A STUDY TO ESTIMATE THE PREVALENCE, RISK FACTORS AND NKX2-5 GENE CORRELATION OF CONGENITAL HEART DISEASES IN KANPUR AND PERIPHERY

INTRODUCTION
The term Congenital is derived from the Latin Word (‘con’ – means together and ‘genitus’ means born) referring to ‘present at birth’. Congenital Heart Diseases (CHD) are defined as an abnormality in ‘Cardiocirculatory’ structure or function that is present since birth, even though it discovered later\(^1\).

Congenital Heart Diseases (CHD) are structural anomalies of the heart arising from abnormal formation of the heart or major blood vessels. At least 18 distinct types of congenital heart defects are recognized, with many additional anatomic variations. These structural defects are the result of abnormal embryonic heart development and can be anatomically classified into abnormalities of the septa, the heart valves, and inflow or outflow tract of the heart. These structural defects most often have functional consequences, but of varying degree and significance. Some structural defects do not have important functional significance in early life such as the aortic valve with two leaflets, the prolapsing mitral valve, a small persistent patency of the arterial duct, small septal defects and patency of the oval foramen.\(^2\)

The common defects are classified according to: a) side of the affected heart, b) communication or short circuit between both hearts chambers and c) Presence or absence of cyanosis\(^7\). According to the Merck manual of Diagnosis the first eight CHDs are the common lesions, which account for 85% of all cases and the remaining (15%) account for variety of rare and complex CHDs\(^17\).

1) Atrial Septal Defect (ASD): A septal defect is a hole in different part of the atrial septum which lets some amount of blood from the left atrium to right atrium instead of flowing into left ventricle. The frequency of ASD in national level is 26.6\(^3\)

2) Ventricular Septal Defect (VSD): A septal defect is a hole, existing between the lower chambers of the heart. Oxygen rich blood from the lungs is pumped into the aorta from the left ventricle. During this process with VSD some amount of blood is passed into the right ventricle and into the pulmonary artery back to the lungs. It is classified into 2 main types according to their location relative to the components of the septum. The common types are perimembranous and trabecular. The frequency of VSD in national level is 30.3\(^3\)
(3) **Patent Ductus Arteriosus (PDA):** Ductus arteriosus, the temporary duct connecting the left pulmonary artery to the aorta in the fetal heart, fails to close after birth. The frequency of PDA in national level is 15.4^3

(4) **Pulmonary Stenosis (PS):** Narrowing of the pulmonary valve between right ventricle and the pulmonary artery is called pulmonary stenosis. The frequency of PS in national level is 6.9^3

(5) **Aortic Stenosis (AS):** Narrowing of the aortic valve between the left ventricle and the aorta is called aortic stenosis. The frequency of AS in national level is 4.1^3

(6) **Coarctation of the aorta (COA):** It is a constricted segment of the aorta that obstructs blood flow to the lower part of the body and increases blood pressure above the constriction. The frequency of COA in national level is 1.8^3

(7) **Tetralogy of Fallot (TOF):** TOF is made up of 4 separate components: a) VSD, that lets blood pass from the right to the left ventricle without going through the lungs and b) a narrowing (stenosis) at or just beneath the pulmonary valve. This narrowing partially blocks the blood flow from the right side of the heart to the lungs. c) The right ventricle is more muscular than normal, and d) the aorta lies directly over the VSD. Collectively, this results in cyanosis or blue baby, which may appear soon after birth, in infancy or later in childhood. The frequency of TOF in national level is 10.4^3

(8) **Transposition of the great arteries (TOA):** The great arteries are pulmonary artery and the aorta. The normal positions of the arteries are reversed in this type of defect. This defect is commonly associated with VSD, PS, heart block and an Ebstein like malformation of the tricuspid valve, which helps in communicating the oxygen rich blood to different parts of the body^3.

(9) **Atrioventricular Septal Defect (AVSD):** A large hole in the centre of the heart exists where the wall between the upper chambers joins the wall between the lower chambers. This is called as a complete AVSD. ^3

(10) **Persistent truncus arteriosus:** It is a complex malformation where only one artery arises from the heart and forms the aorta and pulmonary artery. That means the pulmonary arteries then branch off this common artery. This defect is found in association with VSD. ^3
(11) **Tricuspid Arteria (TA):** The valve between the right atrium and the right ventricle is missing. This defect is found in association with ASD, VSD and PDA. The frequency of TA in national level is $1.5^3$.

