A novel mutation in *GATA4* gene associated with dominant inherited familial atrial septal defect

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Objective: Atrial septal defect (ASD) is a common congenital heart disease (CHD). Although most cases are sporadic, familial cases have been reported. The transcription factors NKX2.5 and GATA4 play important roles in the pathogenesis of ASD. Mutations in either gene have been identified in familial cases of ASD. Here, we examine a Chinese family with isolated ASD to find out whether there is any mutation in NKX2.5 or GATA4 accounting for the etiology.

Methods: We identified kindred spanning 3 generations in which 8 of 31 (38%) individuals had ASD. One hundred seventy unrelated individuals were included as controls. Peripheral blood samples were collected and genomic DNA was extracted from the leukocytes. NKX2.5 and GATA4 were amplified by polymerase chain reaction (PCR) with specific primers. The sequences of PCR products were compared between affected members and unaffected members, as well as controls.

Results: Direct sequencing of NKX2.5 from the genomic DNA of family members failed to identify mutations, whereas sequencing of GATA4 identified an A-to-G transition at nucleotide 928 in exon 5 that predicted a methionine to valine substitution at codon 310 (M310V) in the NLS region. All affected members and a patriarch of the family who was recognized as a carrier exhibited this mutation, whereas the other unaffected family members or control individuals did not. This mutation has not been reported previously in either familial or sporadic cases of CHD.

Conclusions: We identified a novel M310V mutation in GATA4 gene that is located in the NLS region and leads to hereditary ASD in a Chinese family. In this family, we identified a carrier with incomplete penetrance and 8 patients with variable expressivity. However, the mechanism by which this mutation contributes to the development of a congenital heart defect remains to be ascertained. (J Thorac Cardiovasc Surg 2010;140:684-7)

Atrial septal defect is a common congenital heart disease, and although most cases are sporadic, familial cases have been reported. The transcription factors NKX2.5 and GATA4 play important roles in the pathogenesis of atrial septal defect. Mutations in the *NKX2.5* gene have been identified in familial cases of atrial septal defect with atrioventricular conduction abnormalities, and mutations in *GATA4* have been found in familial cases of isolated atrial septal defect. Here, we present 8 cases from a large Chinese family with 3 generations of isolated atrial septal defect. Disease transmission in this family was in accordance with an auto-

somal dominant mode of inheritance. A novel heterozygous M310V missense mutation in the nuclear localization signal region of exon 5 of *GATA4* was detected in all investigated patients with atrial septal defect in this family. The mutation was also found in a patriarch of the family who was recognized as a carrier, but it was not seen in other unaffected family members or any control individuals.

Atrial septal defect (ASD) is a common form of congenital heart disease (CHD), and the prevalence of familial ASD is significantly higher than that of other familial CHDs. Recently, both the genetic basis of familial ASD and the role of transcription factors as mediators of cardiac morphogenesis have been investigated.¹⁻³ Genetic factors have been found to play a predominant role in the pathogenesis of familial ASD, and heterozygous mutations in 2 transcription factor genes, *NKX2.5* (or *CSX*) and *GATA4*, have been identified as causative factors of familial ASD.⁴⁻⁶ Mutations in *NKX2.5* have been detected in families with inherited autosomal dominant ASD (secundum type) with atrioventricular conduction block,⁶⁻⁸ whereas mutations in *GATA4* are more likely to be associated with cardiac malformations than with disturbances in the conduction system.

GATA4, which encodes a zinc-finger transcription factor, is essential for endodermal differentiation and ventral

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Abbreviations and Acronyms	
ASD	= atrial septal defect
CHD	= congenital heart disease
CZf	= C-terminal zinc finger
NLS	= nuclear localization signal
NZf	= N-terminal zinc finger

morphogenesis.⁹⁻¹² The GATA4 protein binds the GATA motif consensus sequence to modulate the transcription of target genes.¹³ *GATA4* has 442 amino acids with two trans-activation domains; two zinc fingers, one at the N-terminus (NZf) and the other at the C-terminus (CZf); and a nuclear localization signal (NLS), for import into the nucleus. The CZf and the adjacent basic domain of GATA4 have dual functions in both DNA binding and nuclear localization.¹⁴ The amino acids 270–324 fragment within the DNA-binding domain at amino acids 216–324 has been identified as the minimal, functional NLS region for actively transporting GATA4 into the nucleus of both HeLa cells and cardiac myocytes.¹⁵

We identified a large Chinese family with familial ASD after performing surgical repairs on a mother and 2 daughters who had the same congenital ASD. To identify the genetic basis for the familial ASD in this family, we conducted a clinical and genetic analysis, diagnosing a total of 8 family members with congenital ASD (secundum type). A novel missense mutation of *GATA4* was found in the NLS region. A patriarch with this mutation did not have ASD but was found to be a carrier. The 8 pedigree members with complete penetrance strongly suggest that, in this family, ASD may be caused by a single-gene defect and dominantly inherited.

