



RAPID COMMUNICATION

Haploinsufficiency of syncoilin leads to hypertrophic cardiomyopathy



Here we reported a *SYNC* nonsense variant in a Chinese family with hypertrophic cardiomyopathy (HCM) and firstly linked syncoilin (*SYNC*) to HCM.

HCM is an inherited cardiovascular disease, affecting approximately 1:500 people, that is characterized by thickening of left ventricle (LV), especially the interventricular septum (IVS), and diastolic ventricular failure.¹ To date, more than 15 genes of two groups underlying HCM have been identified.¹ About 35%–60% HCM patients present autosomal dominant inheritance and carry a pathogenic variant in sarcomeric protein genes, such as β -myosin heavy chain (*MYH7*), myosin binding protein C (*MYBPC3*), and Troponin T (*TNNI3*).¹ In addition, non-sarcomeric genetic causes of disease have also been observed in about 25% HCM patients, mainly related to metabolic storage diseases, mitochondrial cardiomyopathies, inborn errors of metabolism etc.¹

Syncoilin is a member of the intermediate filament protein family, which is highly expressed in skeletal and cardiac muscle. As a part of dystrophin-associated protein complex at the sarcolemma, *SYNC* provides a link between the extracellular matrix and the cytoskeleton through its interaction with α -dystrobrevin and desmin.^{2,3} Previous studies have revealed that the expression of *SYNC* is increased in the muscle of patients with neuromuscular disease and decreased in patients with congenital myopathy,² which indicates that variants in *SYNC* may be involved in some forms of muscle diseases. Abnormally high levels of syncoilin have been shown to be a characteristic of neuromuscular wasting diseases such as desminopathy and muscular dystrophy. However, whether variants of *SYNC* have effects on cardiomyocytes have remained elusive.

In this study, we enrolled a Chinese family with cardiomyopathy (Fig. 1A). The proband (III-2), a 13-year-old girl with “cardiac murmur” for five years, hospitalized because of fainting on the stairs. Further computed tomography (CT) scan, 12-lead electrocardiogram and echocardiography

found that she suffered from left ventricular hypertrophy, abnormal repolarization, LV outflow tract obstruction and mitral regurgitation (Fig. 1B). CT presented significant hypertrophy of LV myocardium and interventricular septum with a maximum value of 39 mm and LV outflow tract obstruction. Echocardiography showed that the thickness of the base of the interventricular septum was 16 mm $\pm$, the thickness of the middle was 30 mm $\pm$, the posterior wall thickness of LV was 10 mm $\pm$, the inner diameter of LV outflow tract was 4 mm $\pm$ at the narrowest point and positive systolic anterior motion (SAM). Then the proband accepted the surgery to reconstruct LV outflow tract and mitral valvuloplasty. Postoperative echocardiography showed the inner diameter of LV outflow tract was 16 mm $\pm$ and negative SAM. Pathological sections with hematoxylin and eosin staining and Masson staining showed cardiomyocyte hypertrophy and interstitial fibrous hyperplasia (Fig. 1C). Family history investigation indicated that her father (II-3) and one aunt (II-1) also suffered from cardiomyopathy, which type and clinical data are unknown.

After written informed consent by all the participates and approved by institutional ethics committee of Central South University, exome sequencing was applied to analyze the genetic lesion of family. A novel heterozygous nonsense variant (NM_001161708, c.769C > T/p. Q257X) of *SYNC* was identified in the proband. Sanger sequencing validated that the nonsense variant was present in the affected individuals (II-1, II-3 and III-2) and absent in the healthy members (I-1, II-5, II-7, III-1 and III-3) (Fig. 1D). Moreover, the novel variant was located in the conserved domain and was also absent in 1000 Genomic, ExAC database and 200 unrelated ethnically matched healthy controls with an allele frequency of 0.00005077 in gnomAD. The controls were individuals presenting for routine health checkups or volunteers without similar symptoms or any positive family history of cardiovascular disorders (male/female: 100/100, mean age 36.1 $\pm$ 4.3 years). According to ACMG guidelines, the novel variant met the criteria of pathogenic variant (PS3+PM2+PM4+PP1+PP3+PP4).

