The most frequent c.239A>G SNP of *NKX2.5* is not involved in Congenital Heart Disease

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Abstract: Congenital heart disease (CHD) is the most developmental errors in humans, affecting 8 out of 1000 newborns. Cardiac septal defects (CSD) constitute the majority of CHD, however, the etiology of CSD remains unclear. About 85 single nucleotide polymorphisms (SNPs) of *NKX2.5* have been associated with various forms of CHD. A total of 150 CHD patients and 70 controls were screened for *NKX2.5* SNPs by PCR amplification and direct sequencing, as well as Mass array techniques. The sequence analysis was done by using Accelerys gene software. In this study, six SNPs, c.239A>G (48.66%), c.608A>G (0.6%), c.646C>T (25.33%), c.852G>A (3%), c.896C>A (0.6%) and 1212G>T (40%) were identified, of which c.239A>G was the most frequent in both cases and controls. The SNP c.239A>G (rs2277923), leads into synonymous change of glutamine, in turn, the effect is neutral, thereby its frequency is almost equal in both controls and cases. Therefore, this SNP is not involved in the manifestation of CHD. [New York Science Journal 2010;3(8):43-47]. (ISSN: 1554-0200).

Keywords: *NKX2.5*; Congenital Heart Disease, Mass array, Direct sequencing, Accelerys gene software.

1. Introduction

Congenital heart disease (CHD) is the most common developmental errors in humans, affecting 8 out of 1,000 newborns (Posch et al., 2008). Cardiac septal defects (CSDs) constitute the majority of CHD, however, the etiology of CSD remains unclear. The available data suggest that fetal heart development is regulated by a group of highly conserved transcription factors, including NKX2.5, TBX5, GATA4, TBX1, ZIC3, TFAP2B, FOG2, and others (Ramegowda and Ramachandra, 2005; Clark et al., 2006; Chen et al., 2010). *NKX2.5*, a vertebrate homologue of *Drosophila* tinman, is a homeobox transcription factor highly expressed in early progenitors and in adult cardiomyocytes (Posch et al., 2008). Targeted disruption of *NKX2.5* in rodent models results in early embryonic lethality due to failed cardiac looping and defects in chamber formation (Lyons et al., 1995; Posch et al., 2008).

NKX2.5 was the first gene identified as a relevant disease gene for non-syndromic human CHD (Posch et al., 2008). Schott et al., (1998) described heterozygote mutations in *NKX2.5* that resulted in atrioventricular (AV) conduction block; many genotype-positive individuals also had a secundum atrial septal defect (ASD). Subsequent family studies identified extensive variable expressivity, in addition to AV block and ASD, genotype positive individuals also had ventricular septal defect (VSD), tetralogy of fallot (TOF), double outlet right ventricle (DORV), and tricuspid valve abnormalities, including Ebstein anomaly (Benson, 2010). These results suggest an essential role for *NKX2.5* in atrial, ventricular, and conotruncal septation, AV valve formation, and maintenance of AV conduction. These findings have been confirmed by numerous other studies (Benson, 2009).

About 85 Single nucleotide polymorphisms (SNPs) of *NKX2.5* have been associated with various forms of CHD (Schott et al., 1998; Benson et al., 1999; Hosoda et al., 1999; Kasahara et al., 2000; Goldmuntz et al., 2001; Ikeda et al., 2002; Watanabe et al., 2002; Elliot et al., 2003; McElhinney et al., 2003; Kasahara et al., 2004; Hirayama-Yamada et al., 2005; Sarkozy et al., 2005; Gutierrez-Roelens et al., 2006; Konig et al., 2006; Pabst et al., 2008). *NKX2.5* sequence variants have also been found in sporadic CHD, although these variants' contribution to the disease phenotype remains uncertain (Zhang et al., 2009). Here we made an attempt to investigate the role of c.239A>G SNP of *NKX2.5* in the manifestation of CHD.

2. Materials and Methods

A total of 150 CHD patients at K.R Hospital and J.S.S Hospital, Mysore (South India) were diagnosed by their past histories, physical examination and echocardiograms by experts. This study includes, 56 patients with VSD, 21 with ASD, 10 with ASD-VSD, 4 with TOF and 8 patients with Patent Ductus Arteriosus (PDA) and remaining with specific CHD phenotypes and complex forms. Informed consent was obtained from their parents or guardians. 70

individuals with the same ethnic background as well as no clinical history of CHD were used as controls. The research protocols and procedures were approved by the research ethics committee of both Hospitals and the ethics committee of University of Mysore.

EDTA tubes were used for venous blood collection. Genomic DNA was extracted from peripheral leukocytes by using Wizard genomic DNA purification kit (Promega). Both the exons of human *NKX2.5* (Gene Bank Accession Number: NM004387) were amplified through polymerase chain reaction (PCR) using exon specific primers (Benson et al., 1999). The PCR was performed in a 20µl reaction mixture containing 10x assay buffur, 60ng of genomic DNA, 0.2mM of each dNTP, 0.5µM of each primer and 1U taq DNA polymerase. The PCR condition was as follows: 95°C of initial denaturation for 3 minutes followed by 95°C for 30 seconds, annealing temperature of 68–72°C for 30 seconds, and extension at 72°C for 45 seconds for 35 cycles. A final extension was conducted at 72°C for 5 minutes. Further, the amplified products were purified using the Bangalore genei PCR purification kit (Cat # 117310) and sequenced for both the forward and reverse strands. The sequence analysis was done by using Accelerys gene software. The SNPs identified through these investigations in patients and controls were reconfirmed through resequencing. Mass array analysis was carried out by using Sequenom-iPLEXR Gold SNP genotyping platform with Spectro CHIPR and MALDI-TOF Mass spectrometer (Sladek et al., 2007; Wilke et al., 2009).