PATIENTS AND METHODS

Study Population

We identified a large kindred spanning 3 generations, in which 8 individuals had ASD (secundum type) (II-3, II-4, II-6, II-7, III-3, III-4, III-5, III-7;

TABLE 1. CHD types and need for surgical repair

Patient ID	CHD	Surgery required
II-3	ASD	+
II-4	ASD	+
II-6	ASD+PS	+
II-7	ASD	–
III-3	ASD+PS	+
III-4	ASD+PS	+
III-5	ASD	+
III-7	ASD	–

CHD, Congenital heart disease; ASD, atrial septal defect; PS, pulmonary stenosis. II-3, III-3, and III-4 underwent surgery previously.

Figure 1); the incidence of ASD in the members surveyed was 38% (8/21). Clinical manifestations were varied, with 6 patients (II-3, II-4, II-6, III-3, III-4, III-5) requiring surgical repair and 3 (II-6, III-3, III-4) who had pulmonary stenosis in addition to ASD (Table 1). In all cases, the diagnosis was made by a cardiologist and a cardiac surgeon on the basis of medical and surgical histories, physical signs, 12-lead electrocardiograms, and color Doppler echocardiograms. The patent foramen ovale was excluded. The cardiac conduction system and the other organs were unaffected in the patients with ASD, suggesting that CHD in this family was isolated, without syndromic disease. As controls, we included 170 unrelated individuals, 100 of whom were healthy and 70 of whom had various forms of sporadic CHDs, including 20 cases of ASD. Informed consent was obtained from each participant, and the research protocols and procedures were approved by the Institutional Research Ethics Committee of Peking University People’s Hospital. Peripheral blood samples were collected and DNA was extracted from the leukocytes.

Genetic Analysis

Exons 2 to 7 of *GATA4* (accession No. NM_002052) and exons 1 to 2 of *NKX2.5* (NM_004387) were amplified by polymerase chain reaction with specific primers, as described previously.^{5,16} Genomic DNA (50 ng) was amplified in a 50-μL reaction. The touchdown polymerase chain reaction program for *GATA4* was 5 minutes at 94°C, 35 cycles of 30 seconds at 94°C, 30 seconds at 58°C to 53°C (touchdown temperature of 53°C), 1 minute at 72°C, and 5 minutes at 72°C. Amplification of *NKX2.5* was 5 minutes at 94°C, 35 cycles of 45 seconds at 94°C, 30 seconds at 63°C to 58°C (touchdown temperature of 58°C), 45 seconds at 72°C, and 5 minutes at 72°C. Products were run on 1% agarose gels to verify amplification, and purified products were sequenced in the sense direction with an ABI 3730XL DNA Analyzer (Applied Biosystems, Inc, Foster City, Calif) by SinoGenoMax Co (Beijing, China).

FIGURE 1. Pedigree of a large Chinese family with heart defects. Participating members of each generation are indicated numerically. Secundum atrial septal defect (ASD) was observed in 8 members and pulmonary stenosis (PS) in 3.

FIGURE 2. All members with the heart defects as well as the individual I-I had a *GATA4* mutation, an A-to-G transition at nucleotide 928 in exon 5 of *GATA4*. Other unaffected family members with wild type alleles of *GATA4* (top) and an unaffected member heterozygous for a mutated allele (bottom) are shown. Wild type (WT) and mutated (MU) amino acid sequences of *GATA4* protein. The A928G transition creates a methionine to valine substitution at codone 310.

