Reduced Penetrance, Variable Expressivity, and Genetic Heterogeneity of Familial Atrial Septal Defects

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Background—Secundum atrial septal defect (ASD) is a common congenital heart malformation that occurs as an isolated anomaly in 10% of individuals with congenital heart disease. Although some embryological pathways have been elucidated, the molecular etiologies of ASD are not fully understood. Most cases of ASD are isolated, but some individuals with ASD have a family history of this defect or other congenital heart malformations.

Methods and Results—Clinical evaluation of three families identified individuals with ASD in multiple generations. ASD was transmitted as an autosomal dominant trait in each family. ASD was the most common anomaly, but other heart defects occurred alone or in association with ASD in individuals from each kindred. Genome-wide linkage studies in one kindred localized a familial ASD disease gene to chromosome 5p (multipoint LOD score 3.6, \( \theta = 0.0 \)). Assessment of 20 family members with the disease haplotype revealed that 9 had ASD, 8 were clinically unaffected, and 3 had other cardiac defects (aortic stenosis, atrial septal aneurysm, and persistent left superior vena cava). Familial ASD did not map to chromosome 5p in two other families.

Conclusions—Familial ASD is a genetically heterogeneous disorder; one disease gene maps to chromosome 5p. Recognition of the heritable basis of familial ASD is complicated by low disease penetrance and variable expressivity. Identification of ASD or other congenital heart defects in more than one family member should prompt clinical evaluation of all relatives. (Circulation. 1998;97:2043-2048.)

Key Words: echocardiography ▪ genetics ▪ heart septal defects

Secundum atrial septal defect (ASD) is a common congenital heart malformation accounting for 10% of isolated congenital heart disease. Uncorrected ASD can cause pulmonary overcirculation, right heart volume overload, and premature death. Models of cardiac embryogenesis have suggested that this defect is caused by malformation of the septum primum, resulting in incomplete coverage of the ostium secundum (fossa ovalis). However, neither the cellular mechanisms nor molecular signals directing these processes are known.

Some individuals with secundum ASD have a family history of this defect or other congenital heart malformations, and coexisting heart block has been observed in some familial ASD kindreds. The genetic basis, if any, for these clinical observations remains unclear. Although an autosomal dominant mode of inheritance for familial ASD has been described in a few families, the incidence of ASD in siblings and offspring of affected individuals is often less than that expected for single gene defects, and multifactorial models of inheritance have also been postulated.

To identify the genetic basis for familial ASD, we clinically evaluated and genetically studied three kindreds with ASD inherited as an autosomal dominant trait. We report a disease locus for familial ASD on the telomeric region of chromosome 5p and demonstrate that this disorder is genetically heterogeneous. Disease penetrance was incomplete (the absence of a clinical phenotype in individuals bearing the gene mutation), and some genetically affected individuals exhibited other structural heart defects. We suggest that incomplete penetrance and variable expressivity of familial ASD may result in a significant underestimate of the heritable nature of this condition.

Methods

Clinical Evaluation

Informed consent was obtained from all participants in accordance with the Brigham and Women’s Hospital Committee of the Protection for Human Subjects From Research Risks. Family members in three unrelated kindreds were evaluated by history, review of medical records, physical examination, 12-lead ECG, and two-dimensional transthoracic echocardiography with color flow Doppler interrogation in four standard and subcostal views. Cardiac catheterization and transesophageal echocardiography had been performed in some individuals. ASD and other malformations were diagnosed by

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standard criteria; atrial septal aneurysms were diagnosed by previously established criteria. Clinical studies were performed without knowledge of genotype.

Genetic Analysis
Genomic DNA was isolated from peripheral lymphocytes as previously described. Polymorphic short tandem repeat sequences with heterozygosity >0.7 and four or more alleles were chosen from each chromosome at ~30-centimorgan (cM) intervals and amplified from genomic DNA by the polymerase chain reaction. In brief, 100 ng of genomic DNA was amplified in a volume of 10 μL containing 40 ng of unlabeled oligonucleotide primer, 40 ng of primer end-labeled with 3²P, 200 mmol/L each of dATP, dCTP, dGTP, and dTTP, and Taq polymerase. The samples were denatured for 2 minutes at 95°C, then processed through 35 cycles including denaturation at 95°C for 20 seconds, primer annealing at 58°C for 30 seconds, and primer extension at 72°C for 40 seconds. The amplified products were electrophoresed on 6% polyacrylamide sequencing gels and visualized by autoradiography.

Two-point linkage analyses were performed by use of MLINK (version 5.1) with allele frequencies determined from family members and a phenocopy rate of 0.001. Multipoint analysis was performed with LINKAGE. Genetic heterogeneity for familial ASD in the study kindreds was assessed by use of HOMOG program.