3. Results

Figure 1 presents the organization of *NKX2.5*, which is 3.125 Kb with two exons that code for 324 amino acid residues having transcript length of 1.580 bp. It has homeodomain (HD), TN domain and NK2 specific domain (NK2-SD). Of the 85 CHD specific SNPs reported till date, only six SNPs, c.239A>G (48.66%), c.608A>G (0.6%), c.646C>T (25.33%), c.852G>A (3%), c.896C>A (0.6%) and 1212G>T (40%) were identified in the present study. Among these, c.239 A>G is the most frequent (48.66%) SNP observed in the present study.

The SNP c.239A>G was observed in 73 cases out of 150 patients with CHD. Of which, 14 patients with cardiac septal defects (10 ASD, 27 VSD, 5 ASD with VSD) and 8 with PDA. In addition, this SNP was also observed in 36 out of 70 controls.

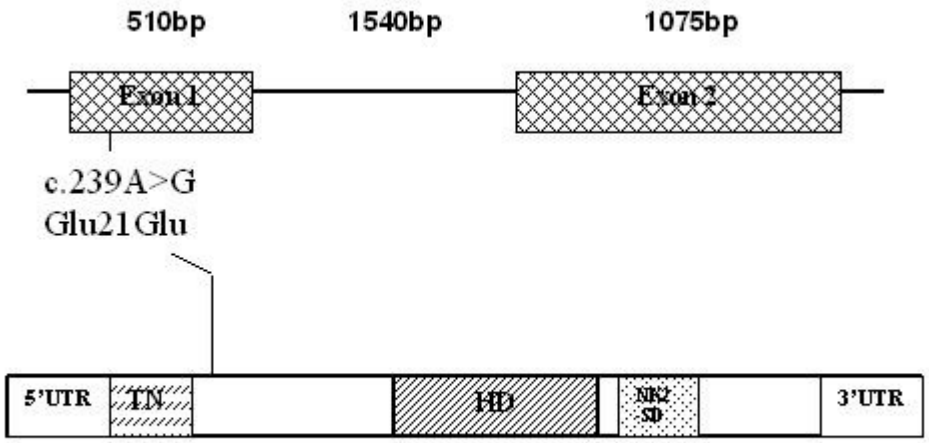

Fig.1 NKX2.5 gene showing the most frequent SNP c.239A>G.

4. Discussion

Mutations in *NKX2.5* cause a spectrum of congenital heart defects, including ASD, VSD, and cardiac conduction abnormalities (Srivastava et al., 2000; Posch et al., 2008; Benson, 2010). Of the 85 SNPs, most of them found only in CHD cases and not in the control samples (Benson et al., 1999; Goldmuntz et al., 2001; McElhinney et al., 2003; Reamon-Buettner et al., 2004; Hirayama-Yamada et al., 2005; Bjornstad et al., 2009). Perusal of the literature revealed that the c.239A>G SNP of *NKX2.5* is more frequent in both CHD and control samples (Table 1). However, in some cases, it was seen only in controls (Benson et al., 1999) and in some cases, it was observed in some CHD cases (Gutierrez-Roelens et al., 2002). The present study revealed that this SNP was also seen both in CHD cases and controls, which is a synonymous SNP coding for glutamine. Taking together of all these studies into consideration, the frequency of occurrence of this SNP in both CHD

cases (42.48%) and controls (41.36%) is almost same. The inconsistent reports of this SNP in some studies could be the error of sample size. One of the possibilities of occurrence of this SNP in more frequency in both CHD patients and controls could be due to its neutral effect, wherein although changes seen at nucleotide level, at the amino acid level the change is synonymous and no effect in the coding property of glutamine. Therefore, this SNP become more common in the population and not under the selection pressure.

Table1. The available data on c.239A>G SNP of *NKX2.5*, screened in the congenital heart disease patients and controls.

Frequency of c.239A>G SNP		
CHD Cases	Controls	References
-	18/52 (36%)	Benson et al., 1999
0/49 (0%)	53/100 (53%)	Gutierrez-Roelens et al., 2002
55/68 (80%)	17/45 (38%)	Reamon-Buettner et al., 2004
5/12 (41.6%)	-	Khetyar et al., 2008
97/205 (47.3%)	-	Posch et al., 2008
17/28 (60.71%)	17/28 (60.71%)	Draus Jr et al., 2009
107/230 (46.5%)	98/200 (49%)	Zhang et al., 2009
12/99 (12.1%)	3/90 (3.3%)	Ding et al., 2009
11/168(6.5%)	-	Liu et al., 2009
140/208 (67.3%)	-	Hamanoue et al., 2009
73/150 (48.6%)	36/70 (51.4%)	The Present study, 2010
517/1217 (42.48%)	**242/585 (41.36%)**	

Thus, this SNP is seen in almost equal frequency in both CHD cases and controls as well as only in controls, one can state that this SNP is not involved in causing CHD.

Conclusion

Since the change of SNP c.239A>G of *NKX2.5* is synonymous, the effect is neutral, thereby its frequency is almost equal in both controls and cases. Therefore, this SNP is not involved in the manifestation of CHD.

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