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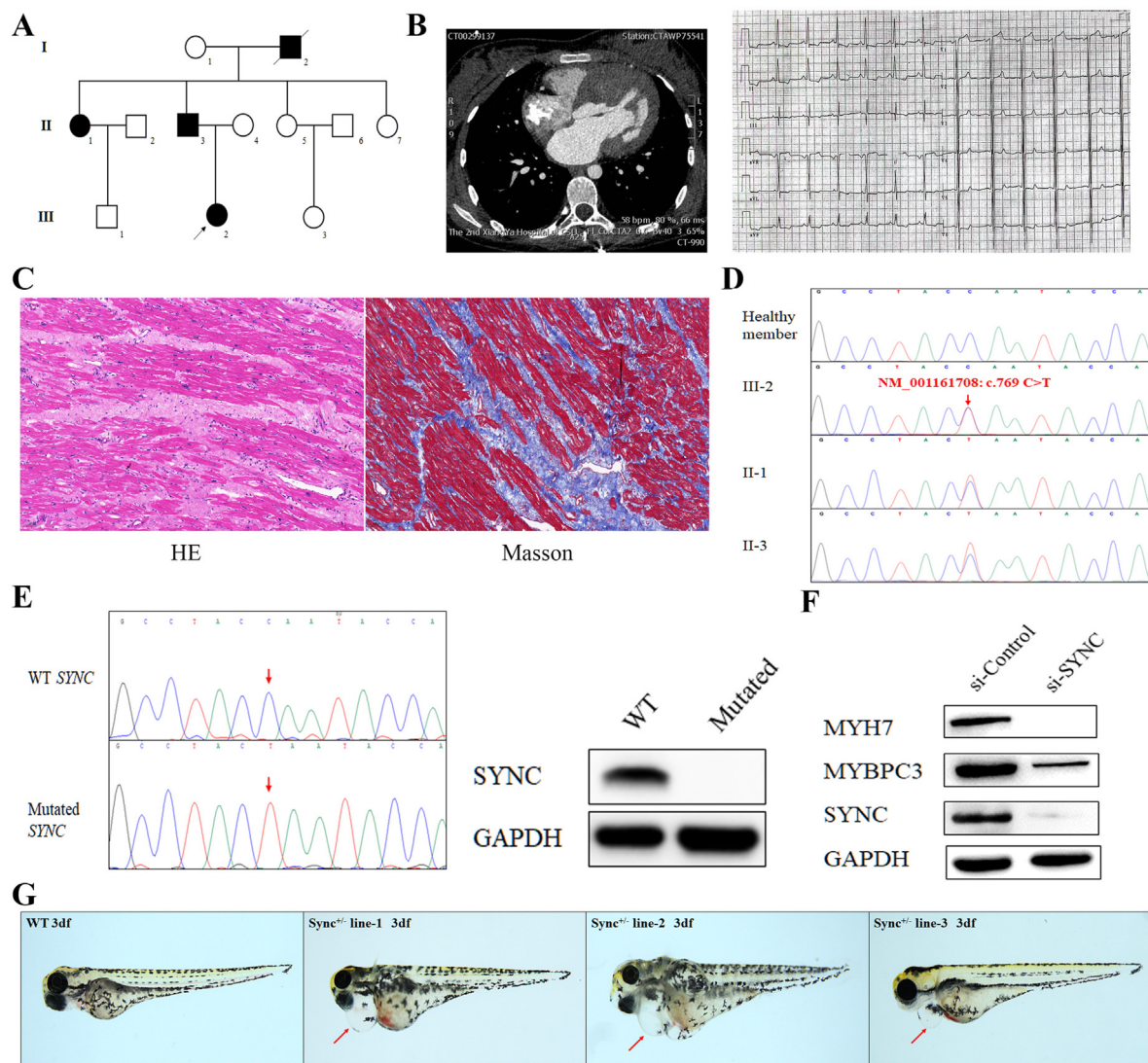


