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# Perspectives in Paediatric Cardiology: Perspektive u pedjatrijskoj kardiologiji

**Mesihović Dinarević, Senka**

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# MOLECULAR GENETICS IN PAEDIATRIC CARDIOLOGY: APPLICATIONS AND CURRENT ADVANCES

*Amina Kurtovic-Kozaric*[1], *Senka Mesihovic-Dinarevic*[2]

[1]Department of Pathology
[2]Pediatric Clinic, Clinical Center of the University of Sarajevo
Bolnicka 25, 71000 Sarajevo, Bosnia and Herzegovina

## Abstract

This presentation is designed to provide the clinician with a summary of our current understanding of the contribution of genetics to the origin of congenital heart disease (CHD) and other heart defects that can occur in childhood. CHD can occur together with other congenital anomalies—there is a number of genetic tests that can help the clinician in diagnosing genetic alterations in the child with CHD like cytogenetic analysis, fluorescence in situ hybridization, microarrays, and DNA mutational analysis. We will discuss several syndromes that are diagnosed using those techniques like Williams-Beuren, Marfan, 22q11 deletion, and Noonan syndromes. Besides CHD, we will discuss the use of molecular genetic testing in diagnosing arrhythmogenic right ventricular cardiomyopathy (ARVC) and hypertrophic cardiomyopathy (HCM), among others. Furthermore, we will look at the newest molecular approaches like whole genome sequencing in diagnosing children with heart defects.

## Introduction

Cardiovascular disease affects both children and adults. It includes a wide range of conditions extending from diseases of the vascular system, diseases of the myocardium, diseases of the heart's electrical circuit, and congenital heart disease [1]. In this review, focus will be on the heart diseases commonly found in children, starting with the short description of the various paediatric cardiac defects, then description of their genetic components and types of genetic testing that should be performed in specific cases. At the end, there will be summary of the current advances in genetic research and diagnosis of cardiac defects together with the recent advances in the understanding of human heart development.

## Cardiac defects in children – basics

Cardiac defects in children range from congenital heart defects, which are diagnosed prenatally or within the first year of life, to paediatric cardiomyopathies, which can be diagnosed within the first year or in the teenage period. In this section, basic cardiology of congenital heart defects and paediatric cardiomyopathies will be reviewed with the special emphasis to the genetic basis of those diseases.

## Congenital heart defects – definition

Congenital heart disease encompasses cardiac malformations present at birth. It is one of the leading causes of death in the first year of life with prevalence of 4 to 50 per 1000 live births [2, 3, 4]. Current estimates are closer to 50 per 1000 live births, especially if other malformations like bicuspid aortic valve, left ventricular outflow tract obstructive disorders, isolated aneurysm of the atrial septum and persistent left superior vena cava are included [5, 6]. About half of the CHD are diagnosed in the first 12 months of life [7].

The underlying causes of the CHD are primarily unknown – however, increasing role of genetics has been crucial in understanding some types of malformations [5]. The determination of the genetic component (deletions, inversions, duplications, or mutations) will help the patients and their family because it can uncover other important organ system involvement, improve the understanding of clinical prognosis, and help other family members with potential reproductive risks [5]. For the explanation of the appropriate clinical diagnostic tests, please refer to another section in this review.

## Paediatric cardiomyopathies – definition

The current definition of cardiomyopathies takes into account the molecular genetic aspects of cardiovascular diseases. By definition given by the Council on Clinical Cardiology, cardiomyopathies are a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction that usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilatation and are present due to a variety of causes that are frequently genetic in nature. Cardiomyopathies are either confined to the heart or are part of generalized systemic disorders, often leading to cardiovascular death or progressive heart failure related disability [8].

