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Original Article

Familial atrial septal defect in the oval fossa with progressive prolongation of the atrioventricular conduction caused by mutations in the NKX2.5 gene

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Abstract *Objective:* To search for a genetic basis in a family with autosomal dominantly inherited atrial septal defect in combination with increasing conduction anomalies. *Design:* We searched for mutations in the NKX2.5 gene by sequencing of desoxyribonucleic acid in a previously investigated family. *Patients:* All family members were included if they, after informed consent, had decided to participate in the genetic testing. A blood sample was sent from local doctors for analysis of potential mutations. Patients with cardiac anomalies were examined in our hospital. For those family members without cardiac anomalies, we relied on local information. *Results:* We identified the mutation Q149X in the NKX2.5 gene on chromosome 5q35 in all patients with atrial septal defect and disturbances of atrioventricular conduction. No family member without an atrial septal defect possessed the mutation, including a member with transposed arterial trunks. *Conclusion:* We have identified a mutation in the NKX2.5 gene responsible for autosomal dominantly inherited atrial septal defect in the oval fossa combined with disturbances of atrioventricular conduction in 7 patients spanning 4 generations.

Keywords: Congenital heart disease; genetics; familial occurrence; dominant inheritance

Through the years, a number of reports have been published where two or more members in one family have been affected with the same or different cardiac defects.[1] Recent publications[2] have studied recurrence in close relatives. Atrial septal defect with prolonged atrioventricular conduction and familial occurrence was repeatedly reported some decades ago.[3–5] We described the findings in such a family in 1974.[6] At least 12 distinctly different mutations of the NKX2.5 gene, mostly outside the homeodomain, have been reported at a rate of zero to 4% in patients with a variety of cardiac lesions, but in almost one-sixth of patients with discordant atrioventricular connections, albeit in none with regular transposition.[7] In recent genetic studies, the NKX2.5 gene on chromosome 5q35 has been reported to be responsible for such defects.[8,9] We therefore re-examined the family we had previously published, screening for mutations of the NKX2.5 gene in the greater part of the family.

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Materials and methods

We were able to track 52 family members, of whom 18 now are dead. We know that there were further descendants of the individuals (II:5-II:10), which we could not retrieve. The first generation was already deceased at the time of our earlier publication. In the meantime, all members of the second generation have also died, and for their details we have relied on the information we collected prior to the former publication. After obtaining written consent, 26 family members in four generations consented to participate in our study. Among these were all family members with documented atrial septal defects. A family member with transposed

