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A novel missense mutation in obscurin gene in a Chinese consanguineous family with left ventricular noncompaction

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ABSTRACT

BACKGROUND Left ventricular noncompaction (LVNC) is an increasingly recognised cardiomyopathy of which a significant percentage are genetic in origin. The purpose of the present study was to identify potential pathogenic mutation leading to disease in a Chinese LVNC family.

METHODS A 3-generation family affected by LVNC was recruited. Clinical assessments were performed on available family members, with clinical examination, ECG, echocardiography and cardiac MRI. The proband (I-2), the proband's daughter (II-1, affected) and mother (III-1, unaffected) were selected for WGS. Sanger sequencing were performed in all of the 4 surviving family members.

RESULTS Combined whole genome sequencing with linkage analysis identified a novel missense mutation in the giant protein obscurin (*OBSCN* NM_001098623, c.C19063T), as the only plausible disease-causing variant that segregates with disease among the four surviving individuals, with interrogation of the entire genome excluding other potential causes. This c.C19063T missense mutation resulted in p.R6355W in the encoded OBSCN protein. It affected a highly conserved residue in the C terminus of the obscurin-B-like isoform between the PH and STKc domains, which was predicted to affect the function of the protein by different bioinformatics tools.

CONCLUSIONS Here we present clinical and genetic evidence implicating the novel R6355W missense mutation in obscurin as the cause of familial LVNC. This expands the spectrum of obscurin's roles in cardiomyopathies. It furthermore highlights that rare obscurin missense variants, currently often ignored or left uninterpreted, should be considered to be relevant for cardiomyopathies and can be identified by the approach presented here. This study also provided new insights into the molecular basis of *OBSCN* mutation positive LVNC.

Left ventricular noncompaction (LVNC) is a relatively rare cardiomyopathy, with or without left ventricular dysfunction, that is characterized by excessively prominent trabeculations and associated deep recesses that communicate with the ventricular cavity.[1] LVNC is one of the unclassified cardiomyopathies according to the genetic cardiomyopathies classified by the American Heart Association.[2] The prevalence of LVNC was estimated to be 0.014%–1.3% depending on the age of the patients. The phenotypic expression and evolution of isolated LVNC are highly variable, and clinical features can range from asymptomatic to symptomatic, with a relatively stable course over several years or an evolution toward severe complications including congestive heart failure, ventricular arrhythmia and sudden cardiac death, atrial arrhythmias, and systemic embolic events. LVNC is supposed to be related to premature arrest of compaction of the loose myocardial meshwork during fetal embryogenesis, with persistent trabeculated myocardium, but the precise pathophysiology remains poorly understood. The predominant mode of inheritance is autosomal dominant, with some cases

with X-linked transmission. Several genes have been identified as causing LVNC disease. The first reported genetic cause of isolated LVNC was the X-linked tafazzin (TAZ) gene, which also causes Barth syndrome.[3] The sarcomere-encoding genes (*MYH7*, *ACTC1*, *TNNT2*, *MYBPC3*, *TMP1*, *TNNI3*) have been found to account for 17%–30% of LVNC.[4,5] Other genes such as *DTNA* (α-dystrobrevin), *NKX2.5*, Z-line protein-encoding *ZASP*/*LDB3*, and lamin A/C (*LMNA*) have been also associated with LVNC.[6] A clinical study by Rowland, *et al*.[7] identified a possible association between LVNC and variants in the obscurin (*OBSCN*) gene. However, the pathogenic involvement of *OBSCN* in LVNC is unclear.

Obscurins (molecular weight about 700 to 900 kDa) are very giant sarcomeric proteins that interact with several other binding partners including titin, myomesmin, and obscurin-like-1 to generate a complex important for myofibrillar M-band function. It plays key roles in myofibrillogenesis and cytoskeletal arrangement.[8,9] A single study in 2007 reported the association of *OBSCN* mutations with hypertrophic cardiomyopathy (HCM).[10] *OBSCN* variants are now considered to be relatively common in inherited cardiomyopathies. For example, Marston, *et al*.[11] found five unique *OBSCN* variants in four hearts from a group of 30 end-stage failing hearts, Xu, *et al*.[12] found six unique *OBSCN* variants in 74 HCM cases, and Rowland, *et al*.[7] found four unique *OBSCN* variants in 335 patients with dilated cardiomyopathy or LVNC, and *OBSCN* variants were associated with three out of the 11 LVNC cases. Chen, *et al*.[13] also identified an *OBSCN* frameshift mutation in a patient with arrhythmogenic right ventricular cardiomyopathy (ARVC) (Table 1). However, in all of these studies, family members of the probands were unavailable for segregation analysis of the *OBSCN* variants.

