EDUCATION FORUM

Familial Atrial Septal Defect and Sudden Cardiac Death: Identification of a Novel *NKX2-5* Mutation and a Review of the Literature

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ABSTRACT

Objective. Atrial septal defect (ASD) is the second most common congenital heart defect (CHD) and is observed in families as an autosomal dominant trait as well as in nonfamilial CHD. Mutations in the *NKX2-5* gene, located on chromosome 5, are associated with ASD, often combined with conduction disturbances, cardiomyopathies, complex CHD, and sudden cardiac death as well. Here, we show that *NKX2-5* mutations primarily occur in ASD patients with conduction disturbances and heritable ASD. Furthermore, these families are at increased risk of sudden cardiac death.

Results. We screened 39 probands with familial CHD for mutations in *NKX2-5* and discovered a novel mutation in one family (2.5%) with ASD and atrioventricular block. A review of the literature revealed 59 different *NKX2-5* mutations in 202 patients. Mutations were significantly more common in familial cases compared to nonfamilial cases ($P = 7.1 \times 10^{-9}$). The majority of patients (74%) had ASD with conduction disturbance. Nineteen patients (15%) of 120 with familial ASD and conduction disturbance died from sudden cardiac death of which nine (8%) were confirmed mutation carriers, and 10 were possible carriers.

Conclusions. *NKX2-5* mutations mainly occur in familial CHD, the signature phenotype is ASD with conduction disturbances and mutation carriers are at increased risk of sudden cardiac death. We suggest that familial ASD patients should be screened for *NKX2-5* mutations and, if they are mutation carriers, implantation of an implantable cardioverter-defibrillator should be considered in these patients.

Key Words. Congenital Heart Disease; NKX2-5; Familial ASD; Congenital Atrioventricular Block; Sudden Cardiac Death

Background

Atrial septal defect (ASD) is the second most common congenital heart defect (CHD) and accounts for approximately 10% of all cardiac malformations.[1,2] Eighty percent of persistent foramen ovale and small ASDs close spontaneously during infancy or childhood, whereas large ASDs or those remaining open into adulthood may cause congestive heart failure, pneumonia, pulmonary vascular disease, atrial arrhythmias, and paradoxical embolism.[3–7] Also, co-occurrence with other cardiac malformations within the same individual is often observed.[8]

ASD is correlated to mutations in the *NKX2-5* gene, located on chromosome 5 (5q34).[9–11] *NKX2-5* is a cardiac transcription factor that plays a significant role in development of the atrioventricular node as well as maintaining function of the node throughout life.[12] In recent years, *NKX2-5* mutations have been reported in

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CHD patients with nonfamilial as well as familial CHD.

Familial atrioventricular block, observed as congenital or adult-onset type, co-occur with ASD.[13–15] Besides ASD,[9–11] congenital complete atrioventricular block co-occur with laterality defects such as levo-transposition of the great arteries (l-TGA) or atrial isomerism.[16–18] As opposed to the adult-onset type, congenital complete atrioventricular block is diagnosed in utero or shortly after birth, and it is associated with mortality rates ranging from 33% to 80% if the heart rate is below 50 or it co-occurs with structural heart disease.[17,18] Conversely, the adult-onset type of familial atrioventricular block is of a progressive nature, and there are several reports of patients with normal ECG or a harmless first-degree atrioventricular block followed by sudden onset of second- and third-degree atrioventricular block or sudden death later in life.[14,15,19]

Sudden cardiac death (SCD) occur in patients with both types of atrioventricular block[15,16,20,21] and in patients with *NKX2-5* mutations.[22,23] SCD have been reported in pediatric as well as adult cases of atrioventricular block,[15,16,20,21] and autopsy studies have shown fibrotic replacement of the AV-bundle,[24] which explains the atrioventricular node malfunctioning in these patients. However, there has been an alarming number of SCDs in obligate carriers and relatives of patients with *NKX2-5* mutations.[22,23,25,26] This, and previous reports of patients with ASD and/or atrioventricular block dying suddenly with a functioning pacemaker, suggest that the myocardium is also involved in the *NKX2-5* phenotype.[15,16] Despite the large efforts in finding *NKX2-5* mutations in CHD patients, there have been no reviews of the existing literature to determine the frequency of the mutation or characterization of the phenotypic appearance of mutation carriers.

We hypothesized, that mutations in *NKX2-5* primarily occur in familial CHD, and that the signature phenotype is ASD with or without conduction disease or arrhythmia (CD/A). Furthermore, we suspected that these carriers were at increased risk of SCD.