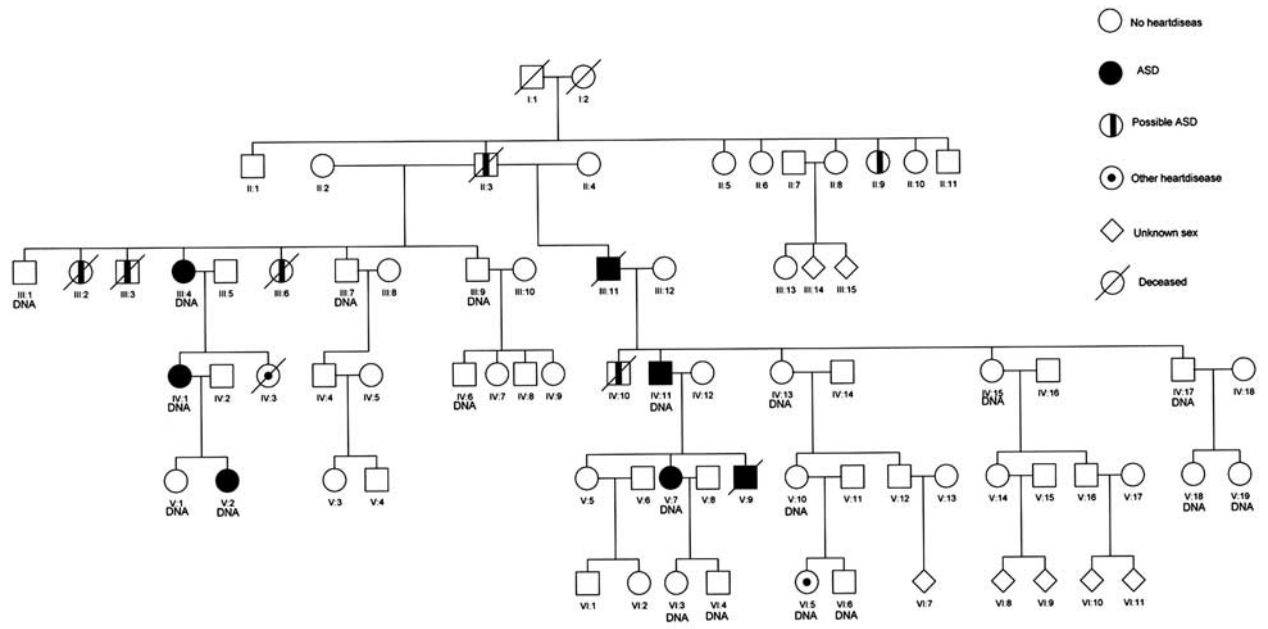

Figure 1.
The tree of the affected family. For explanation of the symbols, see figure. "DNA" indicates each of the 28 family members in whom testing of desoxyribonucleic acid was performed. The NKX2.5 gene segregated completely between those with and without an atrial septal defect.

arterial trunks but an intact atrial septum (VI:7) also participated. Clinical studies have been performed at their home site. The cardiac assessment was made clinically and non-invasively. All patients with cardiac malformations have been studied and treated in our specialised institution. Blood samples were drawn at the premises of their local physicians, for members with and without congenital cardiac disease, and have been studied in our genetic laboratory. Of the family members, 2 (IV:3 and V:10) died following cardiac operations. Paraffin embedded biological material was available from these members, and could be used for the study. The relevant part of the family is depicted in Figure 1.

Genetic studies

The genetic laboratory was blinded with respect to the clinical state of the patients. Desoxyribonucleic acid was extracted from EDTA-containing blood by the use of a BioRobot EZ1 (Qiagen GmbH, Hilden, Germany). In the two deceased patients, genetic material was isolated from paraffin embedded tissue blocks. The two exons of the NKX2.5 gene with flanking intron sequences were amplified by PCR using primers:

Exon 1 forward: 5′-CTGCTGCCCGGACACATCCAGAGCT-3′,
Exon 1 reverse: 5′-GTGTCTCCTCCTCCTGGCCCTGAGT-3′,
Exon 2 forward: 5′-CACGAGGATCCCTTACCATTACTGT-3′,
Exon 2 reverse: 5′-AAATCCAGGGGACTCAGGGTCATGT-3′.

The thermal cycling was preceded by denaturation at 95°C for 10 minutes, and completed by elongation at 72°C for 10 minutes:

Exon 1: 33 cycles of denaturation at 95°C for 30 sec, annealing at 61°C for 30 sec and elongation at 72°C for 30 seconds.
Exon 2: 34 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec and elongation at 72°C for 30 seconds.

After purification of the products of polymerase chain reaction, direct sequencing of the desoxyribonucleic acid was employed to detect mutations. Standard desoxyribonucleic acid sequencing reactions using version 3.1 of Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) were analyzed on a Genetic Analyzer 3100 (Applied Biosystems) using the Secscape 2.1 software (Applied Biosystems). Nucleotide positions of c- desoxyribonucleic acid were numbered according to the published sequence (Accession number NP 004378) with A of the ATG translation initiation codon being nucleotide 1.

If a protein is synthesised from this allele, it will lack a functional homeodomain, and will therefore be unable to bind desoxyribonucleic acid. The sequence is depicted in Figure 2.

Results

The father, I:1, died in his seventies from renal diseases, whereas his wife, I:2 died at the age of

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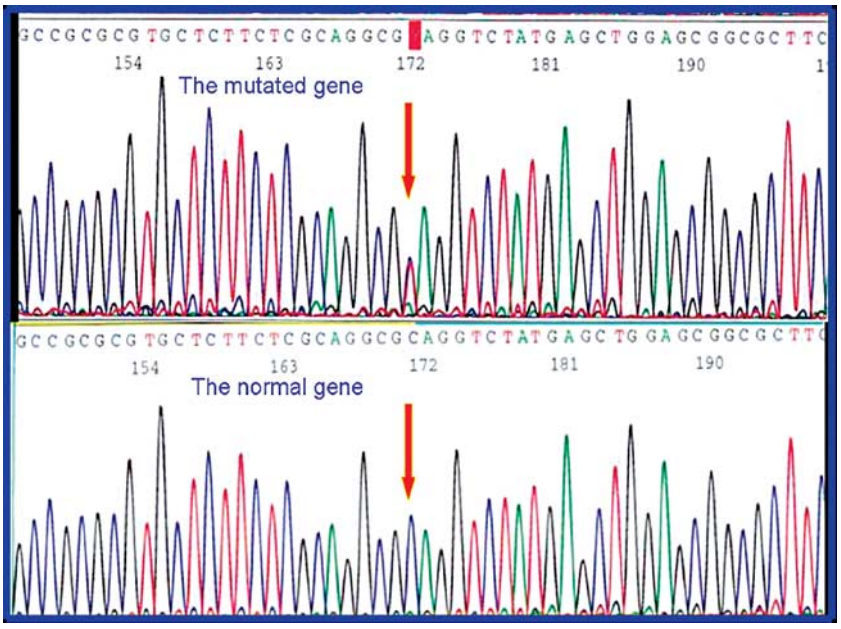