Table 1 Cardiomyopathy-linked *OBSCN* mutations based on the Obscurin B sequence NP_001092093.

	Mutation	CM type	Domain	Patient clinical data	Patient sex	Patient age
Arimura, *et al*.[10]	R4344Q	HCM	Ob58	No avaliable data	Male	19
	A4484T	HCM	Ob59			
Marston, *et al*.[11]	E963K	DCM	Ob9	LVEF10%, NYHA IV; LVEDD 70, VESD 63, FS10%,	Male	43
	V2161D	DCM	Ob21	LVEF20%, LVEDD 73, LVESD 63, FS13%	Male	17
	F2809V	DCM	Ob27			
	R4856H	DCM	Ob47	NYHA IV; IDCM plus mild CAD	Male	56
	D5966N	DCM	PH	Lifelong CM from 8 yr, exercise tachycardia	Male	43
Rowland, *et al*.[7]	T6309R	LVNC	Between Ob66 and Ob67	Dyspnea on exertion; chest pains; NYHA II-III; LVEF21%, LVEDD60mm;	Male	56
	S6990P	LVNC	Between kinase I and Ob69	Shortness of breath; NYHAI-II; LVEF56%, LVEDD43mm;	Female	30
	A6993P	DCM	Between kinase I and Ob69	Shortness of breath; NYHA II-III; LVEF26%, LVEDD70.8mm;	Female	62
	C25367-1G > C	LVNC		Shortness of breath; chest pains; fatigue; NYHA III; LVEF30-33%, LVEDD60mm;	Male	39
Xu, *et al*. [12]	A996fs	HCM	Ob10	No avaliable data	No avaliable data	No avaliable data
	A1088fs	HCM	Ob11	No avaliable data	No avaliable data	No avaliable data
	A1272fs	HCM	Ob13	No avaliable data	No avaliable data	No avaliable data
	A1640fs	HCM	Ob17	No avaliable data	No avaliable data	No avaliable data
	G7500R	HCM	Ob69	No avaliable data	No avaliable data	No avaliable data
Forleo, *et al*.[22]	E268X	HCM	Ig domain	No avaliable data	No avaliable data	No avaliable data
	A5660V	DCM	SH3_Obscurin_like	No avaliable data	No avaliable data	No avaliable data
	R6669H	DCM	STKc_obscurin_rpt1	No avaliable data	No avaliable data	No avaliable data
	A5791P	ARVC	RhoGEF	No avaliable data	No avaliable data	No avaliable data

In the present study, we identified a novel *OBSCN* mutation in a medium-sized Chinese pedigree with LVNC. By performing whole genome sequencing in two family members, filtering against variants seen in normal population cohorts, and using linkage information derived from single nucleotide polymorphism (SNP) arrays of four family members, we identified a missense mutation in *OBSCN* (NM_001098623: c.C19063T:p.R6355W) as the most plausible cause of LVNC in the family.

METHODS

Subjects

The proband (subject II-1), who was clinically diagnosed with LVNC, was admitted to Fuwai Hospital, Beijing, China. The proband's father died of congestive heart failure aged 43 and her daughter was clinically diagnosed with LVNC by cascade screening. Four subjects were enrolled in this study (Figure 1A). This study was approved by the Ethics Committee of Fuwai Hospital and was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from each participant. Physical examination, biochemical evaluation, echocardiography and cardiovascular magnetic resonance imaging were performed for each subject. The diagnosis of LVNC was based on the published criteria from echocardiographic or cardiac magnetic resonance imaging.[14,15] The compaction ratio,

