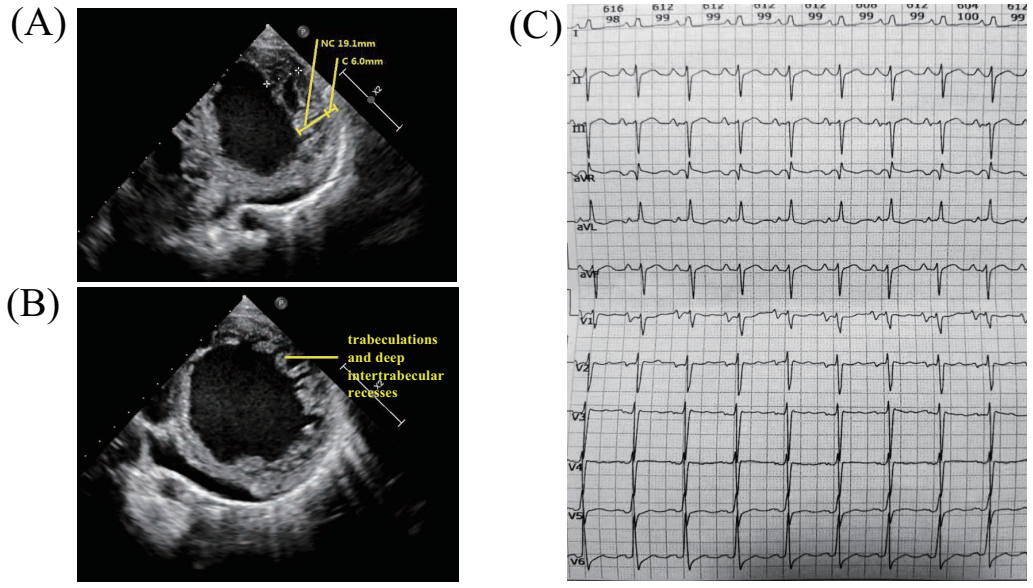


**FIGURE 2** Echocardiography and electrocardiography findings in Patient 2. (A) Echocardiography examination revealed a 3.2:1 ratio of noncompacted-to-compacted myocardium. (B) Trabeculations and deep intertrabecular recesses were observed in echocardiography examination. (C) Electrocardiography revealed sinus rhythm with ST-T changes.

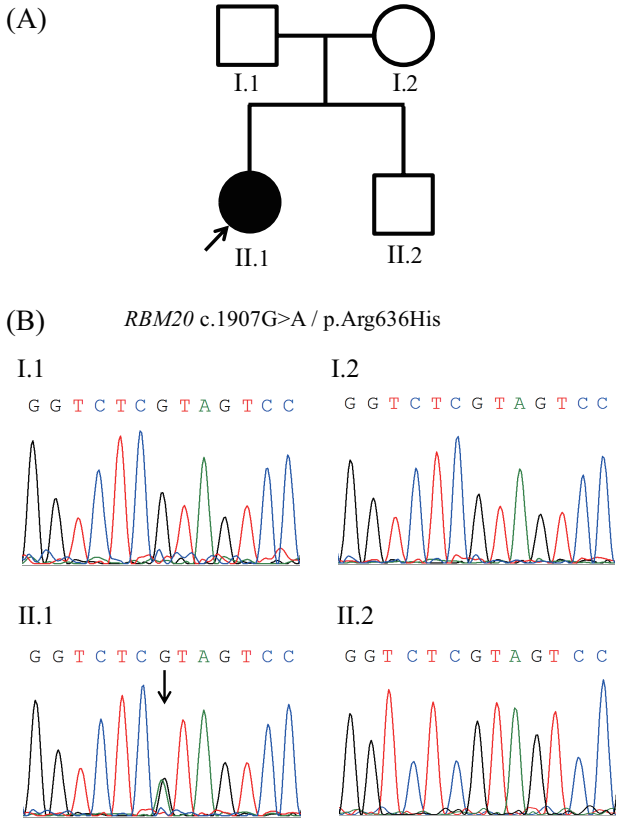


**FIGURE 3** Family pedigree and Sanger sequencing analysis of *RBM20* in Patient 1 and her family members. (A) Pedigree includes Patient 1 (II.1), her unaffected parents (I.1 and I.2), and her unaffected younger brother (II.2). (B) Sanger sequencing analysis of *RBM20*. Arrows indicate mutated nucleotides. The proband (II.1) carries a *de novo* missense variant, c.1907G>A (p.Arg636His), while her younger brother (II.2) does not.