Results
Clinical Evaluation
Seventy individuals from four generations of family MAR were clinically evaluated. In nine individuals, a secundum ASD had been previously identified (Fig 1A and Table). Surgical closure of an ASD had been performed in 7 individuals; surgery occurred between ages 22 and 44 years in 6 individuals and at age 4 years in 1 individual. Secundum ASD was documented at autopsy in individual IV-12 (at age 34 years) and at cardiac catheterization in individual IV-10 (performed at age 63 years). Clinical evaluations in other family members demonstrated an atrial septal aneurysm in individuals V-1 and VI-7 (Fig 3) and a persistent left superior vena cava in individual V-1 (Fig 3). An echocardiographic diagnosis of moderate valvular aortic stenosis was made in individual IV-4; the etiology of aortic stenosis was limited by the extent of valve calcification and technical difficulties. However, given the relatively young age at diagnosis (previously diagnosed at age 54 years), it is most likely that this individual has a bicuspid aortic valve. The clinical status of individual III-1 before his death is unknown.

Eighteen members from three generations of family MBE were clinically evaluated. Six individuals had a secundum ASD (Fig 1B and Table). Diagnosis was made before the age of 5 years in 4 individuals and resulted in surgical closure of the ASD during the first decade of life. Individuals III-2 and IV-8 were diagnosed with an ASD in adulthood and underwent surgical closure at ages 40 and 32 years, respectively. Four individuals had other structural heart defects. A patent ductus arteriosus was diagnosed in individual IV-2 at 6 months (ligated at age 3.5 years) and in individual V-1 (ligated at age 5 years). Individual III-3 had a stenosed bicuspid aortic valve and underwent aortic valve replacement at age 56 years. A bicuspid aortic valve was diagnosed in his son (individual IV-3) at age 37 years.

Twenty-three members from four generations of family MXP were clinically evaluated. Eight individuals (Fig 1C and Table) were previously recognized to have secundum ASD and prolonged AV conduction. One individual (IV-7) had isolated second-degree AV block; transesophageal echocardiography demonstrated normal cardiac structures. Other structural heart abnormalities found in family members included subvalvular aortic stenosis (individual III-3), ventricular septal defect (individuals IV-10 and V-1), tetralogy of Fallot (individuals IV-8 and IV-12), and pulmonary atresia (individual IV-8). Affected family members in generations II and III were diagnosed after age 30 years; however, cardiac malformations in subsequent generations were recognized by age 4 years.

Genetic Analysis
In each family, pedigree evaluations suggested that an autosomal dominant trait caused inherited ASD and other structural heart defects. Only 9 secundum ASDs were identified in the MAR kindred, and disease penetrance appeared incomplete. For example, the incidence of ASD in the offspring of these 9 affected individuals was ~33% and was less than expected for a fully penetrant dominant trait. Furthermore, individual IV-2 provided a clear example of nonpenetrance. Although this woman is clinically unaffected, one son (V-4) had a secundum ASD (Fig 2) and another (V-1) has an atrial septal aneurysm and persistent left superior vena cava (Fig 3).

Linkage studies were performed in family MAR to define the chromosomal location of a mutation causing familial ASD. Because disease penetrance was recognized to be incomplete, initial linkage studies analyzed only the genotypes of individuals with an ASD or individuals whose offspring had an ASD. Accordingly, disease penetrance was set at 100%. A total of 125 polymorphic short tandem repeat sequences distributed across the genome were tested, and ~25% of the genome was eliminated before linkage was detected at DSS208. Linkages to nearby loci on the distal arm of chromosome 5p were then assessed. A maximum 2-point logarithm of the odds (LOD) score of 2.83 (θ=0.0) was detected at DSS406, and a maximum multipoint LOD score of 3.6 was obtained (Fig 4). The familial ASD locus on chromosome 5p was designated ASD1.

A disease haplotype in family MAR was constructed from the genotypes of individuals with ASD at 13 loci near ASD1 and compared with the genotypes of all family members (Fig 1A). The haplotypes of individuals III-1, IV-3, IV-10, and IV-12 were reconstructed from the alleles of offspring and spouses. Twenty-one individuals exhibited the disease haplotype, including all members of family MAR with structural heart disease (9 individuals with secundum ASD, 1 with atrial septal aneurysm, 1 with atrial septal aneurysm and persistent left superior vena cava, and 1 with valvular aortic stenosis). The disease haplotype was also found in 1 deceased individual (clinical status before death unknown) and 8 clinically unaffected individuals. Based on this disease haplotype, the penetrance of secundum ASD was 45%; the penetrance for secundum ASD or atrial septal aneurysm was 55%.

