Original article

A rare HCN4 variant with combined sinus bradycardia, left atrial dilatation, and hypertrabeculation/left ventricular noncompaction phenotype

Marta Alonso-Fernández-Gatta, María Gallego-Delgado, Ricardo Caballero,
Eduardo Villacorta, Elena Díaz-Peláez, Belén García-Berrocal, Teresa Crespo-García,
Beatriz Plata-Izquierdo, Elena Marcos-Vadillo, Luisa García-Cuenllas, Manuel Barreiro-Pérez,
María Isidoro-García, Juan Tamargo-Menéndez, Eva Delpón, and Pedro L. Sánchez

*Servicio de Cardiología, Complejo Asistencial Universitario de Salamanca, Universidad de Salamanca, Salamanca, Spain
Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Salamanca, Spain
Instituto de Investigación Biomédica de Salamanca (IBUSAL), Salamanca, Spain
Centro de Referencia Nacional de Cardiopatías FAMILIARES (CSUR), Salamanca, Spain
Departamento de Farmacología y Toxicología, Facultad de Medicina, Universidad Complutense de Madrid; Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain
Unidad de Genética, Servicio de Bioquímica, Complejo Asistencial Universitario de Salamanca, Universidad de Salamanca, Salamanca, Spain
Servicio de Pediatría, Complejo Asistencial Universitario de Salamanca, Salamanca, Spain

A B S T R A C T

Introduction and objectives: HCN4 variants have been reported to cause combined sick sinus syndrome (SSS) and left ventricular noncompaction (LVNC) cardiomyopathy. This relationship has been proven in few cases and no previous patients have associated left atrial dilatation (LAD). Our objective was to study a familial disorder characterized by SSS, LAD, and hypertrabeculation/LVNC and to identify the underlying genetic and electrophysiological characteristics.

Methods: A family with SSS and LVNC underwent a clinical, genetic, and electrophysiological assessment. They were studied via electrocardiography, Holter recording, echocardiography, and exercise stress tests; cardiac magnetic resonance imaging was additionally performed in affected individuals. Genetic testing was undertaken with targeted next-generation sequencing, as well as a functional study of the candidate variant in Chinese hamster ovary cells.

Results: Twelve members of the family had sinus bradycardia, associated with complete criteria of LVNC in 4 members and hypertrabeculation in 6 others, as well as LAD in 9 members. A HCN4 c.1123C>T(p.R375C) variant was present in heterozygosis in all affected patients and absent in unaffected individuals. Electrophysiological analyses showed that the amplitude and densities of the HCN4 currents (I_{HCN4}) generated by mutant p.R375C HCN4 channels were significantly lower than those generated by wild-type channels.

Conclusions: The combined phenotype of SSS, LAD, and LVNC is associated with the heritable HCN4 c.1123C>T(p.R375C) variant. HCN4 variants should be included in the genetic diagnosis of LVNC cardiomyopathy and of patients with familial forms of SSS, as well as of individuals with sinus bradycardia and LAD.

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Palabras clave: HCN4, Bradicardia sinusal, Hypertrabeculación, Miocardiopatía no compactada, Dilatación de la aurícula izquierda

Una variante rara en HCN4 produce un fenotipo de hipertrabeculación/no compactación ventricular, dilatación auricular izquierda y bradicardia sinusal

RESUMEN

Introducción y objetivos: Se han descrito previamente variantes genéticas del gen HCN4 con el fenotipo combinado de síndrome del seno enfermo (SSE) y miocardiopatía no compactada del ventrículo izquierdo (MCNC). Actualmente hay pocos casos en los que se haya probado esta relación y ningún caso previo se ha relacionado con dilatación de la aurícula izquierda (DAI). El objetivo es estudiar un trastorno familiar caracterizado por SSE, DAI e hipertrabeculación/fenotipo de MCNC para identificar las bases genéticas y electrofisiológicas subyacentes.

Palabras clave: HCN4, Bradicardia sinusal, Hipertrabeculación, Miocardiopatía no compactada, Dilatación de la aurícula izquierda
INTRODUCTION

Tetramers of hyperpolarization-activated cyclic nucleotide-gated channel (HCN4) subunits constitute the ion channels that conduct the hyperpolarization-activated “funny” current (I\textsubscript{f}), which plays a critical role in sinoatrial node pacemaker activity. The involvement of HCN4 loss-of-function variants in familial forms of sinus node dysfunction (SSS) was first noted in 2003. More recently, HCN4 variants have been associated with cardiac abnormalities such as left ventricular noncompaction (LVNC), mitral valve prolapses, and ascending aorta dilatation. We report a large family with inherited SSS associated with left atrial dilatation (LAD) and LVNC caused by the rare p.R375C HCN4 variant, which exhibits complete penetrance and a benign clinical course.

