Cardiology in the Young

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Novel *NKX2.5* variant associated with congenital heart disease and increased risk of malignant arrhythmia and sudden cardiac death

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Original Article

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Abstract

Introduction: The NKX2.5 gene is an important cardiac developmental transcription factor, and variants in this gene are most commonly associated with CHD. However, there is an increased need to recognise associations with conduction disease and potentially dangerous ventricular arrhythmias. There is an increased risk of arrhythmia and sudden cardiac death in patients with NKX2.5 variants, an association with relatively less attention in the literature. *Methods:* We created a family pedigree and reconstructed familial relationships involving numerous relatives with CHD, conduction disease, and ventricular non-compaction following the sudden death of one family member. Two informative but distantly related family members had genetic testing to determine the cause of arrhythmias via arrhythmia/cardiomyopathy gene testing, and we identified obligate genetic-positive relatives based on family relationships and Mendelian inheritance pattern. *Results:* We identified a novel pathogenic variant in the NKX2.5 gene (c.437C > A; p. Ser146*), and segregation analysis allowed us to link family cardiac phenotypes including CHD, conduction disease, left ventricular non-compaction, and ventricular arrhythmias/sudden cardiac death. *Conclusions:* We report a novel NKX2.5 gene variant linking a spectrum of familial heart disease, and we also encourage recognition of the association between NKX2.5 gene and potentially dangerous ventricular arrhythmias, which will inform clinical risk stratification, screening, and management.

The *NKX2.5* gene encodes a cardiac-specific homeobox transcription factor that critically regulates cardiac morphogenesis and development of the cardiac conduction system. Variants in *NKX2.5* have been associated with CHDs with and without conduction disease.[1,2] Other less common associations include left ventricular non-compaction, cardiomyopathy, and atrial fibrillation.[2,3] More recently, *NKX2.5* variants have been associated with ventricular arrhythmias and increased risk of sudden death.[2–6] However, there are limited case reports and family-based case series with ventricular arrhythmia and sudden death risk, and risk of these remains undefined without cohort- or population-based studies of *NKX2.5* patients. Published guidelines of genetic testing for heritable arrhythmias do not specifically mention *NKX2.5*.[7,8] We report a family-based case series in which three distant relatives with variable CHDs, conduction disease, and sudden death are associated with a novel pathogenic *NKX2.5* variant, adding to the growing literature of novel pathogenic *NKX2.5* variants and prompting the question of considering *NKX2.5* in genetic testing guidelines for heritable arrhythmic disease.

Methods

Clinical Evaluations. No written informed consent was required for this study. Deidentified information is reported in accordance with approved Indiana University IRB protocol #1811364611. All clinical evaluations are based on standard electrophysiologic assessments and care.

Genetic Testing. Clinical genetic testing for all cases was completed by a commercial reference laboratory (Invitae, Inc.). Analysis included sequencing and deletion/duplication analysis of 137 genes associated with arrhythmias, cardiomyopathies, and CHD. Genomic DNA obtained from samples was enriched for targeted regions using a hybridisation-based protocol and sequenced using Illumina technology (to ≥ 50x depth, with reads mapped to the GRCh37 reference, including gene coding regions and 20 bp of flanking intronic sequences).

Results

Clinical characteristics

The following family-based case series represents three independent case presentations for evaluation and management of CHDs and conduction disease. Due to limited intra-family communication, the relationships of these individuals were not known prior to completing the genetic testing. The individuals derive from a family with significant history of cardiac disease. Refer to Figure 1 for the pedigree, with reference to each case below as noted in pedigree nomenclature.

Case 1. The index case proband, which prompted genetic investigation, initially presented to electrophysiology clinic in 2021 after her primary cardiologist retired (Individual IV-1, Fig. 1). She is currently a 10-year-old female; status – post-surgical repair of a large secundum atrial septal defect at a local hospital at age 2. She additionally had a persistent left superior vena cava. She was previously noted to have a family history of CHDs and relatives with pacemakers, though details were unclear and genetic testing was not completed by previous providers (Fig. 1). She also had first-degree atrioventricular block. At age 9, a Holter monitor showed a non-sustained run of monomorphic ventricular tachycardia that has been managed on nadolol. Cardiac MRI and echocardiogram were unremarkable aside from the persistent left superior vena cava, and exercise treadmill testing was normal aside from occasional ectopic atrial rhythm. During the initial visit with our electrophysiology clinic, the family disclosed the recent death of a paternal half-sibling with CHD (Case 2 below; Individual IV-2 in Fig. 1) leading to genetic counselling and testing.

