Genetic study on cardiac septal defects in children with congenital heart diseases

Thesis proposal Submitted for fulfillment of PhD in childhood studies
(Special needs)
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Abstract:

Background

Congenital heart defects (CHDs) represent the biggest fraction of morbidity and mortality of all congenital anomalies worldwide. CHDs have complex inheritance patterns and multifactorial aetiologies. Research over the past two decades has established firmly the role of genetics in the development of these congenital defects. CHDs can be clinically classified into syndromic and non -syndromic. Cardiac septal defects account for the greatest proportion of all CHDs in humans and represent approximately about 50% of all CHD. Cardiac septal defects include atrial septal defects (ASD), ventricular septal defects (VSD), and atrioventricular septal defects (AVSD).

Objective: The main aim of the work is to determine whether NKx2.5 gene mutations were the cause of the cardiac phenotype among patients of the studied sample.

Subjects & Methods: In our study, 30 patients with congenital cardiac septal defects were included. Detailed history taking, clinical examination, echocardiography and karyotyping were done to all patients. Molecular screening of NKX2.5 gene was done to all patients.

Results: Screening results revealed 9 mutations in NKx2.5 gene in the form of 4 disease causing mutations (3 of them were novel mutations) and five detected variants which turned to be polymorphisms.

Conclusion: VSD is the commonest phenotype among our patients. NKx2.5 gene mutations might be the cause of cardiac septal defect among patients of the studied sample.

Recommendation: Aiming for proper genotype/ phenotype correlation; larger sample size and screening of all genes known to cause cardiac septal defects are recommended.

Keywords: Congenital heart Diseases-Cardiac septal defects- NKx2.5 gene

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List of Abbreviations

- a CGH: array Comparative Gene Hybridization
- A.D.A.M.: Animated Dissection of Anatomy for Medicine, (online medical encyclopedia)
- AAP: American Academy of Pediatrics
- ACCF: American College of Cardiology Foundation
- AHA: American Heart Association
- AI: Aortic Insufficiency
- AP window: Aortopulmonary Window
- AS: Aortic Stenosis
- ASD: Atrial septal defect
- AV fistulae: Atrio-ventricular fistulae
- AVSD: Atrio-ventricular septal defect
- AzV: azygous vein
- BAS: Balloon atrial septostomy
- BAV: Bicuspid Aortic Valve
- C.O.P: Cardiac output
- CAT: Common Arterial Trunk
- CHARGE syndrome: C-Coloboma of the eye, central nervous system anomalies, H - Heart defects, A - Atresia of the choanae, R - Retardation of growth and/or development, G - Genital and/or urinary defects (Hypogonadism),E - Ear anomalies and/or deafness
- CHD: Congenital heart disease
- CHD: congenital heart disease
- CHMs: Congenital heart malformations
- CNV: copy number variation
- CoA: Coarctation of the Aorta
- COFT: Cardiac outflow tract
- CS: Caesarean section
- CSC: Cardiac stem cell
- CUCH: Cairo University children hospital
- DCRV: Double Chambered Right Ventricle
- DILV: Double Inlet Left Ventricle
- DIRV: Double Inlet Right Ventricle (DIRV)
- DM: Diabetes mellitus