METHODS

Genetic testing

A genetic study including 173 genes was conducted in the index patient using targeted next-generation sequencing (Illumina HiSeq; Applied Biosystems 3730 DNA Analyzer, Thermo Fisher Inc, United States). Candidate variants were considered according to the usual pathogenicity criteria. DNA was obtained from blood samples in adults and from saliva in pediatric patients. Segregation was considered to be present if a variant was found in all affected individuals and if it was absent in individuals without phenotype. Significant cosegregation was defined using a cutoff of N1/32.

Cell culture and electrophysiological analysis of the candidate variant

HCN4 currents (I\textsubscript{HCN4}) were recorded using the whole-cell patch-clamp technique in Chinese hamster ovary cells transiently transfected with either wild-type (WT) or mutated HCN4 channels, as previously described. Current densities were calculated by normalizing the current amplitude to the capacitance of the cell. The voltage-dependence of HCN4 channel activation was analyzed and Boltzmann functions were fitted to the data to calculate the membrane potential at which 50% of the channels were activated (V\textsubscript{h}) and the slope (k) of the curve. The ion selectivity of the HCN4 channels was assessed by measuring the I\textsubscript{HCN4} density relationships of fully activated channels.

Patients and clinical assessment

The clinical evaluation and genetic study of all family members conformed to the principles outlined in the Declaration of Helsinki. Each patient was informed about the objectives of the research. In the case of minors, the consent of the parents was always required. All individuals were evaluated in our inherited cardiovascular disease unit. The initial assessment included 12-lead electrocardiography and transthoracic echocardiography.

We considered individuals as being possibly affected if their heart rate (HR) was below 60 beats per minute (bpm) (or according to the age limit reference in pediatric patients) and/or if they exhibited hypertrabeculation/noncompaction on transthoracic echocardiography according to the standard criteria. The study was conducted with cardiac magnetic resonance (CMR), 24-hour Holter electrocardiography, and cardiopulmonary exercise testing combined with transthoracic echocardiography. LVNC was diagnosed in patients who met Petersen and/or Jacquier criteria. In pediatric patients, the indications for complementary tests were individualized.

Statistical analyses

All statistical analyses were performed using IBM SPSS version 20.0.0 (IBM Corp, United States). Qualitative variables are expressed as absolute number (n) and percentage (%) and continuous values are reported as mean ± standard deviation or median and range.

Results of the functional study are expressed as mean ± standard error of the mean and were compared using t test, ANOVA followed by Tukey test, or Mann-Whitney U test, as appropriate, as well as using multilevel mixed-effects models. P < .05 was considered statistically significant for all analyses. More details on the methodology can be found in the supplementary data.
RESULTS

Clinical assessment in the index patient

The index patient was an 18-year-old man (III.19) with sinus bradycardia (SB) and no relevant medical history. Resting electrocardiography showed narrow QRS and normal PR and QT intervals with a J-point elevation in the inferior leads (figure 1A). Holter recordings demonstrated minimum and average HRs of 32 and 44 bpm, respectively. Exercise testing showed excellent functional class. Transthoracic echocardiography revealed LAD, left ventricular dilatation with hypertrobraculation, and a normal ejection fraction (figure 1B,C). LVNC diagnostic criteria were confirmed by CMR (figure 1D,E).

Family history and clinical screening of relatives

There was no family history of pacemaker implantation or sudden cardiac death. All relatives were asymptomatic at the time of the initial evaluation, except the index patient’s mother (II.10). She had a history of paroxysmal AF and underwent successful pulmonary vein ablation. The maternal grandmother (I.2) died of stroke at 85 years old and the grandfather (I.1) died of heart failure of unknown origin at 72.

Evaluation of 22 individuals was possible: 2 first-degree relatives (mother and sister) and 20 other relatives in the maternal line (figure 2). In the first evaluation, 12 individuals seemed to be affected: all had SB. This was later confirmed by a positive genetic test.