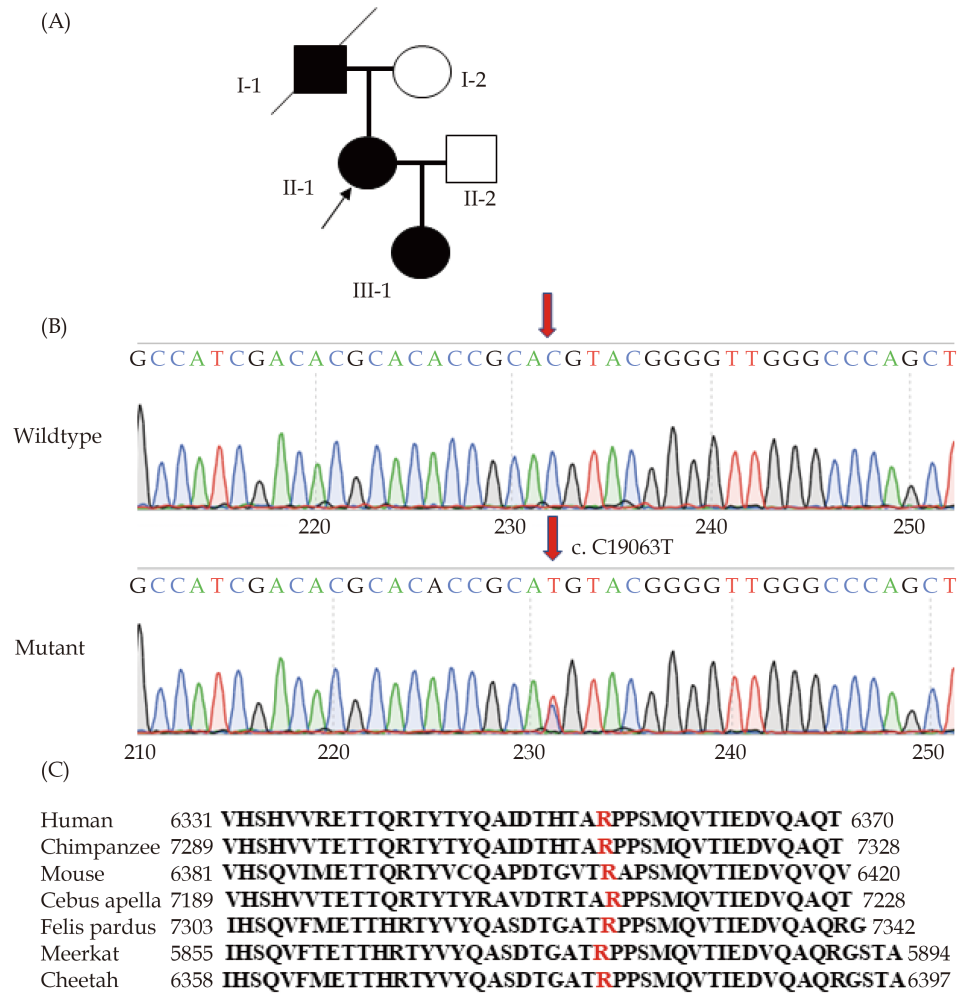

Figure 1 Inheritance characteristics of the proband and her family. (A): Pedigree of the family: black filled symbols- affected subjects; empty symbols- unaffected subjects; gray filled symbols- not avaliable; black arrow indicates the proband; (B): Sanger sequencing of an unaffected control subject and of the proband showing the *OBSCN* (NM_001098623) c.C19063T mutation; and (C): the identified mutation occurs in a highly conserved residue of the vertebrate obscurin protein.

defined as the ratio of the thickness of noncompacted to compacted myocardium > 2.3 measured by magnetic resonance imaging in diastole or > 2.0 measured by echocardiography in systole, was used to diagnose LVNC.

Genetic Screening

Genomic DNA was extracted from venous blood using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) and standard protocols. All DNA samples were quantified using a Nanodrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE). The enriched libraries were sequenced on an Illumina-Solexa HiSeq 2000 platform. Low-quality reads, adapter sequences, and duplicates were removed using the SolexaQA tool, cutadapt program, and Picard software. The clean reads were aligned to the human reference genome (hg19) using the Burrows–Wheeler Aligner. SNPs were detected using SOAP (Short Oligonucleotide Analysis Package) (http://soap.genomics.org.cn/soapsnp.html), and insertions and deletions were identified using GATK (Genome Analysis Toolkit) (https://software.broadinstitute.org/gatk/). After annotation, all the variants were viewed using Magic Viewer. All candidate variants were re-sequenced on an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA). Members of the participating family with LVNC were screened for the *OBSCN* missense mutation (NM_001098623: c.C19063T: p.R6355W) by Sanger sequencing.