In general, cardiomyopathies are divided into two major groups based on predominant organ involvement: a) primary cardiomyopathies (genetic, non-genetic, acquired) are those solely or predominantly confined to heart muscle and are relatively few in number and b) secondary cardiomyopathies which show pathological myocardial involvement as part of systemic disorders [8]. Primary cardiomyopathies that are genetic in origin are: hypertrophic cardiomyopathy (HCM), Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D), LV Noncompaction,

Conduction System Disease, and Ion Channelopathies. The primary cardiomyopathies of mixed origin are: Dilated Cardiomyopathy (DCM) and Primary restrictive cardiomyopathy [8].

Here we will discuss cardiomyopathies that can be found in children. Briefly, paediatric cardiomyopathy, or disease of the heart muscle, is a chronic and occasionally progressive disease of the myocardium (heart muscle), resulting in abnormally stiff and enlarged heart that cannot contract and relax properly. The consequence of the abnormal heart function is inability to pump blood to different parts of the body. Cardiomyopathy is the leading cause for sudden deaths heart transplants in children [9]. According to the US Pediatric Cardiomyopathy Registry, 1 in 100.000 children is diagnosed with cardiomyopathy. There are different types of primary cardiomyopathies that can be found in children, which include: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC).

Dilated or congestive cardiomyopathy (DCM) is diagnosed when the heart is dilated and the heart chambers contract poorly. DCM is the most common form of cardiomyopathy, accounting for about 60% of all paediatric cardiomyopathies [10]. Causes can be both genetic and infectious/environmental, where about 30% of children with DCM have a relative with the disease, although they may not have the symptoms. If no other cause is found like viral infection (idiopathic DCM), specialized genetic testing can be performed to look for mutations commonly found in patients with DCM.

Second most common paediatric cardiomyopathy is hypertrophic cardiomyopathy (HCM), comprising about 35% of paediatric cardiomyopathies. HCM also affects adults, where the children under age 12 account for 10% of all cases. According to the Pediatric Cardiomyopathy Registry, HCM occurs in five per million children. It is typically diagnosed in infancy or adolescence. HCM patients have abnormal growth of heart muscle fibres, making the muscle stiff, resulting in difficulty to relax and fill the heart chambers. HCM usually affects the left ventricle with thickening of the septum, posterior wall or both. Majority of patients with HCM have a family relative with the disease. Genetic testing is available for some, but not for all forms of HCM.

Restrictive cardiomyopathy (RCM) is a rare form of cardiomyopathy, characterized by restrictive ventricular filling – contractile function and wall thickness is mostly normal, but the relaxation (filling phase) is abnormal. The ineffective relaxation and filling with blood results in the backup of blood into the atria, lungs and body, causing the symptoms of heart failure. About one third of all RCM patients have family history.

Arrhythmogenic right ventricular dysplasia (ARVC/D) is a rare form of cardiomyopahy which is characterized by dilated right ventricles which function poorly and contain fatty deposits within the walls. Patients are at the risk of fast heart rhythms

(ventricular tachycardia). Prevalence is about 1:1000 [11], and is more often seen in Europe, specifically Italy, than in the USA [12, 13, 14]. About one third of AVRD patients are familial [15, 16]. Most familial AVRD are transmitted in the autosomal dominant fashion. However, autosomal recessive form exists and is called Naxos disease. Patients with this disease have hyperkeratosis of the palms and soles and woolly hair.

## Overview of basic genetics

The cause of most paediatric cardiomyopathies is still not well understood, but in a portion of children the cause is an error in a gene. Genes are encoded in DNA, which is situated within each cell of the body. Genes have many functions; one of them is to give instructions that determine how the parts of our bodies will be formed and how they will function. Mistakes in the coded instructions (DNA) are called mutations and they can cause problems with the formation and function of different organs. Many genes code for proteins that carry out various functions within the body including building organs or metabolizing various substances. Therefore, mutations may fail to produce the right amount of the protein or may produce a protein that does not function properly. If we focus on cardiomyopathies, mistakes in DNA can lead to isolated cardiomyopathy without other problems. In other cases, a DNA mutation can lead to cardiomyopathy, which is associated with other medical problems such as learning disabilities, muscle weakness, or poor growth.