Genetic testing was completed through a commercial clinical genetic testing laboratory (Invitae, Inc.). Analysis identified a pathogenic variant in *NKX2.5* [NM_004387.3], denoted as c.437C > A (p. Ser146*). This variant occurs in exon 2 and creates a premature stop signal not expected to cause nonsense-mediated RNA decay but would truncate the last 179 amino acids of the NKX2.5 protein. The homeobox domain and the NK2 domains would be disrupted by this variant. This variant is absent from large population databases (ExAC/gnomAD), and other C-terminal variants in *NKX2.5* have been reported in *NKX2.5*-associated disease.[9] Based on the type of variant and consistency with the patient's phenotype, this variant was interpreted clinically as pathogenic. There were additional variants of uncertain significance in the following genes, though these are not felt to be clinically significant based on current review: *CBL*, *CHD7*, and *DSP* (see Supplement 1).

After confirmation of the *NKX2.5* variant in the setting of non-sustained VT and family history of sudden death, discussion regarding implantable cardioverter-defibrillator took place with the family. Given unclear benefit of implantation, the decision was made to implant a loop recorder for long-term rhythm monitoring. There has been no further arrhythmia to date. Following the identification of the *NKX2.5* variant, the family history was reassessed, and family risk counselling was provided. Due to family dynamics, early cascade gene testing was challenging to complete.

Case 2. This individual (Individual IV-2, Fig. 1) had been previously evaluated at our institution, but the relationship with the proband (Case 1) was not known in prior evaluations. She presented to our centre in 2017 from her primary cardiologist for consideration of pacemaker placement due to an underlying diagnosis of first- and second-degree atrioventricular block as well as third-degree atrioventricular block in the setting of her secundum atrial septal defect (surgically repaired). The atrial septal defect was first diagnosed at age 7 and closed surgically by a local hospital, and she was noted to have sinus node dysfunction of unclear cause. The patient had been lost to follow-up for 4 years before presenting to a new local cardiologist.

When she presented to our clinic, Holter monitoring showed first-degree atrioventricular block with periods of second-degree and third-degree atrioventricular block as long as six seconds. No specific cardiovascular symptoms, however, were described. An echocardiogram also demonstrated a persistent left superior vena cava to coronary sinus with no right superior vena cava (similar to Case 1 above), as well as left ventricular non-compaction (Fig. 2). Given her lack of symptoms we completed an exercise treadmill test which showed non-conducted beats with elevated sinus. Left ventricular non-compaction was identified on cardiac MRI, and after discussion with the family, we implanted a dual-chamber epicardial pacemaker. This surgery was uncomplicated though post-operatively she developed incessant atrial flutter not amenable to cardioversion requiring flecainide antiarrhythmic therapy. No genetic evaluation nor genetic testing was completed prior to hospital discharge. The family did not present for scheduled post-operative follow-up in pacemaker clinic. We planned to continue flecainide and warfarin treatment for six months post-surgery and attempted care coordination with local cardiologists after multiple no-shows to our clinic. About one year after pacemaker implant, she presented to local emergency services after cardiac arrest and could not be resuscitated. A post-mortem device interrogation by a local device representative showed ventricular tachycardia events at the time of arrest (although we requested records, they were never sent). No post-mortem genetic testing or molecular autopsy was available.

Case 3. This 19-year-old family member (Individual III-5, Fig. 1) has been followed jointly by her local cardiologist and our centre since birth prior to the others. Her relationship with the first two individuals was not connected until about seven months following the evaluation and genetic testing of Case 1. Upon admission to our institution in the setting of recent onset of syncope, the relationship between Case 3 and Case 1 was made.