RESULTS

Direct sequencing of *NKX2.5* from the genomic DNA of family members failed to identify mutations, ruling out *NKX2.5* as the genetic basis of ASD in this family. Direct sequencing of *GATA4* identified an A-to-G transition at nucleotide 928 in exon 5 that predicted a methionine to valine substitution at codon 310 (M310V) in the NLS region (Figure 2). To the best of our knowledge, this mutation has not been reported previously in either familial or sporadic cases of CHD. In fact, reports of mutations in the *GATA4* NLS region are rare. We therefore believe we have identified a novel, CHD-associated mutation. All affected members, including the family patriarch, exhibited the M310V mutation, suggesting high penetrance of the disease phenotype. Absence of a clinical phenotype in the patriarch with the mutation showed this disease has incomplete penetrance. This mutation was not found in the other unaffected family members or in the 170 control individuals.

DISCUSSION

To date, only four mutations in *GATA4* have been reported to be associated with isolated ASD in unrelated families.^{4,5,17} These mutations include one between the

CZf and the NLS (G296S), one missense mutation (S52F) in transactivation domain 1, and two frameshift mutations, 1074 (delC) and 1075 (delG), in the C-terminal region. Garg and associates⁴ reported that the G296S mutation disrupts the DNA-binding and transactivation activity of *GATA4* and destroys the synergy in transcriptional activation between *GATA4* and its cofactor *TBX5*, resulting in heart anomalies such as pulmonary stenosis, atrioventricular septal defect, aortic regurgitation, and patent ductus arteriosus, in addition to ASD.

Here, we present the first report of an M310V mutation in the NLS region, which is necessary and sufficient for the nuclear localization that is required for *GATA4* transcription factor activity (Figure 3). This mutation affected a highly conserved residue and was not detected in any of 170 unrelated control individuals. Mutations in *GATA4* that change the NLS might lead to reduced transcriptional activity by altering the structure of the protein in a way that impairs import into the nucleus, thereby affecting transcriptional activation. M310V mutation is also immediately adjacent to the CZf, which is essential for DNA-binding and interaction with cofactors.¹⁴ Therefore, it may also disrupt the interaction between *GATA4* and *TBX5* or *NKX2.5*, which is

FIGURE 3. Conserved domains of *GATA4* protein indicate transactivation domains (TAD), N-terminal zinc finger (NZf), C-terminal zinc finger (CZf), and nuclear localization signal (NLS). The location of M310V is shown.

important in cardiogenesis, resulting in the observed pulmonary stenosis seen in 3 affected members of this family.

Although pedigree analysis (Figure 1) showed an autosomal dominant trait with high penetrance in this family, incomplete penetrance and variable expressivity accounted for the diversity in clinical status in this family, whose members varied in the presence of a clinical phenotype, surgical requirements, and the presence of other defects.¹⁸ Individual I-2 was clinically unaffected, even though he had the M310V mutation. We explain his case with several assumptions. First, incomplete penetrance is a common phenomenon in autosomal dominant phenotypes. Heterozygotes with functional haploinsufficiency who bear the gene mutation can be free of clinical phenotype, leading to observations of atavism in these families. Second, the diagnostic techniques used in this study could have contributed to an underestimation of the phenotype, especially in family members who did not undergo surgery. Transthoracic echocardiography is the only available objective diagnostic modality, but it is thought to have lower sensitivity in identifying cardiac abnormalities than do transesophageal echocardiography or magnetic resonance imaging^{19,20}; however, it is unlikely to have changed the principal findings of this study. Finally, ASD is one of several congenital malformations that can resolve spontaneously,^{21,22} so that evaluations performed after this event will show a reduced gene penetrance. Therefore, in the absence of information on individual I-2's predecessors, we suggest that incomplete penetrance, inaccurate diagnosis, and age-dependent phenotype explain his clinical status. We plan to continue monitoring the members of this family, including evaluation of additional siblings and offspring, particularly those of affected individuals, to determine whether the ASD in this family is caused by a single-gene defect.

In conclusion, we identified a novel M310V mutation in GATA4 that is located in the NLS region and leads to hereditary ASD in a Chinese family. In this family, we identified a carrier with incomplete penetrance and 8 patients with variable expressivity. However, the mechanism by which this mutation contributes to the development of a congenital heart defect remains to be ascertained.

With the development of gene therapy and genomic strategies for cardiovascular surgery, further investigation of GATA4 mutations in humans with familial congenital heart defects will provide new avenues for the prevention and therapy of inherited cardiovascular diseases.²³

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