The disease interval defined by the haplotypes of individuals with secundum ASD spans an 11-cM region between DSS208 and DSS807 (Fig 4). However, because the disease haplotype was identified in two individuals (VI-7 and V-1)
with atrial septal aneurysms, we hypothesized that this pathological condition might represent a form fruste or spontaneous closure of an unrecognized ASD. The haplotype of individual V-1 exhibits a recombination event that refines the disease interval to a 4-cM region between $D5S635$ and $D5S807$. LOD scores were also calculated including individuals with atrial septal aneurysms as affected. This maximum 2-point LOD score was 2.83 ($\theta=0$) at $D5S208$ and the maximum multipoint LOD score 3.9, indicating odds of $\approx 8000:1$ that the disease gene in family MAR is genetically linked to loci on chromosome 5p.

To determine whether the disease gene in family MAR was also responsible for the heritable cardiovascular disorders in families MBE and MXP, linkage was assessed at the $ASD1$...

Figure 1. Pedigrees of kindreds with familial secundum atrial septal defect (ASD). Secundum ASD (solid symbols) was present in nine individuals from family MAR (A). Three individuals had other cardiac malformations (partially solid symbols). Individuals without structural heart defects (open symbols) or with unknown clinical status (shaded symbols) are shown. Genotypes for 13 polymorphic loci on chromosome 5 are provided. Haplotype segregating with ASD is enclosed in a box. Secundum ASD in family MBE (B) and family MXP (C) was also inherited as an autosomal dominant trait. Affection status is denoted as in A.
locus only in individuals with ASD or individuals whose offspring had an ASD. Two-point LOD scores achieved in family MBE and family MXP were less than \(-2.0\) across the interval between \(D5S2088\) and \(D5S630\) (Fig 4), indicating that the ASD gene in these families did not map to the \(ASD1\) locus. The HOMOG program provided further evidence of heterogeneity, because heart defects in families MBE and MXP are unlikely to be due to mutations in \(ASD1\) on chromosome 5p (\(P<.001\), data not shown).

**Discussion**

We demonstrate that familial ASD can be caused by a gene mutation on chromosome 5p. This disorder is genetically heterogeneous and can also be caused by defects in other undefined genes. In addition to secundum ASD, clinical manifestations of the \(ASD1\) gene include other frequently occurring cardiovascular malformations; atrial septal aneurysms, venous anomalies (persistent left superior vena cava), and aortic valve disease (bicuspid aortic valve) occur in \(\approx 0.5\%\) to \(1\%\) of the population and could have occurred by chance in some family members. However, the chance of three cardiac anomalies arising concurrently in three family members with the \(ASD1\) gene by chance alone is very small. We suggest that mutations in familial ASD genes cause a broad spectrum of hereditary congenital cardiovascular disorders.

Diversity of clinical phenotype combined with generational skips has led to the hypothesis that the etiology of congenital heart disease is multifactorial and due to interaction of complex (polygenic) traits and environmental factors. The three families studied here typify the difficulties of assessing the heritable nature of congenital heart disease. Although pedigree analyses (Fig 1) suggested that an autosomal dominant trait segregated in each family, this model also requires incomplete penetrance and variable expressivity to account for the clinical status of all family members. The disease haplotype (Fig 1A) defined by linkage studies of affected members in family MAR confirmed this model. Incomplete penetrance, inaccurate diagnosis, or age-dependent phenotype are assumed to account for eight clinically unaffected individuals who carried the disease haplotype.

Diagnostic techniques used in this study could have contributed to an underestimate of phenotype. For example, transesophageal echocardiography is recognized to be more sensitive and specific than transthoracic echocardiography in identifying abnormalities of the atrial septum, especially in large adults. Although transesophageal echocardiography may have increased the diagnostic yield for subtle malforma-

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**Clinical Characteristics of Familial ASD**

<table>
<thead>
<tr>
<th>Family</th>
<th>MAR</th>
<th>MBE</th>
<th>MXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, y (mean)</td>
<td>2-63 (30)</td>
<td>0.3-55 (15)</td>
<td>0.0-59 (15)</td>
</tr>
<tr>
<td>No. affected</td>
<td>12</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>ASD</td>
<td>9</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>1, ASA</td>
<td>2, PDA</td>
<td>1, 2° AV block</td>
</tr>
<tr>
<td></td>
<td>1, ASA+LSVC</td>
<td>1, Bicuspid AV</td>
<td>1, ASD+sub AS</td>
</tr>
<tr>
<td></td>
<td>1, AS</td>
<td>1, AS/bicuspid AV</td>
<td>1, ASD+VSD</td>
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<td></td>
<td></td>
<td></td>
<td>1, VSD</td>
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<td></td>
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<td></td>
<td>1, TOF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1, TOF+PA</td>
</tr>
</tbody>
</table>

ASD indicates atrial septal defect; ASA, atrial septal aneurysm; LSVC, left superior vena cava; PDA, patent ductus arteriosus; bicuspid AV, bicuspid aortic valve; AS, aortic stenosis; 2° AV block, second-degree atrioventricular block; sub AS, subvalvular aortic stenosis; VSD, ventricular septal defect; TOF, tetralogy of Fallot; and TOF+PA, TOF with pulmonary atresia.