Here, we report a novel truncating mutation in six members of a family with autosomal dominant transmission of ASD (n = 5) co-occurring with atrioventricular block and complex CHD (n = 1). By reviewing the literature, we show that the majority of *NKX2-5* mutation carriers are patients with familial ASD and conduction disturbances, and we report an alarming large number of SCDs in such families.

This finding has important implications for the management of patients with familial ASD, because they could be carriers of a *NKX2-5* mutation with an increased risk of SCD. We suggest that a preventive implantable cardioverter-defibrillator should be considered in such patients.

Methods

We screened 39 Danish CHD families for *NKX2-5* mutations. Diagnoses of probands and their affected relatives were verified by a review of the patient file, and a diagnosis was considered confirmed if it was found during echocardiography, heart catheterization, surgery, or autopsy (Relations and diagnoses shown in Supporting Inforamtion). A total of 100 Danish unaffected individuals, unrelated to the study subjects, were used as controls to investigate population frequency of the identified mutation. Genomic DNA was extracted from peripheral leukocytes. The coding regions of *NKX2-5* were amplified by the polymerase chain reaction. Polymerase chain reaction products were sequenced bidirectionally with BigDye Terminator v. 1.1 reagents (Applied Biosystems, Naerum, Denmark) and analyzed using an ABI 3130xl Genetic Analyzer.

Also, a systematic search with the words "*NKX2-5/CSX*" and "Congenital heart disease/ASD/atrioventricular block/heart block" was conducted in Pubmed and OMIM. Mutations annotated in HGMD (www.hgmd.org) were also included. Papers published in English peer-reviewed journals investigating germ-line mutations were included, supplemented with literature cited in key papers.

The study protocol was reviewed and approved by the local ethics committee and written informed consent was obtained from all participants or their legal guardians prior to investigation.

Results

NKX2-5 Mutation in a Family with ASD, Complex Malformation, and Atrioventricular Block

We identified a single nucleotide deletion at position 112 in exon 1 of *NKX2-5* in one family segregating autosomal dominant CHD in three generations (Figure 1). The mutation was not present in 100 Danish controls or the ExAC database of variants in the exome (http://exac.broadinstitute.org/) supporting that 112delG is a rare variant.

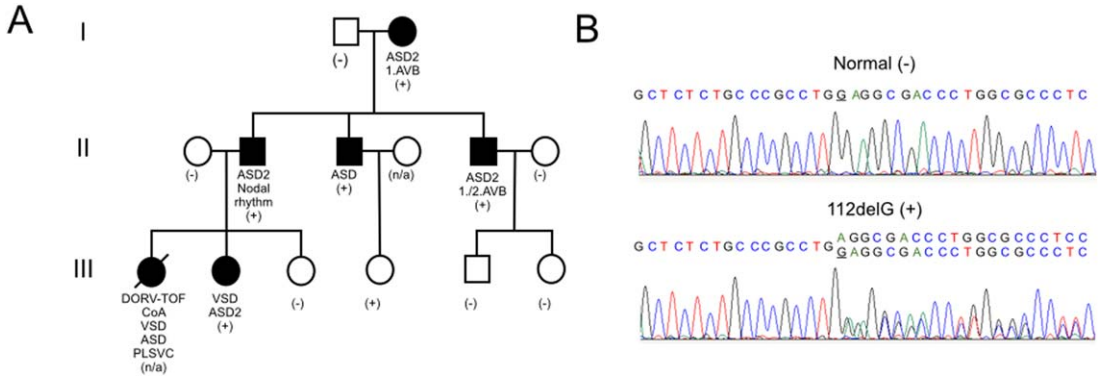

Figure 1. (A) Pedigree of Danish family with six affected individuals. I:2 (37 years old) had surgical closure of a secundum atrial septal defect (ASD2). ECG showed 1. degree atrioventricular block. II:2 (6 years old) had surgical closure of an ASD2. Twenty-year-old male had two episodes of dyspnea, retrosternal pain, and vertigo. ECG showed junctional rhythm with a heart rate of 49 beats per minute (bpm). ASD. II:3 (5 years old) had surgical closure of an ASD2. II:5 (6 years old) had surgical closure of an ASD2 with intermittent 1. and 2. Degree atrioventricular block, Wenckeback type, postoperatively. III:1 (8 months old) had complex CHD [double outlet right ventricle, fallot type (DORV-TOF), coarctation of aorta (CoA), persistent left superior vena cava (PLSVC), and ASD]. She died from respiratory failure. III:2 had an insignificant muscular ventricular septal defect (VSD) and a small ASD2 at birth. III:4 was a healthy carrier of the mutation, but an echocardiogram had never been done by wish of the parents. (+/−) Indicates presence/absence of mutation, respectively. AVB, atrioventricular block. (B) Section of the nucleotide sequence of *NKX2-5* gene located on chromosome 5 (5q34). Top, normal individual. Bottom, affected individual. The deletion of a single nucleotide at position 112 causes a frameshift, resulting in a truncated protein and a premature stop codon.