- DOLV: Double Outlet Left Ventricle
- DORV: Double Outlet Right Ventricle
- DRRS: Duane radial ray syndrome
- ECG: Electrocardiography
- ED: ventricular end-diastolic pressure
- EvC: Ellis van-Creveld syndrome
- EVS: Exome Variant Server
- EVS; Exome variants server
- ExAC: The Exome Aggregation Consortium
- FT: full term
- gDNA position: Genomic DNA position
- HGMD; Human genome mutation database
- HGVS: Names: Human Genome Variation Society
- HLHS: Hypoplastic Left Heart Syndrome
- HOS: Holt-Oram syndrome
- IAA: Interrupted Aortic Arch
- IAA: Interrupted Aortic Arch
- IPCCC: International Paediatric and Congenital Cardiac Code
- ISNPCHD: International Society for Nomenclature of Paediatric and Congenital Heart Disease
- IVC: Inferior Vena Cava
- LHV: left hepatic veins)
- LLPV: left lower pulmonary vein
- LOF: Loss of function
- LPA: left pulmonary artery
- LUPV: left upper pulmonary vein
- LV: Left ventricle
- MAF: Minor allele frequency
- MAPK: Mitogen activated protein kinase
- MCA: multiple congenital anomalies
- MPA: main pulmonary artery
- MR: Mitral Regurgitation
- MRI: Magnetic Resonance Imaging
- MS: Mitral Stenosis
- MT: MutationTaster

- MVP: Mitral Valve Prolapse
- NA: Not available D
- NAD: no abnormality detected
- NF; Not found
- NGS: Next generation sequencing
- NRC: National research centre
- NVD: normal vaginal delivery
- PA: pulmonary atresia
- PAPVC: Partially Anomalous Pulmonary Venous Connection
- PAS: Pulmonary Artery Stenosis
- PDA: Patent ductus arteriosus
- PDB: Protein Data Bank
- PFO: Patent foramen ovale
- PGD: Preimplantation genetic diagnosis
- PKU: Phenyl ketonuria
- Poly-phen: Polymorphism Phenotyping
- PRCM: Polish Registry of Congenital Malformations
- PS: Pulmonary Stenosis
- RA: right atrium
- Ref SNP Alleles: Reference Single Nucleotide Polymorphism
- RHV: right hepatic vein
- RLPV: right lower pulmonary vein
- RPA: right pulmonary artery
- RUPV: right upper pulmonary vein
- RV: Right ventricle
- SCA: Selective coronary angiography
- SD: Standard deviation
- SIFT Allele frequency: Sorting Intolerant From Tolerant
- SLOS: Smith–Lemli–Opitz syndrome
- SNP: Single nucleotide polymorphism
- SVC: Superior Vena Cava
- SVS: Supravalvular Aortic Stenosis
- TA: Truncus Arteriosus
- TAPVC: Total Anomalous Pulmonary Venous Connection
- TAPVC: Total Anomalous Pulmonary Venous Connection

- TGA: Transposition of great arteries
- TGP):Thousand genome project
- TGP; Thousand genome project
- TOF: Tetrology of Fallot
- TR: Tricuspid Regurgitation
- TS: Tricuspid Stenosis
- ULD: Upper limb deformity
- VCFS: Velocardiofacial syndrome
- VSD: Ventricular septal disease

Introduction

Congenital heart disease (CHD) is a complex trait, as both environmental and genetic factors have been implicated in its pathogenesis. The aetiology of CHD has a strong genetic component, as shown by extensive epidemiological studies in large series of consecutive births (Burn et al., 2002).

CHD are the most common developmental errors in humans, affecting about 5- 8 out of 1,000 new-borns and are estimated as a major cause of prenatal birth losses (Posch et al., 2010).

CHD is a major cause of infant morbidity and mortality. The clinical outcome is largely dependent on the severity of the defect, the presence of extra cardiac anomalies, and surgical complications (Breckpota et al., 2011).

Defects of cardiac septation are the most common forms of CHDs and account for approximately 50% of all CHDs. Cardiac septation defects include atrial septal defects (ASDs), ventricular septal defects (VSDs), and atrioventricular septal defects (AVSDs) (Posch et al., 2010).

Understanding the aetiology of CHD will help clinicians in managing the patient. It may help in identifying possible complications of surgery or treatment, as patients with genetic syndromes are generally at higher risk of operative mortality and morbidity (Formigari et al., 2009). Also helping geneticists in offering proper counselling for CHD families and possibly providing them with accurate prenatal diagnosis for future pregnancies (Landis, et al., 2013).