Families which have a child diagnosed with cardiomyopathy are at increased risk for having another child or family member with cardiomyopathy. For that reason, it is crucial that once a cardiomyopathy is diagnosed within the family, all of the family members (brothers, sisters, and parents) are screened with an echocardiogram. Depending on the type of cardiomyopathy diagnosed, it may be necessary to repeat the echocardiogram periodically for younger children (childhood to mid-adult life). This is important specifically if close relatives were diagnosed with cardiomyopathy at an older age.

Cardiomyopathy in children can be caused by many different DNA mutations. In children with isolated hypertrophic cardiomyopathy, the disease is typically due to DNA mutations in genes that code for proteins found in the heart cell (e.g. sarcomere proteins). The diagnosis in such children is made by the confirmation of the mutation by molecular genetic testing. For many of those children, either the mother or father also have the same DNA mutation which they have passed on to their child. Such type of inheritance, where one parent and one child are both affected with the same disease is called autosomal dominantly inherited disease.

Here is a brief explanation of how genes (DNA) are passed from parent to offspring.

Each individual carries two copies of both genes (humans are diploid organisms) – one copy of each gene was passed on from mother and one from father. For autosomal

dominant diseases, a parent has a DNA mutation in one copy of the gene, while the other copy is normal (wild type). Therefore, the offspring of that parent has a 50–50 chance to inherit the mutation. In other words, autosomal dominant inheritance is such that a mutation in one gene of the parent is sufficient to cause the disease. In a family with an autosomal dominant condition, offspring have a 50% chance of having the disease. The reverse is also true – if an individual in affected family does not carry the mutation, the offspring will not have the disease.

If we focus on infants, it is important to remember that cardiomyopathies in that age group can be caused by inborn errors of metabolism. Most of these inborn errors of metabolism are inherited in an autosomal recessive fashion (e.g. fatty acid oxidation disorders and glycogen storage disorders such as Pompe disease will be discussed below). In autosomal recessive disorders, only when child has a mutation in both copies of the gene, the disorder can be observed. If a child has a mutation in one copy of the gene, he is the carrier but does not express the disease, in other words, having one abnormal copy does not cause disease because the other copy of the gene is sufficient to allow the normal heart function. Therefore, children with autosomal recessive diseases have mutations in both copies of a specific gene which they have inherited from both of their parents (both of their parents have the mutation in one copy of the gene and are thus called carriers). A child with an autosomal recessive cardiomyopathy, some of which are associated with inborn errors of metabolism, inherits a defective gene from each of his carrier parents. If both parents are carriers (have the mutation in one copy of the gene) the risk that other children (or future children) will have the cardiomyopathy is one in four or 25%. Usually the only individuals in the extended family at risk are brothers and sisters of the child with the cardiomyopathy.

Besides inborn errors of metabolism, there are other rare conditions that cause cardiomyopathies that are found in males. They are Duchenne or Becker muscular dystrophy or Barth syndrome. The cardiomyopathies in these conditions can occur in two ways: a) they may occur sporadically, i.e. not inherited from the parents or b) they may be inherited from their mother (X-linked). Mothers are females and thus have two X chromosomes. Usually they do not have any problems with X-linked diseases because the mutation is carried on one gene on the X chromosome while the other gene on the other X chromosome is normal. Males (boys) have only one X chromosome, so they have a 50% chance to inherit a mutated gene on the X chromosome from their mother. Since they have no second copy of the X chromosome, they may develop the disease. The daughters, on the other hand, will not have the condition although they may be carriers and could pass it on to their sons in the future.

## Congenital heart disease – genetic basis

Even though Mendelian inheritance of CHD has been reported, the current hypothesis is that CHD is of multi-factorial aetiology – the interaction between the

environment and the genetic predisposition [5, 17]. Even with the accumulating molecular data on CHD, only a minority of patients has an identifiable genetic defect [18]. The proposed approach to newly diagnosed CHD patient is the routine examination of relatives with potential genetic contribution in order to obtain the accurate medical history [5]. The reasoning behind this approach is that although autosomal dominant pattern is theoretically easy to recognize, the problem lies with incomplete penetrance, like in the case of familial bicuspid aortic valve, which has reduced penetrance.