The deletion causes a shift of the reading frame, leading to a deduced protein with abnormal amino acid sequence from amino acid 37 and a premature stop at amino acid 175.

In the Danish family, the deletion segregated with CHD and was observed in 5/5 affected individuals, where a blood sample was available, and in one apparently healthy individual, who, however, has not been thoroughly investigated for CHD. ASD was diagnosed in all six individuals affected with CHD (five live with documented CHD, and one deceased with complex CHD), three also had conduction disease, one a ventricular septal defect (VSD) and one individual (III:1) had ASD in complex CHD (Figure 1). III:1 was diagnosed with double outlet right ventricle, fallot type (DORV-TOF), coarctation of aorta, ASD, and persistent left superior vena cava. The patient died eight months old after complicated staged surgery and atrioventricular block or arrhythmia was not observed.

Review of Published NKX2-5 Mutations

Fifty-nine different *NKX2-5* mutations were reported and confirmed in 202 patients in 31 papers.[2,18,19,22–46,51–56] Thus, including the mutation we identified in six Danish individuals, a total of 60 *NKX2-5* mutations have been identified in 208 patients.

NKX2-5 Mutations Are Significantly more Frequent in Familial CHD Compared to Nonfamilial CHD

A total of 198 index patients from CHD families (ASD = 117; atrioventricular block = 9; HLHS = 12; TOF = 3, mixed CHD = 57) have been screened for mutations and in 18 (9.1%) a *NKX2-5* mutation was found co-segregating with the malformation. In comparison 1037 nonfamilial cases (ASD = 137; atrioventricular block = 4; HLHS = 7; TOF = 290; mixed CHD = 599) have been screened in which 17 (1.6%) had a *NKX2-5* mutation (Table 1). A Fisher's exact test showed that this difference is significant ($P = 7.1 \times 10^{-9}$). Studies by Costa et al., Belvis et al., and Xie et al. were excluded (Table 1), because the index patients did not have CHD, but cardiomyopathy, stroke and atrial fibrillation, respectively. If these are included, the frequencies are 4.4% and 1.8% for familial and nonfamilial cases, respectively, and the difference is still significant ($P = .0032$). Studies, in which an actual screening of affected individuals was undertaken and where the text stated the number of screened familial and sporadic cases, was included in the calculations.

The Signature Phenotype of *NKX2-5* Mutation Carriers is ASD with CD/A

The 208 patients were grouped according to diagnosis and presence/absence of CD/A (Figure 2).

Table 1. Number of Screened Familial and Sporadic Cases Published

Author (Reference)	Number of Families Screened (Number of Index Cases With Mutation)	Mixed Familial/ Nonfamilial CHD	Number of Nonfamilial Cases Screened (Number of Individuals with Mutation)
Schott et al. (1998)[25]*	4 (4)		
Elliott et al. (2003)[31]*	25 (1)‡		121 (0)‡
Hirayama-Yamada et al. (2005)[36]*	16 (2)‡		
Hosoda et al. (1999)[26]*	1 (1)		
Stallmeyer et al. (2010)[42]	(2)	121	
Sarkozy et al. (2005)[41]*	16 (2)‡		13 (1)‡
Gutierrez-Roelens et al. (2002)[34]*	2 (2)		
Gutierrez-Roelens et al. (2006)[35]*	3 (1)‡		4 (0)‡
Benson et al. (1998)[24]	14 (4†)‡		22 (1)‡
McElhinney et al. (2003)[50]		474	(11)
Rifai et al. (2007)[40]*	1 (1)		
Kasahara et al. (2004)[37]*	2 (2)		
König et al. (2006)[38]*	1 (1)		
Liu et al. (2011)[51]*	58 (3)‡		
Perera et al. (2014)[22]*	1 (1)		
Costa et al. (2013)[29]	220 (1)†		
Watanabe et al. (2002)[44]*	2 (2)		
Ouyang et al. (2011)[23]*	1 (1)		
Pabst et al. (2008)[52]*	1 (1)		
Ikeda et al. (2002)[53]*	(1)	109	
Xie et al. (2013)[45]	48 (1)†		88 (1)
Wang et al. (2010)[43]	(1)	136	
Peng et al. (2010)[39]			135 (1)‡
Belvis et al. (2009)[28]			100 (3)
Goldmuntz et al. (2001)[33]			114 (6)‡
Draus et al. (2009)[30]			28 (1†)‡
Esposito et al. (2009)[32]	3 (1)‡		119 (5)‡
Kodo et al. (2012)[54]			256 (1)‡
Akçaboy et al. (2008)[27]			72 (1)‡
Abou Hassan et al. (2015)[46]	25 (3)‡		153 (0)‡
Ellesøe et al. (2015) (present study)	39 (1)‡		
Frequency	**18/198 (9.1%)**		**17/1037 (1.6%)**