In Silico Prediction

To assess the pathogenicity of the identified *OBSCN* missense mutation, the effects of the mutation on protein function were predicted using four bioinformatics tools: SIFT (http://sift.jcvi.org),[16] PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2),[17] PROVEAN (http://provean.jcvi.org/index.php),[18] and MutationTaster (http://www.mutationtaster.org).[19] SIFT is a sequence homology-based algorithm that qualifies amino acid substitutions caused by nonsynonymous SNPs; PolyPhen-2 is a Bayesian classifier that roots on eight sequence-based and three structure-based predictive features; PROVEAN is a web-based tool that classifies missense SNPs (and other variant typologies) using a two-step approach consisting of homologous sequence retrieval and calculation of an amino acid substitution matrix-based score; and MutationTaster integrates information from different biomedical databases and uses established analysis tools to evaluate evolutionary conservation, splice-site changes, loss of protein features, and changes that may affect the amount of mRNA.[20] A multiple sequence comparison was performed for 11 species using Clustal Omega (European Bioinformatics Institute, Hinxton, UK).

RESULTS

Clinical Feature

The proband was a 40-year-old female who had short stature. She was diagnosed with LVNC and dilated cardiomyopathy (DCM) by echocardiography imaging (Figure 2) The electrocardiogram and 24-hour Holter monitoring showed no abnormalities. She was treated with 75 mg irbesartan once daily for her congestive heart failure, with average BP 90/60 mmHg. The proband's father died of congestive heart failure when he was 43 years old. The proband's daughter presented with no clinical symptoms, but was diagnosed with LVNC by clinical cascade screening when she was 10 years old (Figure 3). Other family members presented with no clinical symptoms.

Molecular Genetic Analysis

Next-generation sequencing and Sanger sequencing analysis detected a heterozygous mutation in *OBSCN* (c.C19063T) in the proband (Figure 1B). This missense mutation was located in exon 82 and caused a change from arginine to tryptophan at position 6355 (p.R6355W) in the amino acid sequence This mutation was not detected in the healthy family members. This mutation was not archived in the following public databases as of August 10th 2021: Exome Aggregation Consortium (http://exac.broadinstitute.org/), 1000 Genomes Project (http://browser.1000genomes.org), Genome Aggregation Database (http://gnomad. broadinstitute.org), and Exome Variant Server (https://evs.gs.washington.edu/EVS/).