When faced with congenital heart defect, the clinician should request chromosomal analysis and possibly FISH testing for specific deletion syndromes – if chromosomal abnormality is found, the family has a clear explanation of the cause of CHD, which allows for the appropriate assessment of the recurrence risk. Furthermore, specific assessment of physical features should be done together with the geneticist (e.g. dysmorphic facies, eye and ear abnormalities, limb reduction defects, polydactyly, other skeletal defects, urologic defects, and neurological status – specific care should be paid to skeletal defects, cardiac aortic arch, pulmonary, liver and stomach situs).

In the next section, some of the most common genetic syndromes with congenital heart disease are presented.

## Examples of genetic syndromes with heart disease

### *22q11 Deletion Syndrome – DiGeorge Syndrome*

Patients with DiGeorge syndrome present with CHD, hypocalcemia, immunodeficiency, and facial dysmorphism. More than 90% of patients with the DiGeorge phenotype have a microdeletion on chromosome 22 [19]. Diagnosis is made by FISH because the deletion is small and can be missed on the karyotype. DiGeorge syndrome is part of the 22q11 deletion syndrome; the clinical features can vary among affected individuals, but most common characteristics are cardiovascular anomalies and palate anomalies, facial dismorphism, learning disability, feeding difficulty, renal anomalies and behavioural difficulties. A 22q11 deletion can be inherited from a parent in approximately 6% to 28% of cases [20]. The deletion in the parent is identified after the child's diagnosis and that parent often have subtle syndromic features. Such information is important for parental reproductive planning, since the implication is that half of future pregnancies will carry the deletion [21].

The most common cardiovascular defects associated with a 22q11 deletion include tetralogy of Fallot (8–35%), interrupted aortic arch type B (50–89%), truncus arteriosus (34–41%), VSDs (with aortic arch anomaly 45%), and aortic arch anomalies (24%) [5]. However, pulmonary stenosis, atrial septal defects, and hypoplastic left heart syndrome have also been reported. The importance of proper diagnosis of CHD child with a 22q11 deletion is augmented by several reasons: a) timely evaluation of

non-cardiac phenotypes for proper care, b) higher mortality of children with 22q11 deletion due to abnormalities of calcium metabolism and immunodeficiency [22].

### *Down's syndrome*

About one half of Down syndrome patients have atrial-ventricular canal defects, ventricular septal defects, patent ductus arteriosus, atrial septal defect, and tetralogy of Fallot [53]. Diagnosis is made by conventional karyotype, but FISH can also be ordered if a rapid diagnosis is required.

### *Turner Syndrome*

Turner syndrome is characterized by coarctation of the aorta. The diagnosis is evident clinically in the majority of cases. About half of the cases have 45, X karyotype, and the rest is due to mosaics, deletions, translocations and other cytogenetic aberrations.

### *Marfan syndrome*

Marfan syndrome is a connective tissue disorder resulting in skeletal, ocular, and cardiovascular defects. The diagnosis is based on clinical parameters [54, 55]. The syndrome is inherited as a dominant trait, carried by the gene FBN1, which encodes the connective protein fibrillin-1. Because it is dominant, people who have inherited one affected FBN1 gene from either parent will have Marfan syndrome. Mutations in the fibrillin-1 gene on chromosome 15 (15q15-21.3) are responsible for 50–60% of cases with autosomal dominant inheritance, high penetrance and clinical variability [56]. The most fatal cardiovascular consequence is aortic dissection resulting from progressive aortic root dilatation in classic Marfan syndrome [55], but different clinical variances such as the MASS phenotype also result in mitral valve prolapse [58]. A Cystine to Arginine amino acid substitution in the fibrillin-1 gene has been associated with a more severe phenotype and may have cardiovascular implications: aortic root dissection, mitral valve prolapse (MVP), and others [55].

