

## **Mutation Analysis of Nkx2.6 Gene in a Cohort of Patients with Conotruncal Defects**

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Conotruncal defects are a subcategory of congenital heart defects, involving the truncus arteriosus and its development into the definitive aorta and pulmonary trunk. A variety of congenital heart defects are considered to be conotruncal defects, including tetralogy of Fallot, persistent truncus arteriosus and double outlet right ventricle. Recent literature suggests a mutation in the homeobox region of the Nkx2.6 gene causes persistent truncus arteriosus, a conotruncal defect. To determine whether NKX2.6 mutations might cause other types of conotruncal defects, we conducted mutation analysis on 20 probands with conotruncal defects. Genomic DNA was isolated from blood lymphocytes or fibroblasts of study participants, and the polymerase chain reaction (PCR) was used to amplify the coding region and flanking intronic sequence of NKX2.6. Sequencing reactions were performed in the presence of fluorescence-labeled dideoxynucleotides and additional primer for exon specific sequencing in both sense and antisense direction on isolated PCR product. Sequence results were analyzed with SeqMan software and the Ensembl website.

We found 8 notable alterations, including 3 non-synonymous SNPs. Of these, R21L was 1 aa downstream from the TN domain, A129E was 4 aa upstream from the homeobox region, and L138P was within the homeobox. These results, along with the Heathcote 2005 paper and other ongoing work in the Benson Lab highlights the importance of NKX2.6 in conotruncal defects.