Overview of the number of familial/sporadic index cases with CHD screened for *NKX2-5* mutations. Only the number of index cases screened and the number of index cases () with mutations are displayed. For example, in this study 39 index cases from 39 families were screened and one subject had a mutation. Bold indicate the total of the two columns.
*Indicate that the index cases in the study had ASD.
†Indicate that mutation carriers found had ASD (e.g., in the study by Costa et al., where index patients had familial dilated cardiomyopathy, or Xie et al., where index patients had familial atrial fibrillation).
‡Indicate that the study was included in the frequency calculation.

Patients with several malformations are part of several groups (e.g., a patient with ASD and VSD were included in the ASD and in the VSD column, respectively). ASD was present in 145 (70%) of the mutation carriers and 112 (54%) also had CD/A. In addition, 17 patients had VSD and CD/A, however, 16 of these also had an ASD. None of the three and thirteen patients with HLHS or TOF, respectively, had CD/A. Lastly, 11 patients had cardiomyopathy (left ventricular noncompaction, left ventricular hypertrophy, dilated cardiomyopathy) co-occurring with CD/A in six.

The Majority (94%) of Mutation Carriers with ASD and CD/A are Familial Cases

In a total of 112 patients with ASD and CD/A, 27 mutations were reported in 105 cases of familial CHD, whereas only seven mutations were found in cases with nonfamilial CHD. One mutation (Gln198ter) was found in both groups.

SCD Occurred in 15% of Patients with Familial ASD and CD/A

There were 19 SCDs in nine families with ASD and CD/A, and cardiomyopathy was also present in four (44%) of these nine families. A mutation (p.512insGlyCys) was documented in only one of these patients, however, additionally eight patients were obligate carriers (Supporting Information Figures II–IV). The remaining 10 patients were all part of pedigrees with dominant traits of *NKX2-5* mutations co-segregating with the malformations, and they all died suddenly before the age of 50. Assuming that these 10 were carriers of the mutations transmitted in their families, the total number of patients with familial ASD with CD/A would be 130 (112 + 8 obligate +10 possible carriers),

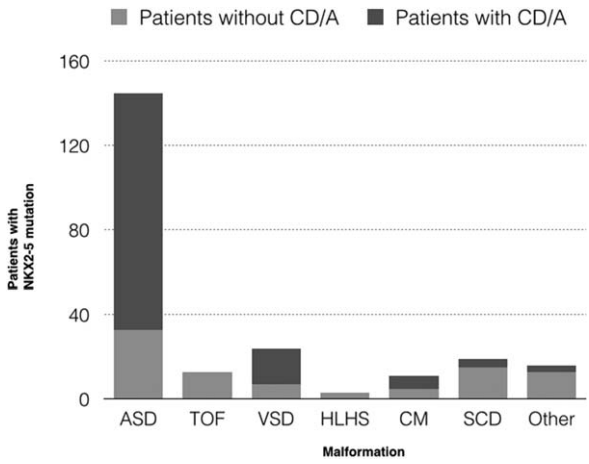

Figure 2. The majority of patients with a confirmed mutation in *NKX2-5* has ASD with CD/A. ASD, atrial septal defect; TOF, tetralogy of Fallot; VSD, ventricular septal defect; HLHS, hypoplastic left heart syndrome; CM, cardiomyopathy (left ventricular noncompaction, dilated cardiomyopathy, left ventricle hypertrophy); SCD, sudden cardiac death (sudden death in otherwise healthy individual before age 50); Other: Interrupted aortic arch = 1; truncus arteriosus = 1; levo-transposition of the great arteries = 1; coarctation of aorta = 2; double outlet right ventricle = 1; tricuspid valve anomaly (atresia, Ebstein) = 4; anomalous pulmonary venous return = 1; heterotaxy = 1.

corresponding to SCDs in 8% of mutation carriers with familial ASD and CD/A and 15% if the possible carriers are included.