She was diagnosed with a large atrial septal defect and valvar pulmonary stenosis at birth and underwent transcatheter septal defect device closure at 8 months of life. She had neonatal supraventricular tachycardia though this spontaneously resolved. At age 6, she had symptoms of chest pain and fluttering with an ECG showing a severe PR prolongation and intra-ventricular conduction delay. In the setting of her paternal family history of pacemakers and sudden cardiac arrest of unknown cause, a Holter showed a wide-complex tachycardia suspicious for aberrant supraventricular tachycardia vs. ventricular tachycardia. She underwent electrophysiologic study in 2009, though the findings from this study are not available. At age 7, due to progressive fatigue, evidence of rare second- and third-degree atrioventricular block on Holter monitor, and an unresolved family history of CHDs and malignant arrhythmia, it was decided to place a dual-chamber epicardial pacing system. She continued to demonstrate palpitations and have evidence of atrial and ventricular ectopy prompting initiation of atenolol treatment, which was taken irregularly due to dizziness. Device evaluations showed ventricular pacing percentages as high as 70% during this time. At age 12, she remained symptomatic, showing evidence of AV node dysfunction, and continued palpitations with no recorded arrhythmias, and non-medicinal treatment for anxiety lead to improvement. In the following years, device evaluations showed periodic episodes of non-sustained supraventricular ectopy not associated with

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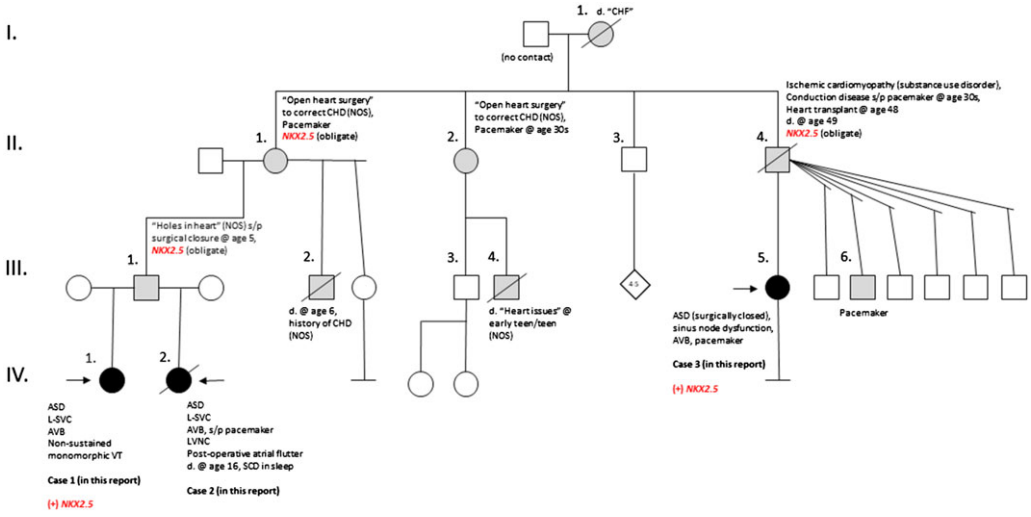

Figure 1. Case series family pedigree. Dark-shaded symbols indicate verified phenotypes and grey-shaded symbols indicate unverified phenotypes likely associated with *NKX2.5*-related disease. Acronyms: ASD=atrial septal defect, AVB=atrioventricular block, CHD=congenital heart disease, CHF=congestive heart failure, L-SVC=persistent left superior vena cava, LVNC=left ventricular non-compaction, NOS=not otherwise specified, SCD=sudden cardiac death, VT=ventricular tachycardia. Based on family variant segregation and family relationships, several relatives can be determined to have the *NKX2.5* variant (in obligate fashion). Note: (+) indicates genetic testing confirmation; otherwise, obligate carriers for the *NKX2.5* are noted.