Patient 2 carried a *de novo* missense variant, c.1909A>G (p.Ser637Gly, rs267607005), in *RBM20* (Figure 4). Sanger sequencing was performed in the proband and his parents. A blood sample from the proband's elder sister was not available. c.1909A>G was also located in exon 9 and not present in gnomAD v2 or the 1000 Genomes Project Database. This variant was considered to be "damaging" by multiple lines of computational evidence (SIFT, PolyPhen-2, MutationTaster, and CADD). In accordance with the ACMG guidelines, c.1909A>G was also classified as a pathogenic variant. Also no pathogenic or likely pathogenic variants in any other cardiomyopathy disease genes were identified in Patient 2.

## DISCUSSION

We identified two *de novo* heterozygous variants in *RBM20*, c.1907G>A and c.1909A>G, in two unrelated patients with LVNC and DCM. Although these variants (c.1907G>A[7,13-15] and c.1909A>G[7]) have been previously identified in patients with DCM, none of those patients have been reported to exhibit concurrent LVNC.

*RBM20* is a key cardiac splice regulator that controls the processing of several important transcripts predominantly expressed in striated muscle, with the highest levels in heart tissue.[16,17] *RBM20* mutations can cause mis-splicing of several targets and often lead to progressive DCM with conduction diseases, as well as atrial or malignant ventricular arrhythmia.[7,17] Multiple human *RBM20* pathogenic variants that cause DCM have been identified thus far, including a mutational hot spot in an arginine/serine-rich domain.[7,15,18] Recurrent pathogenic missense variants in the arginine/serine-rich region disrupt binding

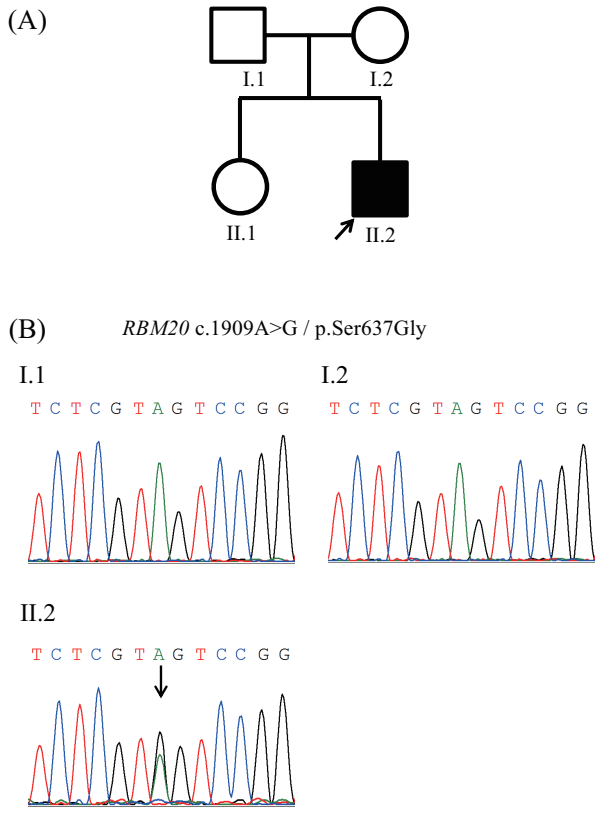


**FIGURE 4** Family pedigree and Sanger sequencing analysis of *RBM20* in Patient 2 and his family members. (A) Pedigree includes Patient 2 (II.2), his unaffected parents (I.1 and I.2), and his unaffected elder sister (II.1). (B) Sanger sequencing analysis of *RBM20*. Arrows indicate mutated nucleotides. The proband (II.2) carries a *de novo* missense variant, c.1909A>G (p.Ser637Gly). The proband's elder sister did not undergo genetic testing

with other splicing factors and alter transcript processing, causing highly penetrant DCM.[16]