Testing is most useful in familial cases and can be used to identify family members who may have the defective gene and be at risk of cardiovascular complications.

### *Williams-Beuren Syndrome*

Williams-Beuren Syndrome (WBS) is a connective tissue and brain disorder characterized by elfin facies, mental retardation, gregarious personality and congenital heart defects such as supravalvular aortic stenosis, supravalvular pulmonic stenosis, VSD, PDA and systemic hypertension [57], as well as diffuse arterial wall thickening including the coronary arteries. The cardiac defects often escape detection until developmental abnormalities bring this disorder to attention at about 6 years of age. Supravalvular aortic stenosis, which can be present in about 2/3 of cases [57] is associated with disruption of the elastin gene either from a deletion at 7q11.23 in the

majority of cases or from an autosomal dominant 6;7 translocation on chromosome 7 [59].

### *Ehler's Danlos Syndrome Type IV*

Ehler's Danlos Syndrome Type IV is an autosomal dominant connective tissue disorder. There is hyperextensibility of the skin and hypermobility of the joints, but the disorder can present initially as a fatal event from aortic (or other arterial) rupture, bowel rupture or uterine rupture during pregnancy. The defect is due to mutations in the COL3A1 gene at 2q31-q32 which encodes for type III collagen [60, 61]. Because there are different mutations (polymorphisms), siblings may not be equally affected.

## Paediatric cardiomyopathies – genetic basis

The genetic basis of different functional types of cardiomyopathy is given in this section, with emphasis on the specific genetic mutations responsible for different forms of cardiomyopathy and clinical implication of those mutations. In a population-based study of paediatric cardiomyopathies, DCM was found in 51% of the cases, while HCM was found in 42% of cases [23]. Therefore, we will focus on those types of cardiomyopathy.

### *Genetics of hypertrophic cardiomyopathy*

Hypertrophic cardiomyopathy, HCM, can be caused by mutations in one of 14 known genes that encode different sarcomere components – if caused by those mutations, HCM is characterized by LVH, left ventricular hypertrophy without predisposing cardiovascular conditions like aortic stenosis or long-standing hypertension [24]. Commonly the LVH is recognized in adolescence, but it can be seen at any age [25]. Familial HCM can be diagnosed based on the family history as well as molecular testing of the known 14 genes encoding sarcomere components. About 80% of mutations are found in two genes, MYH7 and MYBPC3, beta-myosin heavy chain (the first identified) and myosin binding protein C. The other genes appear to account for far fewer cases of HCM and include genes troponin T and I, regulatory and essential myosin light chains, titin, alpha-tropomyosin, alpha-actin, alpha-myosin heavy chain, and muscle LIM protein. It is important to mention nonsarcomeric mutations in two genes involved in cardiac metabolism responsible for regulating cardiac glycogen storage, which are found in older children and adults, but are clinically indistinguishable from other HCM. One gene encodes the gamma-2-regulatory subunit of the AMP-activated protein kinase (PRKAG2), associated with variable degrees of LV hypertrophy and ventricular pre-excitation [25]. The second gene is lysosome-associated membrane protein 2 (LAMP-2), resulting in Danon-type storage disease [8]. Clinical manifestations are confined to the heart abnormalities, presenting with massive degrees of LV hypertrophy and ventricular pre-excitation. These disorders are now part of a subgroup of previously described infiltrative forms of LV hypertrophy such as Pompe disease (discussed below), and Fabry's disease, an X-linked

recessive disorder of glycosphingolipid metabolism caused by a deficiency of alpha-galactosidase A [8].