Congenital heart defects arise from an abnormal heart development, induced either by environmental influences, altered gene dosage / function or by combinations of both (Bruneau, 2008).

About 20% of cases are genetic in origin and can be attributed to multifactorial causes (most sporadic isolated cases); chromosomal anomalies (numerical or structural), Mendelian syndromes (as Holt–Oram syndrome), non-syndromal single gene disorders (as CHD caused by mutations in NKX2-5 and GATA4, TBX5 genes). While 80% of cases are due to environmental factors as; maternal diabetes, low peri-conceptional folate, maternal febrile illness (e.g. rubella) and teratogenic drugs (e.g. Retinoic acid and maternal antidepressants) (Bruneau, 2008).

Chromosomal anomalies account for about 8%–10% of cases presenting with syndromic CHD. Down syndrome being the most common chromosomal anomaly seen, followed by Velocardiofacial syndrome (VCFS) (Roos-Hesselink et al., 2005). CHD could be divided into Syndromic and non-syndromic. CHD patients with a second major anomaly, developmental delay or dysmorphism are considered syndromic and represent about 22- 25% of all CHDs (Oyen et al., 2009).

CHD can be associated with extra- cardiac anomalies and in some cases can be diagnosed as being part of a syndrome. About 3%–5% of CHD can be attributed to Mendelian syndromes where a single mutation in the DNA results in pathological consequences, following a Mendelian inheritance pattern. Examples are Noonan Syndrome and RASopathies, Connective Tissue Disorders as Marfan syndrome, Ehlers-Danlos syndromes, rare Genetic Syndromes as 1p36 deletion syndrome, Jacobsen syndrome and finally single gene syndromic disorders as CHARGE syndrome, Kabuki syndrome (Soemedi et al., 2012).

Most recently Chromosome microarray analysis has provided a new tool to understand the genetic basis of syndromic CHD resulting from microdeletion or microduplication of genetic material, allowing the delineation of new syndromes. Improvements in sequencing technology have led to increasingly comprehensive testing for aortopathy, cardiomyopathy, single gene syndromic

disorders, and Mendelian-inherited congenital heart disease (Ware and Jefferies, 2012).

Human genetic studies have discovered numerous numbers of mutations causing isolated or syndromic CHD. Thus, nucleotide mutations in over 20 -30 genes have been implicated in sporadic or familial non-syndromic CHD and over twice as many in syndromic CHD. To date the contribution of many of these genes in CHD pathogenesis still remains doubtful (Barriot et al., 2010).

Chromosomal loci and genes implicated in non-syndromic septal defects include 5p, 8p23.1 GATA4, 14q11.2 MYH6,7p14.2 TBX20,15q14 ACTC1,4q32.3 TLL1,5q35.1 NKX2.5, 6q24.1 CITED2, 8p23.1 GATA4, 6q24.1CITED2, 5q35.1 NKX2.5, 1p31-21 unknown, 3p25.1 RELD1, 6q22.31 GJA1 (Pierpont et al., 2007). Genes that encode transcription factors have been found to be associated with cardiac septal defects and include TBX5, NKX2.5, and GATA4 (Posch et al., 2010).

Increasing evidence demonstrates that genetic variation in the NKX2.5 gene, which encodes a homeobox-containing transcription factor crucial to cardiogenesis, is an important molecular determinant for CHD (Wage et al., 2011).

Indeed, more than 40 heterozygous NKX2.5 germline mutations have been observed in individuals with CHD, and these are spread along the coding region, with many shown to impact protein function. Thus, NKX2.5 appears to be hyper mutable, yet the overall detection frequency in sporadic CHD is about 2% (Reamon-Buetlner and Borlak, 2010).

The NKX2.5 gene was cloned in 1996 and since then, it was shown to be one of the most common known genetic causes of human CHD. NKX2.5 plays critical roles in later stages of cardiac development, especially cardiac septation and development of the conduction system. Mutations in NKX2.5 gene cause