Recently, *RBM20* was identified as a novel LVNC-associated gene.[8] To the best of our knowledge, only five LVNC-associated variants in *RBM20* have been reported thus far, including three missense variants: c.1901G>T (p.Arg634Leu),[8] c.3416C>T (p.Pro1139Leu),[6] and c.3584C>A (p.Ser1195Tyr)[6]; a splicing region variant: c.192-3C>T[6]; and a frameshift variant: c.846_853delTTACGGAC (p.Tyr283Glnfs*14).[5] However, the ACMG guidelines indicate that only two of these variants, c.1901G>T (p.Arg634Leu) and c.846_853delTTACGGAC (p.Tyr283Glnfs*14), can be classified as pathogenic or likely pathogenic. The two variants identified in this study, c.1907G>A and c.1909A>G, were both located in the arginine/serine-rich region of *RBM20*. c.1907G>A has been reported to segregate with DCM in three families[7,13,15]; DCM was most noticeable during adolescence or young adulthood in each family. c.1909A>G was identified in four patients from a family with DCM, in which the age of diagnosis ranged from 20 to 44 years.[7] However, none of the patients in these families were reported to exhibit LVNC. Our work extends the phenotypic spectra of these two variants by reporting their associations with the LVNC phenotype.

*RBM20* is an important splice regulator of *TTN*; notably, *RBM20* regulates alternative splicing of the *TTN* gene by exon skipping or exon shuffling. Functional studies have shown that the c.1901G>T variant in *RBM20*, identified in patients with LVNC, leads to an abnormal giant isoform of *TTN*.[8] *TTN* is the most prevalent disease-causing gene in patients with DCM and LVNC. It has been reported that variants in a single gene (e.g., *SCN5A* or *PKP2*) can lead to apparently unrelated phenotypes in inheritable cardiac disorders. Mutations in *SCN5A* have been previously identified in patients with DCM and long QT syndrome.[19] Various phenotypes, ranging from purely arrhythmogenic (Brugada syndrome or catecholaminergic polymorphic ventricular tachycardia) to purely structural (DCM), as well as arrhythmogenic cardiomyopathy, could result from dysfunction in the *PKP2* gene.[20] In this study, the two probands exhibited clinical phenotypes distinct from those of previously described patients with identical variants in *RBM20*. There may be an underlying mutation in other genes that contributes to the LVNC phenotype (i.e., a causative mutation or genetic modifier). However, it remains unclear why these two patients exhibited LVNC with DCM, rather than DCM alone.

In conclusion, we have described two patients with distinct *RBM20* variants that were not previously associated with LVNC; no pathogenic or likely pathogenic variants in any other cardiomyopathy disease genes were identified in either patient, which might have contributed to the deviant phenotype. This study extended the clinical spectrum of phenotypes associated with variants in *RBM20*. Furthermore, although the mechanism remains unknown, we have shown that the same variant in *RBM20* can cause distinct cardiac phenotypes.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Arbustini E, Weidemann F, Hall JL. Left ventricular noncompaction: a distinct cardiomyopathy or a trait shared by different cardiac diseases? J Am Coll Cardiol. 2014;64:1840-1850.
2. Jefferies JL, Wilkinson JD, Sleeper LA, Colan SD, Lu M, Pahl E, et al. Cardiomyopathy phenotypes and outcomes for children with left ventricular myocardial noncompaction: Results from the pediatric cardiomyopathy registry. J Card Fail. 2015;21:877-884.
3. Towbin JA. Left ventricular noncompaction: a new form of heart failure. Heart Fail Clin. 2010;6:453-469.
4. Brescia ST, Rossano JW, Pignatelli R, Jefferies JL, Price JF, Decker JA, et al. Mortality and sudden death in pediatric left ventricular noncompaction in a tertiary referral center.