(12) **Pulmonary Arteria (PA):** In this case no pulmonary valve exists; therefore blood cannot flow from the right ventricle into the pulmonary artery and on to the lungs. $^3$

(13) **Total anomalous pulmonary venous connection (TAPVC):** In this case, all the pulmonary veins drain into the right atrium instead of left atrium, which brings the mixing of the blood. In addition to this, there is also presence of ASD and VSD, which results in cyanosis. $^3$

(14) **Hypoplastic left heart syndrome (HLHS):** In this condition the left ventricle and the aorta are small and underdeveloped. Therefore, the mitral and aortic valves are usually tiny or absent. It is one of the top three heart abnormalities to cause problems in the newborn. The frequency of HLHS in national level is $1.5^3$

(15) **Double outlet right ventricle (DORV):** It is a most uncommon defect in which both the pulmonary artery and aorta arises from the right ventricle, each with its own outflow tract and valve. $^3$

(16) **Single ventricle / univentricular heart:** It refers to a congenital malformation in which two atria are related to one ventricle that qualifies as left, right or indeterminate ventricle on purely morphologic ground$^3$.

(17) **Ebstein’s anomaly (EA):** In this case there is a downward displacement of the tricuspid valve into the right ventricle. It is usually associated with an ASD. The frequency of EA in national level is $1.5^3$

(18) **Dextrocardia (heart on the right):** If the developing heart tube bends to the left instead of the right, then the heart is displaced to the right and develops in a mirror image of its normal state. This is a condition called situs inversus. In many a cases Dextrocardia heart functions normally unless there are no associated vascular abnormalities. In cases where the heart is the only organ, which is transposed, known as isolated Dextro-cardia, there are usually other severe cardiac abnormalities associated with it. The frequency of Dextrocardia in national level is $1.5^3$.
The environmental and genetic risk factors may play a key role in congestive heart diseases. They include having rubella during pregnancy, pregestational diabetes, Certain medications taken during pregnancy like (thalidomide, angiotensin-converting enzyme (ACE) inhibitors, statins, the acne medication isotretinoin and lithium), smoking, Drinking alcohol during pregnancy, and Heredity. Some studies have reported associations between maternal smoking and heart defect groups, including ASDs, atrioventricular septal defects, and tetralogy of Fallot. 

As Helen Taussig stated 50 years ago “Our next great step forward will come in the field of cause and prevention of malformations.” to Causes of CHD are often divided into genetic and non-genetic influences. The advantage of contemporary genomic technologies including single-nucleotide polymorphism (SNP) arrays, next-generation sequencing, and copy-number variant platforms are accelerating the discovery of genetic causes of CHD. “Genome-Wide Association Studies and Meta-analysis for Congenital Heart Defects” are important studies performed using distinct patient cohorts from multiple sites. We are now looking for the complex multigenetic explanations for CHD in a multifactorial scheme, including epigenetic and environmental factors.

CHDs include a broad spectrum of malformations that differ with respect to morphology, physiology, and clinical outcome. Although CHD risk is thought to be influenced by both environmental and genetic factors, relatively few specific CHD risk factors have been identified and the extent to which the etiology of different CHDs differ or overlap is unknown. Large epidemiological studies, such as the National Birth Defect Prevention Study; have identified a few non-genetic risk factors for CHDs including maternal pre-gestational diabetes, obesity, and smoking.

The NKX2-5 gene on chromosome 5q34 consists of two exons which encode a 324 amino acid protein. This homeobox transcription factor is expressed during early cardiac morphogenesis and serves as a master regulatory protein. Because of its critical role in cardiogenesis, NKX2-5 has been a prime candidate in studies to identify the genetic basis of structural Congenital Heart Defects. 

CSX/ NKX2.5 the earliest molecule marker of the cardiac lineage is NKX2.5 in vertebrates. It is one of the members of NK2 family of homeobox genes and a homolog of the Drosophila tinman. It has highly conserved regions of DNA binding, protein-protein interactions, nuclear translocation, and regulation of other transcription factors. Their homeodomains have a tyrosine at position 54, making it the most unambiguous feature of this class and is a useful classification tool. Homeobox genes
have been found to play a crucial role in regulating tissue specific gene expression. Mutations in this gene have been reported to cause ASD, VSD with atrial ventricular block, TOF and Tricuspid valve abnormalities.\textsuperscript{16}

NKX2-5 mutations display diverse cardiac abnormalities that include septal defects, conotruncal malformations, hypoplastic left heart, dilated cardiomyopathy, and atroventricular conduction block (5)\textsuperscript{17}. Most reported NKX2-5 mutations were in the homeodomain, a critical part of the protein that interacts with deoxyribonucleic acid (DNA), and were typically associated with cardiac conduction anomalies\textsuperscript{18}. 