Figure 1 Clinical data, genetic analysis and functional research of this study. **(A)** Pedigree of the HCM family. The pedigree shows three generations of the family. Roman numerals refer to generations. Circles refer to female subjects. Squares refer to male subjects. Solid symbols refer to affected subjects. Crossed-out symbols refer to deceased subjects. The arrow indicates the proband. **(B)** The CT scan and 12-lead echocardiograms testing of the proband. **(C)** The HE and Masson staining of the proband's myocardial tissue. **(D)** Sanger sequencing analysis of the variant (NM_001161708, c.769C > T/p. Q257X) of SYNC in the proband, their family members, and the controls. **(E)** Sanger sequencing validates the construction of mutated SYNC plasmid and Western blot confirms that the mutated SYNC may not express in HEK-293 cell line. **(F)** Western blot detects the expression of MYH7 and MYBPC3 in AC16 cell line transfected with si-Control and si-RNA of SYNC. **(G)** The zebrafish with *sync*^{+/+} presents with pericardial effusion. The arrows indicate cardiac enlargement and pericardial effusion.

We then constructed the WT and Q257X mutated SYNC CDS plasmids. *In vitro* transfected HEK-293 cell lines indicated that this novel variant leads to loss of Syncoilin expression, possibly due to nonsense-mediated mRNA decay (Fig. 1E). Next, we used si-RNA to knockdown the expression of SYNC to simulate this new variant in AC16 cell lines, Western blot suggested that the expression of MYH7 and MYBPC3 was significantly reduced (Fig. 1F).

Finally, we constructed the *sync* knockout zebrafish model by CRISPR/Cas9; three days after birth, the zebrafish with loss of heterozygosity of SYNC showed obvious cardiac enlargement and pericardial effusion (Fig. 1G).

The intermediate filament (IF) proteins play a crucial role in the development of the sarcomere and the pathogenesis of cardiomyopathy.³ More than ten genes encoding IF related proteins have been identified in cardiomyopathy, such as *desmin*, *lamin A/C*, *synemin*, etc.³ Synemin, syncoilin and paranemin are expressed throughout cardiac development and may contribute to the stability of the desmin IF network.³ Furthermore, syncoilin can bind to α -dystrobrevin, a component of the dystrophin-associated protein complex, it has previously been demonstrated that variants in this complex led to major skeletal and cardiac diseases.³ Differences in the expression levels of

syncoilin in neurogenic disorder, congenital myopathy and control human muscle suggests that it may play either a causal or secondary role in these disorders.²

SYNM (synemin) null mice presented with LV systolic dysfunction, LV hypertrophy, and dilation.⁴ In addition, the isolated cardiomyocytes from *SYNM* null mice showed alterations in calcium transients.⁴ In our study, the patient with the novel variant (NM_001161708, c.769C > T/p. Q257X) of *SYNM* also presented with LV hypertrophy, and dilation. In addition, we also found that the reduction of *SYNM* may down-regulate the expression of *MYH7* and *MYBPC3*, which have been proved as the HCM-causing genes.⁵ Our study together with previous animal research proved that *SYNM* may be a new HCM-causing gene.

In summary, we identified a novel variant (NM_001161708, c.769C > T/p. Q257X) of *SYNM* in a Chinese family with HCM. Functional studies in AC16 cells and zebrafish proved that *SYNM* may be a new HCM-causing gene. Our research not only expands the spectrum of HCM-causing genes but also contributes to the genetic counseling of HCM patients and provides a better understanding of the pathogenesis of HCM.

Ethics declaration

The studies involving human subjects were reviewed and approved by Ethics Committee of the Second Xiangya Hospital of the Central South University and conformed to the principles outlined in the Declaration of Helsinki.

The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Second Xiangya Hospital of the Central South University. All procedures conform to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and the NIH Guide for the Care and Use of Laboratory Animals. No animals were euthanized.

Author contributions

Conceptualization: Z.T.; Data curation: Z.T., L.F., Z.Y., H.Z.; Formal Analysis: Z.T., L.F., Z.Y.; Funding acquisition: Z.T., L.F.; Investigation: L.F., Z.Y., Z.J.; Validation and methodology: Z.Y., Y.Y.; Writing – original draft: Z.T., L.F., Z.Y.; Writing – review & editing: Z.T., L.F., Z.Y., Z.J., H.Z., Y.Y.

Conflict of interests

Authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgendis.2022.02.011>.

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