Circulation. 2013;127:2202-2208.
5. van Waning JI, Caliskan K, Hoedemaekers YM, van Spaendonck-Zwarts KY, Baas AF, Boekholdt SM, et al. Genetics, clinical features, and long-term outcome of noncompaction cardiomyopathy. J Am Coll Cardiol. 2018;71:711-722.
6. Miszalski-Jamka K, Jefferies JL, Mazur W, Glowacki J, Hu J, Lazar M, et al. Novel genetic triggers and genotype-phenotype correlations in patients with left ventricular noncompaction. Circ Cardiovasc Genet. 2017;10:pii:e001763.
7. Brauch KM, Karst ML, Herron KJ, de Andrade M, Pellikka PA, Rodeheffer RJ, et al. Mutations in ribonucleic acid binding protein gene cause familial dilated cardiomyopathy. J Am Coll Cardiol. 2009;54:930-941.
8. Sedaghat-Hamedani F, Haas J, Zhu F, Geier C, Kayvanpour E, Liss M, et al. Clinical genetics and outcome of left ventricular non-compaction cardiomyopathy. Eur Heart J. 2017;38:3449-3460.
9. Jenni R, Oechslin E, Schneider J, Attenhofer Jost C, Kaufmann PA. Echocardiographic and pathoanatomical characteristics of isolated left ventricular non-compaction: a step towards classification as a distinct cardiomyopathy. Heart. 2001;86:666-671.
10. Guo J, Li Z, Hao C, Guo R, Hu X, Qian S, et al. A novel de novo *CASZ1* heterozygous frameshift variant causes dilated cardiomyopathy and left ventricular noncompaction cardiomyopathy. Mol Genet Genomic Med. 2019;7:e828.
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-424.
12. Harakalova M, Kummeling G, Sammani A, Linschoten M, Baas AF, van der Smagt J, et al. A systematic analysis of genetic dilated cardiomyopathy reveals numerous ubiquitously expressed and muscle-specific genes. Eur J Heart Fail. 2015;17:484-493.
13. Li D, Morales A, Gonzalez-Quintana J, Norton N, Siegfried JD, Hofmeyer M, et al. Identification of novel mutations in *RBM20* in patients with dilated cardiomyopathy. Clin Transl Sci. 2010;3:90-97.
14. Walsh R, Thomson KL, Ware JS, Funke BH, Woodley J, McGuire KJ, et al. Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. Genet Med. 2017;19:192-203.
15. Wells QS, Becker JR, Su YR, Mosley JD, Weeke P, D'Aoust L, et al. Whole exome sequencing identifies a causal *RBM20* mutation in a large pedigree with familial dilated cardiomyopathy. Circ Cardiovasc Genet. 2013;6:317-326.
16. Maatz H, Jens M, Liss M, Schafer S, Heinig M, Kirchner M, et al. RNA-binding protein RBM20 represses splicing to orchestrate cardiac pre-mRNA processing. J Clin Invest. 2014;124:3419-3430.
17. Guo W, Schafer S, Greaser ML, Radke MH, Liss M, Govindarajan T, et al. *RBM20*, a gene for hereditary cardiomyopathy, regulates titin splicing. Nat Med. 2012;18:766-773.
18. Millat G, Bouvagnet P, Chevalier P, Sebbag L, Dulac A, Dauphin C, et al. Clinical and mutational spectrum in a cohort of 105 unrelated patients with dilated cardiomyopathy. Eur J Med Genet. 2011;54:e570-575.
19. Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, et al. Atlas of the clinical genetics of human dilated cardiomyopathy. Eur Heart J. 2015;36:1123-1135a.
20. Cerrone M, Remme CA, Tadros R, Bezzina CR, Delmar M. Beyond the One Gene-One Disease Paradigm: Complex genetics and pleiotropy in inheritable cardiac disorders. Circulation. 2019;140:595-610.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Sun Q, Guo J, Hao C, Guo R, Hu X, Chen Y, et al. Whole-exome sequencing reveals two *de novo* variants in the *RBM20* gene in two Chinese patients with left ventricular non-compaction cardiomyopathy. Pediatr Invest. 2020;4:11-16. https://doi.org/10.1002/ped4.12183