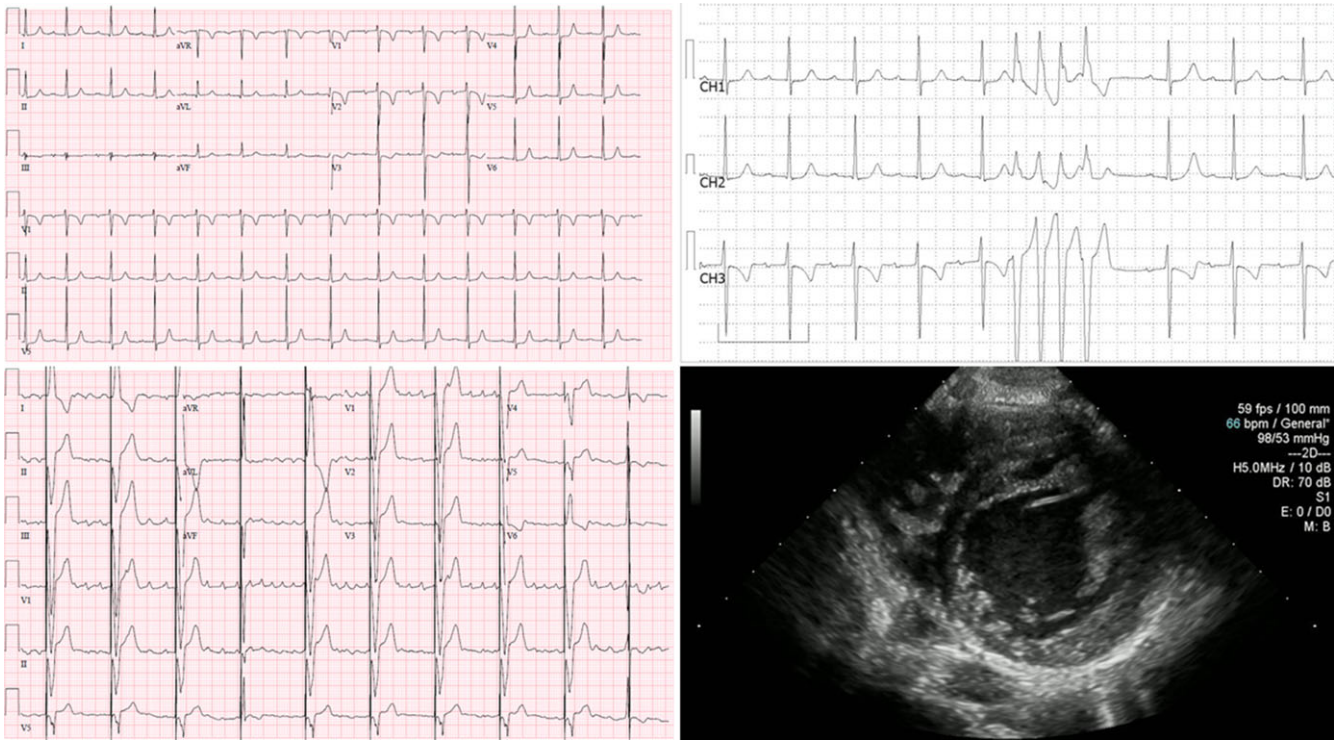

Figure 2. Clockwise from upper left – baseline ECG in Case 1 showing PR prolongation > 220 msec; non-sustained VT on Holter in Case 1; echocardiography of LV non-compaction in Case 2; and Case 2 atrial flutter with ventricular pacing during mode switch to DDI.

symptoms and ventricular pacing < 20%. She remained on atenolol treatment by personal choice rather than discontinuing to assess for her underlying burden or transitioning to a different agent. At 14 years old, she experienced an atrial lead fracture resulting in dizziness. We revised her pacing system to a dual-chamber transvenous system. She continued to ventricularly pace around 15% with minimal evidence of atrial or ventricular arrhythmia such that atenolol was stopped with no evidence of increased arrhythmia. In the following years, she had progressively decreased ventricular pacing from 8–15% and rare episodes of non-sustained atrial tachycardia and wished to remain off medication. In the setting of the COVID-19 pandemic, she did not present for scheduled follow-up evaluation for nearly two years with remote monitoring showing continued rare atrial ectopy. She experienced an episode of unexplained syncope at school, which prompted transfer to our hospital for observation. The team elected to complete a comprehensive cardiomyopathy/arrhythmia/CHD gene panel instead of targeted *NKX2.5* analysis given the distance in familial relationship.