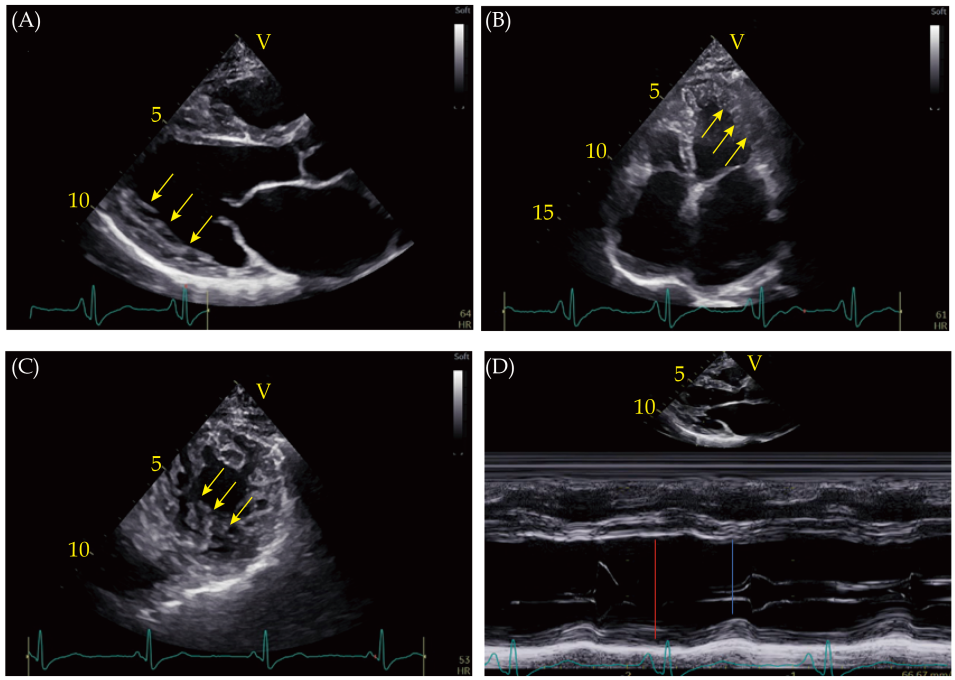

Figure 2 Echocardiogram images showing the characteristic spongy appearance of noncompaction (indicated by yellow arrows）and left ventricular dilation in the proband (A); long axis section of left ventricle (B); apical four chamber section (C); and short axis section of left ventricle (D) M mode. LVEDD indicated by red line, LVESD indicated by blue line. LVEDD: left ventricle end diastolic dimension; LVESD: left ventricular end-systolic diameters.

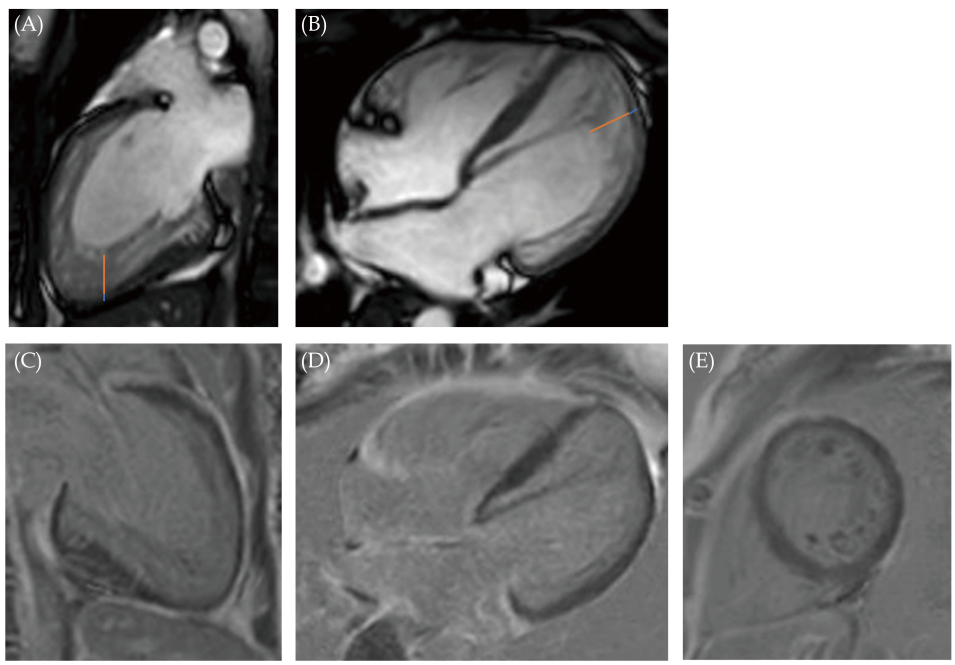

Figure 3 Cardiac nuclear magnetic images from individual IV-1showing an extensive noncompacted myocardial layer lining the cavity of the LV. (A): SSFP sequence: vertical long axis (2-chamber) view; (B): SSFP sequence: horizontal long axis (4-chamber) view; (C): PSIR sequence: vertical long axis (2-chamber) view; (D): PSIR sequence: horizontal long axis (4-chamber) view; and (E): PSIR sequence in the basal short-axis image showing the apex of the LV completely filled by trabeculated myocardium. The orange line identifies the thickness of the trabeculated layer; the blue line identifies the thickness of the compacted layer. LV: left ventricle; SSFP: steady-state free precession; PSIR: phase sensitive inversion recovery.

In Silico Mutation Analysis

In silico mutation analyses were performed using four bioinformatics tools (SIFT,(16) PolyPhen-2,[17] PROVEAN,[18] MutationTaster). They all predicted a deleterious effect of p.R6355W on OBSCN function (Table 2). Furthermore, the multiple alignment of vertebrate OBSCN sequences showed that p.R6355W was in a highly conserved region (Figure 1C), of implying that this residue may play a crucial role in OBSCN function.

