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Investigation of *NKX2.5* Gene Mutations in Congenital Heart Defects in an Indian Population

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Background and Aim: Mutations in the *NKX2.5* gene, a cardiac transcription factor, have been implicated in various types of congenital heart defects (CHD) and it is known that optimal expression levels of this gene are crucial for proper cardiogenesis. However, most of the mutations have been identified in cases of syndromic CHD, and the functional significance of other mutations in this gene has not been studied. We describe in this study the mutational and expression analysis of the *NKX2.5* gene in nonsyndromic CHD patients. *Methods:* In this study, exon 1 of the *NKX2.5* gene was sequenced from 50 probands with sporadic CHD and 50 healthy volunteers. *NKX2.5* gene expression levels in blood and cardiac tissue samples were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) in the probands. *Results:* No new mutations were identified; however, a previously reported variant A63G (rs2277923) was found to be present at significantly higher levels in the CHD population than in the control group. Changes in expression between the blood and tissue samples were seen in 37 out of the 50 CHD patients. *Conclusion:* Multiple factors, in addition to *NKX2.5* gene mutations, may cause CHDs. *NKX2.5* gene mutations may be mosaic in nature, therefore warranting investigation in both blood and tissue samples.

Introduction

CONGENITAL HEART DEFECTS (CHDs) are the most commonly reported type of birth defects with an incidence of 2.25–5.2 per 1,000 live births in the Indian population (Saxena, 2005). Etiological analysis for these defects has proven a definite involvement of both genetic and environmental factors (Wren *et al.*, 2003; Kopf and Walker, 2009; Zhu *et al.*, 2009). Chromosomal disorders such as aneuploidies (Pont *et al.*, 2006; Bondy, 2008) and structural defects are common attributes of congenital heart disease. However, the vast majority of CHDs are isolated or nonsyndromic in nature and occur sporadically. Monogenic alterations (Ware *et al.*, 2004; Lenhart *et al.*, 2013) have been identified in accordance with these isolated CHDs. Of the various genes implicated, mutations in cardiac transcription factors like *NKX2.5* (Goldmuntz *et al.*, 2001; Reamon-Buettner *et al.*, 2004) and *GATA-4* (Sarkozy *et al.*, 2005; Tomita-Mitchell *et al.*, 2007) have been identified in both familial and sporadic CHD cases.

The *NKX2.5* gene is the master regulator of the process of cardiogenesis. It belongs to the NK2 family of homeobox genes and is a homolog of the *Tinman* found in *Drosophila melanogaster*. The gene maps to chromosome 5q34 and is made up of two exons with the homeodomain region being present on exon 2. Two other domains, the transactivation and NK-2-specific domains, are highly conserved and have autoregulatory functions (Inga *et al.*, 2005).

A wide range of mutations, including both synonymous and nonsynonymous, have been identified in the *NKX2.5* gene. Till date, over 40 heterozygous mutations (McElhinney *et al.*, 2003; Reamon-Buettner and Borlak, 2004) have been identified in the *NKX2.5* gene, resulting in various CHD phenotypes. In addition, a few single-nucleotide polymorphisms (SNPs) have been reported in exon 1, which appear to be specific to Asian populations with CHD and not seen frequently elsewhere (Dinesh *et al.*, 2010).

Previous studies have revealed that *NKX2.5* gene mutations may be mosaic in nature, meaning that they are confined to the affected tissue and, hence, not detected in a simple peripheral blood analysis (Reamon-Buettner and Borlak, 2004). During cardiovascular development, optimal *NKX2.5* gene expression is an important requisite (Pabst *et al.*, 2008), owing to the dosage interdependence between cardiac transcription factors, and hence, the proper development of cardiac structures. In a recent study by Sheng *et al.*, mRNA levels of the *NKX2-5* gene were significantly lower in Tetralogy of Fallot (TOF) patients compared to controls (Sheng *et al.*, 2013). Hence, the aim of this study was to analyze for the presence of sequence variants in the exon 1 region of the *NKX2.5* gene in the peripheral blood samples of 50 CHD patients. We also investigated the variations seen in gene

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