There were no sudden deaths reported in the nonfamilial cases.

Discussion

Mutations in the *NKX2-5* gene have been reported several times in CHD patients, but a review of the phenotypic characteristics of the mutation carriers has not been presented. In this study, we identified a novel *NKX2-5* mutation in a Danish family and through a review of the literature, we found, that *NKX2-5* mutations usually occur in familial cases, the signature phenotype is ASD with CD/A and there is an alarming number of SCDs in the mutation carriers and their relatives.

In a Danish family with autosomal dominant transmission of ASD, atrioventricular block and complex heart defect, we identified of a novel mutation in 5/5 affected individuals. The mutation causes a frameshift and is expected to cause haploinsufficiency, due to nonsense mediated mRNA decay or production of a truncated version of the protein. The mutation co-segregated with CHD in the family, and all but one of the healthy individuals was negative for this mutation. We cannot exclude the possibility, that the unaffected carrier (III:4) had an insignificant ASD that closed early, or that she later in life develops an adult-onset atrioventricular block. It could also be caused by nonpenetrance of the mutation. Nonpenetrance has been reported for a few mutations in *NKX2-5* of which one (Arg25Cys) recently was suggested not to be causative due to the increasing number of unaffected relatives or controls carrying this mutation.[27,51] The mutation reported in the present study has never been found in any healthy individuals and we strongly believe this mutation is causative. Due to the lack of guidelines in this area, we decided to enrol the Danish family in 5 yearly checkups to monitor their heart rhythm.

Including the Danish family, 60 different mutations have been reported in 208 CHD patients and 74% of the patients had ASD, whereas only three documented cases of HLHS and 13 of TOF was reported. We found a highly significant increased frequency of mutations in familial cases compared to nonfamilial cases ($P = 7.1 \times 10^{-9}$). Lastly, we found that nine mutation carriers and 10 relatives died suddenly before the age of 50, two of which had functional pacemakers at time of death. SCD in individuals without progressive heart failure and with functional pacemakers can be assumed to be caused by tachyarrhythmias. These tachyarrhytmias can either originate in the myocardium or in the conduction system. However, the presence of cardiomyopathy in some of these individuals strongly suggests that this is a disease of the myocardium.

We hypothesized, that *NKX2-5* mutations are correlated to ASD with CD/A and that the disease-related mutations predominantly occur in CHD families, rather than in nonfamilial CHD, and we confirmed this by review of the existing literature.

NKX2-5 is necessary for cardiac development as well as maintaining proper function of the AV-node and myocardium throughout adult life.[12,47] *NKX2-5* mutations in patients with ASD and atrioventricular block has been reported sporadically, however, recent studies have also reported healthy mutation carriers exhibiting runs of nonsustained ventricular tachycardia, ventricular fibrillation, and paroxysmal atrial fibrillation during Holter-monitoring or recordings from implantable cardioverter-defibrillators.[22,35,45] During cardiogenesis *NKX2-5* signal the heart to develop from primary slowly conducting into fast conducting working

myocardium.[48,49] However, in the areas of the developing conduction system timed repression of *NKX2-5* is crucial for proper formation of the sinus node, the AV node and the peripheral conduction system.[49]

Our study is limited by the retrospective design and should be interpreted with certain precautions. First, we only included studies in which a clear screening procedure was reported, as well as studies in which the number of screened familial/nonfamilial cases was stated clearly. This could have biased our results; however, when we included three screening studies of patients without CHD (cardiomyopathy, atrial fibrillation, and stroke) the difference was still significant.

Second, we have proof of one mutation carrier and eight obligate carriers dying from SCD, and the inclusion of the remaining 10 in the cohort as assumed mutation carriers could be considered as speculative. However, they were all part of pedigrees with confirmed mutations segregating with CHD, which support our theory, that they were also mutation carriers.

With this review, we have established a connection between SCD, cardiomyopathy and familial ASD, that necessitates clinicians not merely to see patients with familial ASD as cured after successful ASD repair, but as possible carriers of a mutation associated with increased risk of developing progressive arrhythmias, cardiomyopathy, and SCD.[23,25,29,41] We found that 44% of families with SCD cases had *NKX2-5* mutations combined with cardiomyopathy. Due to the small number of reported cases, we can only speculate whether this combination of *NKX2-5* mutation and cardiomyopathy increases the risk of SCD. Further studies are needed to confirm this theory, but in the meantime, we suggest, that patients with familial ASDs should be screened for mutations in *NKX2-5* to assess the risk of malignant arrhythmias and sudden deaths. If they are mutation carriers, we suggest that a preventive implantable cardioverter-defibrillator should be considered in these patients, especially if there is also a family history of cardiomyopathy.

