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Clinical and Genetic Investigation of Atrial Septal Defect with Atrioventricular Conduction Defect in a Large Consanguineous Tunisian Family

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Background. Atrial septal defect (ASD) is an autosomal dominant disease characterized by left-to-right shunting and increased right ventricular output. Approximately 5–10% of congenital heart diseases (CHD) are due to ASD, which is one of the most frequent CHD found in adults. The gene responsible for ASD was mapped to chromosome 5q35 encoding the transcription factor NKX2–5 that plays an important role for the regulation of septation during cardiac morphogenesis.

Methods. A Tunisian family including four affected members was investigated. Individuals were genotyped using the polymorphic microsatellite markers D5S394 and D5S2069 overlapping the *NKX2–5* gene.

Results. We report here clinical and molecular investigation of a Tunisian consanguineous family with four affected members. Two presented with ASD associated with prolonged PR interval, whereas the other two presented only a prolonged PR interval. We also identified five asymptomatic individuals in the same family with ventricular preexcitation. Although the patients were products of a consanguineous marriage, no other abnormalities were observed in this family. Genotyping and linkage analysis showed exclusion of linkage between the gene responsible for ASD in this family and *NKX2.5* gene.

Conclusions. Our results further confirm the genetic heterogeneity of ASD. © 2008 IMSS. Published by Elsevier Inc.

Key Words: Atrial septal defect (ASD), *NKX2–5*, Linkage analysis, Conduction defect, Preexcitation.

Introduction

Atrial septal defect (ASD) is a common congenital heart malformation affecting 1/1500 live births and accounting for 10% of isolated congenital heart diseases (CHD) (1,2). ASD refers to a communication between the right and left atria, anatomically classified into the deficient atrial septum structure. ASD ostium secundum is the prevalent defect, representing 85% of all ASD (3). ASD may present as an isolated defect or with conduction and skeletal abnormalities. An uncorrected ASD can cause pulmonary overcirculation and generates right heart volume overload. Increased pulmonary resistance and pressure due to histopathological pulmonary arteriolar changes lead to reduced functional capacity; cyanosis, arrhythmia, stroke and premature death may result during adult life (4).

An autosomal dominant mode of inheritance for familial ASD has been described in a few families (5). The

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incidence of ASD in these families is often less than expected for single gene defects, and for this reason multifactorial model of inheritance has also been postulated (6).

Models of cardiac embryogenesis have suggested that this defect is caused by a malformation of the septum primum resulting in incomplete coverage of the ostium secundum (7).

Molecular signals regulating cardiac septation are largely unknown, although recent studies have shown that mutations within genes that encode for transcription factors are responsible for ASD. *NKX2–5* (8,9) is one of the known monogenic contributors to non-syndromic CHD along with *GATA4*, *TBX5*, *MYH6* and others genes (10–12).

Atrial septal defect with atrioventricular (AV) conduction defect (MIM #108900) is the most frequent phenotype of patients carrying mutations in the transcription factor NKX2–5.

NKX2–5 is essential in mammalian heart development (13) and occupies a crucial position in the hierarchy of cardiac determinant (14). Studies in animal models showed that *nkx2–5* deficiency in mice causes lethality due to impaired cardiac looping, thus indicating that NKX2–5 is essential not only in the morphogenesis of the heart but also in the physiological function of the cardiac conduction system (15,16).

The progressive nature of the electrophysiological abnormalities in individuals with normal heart structure or in patients following the spontaneous closure or surgical correction of ASD, in addition to the elevated incidence of sudden death in affected individuals without pacemakers, indicates that normal levels of NKX2–5 are essential for physiological AV node function during life, suggesting the importance of its role (4).

In the present study we report clinical and molecular investigation of ASD in a Tunisian consanguineous family.

Materials and Methods

Patients and ASD Diagnosis

We investigated a large consanguineous family originating from the South of Tunisia. Subjects were evaluated by history, review of medical records, physical examination, 12-lead electrocardiogram (ECG), and two-dimensional transthoracic echocardiography.

Genotyping and Mutation Screening

After obtaining informed consent, blood samples were collected from each family member, and genomic DNA was extracted from peripheral blood leucocytes by standard procedures. Genotyping analysis was performed as described in Charfeddine et al. (17). Two polymorphic microsatellite markers corresponding to the closest markers to the *NKX2–5* gene were selected. Microsatellite markers span a 0.5 cM interval according to the Genethon mapping panel and the genetic maps available on NCBI and Ensembl genome browsers (www.ncbi.nlm.nih.gov and www.ensembl.org).

Intronic oligonucleotide primers flanking the two *NKX2–5* coding exons were designed as previously described in Benson et al. (9) and Goldmuntz et al. (18). PCR conditions were carried out as previously described in Benson et al. (9), and mutation screening was performed by direct sequencing of the corresponding PCR products.

Genetic Analysis

Linkage analysis was performed using the computer program Genehunter v2.1 (19), assuming autosomal dominant inheritance, complete penetrance, a disease allele frequency of 1/100,000 and equal allele frequencies for the markers. Multipoint parametric LOD scores were calculated and the haplotypes of all the pedigree members checked by visual inspection and were confirmed using Genehunther. A LOD score ≤ -2 was considered significant against linkage.

Results

Clinical Report

Clinical and pedigree investigation allowed the identification of a familial form of ASD. Clinical records revealed that the ancestor (II–1) of family ASD-BEN died of heart failure at 60 years of age. Table 1 regroups the characteristics and clinical data of affected ASD-BEN family members.

Case III–1

A 66-year-old woman presented with dyspnea. A systolic murmur at the pulmonic area was noted. Electrocardiogram revealed atrial fibrillation, and echocardiogram (ECG) showed a wider secundum ASD with moderate pulmonary hypertension. She then underwent surgical repair.

Table 1. Characteristics and clinical data of affected family members ASD-BEN

Cases	Age (years)	Sex	Type of heart disease	Diagnosis confirmed	PR interval (sec)
III–1	70	F	SASD		
IV–1	47	F	SASD + CD	S	0.22
IV–5	38	M	CD	CL	0.22
IV–9	35	M	CD	CL	0.22
IV–10	36	M	VP	CL	<0.12
IV–11	33	F	VP	CL	<0.12
IV–13	33	F	VP	CL	<0.12
V–13	12	F	VP	CL	<0.12
V–15	10	F	VP	CL	<0.12
V–10	13	M	RVH + PS	CL	

S, surgery; CL, clinical diagnosis; SASD, secundum atrial septal defect; CD, conduction defect; RVH, right ventricular hypertrophy; PS, pulmonary stenosis; VP, ventricular preexcitation.