DISCUSSION

We identified a novel heterozygous mutation p.R6355W in OBSCN in a Chinese pedigree with LVNC. By combining whole genome sequencing with linkage analysis in a three-generation family affected by autosomal dominant LVNC, we associated a missense mutation in *OBSCN* with this disease. This study contributes to the spectrum of *OBSCN* genotypes and phenotypes in the Chinese population.

The two affected family members had sufficient noncompaction to meet the diagnostic criteria for LVNC. The proband presented with consistent heart failure symptoms and was diagnosed with LVNC at the age of 28, whereas the proband's daughter had no clinical symptoms and was only diagnosed with LVNC by cascade screening. Both patients carried the c.C19063T heterozygous mutation in *OBSCN*. The *OBSCN* c.C19063T missense variant was not recorded in the 1000 Genome, ClinVar, and Exome Variant Server databases, which include both normal and pathological genomes. The multiple alignment of vertebrate OBSCN sequences showed that this mutation was in a highly conserved region of the OBSCN sequence, which indicated it would affect the function of OBSCN (Figure 1C). Therefore, we hypothesized the *OBSCN* c.C19063T:p.R6355W was a potential disease-causing mutation. In previous studies, *OBSCN* mutations have been found to be associated with cardiomyopathies, with variations in their penetrance and phenotypic representation.[21] In a cohort of 38 unrelated patients with cardiomyopathies, heterozygous mutations in *OBSCN* were identified in 1 of 14 patients with HCM, 1 of 8 patients with ARVC, and 2 of 16 patients with DCM.[22] Similarly, in a cohort of 335 patients with cardiomyopathy, 1 of 325 patients with DCM and 3 of 10 patients with LVNC had *OBSCN* frameshift or splicing variants.[7] However, none of these studies examined family histories to determine if these variants segregated with the disease, which is required to confirm causality.

The *OBSCN* missense mutation identified in the present study was located in the C terminus of the obscurin-B-like isoform between the PH and STKc domains. The possible pathogenic role of this novel mutation is supported by the finding that the variation was in a highly conserved region of OBSCN sequences from many species. Our results are in agreement with the association between *OBSCN* variants and the LVNC phenotype. For example, Teisha, *et al*.[7] reported two *OBSCN* frameshift (fs) variants (p.Thr7266ArgfsTer53 and p.Ser7947ProfsTer82) and one splicing variant (c.253671G>C) in three patients with LVNC. These three OBSCN variations were located in the C terminus of the obscurin-B-like isoform and were upstream of the fibronectin type-III 4 and protein kinase 2 domains, which were predicted to be absent in the frameshift variants.[7] Arimura, *et al*. reported an OBSCN R4344Q mutation in one patient with HCM and proposed that the missense mutation affected binding of OBSCN to Z-line titin (domains Z9 and Z10).[10] Marston, *et al*.[11] associated five *OBSCN* missense variants with four patients with DCM and noted *OBSCN* haploinsufficiency as a potential mechanism for development of the DCM phenotype, which also supported *OBSCN* mutations as a significant causal factor for DCM pathogenesis.

Clues to the physiological function of OBSCN

Table 2 *In silico* prediction of the consequences of the p.R6355W mutation in *OBSCN*.

Prediction tools	Score	Consequencen of mutation
SIFT	0.01	Damaging
Polyphen-2	0.993	Damaging
PROVEAN	−3.764	Deleterious
MutationTaster	1	Disease causing

have also been obtained using *OBSCN* knockout mouse models.[23,24] The absence of OBSCN in skeletal muscle resulted in disorganization of microtubules at the sarcolemma and delocalization of dystrophin at costameres.[23] More striking abnormalities appeared with intense exercise, including fiber damage, atrophy, and degeneration, and they reverted when exercise was stopped.[24] The effects of *OBSCN* knockout have been fully studied only in skeletal muscle and not in cardiac muscle. Further functional studies of cardiac muscle are needed to establish a clearer understanding of the role of OBSCN in cardiomyopathies.

In this study, we reported a novel *OBSCN* missense mutation that is associated with LVNC in a Chinese pedigree. Our results indicate that molecular screening for *OBSCN* mutations should be considered in patients with LVNC and other inherited cardiomyopathies.

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