Author Contributions

All authors contributed to design of the study, data analysis as well as publication review. SGE contributed to the data collection, analysis and drafting of the article. In addition, MMJ and LAL contributed to sequencing of the study subjects.

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References

1 Zhao QM, Ma XJ, Jia B, Huang GY. Prevalence of congenital heart disease at live birth: an accurate assessment by echocardiographic screening. *Acta Paediatr Int J Paediatr.* 2013;102(4):397–402.
2 Van der Linde D, Konings EEM, Slager MA, et al. Birth prevalence of congenital heart disease worldwide: a systematic review and meta-analysis. *J Am Coll Cardiol.* 2011;58(21):2241–2247.
3 Cantinotti M, Assanta N, Murzi B, Lopez L. Controversies in the definition and management of insignificant left-to-right shunts. *Heart.* 2014;100(3):200–205.
4 Azhari N, Shihata MS, Al-Fatani A. Spontaneous closure of atrial septal defects within the oval fossa. *Cardiol Young.* 2004;14(2):148–155.
5 Wilmshurst PT, Pearson MJ, Nightingale S, Walsh KP, Morrison WL. Inheritance of persistent foramen ovale and atrial septal defects and the relation to familial migraine with aura. *Heart.* 2004;90(11):1315–1320.
6 Nyboe C, Olsen MS, Nielsen-Kudsk JE, Johnsen SP, Hjortdal VE. Risk of pneumonia in adults with closed versus unclosed atrial septal defect (from a Nationwide Cohort Study). *Am J Cardiol.* 2014;114(1):105–110.
7 Nyboe C, Olsen MS, Nielsen-Kudsk JE, Hjortdal VE. Atrial fibrillation and stroke in adult patients with atrial septal defect and the long-term effect of closure. *Heart.* 2015;101:706–711.
8 Caputo S, Capozzi G, Russo MG, et al. Familial recurrence of congenital heart disease in patients