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**Figure 2.** Transesophageal echocardiogram in individual V-4 (family MAR). Two-dimensional echocardiographic images demonstrated secundum atrial septal defect (ASD) (A) with left atrial to right atrial blood flow on color Doppler interrogation (B). Ao indicates aortic root; LA, left atrium; and RA, right atrium.
tions such as atrial septal aneurysm, it is unlikely to have changed the principal findings of this study. Alternatively, the clinical status of individuals carrying mutations at ASD1 may change. For example, because ASD is one of several congenital malformations that can “spontaneously” resolve,8,16 evaluations performed after spontaneous closure would inaccurately assign an unaffected status and thereby contribute to the impression of reduced gene penetrance. The basis for reduced penetrance has not been established, but this phenomenon is associated with other congenital cardiac malformations.17,18

Genetic studies in family MAR further indicated that the ASD1 gene mutations accounted for several other cardiovascular malformations present in family members. Although secundum ASD was the most common cardiac malformation in family MAR and two other study kindreds, patent ductus arteriosus, ventricular septal defect, atrial septal aneurysm, left superior vena cava, tetralogy of Fallot, bicuspid aortic valve, valvular or subvalvular aortic stenosis, and AV conduction abnormalities were also observed. These families are representative of other kindreds with familial ASD19–24 in which as many as 40% of individuals had additional or other cardiac anomalies. The identification of a common haplotype in family MAR individuals with distinct congenital heart defects may indicate that variable expressivity of a single gene defect can account for clinical diversity of congenital heart disease in a family. Diverse cardiac malformations are recognized in other monogenic human disorders, including Holt-Oram syndrome25–28 and chromosome 22q11 microdeletions,29 and also occur in retinoic X receptor–deficient mice.30 Although variable expressivity is not a feature predicted by classic embryological models of cardiac development, heritable monogenic mutations can clearly cause pleiomorphic cardiovascular defects.

Although ASD causes no obvious deleterious effect during fetal development, the consequences of these defects during postnatal life are variable.2 Uncorrected ASD can lead to symptoms due to pulmonary overcirculation and right heart volume overload. However, spontaneous closure in the first few years of life has been well documented, with no apparent long-term sequelae.8,16 The association of atrial septal aneurysm and secundum ASD has led to the suggestion that aneurysms are a congenital malformation of the septum3 that may play a role in postnatal ASD closure.3 Genetic studies in family MAR support this cause-and-effect relationship in that a disease haplotype identified in individuals with secundum ASD was also found in two individuals (V-1, V-7) with atrial septal aneurysm. Serial studies of these individuals may help to define postnatal changes in the atrial septum.

Figure 3. Transesophageal echocardiography in individual V-1 (family MAR) demonstrated atrial septal aneurysm (A). B, Injection of saline into left brachial vein identified flow into coronary sinus (CS) and right atrium (RA), indicating persistent left superior vena cava (LSVC) (diagrammed in C). LA indicates left atrium; RV, right ventricle.

Figure 4. Multipoint LOD score in family MAR defines ASD1 locus between D5S2088 and D5S807 (top). Cardiac defects in family MBE or family MXP are not caused by mutations at this locus (LOD scores < –2.0). Haplotype analysis of individuals with secundum ASD (V-4, III-3) and atrial septal aneurysm (V-1) indicate recombination events (solid box) that refine the ASD1 locus to a 4-cM interval between D5S635 and D5S807.
Embryological models of secundum ASD have emphasized abnormal development of the septum primum, but little is known of the molecular basis of atrial septation. No obvious candidate genes have been mapped to ASD1, but it is noteworthy that large deletions of chromosome 5p cause cri-du-chat syndrome, a contiguous-gene syndrome occasionally associated with congenital heart disease.11 Definition of the ASD1 gene may also help to elucidate the molecular basis for cardiac malformations in this syndrome. Identification of this and other familial ASD genes should provide new insights into the important steps in cardiac morphogenesis leading to atrial septation.

The contribution of familial ASD gene defects to the incidence of congenital heart disease remains unknown. Given the variable expression and low penetrance of the ASD1 gene mutations described here, an individual bearing one of these gene defects might present as an isolated case. When family history reveals a congenital heart disorder in more than one individual, clinical and genetic evaluations of all family members are recommended.

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