Figure 2.
Display of the nucleotide sequence encoding a part of the homeodomain of the NKX2.5 gene. The lower panel shows the normal sequence, whereas the upper panel shows heterozygosity for the mutation Q149X (CAG → TAG) in exon 2 [arrows indicating the site of the exchanged amino acid (T for C)].

80 years. No cause of her death was given, but in neither case was there any report of cardiac disease. Patient II:8 reportedly suffered from cardiac problems in her 60ies, without anything specifically indicating such a diagnosis the cause still might have been an atrial septal defect. All members of the oldest generation (II) have now died without a specific cardiac diagnosis, including the father of the two different lines of dominant atrial septal defect (II:3). At the age of 55 he had complete right bundle branch block and atrial fibrillation. He might very well have had an atrial septal defect. He died at the age of 63 years. With different degrees of certainty, 3 of the deceased members of the family (III:2, III:3, III:6) could also have had this specific malformation. These patients died at 7, 5, and 20 months of age over sixty years ago in a remote area of Norway. Prior to our first publication their mother described, meticulously observed, the clinical picture of progressive cardiac failure, indicating a possible atrial septal defect and/or atrioventricular block. These patients, however, were never studied. The clinical diagnosis of atrial septal defect had been made in a boy (IV:10) who died untreated in 1958 at the age of 10. A PR interval of 0.54 seconds was measured shortly prior to his death, as shown in our earlier publication[6] (see Table 1).

The atrial septal defect was, in all cases, accompanied by an increasing prolongation of the PR interval. The 2 patients with additional coarctation or tricuspid atresia were probably too young to demonstrate involvement of the axis responsible for atrioventricular conduction. At present, all patients with an atrial septal defect are dependent on a pacemaker. Desoxyribonucleic acid sequencing of the two exons of the index patient (V:8) revealed that she was heterozygous for mutation Q149X (CAG → TAG) in exon 2 of the NKX2.5 gene, thus lacking a functional homeodomain and unable to bind desoxyribonucleic acid.

We tested 26 living family members who agreed to participate for the Q149X mutation. Of these, 5 living family members in 3 generations were heterozygous for the mutation. All of them had atrial septal defect, and were dependent on artificial pacing because of complete atrioventricular block. The 2 patients who died following cardiac surgery were also both heterozygous for the mutation. In both cases, the atrial septal defect was described as "big" in the operative report, in the girl with tricuspid atresia with the comment "almost a single atrium". None of the 21 family members, who did not possess the mutation, had deficient atrial septation. Such a mutation was also excluded in the patient with transposition (see Table 1).

Discussion

We have identified mutation Q149X in the NKX2.5 gene on chromosome 5q35 in all affected

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Table 1.

Family members genetically tested who were positive for the NKX2.5 mutation	
Atrial septal defect, treated. Pacemaker.	III:4, IV:1, IV:11, V:2, V:8
Tricuspid atresia and very big atrial septal defect. Died following Fontan operation. †	IV:3
Atrial septal defect. Died after neonatal operation for coarctation of the aorta and open arterial duct. †	V:10
Family members genetically tested and who were negative for the NKX2.5 mutation	
No cardiac disease.	III:1, III:7, III:9, IV:6, IV:7, IV:8, IV:9, IV:13, IV:15, IV:17, V:1, V:6, V:11, V:16, V:17, VI:3, VI:4, VI:5, VI:6, VI:8
Transposition	VI:7
Family members not genetically tested (deceased or unwilling to participate)	
Operated for atrial septal defect. Pacemaker. †	III:11
Clinical diagnosis atrial septal defect. PR interval = 0.54 seconds †	IV:10
Cardiac disease likely, probably atrial septal defect. †	II:3, III:2, III:3, III:6
Cardiac disease, possibly atrial septal defect. †	II:8
No indication of cardiac disease. †	I:1, I:2, II:1, II:2, II:4, II:5, II:6, II:7, II:9, II:10
No indication of cardiac disease.	IV:4, V:3, V:4, V:13, V:14, V:15, VI:1, VI:2