Genetic results and cosegregation analysis

Genetic analysis in the index patient identified 3 variants of uncertain significance in heterozygosity: NM_005477.2:c.1123C>T;(p.R375C) in the HCN4 gene, NM_001281740.1:c.1701C>A;(p.F567L) in the FHOD3 gene, and NM_004415.2:c.4632G>T;(p.R1544S) in the DSP gene. Of these variants, only HCN4 c.1123C>T;(p.R375C) segregated with the familial disease. The p.R375C variant was previously annotated (rs755356387) with a total allele frequency of 0.000003977 (Genome Aggregation Database [gnomAD]). SB had complete penetrance, even in pediatric individuals. Thus, by considering affected and non-affected family members, SB showed very strong cosegregation (n = 1/218). LVNC had incomplete penetrance, with variable expressivity among individuals, but also showed significant cosegregation (n = 1/25). In this case, only affected individuals were taken into account for cosegregation calculation (figure 2). The main characteristics of the HCN4 variant carriers are shown in table 1 and table 2.

Functional characterization of the HCN4 variant

The left panel in figure 3A shows the I_{HCN4} traces generated by applying 2-second pulses from −140 to +20 mV in 10-mV steps from a holding potential of −40 mV to a Chinese hamster ovary cell expressing WT HCN4 channels. Hyperpolarizing pulses generated an inward current that slowly activated until reaching a steady-state level whose amplitude progressively decreased at more positive potentials. Compared with WT channels, p.R375C HCN4 channels generated a smaller and much more slowly activating inward current (figure 3A, middle panel). Considering the heterozygous condition of carriers, cells were cotransfected with WT and p.R375C HCN4 channels in a 1:1 ratio. The I_{HCN4} amplitude generated by the cotransfection was lower than that generated by WT channels (figure 3A, right panel). By analyzing the I_{HCN4} density at the different membrane potentials, we confirmed that the I_{HCN4} densities generated by p.R375C channels were significantly lower than the densities generated by WT+p.R375C and WT channels (n ≥ 16; P < .01; figure 3B). Additionally, the I_{HCN4} decrease was evident both at very negative (−140 mV, figure 3C) and physiological (−60 mV, figure 3D) membrane potentials (n ≥ 16; P < .01). Interestingly, the current density generated by the cotransfection of WT and p.R375C channels was half of that generated by WT channels (n ≥ 16; P < .01). These results suggest that p.R375C channels do not act as “poisoning proteins” with a dominant negative effect.

To quantify the activation kinetics, a monoeponential function was fitted to the activation of traces generated by pulses to −130 mV. Time constants of the I_{HCN4} activation (τ) averaged 495 ± 44, 1960 ± 387, and 727 ± 85 milliseconds for WT, p.R375C, and p.R375C+WT HCN4 channels, respectively (n ≥ 16, P < .01).

An analysis of the voltage-dependence of HCN4 channel activation is shown in figure 3E (see supplementary data: Patch-clamp recordings). p.R375C channels were activated at more negative potentials compared with WT channels, an effect that probably accounts for the reduction in the current density produced by the variant. Indeed, the Vh was significantly hyperpolarized (n ≥ 17; P < .01) (figure 3F). WT+p.R375C channel activation was also shifted to negative potentials compared with WT channels (n ≥ 18; P < .05; figure 3F).

To determine whether the variant alters the ion selectivity of HCN4 channels, we measured the I_{HCN4} density relationships of fully activated channels (figure 3G). The reversal potential (Erev) was calculated from the intersection of the linear regression of the data with the abcissa axis at extracellular and intracellular K⁺ concentrations of 30 and 142 mM, respectively (see supplementary data: Patch-clamp recordings). The reversal potential was not modified by p.R375C channels alone or by their coexpression with WT channels (n ≥ 15; P > .05) (figure 3H,G). These results suggest that the variant did not modify the ion selectivity of HCN4 channels.

Complementary studies in HCN4 variant carriers and follow-up

Because participant II.9 underwent clinical follow-up in another medical center, we do not have further data on this person. All genetic carriers met the criteria for sinus dysfunction and none showed AF in Holter electrocardiography (table 1). Interestingly, IV:8 and IV:9 were 5-year-old twins, but IV:9 was not a genetic carrier and her resting HR was 100 bpm compared with the 75 bpm of IV:8.

Transthoracic echocardiography revealed the hypertrobraculation/LVNC phenotype in 10 of the 12 genetic carriers (83%), LAD in 7 of the 12 genetic carriers (58%), and a normal heart in 2 pediatric individuals (table 1). No other abnormalities were found in any participants.