The same pathogenic *NKX2.5* variant found initially in Case 1 (c.437C > A; p. Ser146*) was identified in Case 3. The results, however, were relatively more complex, with the laboratory reporting an additional likely pathogenic variant in *SCN5A* (c.4978A > G; p. Ile1660Val). Based on in-depth clinical review, this variant is currently interpreted as an inconclusive variant of uncertain significance that will be re-reviewed annually.[10,11] There

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were additional variants of uncertain significance in the following genes: *SCN5A*, *ACTN2*, and *KCNQ1*. Based on current review, these are not felt to be clinically significant at this time but will be reassessed regularly in clinic (Supplement 1).

Family History Summary. Figure 1 summarises key findings about the family. Notably, based on family genetic testing and relationships, we can conclude that individuals III-1, II-1, and II-4 also have the *NKX2.5* variant; this increases the likelihood that Case 2 also had the *NKX2.5* variant even though death preceded confirmatory genetic testing.

Discussion

The *NKX2.5* gene has not received as much attention as other canonical cardiac channelopathy genes in the heritable arrhythmia literature. *NKX2.5* is a critical cardiac developmental gene, and pathogenic variants are associated with variable CHDs and conduction disease. In recent years, there have been case reports describing *NKX2.5*-associated ventricular arrhythmias and sudden death, and additional investigation will be required to understand arrhythmia risk and its mechanisms. Our family-based case series adds to the growing literature in this area by reporting on (1) a novel *NKX2.5* variant expected to cause loss of function; (2) a family with multiple affected relatives with ventricular arrhythmia/sudden cardiac death despite treatment of conduction disease; and (3) multiple affected relatives with phenotypes consistent with *NKX2.5*-associated disease like septal defects, conduction disease requiring pacemaker intervention, ventricular arrhythmias (including risk of sudden death), and persistent left superior vena cava. It is interesting to note that *NKX2.5* is associated with heterotaxy and laterality-spectrum cardiovascular malformations (e.g., persistent left superior vena cava), adding evidence supporting this gene-disease association.[12] While the family phenotype is consistent with previous reports of *NKX2.5*-associated disease, additional research will be needed to understand the variable expressivity of disease.

Each of the three cases described was seen by independent clinicians agnostic to details of the family history. However, a deeper review not only linked these three cases genetically but also allowed a more precise gene-specific diagnostic differential. The constellation of CHD (predominantly septal defects), conduction disease/pacemakers, persistent left superior vena cava, and left ventricular non-compaction in the family history allowed the team to prioritise *NKX2.5* in addition to other CHD-associated genes that can present with conduction disease/arrhythmia risk. Genetic testing strategies that incorporate cardiac developmental genes (like *NKX2.5*) would be more helpful than analyses involving only canonical cardiac channelopathy genes. Additionally, the clinical utility of genetic testing for simplex, non-syndromic left ventricular non-compaction cases has been questioned.[13] However, we would argue for stronger consideration of genetic testing for cases of left ventricular non-compaction accompanied by conduction disease or CHDs or a family history of such, especially for cardiac developmental genes like *NKX2.5*. This case highlights the importance of a comprehensive family history and the utility of knowing the phenotypic spectrum for *NKX2.5* variants to identify the most informative genetic testing strategies.

We particularly want to raise attention regarding the putative increased risk of ventricular arrhythmias and sudden death in *NKX2.5* cases highlighted in this case series and other recent publications.[2–6] While additional research is necessary to quantify risk and understand pathophysiologic mechanisms, this and other reports are useful in prognosis and guiding counselling of patients/families with *NKX2.5*-associated disease. Specifically, management should include long-term surveillance for ventricular arrhythmias and discussion about interventions to reduce this risk. Last, there is a need to quantify arrhythmic and sudden death risk in *NKX2.5* families, and the electrophysiology community is encouraged to collaborate with the goal of developing risk prediction models using cohort- or population-based observational data.

Conclusion

This case series reports on a novel *NKX2.5* gene variant identified in a family with a constellation of cardiac disease including CHDs, conduction disease/sinus node dysfunction, ventricular arrhythmias, and sudden cardiac death. Comprehensive family history review was essential to identify the causative *NKX2.5* gene variant, and the genetic testing results helped inform familial arrhythmia risks.

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Competing interests. None.

Ethical standard. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant United States guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the Indiana University (IU) School of Medicine and IU Health Institutional Review Board (IRB-1811364611) with a waiver of consent. No immediately identifying information is shared in this work in accordance with health privacy laws.

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