Familial congenital heart disease, progressive atrioventricular block and the cardiac homeobox transcription factor gene NKX2.5: identification of a novel mutation

Sirs: Congenital heart disease has the highest incidence of all birth defects affecting about 6–8/1000 live births. These heart malformations are believed to arise during cardiac development, but there is little known about the underlying mechanisms of disease [12]. Cardiac development is a complex process, and in recent years, the role of transcription factors as possible navigators of development has been frequently investigated [7, 10, 19]. The cardiac transcription factor NKX2.5 was identified as the first genetic cause of nonsyndromic congenital heart disease [22]. A total of 33 heterozygous mutations in NKX2.5 have been reported in individuals with a variety of congenital heart malformations, including atrial septal defect, ventricular septal defect, tetralogy of Fallot and abnormalities of the tricuspid valve [2, 6, 8, 9, 11, 13, 14, 16, 18, 21–23]. Specific types of mutations result in progressive atrioventricular (AV) conduction disturbance, requiring pacemaker implantation. These studies have provided important insight into the genetic origins of cardiac malformations and, by providing tools for the developmental biologist, have contributed to improved insights into the pathogenesis of congenital heart disease [3, 5]. As such, these results are important to pediatricians, cardiologists and surgeons who diagnose, treat and provide long-term follow-up to these patients.

Here we report a novel NKX2.5 mutation in a small family where progressive AV block and congenital heart disease was identified in two generations.

Patients

The proband, an 11 year-old boy (III-2, Fig. 1) was followed in our outpatient department after successful surgical closure of a secundum atrial septal defect in his second year of life. First degree AV block was noted during the first year of life prior to surgery (Fig. 2). This conduction disturbance was progressive (Fig. 3); second degree AV block, first seen seven years after surgery, progressed to third degree AV block and a pacemaker was implanted at the age of 10 years.

In the family medical history, his older brother (III-1) died in the 8th week of life because of heart failure due to a complex congenital heart defect with an atrial and ventricular septal defect and coarctation of the aorta (an electrocardiogram is not available for review). His younger sister (III-3) is three years old and free of heart disease.
The father of the proband (II-2) is 45 years old and had had surgical closure of secundum atrial septal defect in his 20th year of life. Because of progressive AV block, he had pacemaker implantation at the age of 38 years. The other family members are clinically without heart disease and have normal electrocardiograms (ECG) (Fig. 1).

**Methods**

Written consent was obtained from the family participants (II-2, III-2, III-3); the other family members declined participation in the genetic study. The investigation conforms with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants in accordance with the Cincinnati Children's Hospital Medical Center Institutional Review Board. Participants were evaluated by medical history, physical examination and ECG; a blood sample was obtained. Medical records including reports of echocardiogram, cardiac catheterization, electrophysiology study and cardiac surgery were reviewed when available. Clinical assessment was performed without knowledge of genotype.

Polymerase chain reaction (PCR) was used to amplify the coding region of \( \text{NKX2.5} \) from genomic DNA, as previously described [2], and PCR products were prepared and sequenced in both the sense and antisense direction through use of an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Nucleotide numbering starts at the adenine nucleotide (A) in the ATG initiation codon of \( \text{NKX2.5} \) (Accession number HSU34962). To clarify a sequence change in the mutant allele, the PCR product of exon 1 was cloned using a TA cloning kit (Invitrogen Corp, Carlsbad, CA) and sequenced.

**Results**

During survey of the \( \text{NKX2.5} \) coding region, a single nucleotide deletion in exon 1, del312G, that produced a frameshift was identified. The deletion frameshift arises in amino acid 105 and is predicted to produce a truncated protein (174 amino acids) without a homeodomain (K104fs70X) (Figs. 4 and 5). The sequence change abolishes a Sty I restriction enzyme site that allowed independent confirmation of the sequence alteration (data not shown). This sequence variant was considered to be a mutation based on its cosegregation with disease status in affected family members, its absence in 200 chromosomes from 100 unrelated normal subjects, and the alteration of highly conserved amino acid residues.