CMR was performed in 8 patients (pediatric patients were excluded) (table 2). Four patients met LVNC diagnostic criteria and 4 other patients showed nonpathological ventricular hypertrobraculation. Five patients had mild left ventricular dilatation and normal right ventricular size, and the index patient (III.19) exhibited severe biventricular dilatation. Three HCN4 variant carriers (III1.1, III.4, and III.6) had borderline LVEF. Regarding myocardial deformation data, just 1 patient (III.1) had both a pathological global longitudinal strain (GLS) and global circumferential strain (GCS). None of the patients exhibited late gadolinium enhancement and all patients showed normal values of the native T1 (965 ± 15.8 milliseconds) and extracellular volume (21.7% ± 1.2%). All patients exhibited LAD, with 89% showing severe
Figure 1. Complementary tests in the index patient. A: electrocardiography indicating sinus bradycardia. B: apical 4-chamber transthoracic echocardiography exhibiting deep apical hypertrabeculation in the left ventricle (asterisk). C: end systolic frame showing left atrial dilatation (arrow). D: CMR showing biventricular hypertrabeculation (arrows) meeting LVNC criteria. E: CMR, with the endsystolic phase showing left atrial dilatation (arrow). CMR, cardiac magnetic resonance; LVNC, left ventricular noncompaction.
enlargement, and all had normal atrial ejection fraction on CMR
(ejection fraction, 56.6%; GLS, 35.8%). Cardiopulmonary exercise testing was performed in all adult
genetic carriers (table 1). All carriers completed the third stage of
the Bruce protocol and 6 developed ventricular ectopic beats.
Stress echocardiograms showed a positive contractile reserve
without other pathological indicators. All patients had a normal
functional class for the theoretical peak VO₂
(111% ± 15%), normal behavior of the O₂ pulse (142% ± 30%), and
VO₂ at the anaerobic threshold higher than predicted (72% ± 15%).
Pediatric carriers were evaluated by a conventional treadmill exercise
test and all had a normal functional class with no significant
arrhythmias.

At the end of this study, all family members were alive (mean
follow-up, 29 ± 9 months) and none had developed heart failure.

Apart from the patient with AF, no other arrhythmias have been
documented. Accordingly, no patient has needed a pacemaker or
implantable cardioverter-defibrillator.

**DISCUSSION**

This study describes a large family with a combined phenotype
of SB, LAD, and LVNC due to the c.1123C > T; (p.R375C) variant in
the HCN4 gene. This variant was previously annotated
(RCV000693215.1; rs755356387) and described in 1 individual
with sudden cardiac death and LVNC/ SB, but no segregation
or functional study was performed.16 According to the consensus
recommendation of the American College of Medical Genetics and
Genomics,7 it is classified as a “variant of uncertain significance”.

![Figure 2. Pedigree of the family with the HCN4 variant. Numbers below the subject symbol denote the family member’s identification. Squares/circles: male/female. Asterisk (*): individuals studied by our clinic team. Slashed symbols: deceased persons. Shaded left: sinus bradycardia. Shaded right: left ventricular noncompaction. Half shaded right: hypertrabeculation without LVNC criteria. Blue shading in the upper left: left atrial dilatation. G1: genotype for HCN4:p.R375C; G2: genotype for FHOD3:p.F567L; G3: genotype for DSP:p.R1544S. +/-: heterozygosis; -/-: homozygosis (not mutated). LVNC, left ventricular noncompaction.](https://www.revespcardiol.org/?ref=872048842, day 17/11/2023. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.)

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AVB, atrioventricular block; bpm, beats per minute; ECG, electrocardiogram; F, female; HR, heart rate; LA, left atrial; LVNC, left ventricular noncompaction; M, male; MHR, maximum heart rate; NYHA FC, New York Heart Association functional class; SR, sinus rhythm; VO₂, oxygen consumption.

* Index case.

b On beta-blockers.

![Table 1 Baseline characteristics and electrocardiogram, exercise test, and echocardiogram data of the p.R375C HCN4 variant carrier’s family.](https://www.revespcardiol.org/?ref=872048842, day 17/11/2023. This copy is for personal use. Any transmission of this document by any media or format is strictly prohibited.)
Table 2
Cardiac magnetic resonance data of the p.R375C HCN4 variant carrier’s family

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<th>Patient</th>
<th>Hypertrabeculation pattern</th>
<th>LVEF, %</th>
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<th>Indexed RVTVD, mL/m²</th>
<th>LGE</th>
<th>GLS-FT, %</th>
<th>GCS-FT, %</th>
<th>Native T1, ms</th>
<th>ECV, %</th>
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CMR, cardiac magnetic resonance; ECV, extracellular volume; GCS-FT, global circumferential strain by feature tracking; GLS-FT, global longitudinal strain by feature tracking; LA, left atrial; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; LVTVD, left ventricular telediastolic volume; RVEF, right ventricular ejection fraction; RVTVD, right ventricular telediastolic volume.