Several other diseases associated with LV hypertrophy involve prominent thickening of the LV wall, occurring in infants and children less than 4 years of age, may resemble HCM caused by sarcomere mutations. These cardiomyopathies include secondary forms such as Noonan syndrome (discussed below), an autosomal dominant cardiofacial condition associated with a variety of cardiac defects

If familial HCM is diagnosed, genetic counselling is strongly recommended because the mode of inheritance is autosomal dominant [26, 27]. Histopathological findings include enlarged, disorganized myocytes, which die prematurely, leading to cardiac fibrosis. Clinical symptoms are highly variable and may include dyspnea, chest pain, palpitations, arrhythmias, and syncope. HCM is the most common cause of sudden death in healthy young individuals [27].

If we focus primarily on children with HCM, findings from the Pediatric Cardiomyopathy Registry [28] identified one disease in each of the following categories that accounted for most affected children: a) inborn error of metabolism (e.g. Pompe disease or glycogen storage disease type II), b) malformation syndrome (Noonan syndrome) and c) neuromuscular disorder (Friedrich's ataxia, FRDA).

*Pompe disease* (three forms: infantile, juvenile and adult-onset) is an autosomal recessive disorder that results from the deficiency in an enzyme acid alpha-glucosidase (GAA), causing the abnormal glycogen accumulation in all tissues [29, 30]. Carriers have no clinical manifestations. Different types of mutations in GAA have been described which lead to loss of protein product, reduced protein activity, reduced amount of protein, etc. The c.-32-13T → G mutation is the most common among adult form of the disease [29] and has not been reported in infants.

*Noonan syndrome* is defined by short stature, webbed neck, chest shape, and variable degree of developmental delay. Most patients with Noonan syndrome have congenital heart disease (pulmonary valve stenosis, atrial and ventricular septal defects, branch pulmonary artery stenosis and tetralogy of Fallot), and 20%–30% have HCM.

Cytogenetics is normal for Noonan patients, but four genes are known to be associated with this syndrome: PTPN11 (~50% of affected individuals), RAF1 (3%–17%), SOS1 (~10%), and KRAS (<5%). It is an autosomal dominant pattern of inheritance.

*Friedreich ataxia* (FRDA) is characterized by progressive ataxia that is diagnosed in teenage period. HCM is present in two thirds of patients. FRDA diagnosis is done by molecular testing of frataxin gene (OMIM 229300) and almost all patients have a mutation that reduces the amount of frataxin protein – the mutation is an intronic GAA repeat, which silences the gene transcription [31]. It is inherited in autosomal recessive fashion.

HCM genotype-phenotype correlations have been attempted, but care must be taken when intending to do genetic testing. In some cases the age of onset of HCM is associated with specific gene mutations, caution should be applied. About half of children with LVH have a mutation in one of the genes encoding components of sarcomere (out of which 49% a single occurrence in a family and 64% of familial cases).

### *Genetics of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia*

ARVC/D is inherited as an autosomal dominant disorder with incomplete penetrance, but autosomal form can also be found (Naxos disease). Clinical genetic studies suggest that about 30 percent of ARVC are familial [15, 16]. Multiple genes have been identified in 8 chromosomal locations, including ryanodine receptor 2 (RyR2), desmoplakin, plakophillin-2 and mutations in regulatory sequences of the transforming growth factor-beta, TGFB gene. Here is the more comprehensive list of the chromosomal locations associated with ARVD: 14q23-q24 (ARVC1), 1q42-q43 (ARVC2), 14q12-q22 (ARVC3), 2q32 (ARVC4), 3p25 (ARVC5), 10p12-p14 (ARVC6), 10q22, 6p24 (ARVC8), and 12p11 (ARVC9) [32-40].

Desmoplakin was the first gene to be identified and associated with ARVD, mapped to 6q24 (ARVC8). The protein product is the key component of desmosomes and adherens junctions that is important for maintaining the tight adhesion of many cell types. Once the junctions are disrupted, cell death and fibrofatty replacement take place. Mutations in desmoplakin have also been associated with left-sided ARVC and with autosomal recessive disease.