Symbol used: † = deceased.

members studied in this family. The same genetic defect was, if tested, found in one of the parents of each affected member. We did not find the mutation in any member without atrial septal defect. A report from Japan[10] found another gene, CSX/NKX2.5 localised to chromosome 5q/34 in 4 patients in 3 generations with familial atrial septal defect and prolongation of the PR interval. We did not find the mutation in the patient with transposition. This is consistent with the report of McElhinney and colleagues.[7] In 86 patients with transposition, they found no mutations in NKX2.5, be it inside or outside the homeodomain. The part of the NKX2.5 gene called "homeodomain", the part of the protein which binds to the desoxyribonucleic acid regulating the gene expression, is located between amino acids number 138 and 197. Mutation Q149X is expected to encode a truncated protein of only 148 amino acids. This results in an incomplete homeodomain, prevents its binding to desoxyribonucleic acid, and makes the protein non-functional.

We restudied the family we published over 30 years ago[6] because of reports on this gene.[7–9] On the basis of the findings, we are confident that this mutation Q149X must be responsible for the cardiac defect. The dominant mode of inheritance postulated in our first report is also seemingly confirmed by these genetic studies. The mutation has also previously been found to underlie atrial septal defect when occurring in autosomal dominant fashion.[11–13] These observations make the mutation responsible for both the cardiac malformation and the involvement of the conduction system. Recently, König and colleagues published a family with 3 affected members in 2 generations.[14] Since the various families are reported from different parts of the world, it is unlikely that there should be any connection between them. The big family reported from our neighbouring country, Sweden,[3] was traced back to the 17th century. We cannot rule out completely a cross-border relationship, but it seems very unlikely.

Our previous study of this family indicated an autosomal dominantly inherited trait. The fact that the oldest persons with documented atrial septal defect in the two affected lines of the family (III:4 and III:11) had a common father but different mothers made it most likely that their common father was the source of their common gene. Since we now have defined the rare, mutated gene itself in both lines, we believe it proves that this common father also had the genetic mutation five generations ago. He presented with atrial fibrillation and right bundle branch block at the age of 55, five years prior to his death. His symptoms of fatigue could also indicate an atrial septal defect. This man had seven siblings. Our earlier study of their scattered and incomplete files gave only vague indications that one younger sister, born in 1913 (II:8), might have had an atrial septal defect. Thus, we think it most likely represents a new mutation, although we cannot completely rule out that the ancestor in question had acquired the genetic mutation from one of his parents.

Additional or different cardiac lesions were found in 3 patients in the family. In one, coarctation was found together with an atrial septal defect (V:10), while another had tricuspid atresia with deficient atrial septation (IV:3). Of these patients, 1 parent of each possessed the mutant gene, and so did their children. The third patient had transposition with

an atrial communication not necessitating a Rashkind procedure (VI:7), but without the mutated gene. Of 7 patients with the gene, 2 having additional cardiac malformations may indicate that the effects of the mutant gene in some patients influence a wider spectrum of cardiac development. Although the main defect caused by the mutation is deficiency of the floor of the oval fossa and prolongation of atrioventricular conduction, the expressivity may differ in the individual patient.

Neither the child with transposition, nor her mother or grandmother, possessed the mutation. We think that her cardiac defect occurring in this family most likely is just a coincidence. We cannot, however, completely rule out that there may be other genetic factors at play.

Our institution is the only hospital in Norway undertaking surgery for children with congenital cardiac defects. Among our patients in approximately $4\frac{1}{2}$ million inhabitants, this is the only family we know of with genetically transmitted atrial septal defect. Only one family is known in Sweden, with double the number of inhabitants. The incidence of such families must be rare, but the presence of an atrial septal defect with increasingly long PR interval should lead to the suspicion that the underlying cause is a mutation in the NKX2.5 gene.

In conclusion, we have shown that autosomal dominant inheritance of atrial septal defect with increasingly prolonged atrioventricular conduction time eventually producing atrioventricular block segregates completely with mutation Q149X in the NKX2.5 gene.

Conflicts of interest: None
Grants and commercial support: None

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