with ostium secundum atrial septal defect. *Eur Heart J.* 2005;26(20):2179–2184.
9 Gelernter-Yaniv L, Lorber A. The familial form of atrial septal defect. *Acta Paediatr.* 2007;96(5): 726–730.
10 Gunal N, Gül S, Kahramnyol Ö. Familial atrial septal defect with prolonged atrioventricular conduction. *Acta Paediatr Jpn Overseas Ed.* 1997;39: 634–636.
11 Nora JJ, Meyer TC. Familial nature of congenital heart diseases. *Pediatrics.* 1966;37(2):329–334.
12 Pashmforoush M, Lu JT, Chen H, et al. Nkx2-5 pathways and congenital heart disease: loss of ventricular myocyte lineage specification leads to progressive cardiomyopathy and complete heart block. *Cell.* 2004;117(3):373–386.
13 Gazes PC, Culler RM, Taber E, Kelly TE. Congenital familial cardiac conduction defects. *Circulation.* 1965;32:32–34.
14 Morgans CM, Gray KE, Robb GH. A survey of familial heart block. *Br Heart J.* 1974;36(7):693–696.
15 Sarachek NS, Leonard JJ. Familial heart block and sinus bradycardia. *Am J Cardiol.* 1972;29: 451–458.
16 Celiker A, Ciçek S, Ozme S. Long-term results of patients with congenital complete atrioventricular block. *Pediatrics.* 1982;69(6):728–733.
17 Groves AM, Allan LD, Rosenthal E. Outcome of isolated congenital complete heart block diagnosed in utero. *Heart.* 1996;75(2):190–194.
18 Michaelsson M, Riesenfeld T, Jonzon A. Natural history of congenital complete atrioventricular block. *Pacing Clin Electrophysiol.* 1997;20(8 Pt 2): 2098–2101.
19 Tsagaris T, Bustamante R, Friesendorff R. Familial heart disease. *Chest.* 1967;52(2):153–158.
20 Reid JM, Coleman EN, Doig W. Complete congenital heart block. Report of 35 cases. *Br Heart J.* 1982;48(3):236–239.
21 Chow LTC, Cook AC, Ho SY, Leung MP, Anderson RH. Isolated congenitally complete heart block attributable to combined nodoventricular and intraventricular discontinuity. *Hum Pathol.* 1998;29(7):729–736.
22 Perera JL, Johnson NM, Judge DP, Crosson JE. Novel and highly lethal NKX2.5 missense mutation in a family with sudden death and ventricular arrhythmia. *Pediatr Cardiol.* 2014;35(7):1206–1212.
23 Ouyang P, Saarel E, Bai Y, et al. A de novo mutation in NKX2.5 associated with atrial septal defects, ventricular noncompaction, syncope and sudden death. *Clin Chim Acta.* 2011;412(1–2):170–175.
24 Benson DW, Sharkey A, Fatkin D, et al. Reduced penetrance, variable expressivity, and genetic heterogeneity of familial atrial septal defects. *Circulation.* 1998;97:2043–2048.
25 Schott JJ, Benson DW, Basson CT, et al. Congenital heart disease caused by mutations in the transcription factor NKX2-5. *Science.* 1998; 281(5373):108–111.
26 Hosoda T, Komuro I, Shiojima I, et al. Familial atrial septal defect and atrioventricular conduction disturbance associated with a point mutation in the cardiac homeobox gene CSX/NKX2-5 in a Japanese patient. *Jpn Circ J.* 1999;63(5):425–426.
27 Akçaboy MI, Cengiz FB, Inceoğlu B, et al. The effect of p.Arg25Cys alteration in NKX2-5 on conotruncal heart anomalies: mutation or polymorphism? *Pediatr Cardiol.* 2008;29(1):126–129.
28 Belvís R, Tizzano EF, Martí-Fàbregas J, et al. Mutations in the NKX2-5 gene in patients with stroke and patent foramen ovale. *Clin Neurol Neurosurg.* 2009;111(7):574–578.
29 Costa MW, Guo G, Wolstein O, et al. Functional characterization of a novel mutation in NKX2-5 associated with congenital heart disease and adult-onset cardiomyopathy. *Circ Cardiovasc Genet.* 2013;6(3):238–247.
30 Draus JM, Hauck MA, Goetsch M, Austin EH, Tomita-Mitchell A, Mitchell ME. Investigation of somatic NKX2-5 mutations in congenital heart disease. *J Med Genet.* 2009;46(2):115–122.
31 Elliott DA, Kirk EP, Yeoh T, et al. Cardiac Homeobox gene NKX2-5 mutations and congenital heart disease. *J Am Coll Cardiol.* 2003;41(11):4–8.
32 Esposito G, Grutter G, Drago F, et al. Molecular analysis of PRKAG2, LAMP2, and NKX2-5 genes in a cohort of 125 patients with accessory atrioventricular connection. *Am J Med Genet Part A.* 2009;149(7):1574–1577.
33 Goldmuntz E, Geiger E, Benson DW. NKX2.5 mutations in patients with tetralogy of fallot. *Circulation.* 2001;104(21):2565–2568.
34 Gutierrez-Roelens I, Sluysmans T, Gewillig M, Devriendt K, Vikkula M. Progressive AV-block and anomalous venous return among cardiac anomalies associated with two novel missense mutations in the CSX/NKX2-5 gene. *Hum Mutat.* 2002;20(1):75–76.
35 Gutierrez-Roelens I, De Roy L, Ovaert C, et al. A novel CSX/NKX2-5 mutation causes autosomal-dominant AV block: are atrial fibrillation and syncopes part of the phenotype? *Eur J Hum Genet.* 2006;14(12):1313–1316.
36 Hirayama-Yamada K, Kamisago M, Akimoto K, et al. Phenotypes with GATA4 or NKX2.5 mutations in familial atrial septal defect. *Am J Med Genet A.* 2005;135(1):47–52.
37 Kasahara H, Benson DW. Biochemical analyses of eight NKX2.5 homeodomain missense mutations causing atrioventricular block and cardiac anomalies. *Cardiovasc Res.* 2004;64(1):40–51.
38 König K, Will JC, Berger F, Müller D, Benson DW. Familial congenital heart disease, progressive atrioventricular block and the cardiac homeobox transcription factor gene NKX2.5:

identification of a novel mutation [7]. *Clin Res Cardiol*. 2006;95(9):499–503.
39 Peng T, Wang L, Zhou SF, Li X. Mutations of the GATA4 and NKX2.5 genes in Chinese pediatric patients with non-familial congenital heart disease. *Genetica*. 2010;138(11):1231–1240.
40 Rifai L, Maazouzi W, Sefiani A. Novel point mutation in the NKX2-5 gene in a Moroccan family with atrioventricular conduction disturbance and an atrial septal defect in the oval fossa. *Cardiol Young*. 2007;17(1):107–109.
41 Sarkozy A, Conti E, Neri C, et al. Spectrum of atrial septal defects associated with mutations of NKX2.5 and GATA4 transcription factors. *J Med Genet*. 2005;42(2):e16.
42 Stallmeyer B, Fenge H, Nowak-Göttl U, Schulze-Bahr E. Mutational spectrum in the cardiac transcription factor gene NKX2.5 (CSX) associated with congenital heart disease. *Clin Genet*. 2010;78(6):533–540.
43 Wang J, Xin Y-F, Liu X-Y, Liu Z-M, Wang X-Z, Yang Y-Q. A novel NKX2-5 mutation in familial ventricular septal defect. *Int J Mol Med*. 2011; 27:369–375.
44 Watanabe Y, Benson DW, Yano S, Akagi T, Yoshino M, Murray JC. Two novel frameshift mutations in NKX2.5 result in novel features including visceral inversus and sinus venosus type ASD. *J Med Genet*. 2002;39:807–811.
45 Xie W-H, Chang C, Xu Y-J, et al. Prevalence and spectrum of Nkx2.5 mutations associated with idiopathic atrial fibrillation. *Clinics (Sao Paulo)*. 2013;68(6):777–784.
46 Abou Hassan OK, Fahed AC, Batrawi M, et al. NKX2-5 mutations in an inbred consanguineous population: genetic and phenotypic diversity. *Sci Rep*. 2015;5:8848.
47 Pashmforoush M, Lu JT, Chen H, et al. Nkx2-5 pathways and congenital heart disease; loss of ventricular myocyte lineage specification leads to progressive cardiomyopathy and complete heart block. *Cell*. 2004;117(3):373–386.
48 Prall OWJ, Menon MK, Solloway MJ, et al. An Nkx2-5/Bmp2/Smad1 negative feedback loop controls heart progenitor specification and proliferation. *Cell*. 2007;128(5):947–959.
49 Miquerol L, Kelly RG. Organogenesis of the vertebrate heart. *Wiley Interdiscip Rev Dev Biol*. 2013; 2:17–29.
50 Jongbloed MRM, Vicente Steijn R, Hahurij ND, et al. Normal and abnormal development of the cardiac conduction system; implications for conduction and rhythm disorders in the child and adult. *Differentiation*. 2012;84(1):131–148.
51 McElhinney DB, Geiger E, Blinder J, Benson DW, Goldmuntz E. NKX2.5 mutations in patients with Congenital Heart Disease. *J Med Genet*. 2003;42(9):1650–1655.
52 Liu X-Y, Wang J, Yang Y-Q, et al. Novel NKX2-5 mutations in patients with familial atrial septal defects. *Pediatr Cardiol*. 2011;32(2):193–201.
53 Pabst S, Wollnik B, Rohmann E, et al. A novel stop mutation truncating critical regions of the cardiac transcription factor NKX2-5 in a large family with autosomal dominant inherited congenital heart disease. *Clin Res Cardiol*. 2008;97(1): 39–42.
54 Ikeda Y, Hiroi Y, Hosoda T, et al. Novel point mutation in the cardiac transcription factor CSX/NKX2.5 associated with congenital heart disease. *Circ J*. 2002;66(6):561–563.
55 Kodo K, Nishizawa T, Furutani M, et al. Genetic analysis of essential cardiac transcription factors in 256 patients with non-syndromic congenital heart defects. *Circ J*. 2012;76(7):1703–1711.
56 Gioli-Pereira L, Pereira AC, Mesquita SM, Xavier-Neto J, Lopes AA, Krieger JE. NKX2.5 mutations in patients with non-syndromic congenital heart disease. *Int J Cardiol*. 2010;138(3): 261–265.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table I. Diagnoses of Danish probands screened for NKX2-5 mutations and their relatives.
Figure I. Pedigrees of three Danish families screened for *NKX2-5* mutations.
Figure II. Pedigrees from published papers reporting sudden cardiac deaths: Schott et al. (1999).[25]
Figure III. Pedigrees from published papers reporting sudden cardiac deaths: Hosoda et al. (1999),[26] Ouyang et al. (2011),[23] and Perera et al. (2014).[22]
Figure IV. Pedigrees from published papers reporting sudden cardiac deaths: Abou Hassan et al. (2015).[46]