<sup>a</sup> Index case.

<sup>b</sup> On beta-blockers.

HCN4 variants were initially linked to inherited SSS without structural heart disease. In 2014, the association between LVNC and SSS was first described in several families with HCN4 variants. Only 68 cases belonging to 16 families have thus far been described. The presence of left ventricular hypertrobrabeculation/LVNC and different degrees of sinus node dysfunction, including tachycardia-bradycardia syndrome with AF, has been constant in all cases described, sometimes associated with other disorders such as mitral valve prolapse or ascending aorta dilatation. Pacemaker need seems high in this group of patients and, among all cases described, 4 patients experienced aborted sudden cardiac death due to ventricular fibrillation and 1 patient experienced sudden cardiac death.

We believe that the pathogenicity of HCN4 c.1123C>T(p.R375C) has been proven according to reference criteria. Family studies show an autosomal dominant inheritance pattern with complete penetrance for SB and incomplete but equally high penetrance for LAD and noncompaction. The high penetrance of SB present in pediatric individuals has previously been reported and could be a marker of the disease. The HCN4 c.1123C>T(p.R375C) carriers in our family showed a benign course compared with previous work. The only complication identified during follow-up was an episode of persistent AF with excellent rhythm control, which was managed using pulmonary vein ablation.

Mechanisms of HCN4 variants underlying structural heart abnormalities

Our results have shown that the p.R375C variant profoundly modifies the voltage-dependence and kinetics of HCN4 channel activation. This significantly decreased the channel availability, diminishing the density of the current generated. The causality of HCN4 variants has been demonstrated via cellular electrophysiological analysis. Mutated HCN4 channels were nonfunctional, resulting in I<sub>f</sub> current reduction. In the present study, the electrophysiological data provided strong evidence for the causality of the p.R375C variant in the pathogenesis of the observed SB.

The p.R375C residue is located in the S4 segment of HCN4 channels and is 1 of the 7 positively charged residues of this channel domain (figure 4). Indeed, the S4 segment is the voltage sensor of the channel, and the variant replaces 1 of the positively charged Arg residues with a polar neutral residue (Cys). This perfectly explains the profound changes produced by the variant in the voltage-dependence and kinetics of channel activation. Similar results were previously described for the p.R377C variant found in a patient with SSS.

Mechanisms of HCN4 variants underlying structural heart abnormalities

LVNC cardiomyopathy has been linked to different variants affecting sarcomeric, cytoskeletal, and nuclear membrane genes. Animal models indicate that the hypertrobrabeculation results from altered regulation of cell proliferation, differentiation, and maturation during the formation of the ventricular wall.

Thus far, at least 8 different HCN4 variants associated with this combined phenotype have been published (P883R, K645X, A485E, G482R, Y481H, 1479 V, A414G, R393H). There may be a relationship between the development of the cardiac conduction system and the myocardium maturation that explains the concomitant presence of cardiomyopathy and specific arrhythmias. These HCN4 variants could be considered the “final common pathway” that leads to a common phenotype, although it remains to be determined how the hypertrobrabeculation develops. Another hypothesis for the occurrence of LVNC is an acquired adaptive remodeling feature in response to SB. Previous studies have reported that genes encoding ion channels (SCN5A and RYR2) are involved in cardiomyopathy pathophysiology. In this study, we found some confusing data regarding whether our family has an acquired phenotypic trait or a true cardiomyopathy. Almost all carriers had normal or borderline LVEF and myocardial
Figure 3. Electrophysiological analysis of the HCN4 variant. A: families of $I_{\text{HCN4}}$ traces generated in CHO cells transiently expressing HCN4 WT, p.R375C, and WT+p.R375C channels by applying the protocol depicted at the top. B: density–voltage relationships generated in cells expressing WT, p.R375C, and WT+p.R375C channels. C and D: $I_{\text{HCN4}}$ density generated by HCN4 WT, p.R375C, and WT+p.R375C channels with the application of pulses at –140 mV (panel C) or –60 mV (panel D). E: tail current densities generated by different channels by applying 1-second pulses to –140 mV were normalized and plotted against the membrane potential of the test pulse. Continuous lines represent the Boltzmann fit to the data. F: the membrane potential activating 50% (Vh) of the WT, p.R375C, and WT+p.R375C HCN4 channels. G: fully activated $I_{\text{HCN4}}$ density–voltage relationships generated by WT, p.R375C, and WT+p.R375C channels. Continuous lines represent the linear regression to the data. H: Erev of the channels calculated from the intersection of the linear regression to the data with the abscissas axis of each individual experiment. In B to H, each point/bar represents the mean ± SEM of ≥ 15 experiments/cells from ≥ 3 dishes. CHO, Chinese hamster ovary; Erev, reversal potential; $I_{\text{HCN4}}$, HCN4 currents; WT, wild-type.

$^a P < .01$ vs HCN4 WT.

$^b P < .05$ vs HCN4 WT.

$^c P < .05$ vs p.R375C.
deformation results, as well as normal functional class and positive contractile reserve. None of the individuals analyzed exhibited late gadolinium enhancement or extracellular volume and T1 mapping alterations.

Interestingly, we found high penetrance of LAD, always with normal atrial contractility. This finding had not previously been associated with HCN4 variants. The mechanism underlying the LAD development is unknown. As previously explained, LAD could be a result of the anatomical structure, because HCN4 contributes to the embryonic development of the left ventricle and both atria, or a physiological adaptation to SB.

**HCN4 variants with SSS, LAD, and LVNC implications**

The association between HCN4 variants and the combined SSS, LAD, and LVNC phenotype seems undeniable. The noncompaction pattern exhibited by the patients with the present HCN4 p.R375C variant in this family suggests low aggressiveness and good prognosis. Left atrial remodeling is an important underlying substrate for AF and could be related to the appearance of the familial AF previously linked to HCN4 variants.

The assessment and follow-up of patients with emerging HCN4 variants are not currently established. The follow-up that we have proposed for this family is mainly focused on the detection of systolic ventricular dysfunction and arrhythmic complications, mainly in phenotypes with LAD and the probability of AF development. We have decided to perform an annual clinical review with electrocardiography and Holter electrocardiography. The echocardiogram will be repeated every 1 to 2 years (except for changes in the clinical situation).

**Study limitations**

This study included only 1 family and assessment of the entire family was not possible. However, a large number of family members have been evaluated. Indeed, our study reports the largest family linked to the HCN4 gene described to date and a comprehensive clinical study, which included cardiopulmonary exercise testing and CMR.

Our study, similar to others, does not explain the mechanism underlying the effect of the HCN4 variant on the development of left ventricular hypertrabeculation or LAD. Nonetheless, this is the first work involving the analysis of patients by CMR with parametric techniques and feature-tracking myocardial deformation, and we have shown the existence of a possible subclinical systolic dysfunction and LAD with potential clinical implications.

**CONCLUSIONS**

The combined phenotype of SSS, LAD, and LVNC is associated with different heritable HCN4 variants. Patients with familial forms of SSS should be studied to rule out the presence of structural heart disease, and HCN4 variants should be included in the genetic diagnosis, even individuals with SB and isolated LAD. In addition, HCN4 variants should be added to other recognized genes in the study of patients with LVNC. We report a family with a HCN4 c.1123C>T; (p.R375C) variant causing the combined phenotype of SSS, LAD, and LVNC with a benign course. Further studies are necessary to elucidate the pathophysiological mechanism of the atrial dilation and hypertrabeculation/noncompaction phenotype, as well as characterize the natural history of patients affected by HCN4 variants and the risk stratification in this specific population.
WHAT IS KNOWN ABOUT THE TOPIC?

- Due to recent evidence, the association between HCN4 variants and the combined SSS, LAD, and LVNC phenotype seems undeniable, with phenotypes appearing to differ according to the underlying variant.
- The main complications related to HCN4 variants are arrhythmic complications, such as AF and sudden death, early pacemaker need, cardioembolic complications, and systolic ventricular dysfunction.

WHAT DOES THIS STUDY ADD?

- We found high penetrance of LAD, always with normal atrial contractility, a novel finding for HCN4 variants. The importance of this result is that this structural abnormality could be related to the appearance of familial AF previously linked to HCN4 variants.
- The noncompaction pattern exhibited by patients with the present HCN4 p.R378C variant suggests low aggressiveness and good prognosis.
- We propose a follow-up of patients with HCN4 variants that is mainly focused on the detection of systolic ventricular dysfunction and arrhythmic complications, mainly in phenotypes with LAD and the probability of AF development.

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CONFLICTS OF INTEREST

The authors have no conflicts to disclose.

APPENDIX. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version available at https://doi.org/10.1016/j.rec.2020.06.019