ARVD/C can also be associated with Naxos syndrome, an autosomal recessive disorder caused by mutations in the plakoglobin protein and characterized by severe problems with the skin, teeth, hair and nails in addition to the heart [41, 42]. Like desmoplakin, plakoglobin is a key component of desmosomes and participates in maintaining tight cell-cell adhesion.

A similar autosomal recessive disorder is Carvajal syndrome and is caused by mutations in the protein desmoplakin and is manifested by woolly hair, epidermolytic palmoplantar keratoderma, and cardiomyopathy [43].

Plakophilin-2, PKP2, (ARVC9) gene mutations can be found in more than one third of AVRC patients [40, 44]. Other genes associated with AVRC are desmoglein-2 gene, DSG2; desmocollin-2 gene; TMEM43 gene; cardiac ryanodine receptor RyR2; and TGF-beta-3 gene.

### *Genetics of Left Ventricular Noncompaction (LVNC)*

Noncompaction of ventricular myocardium (LVNC) is a recently recognized congenital cardiomyopathy [8]. It is characterized by a distinctive morphological appearance of the LV myocardium. The inheritance pattern in LVNC can be autosomal dominant, X-linked or maternally transmitted due to mitochondrial mutations.

LVNC can be caused by mutations in the X-linked gene tafazzin (G4.5) that causes Barth syndrome and mitochondrial disorders, or it can be autosomal dominantly inherited if mutations occur in the alpha-dystrobrevin and ZASP genes (NKX2.5).

### *Genetics of Ion Channelopathies*

Recently there has been a better understanding of the rare congenital arrhythmia disorders which include LQTS, short-QT syndrome (SQTS), Brugada syndrome, and CPVT that are caused by the mutations in ion channel proteins, responsible for membrane transport of sodium and potassium ions [8].

Long-QT Syndrome, the most common of the ion channelopathies, is characterized by two patterns of inheritance: a) a rare autosomal recessive disease associated with deafness (Jervell and Lange-Nielsen syndrome), caused by mutation in genes KCNQ1 and KCNE1, and b) autosomal dominant disease unassociated with deafness (Romano-Ward syndrome), which is caused by mutations in 8 genes – SCN5A (Na1.5, LQT3), KCNQ1 (KvLQT1, LQT1), KCNH2 (HERG, LQT2), ANKB (LQT4), KCNE1 (minK, LQT5), KCNE2 (MiRP1, LQT6), KCNJ2 (Kir2.1, LQT7, Andersen's syndrome), and CACNA1C (Ca1.2, LQT8, Timothy syndrome).

Besides Long QT syndrome, a new disorder was described in 1992, called Brugada syndrome (OMIM 601144), characterized by distinct ECG pattern and an increased risk of sudden cardiac death [45-47]. The syndrome is mostly diagnosed during adulthood, but occurs in infants and children (Antzelevitch, 2005). It is the major cause of sudden unexplained death syndrome (SUDS) and is the most common cause of sudden death in young men in Thailand and Laos [45]. Brugada syndrome is familial autosomal dominant disorder – 20% of patients have been linked to mutations in SCN5A gene (OMIM 600163, the same gene responsible for LQT3), also called Brugada syndrome type 1. However, there is a wide range of genetic heterogeneity in Brugada syndrome patients. There are several types: Brugada syndrome-2 (OMIM 611777) is caused by mutation in the GPD1L gene (OMIM 611778). Brugada syndrome-3 (OMIM 611875) and Brugada syndrome-4 (OMIM 611876) are caused by mutation in the CACNA1C (OMIM 114205) and CACNB2 (OMIM 600003) genes, respectively. Brugada syndrome-5 (OMIM 612838) is caused by mutation in the SCN1B gene (OMIM 600235). Brugada syndrome-6 (OMIM 613119) is caused by mutation in the KCNE3 gene (OMIM 604433). Brugada syndrome-7 (OMIM 613120) is caused by mutation in the SCN3B gene (OMIM 608214). Brugada syndrome-8 (OMIM 613123) is caused by mutation in the HCN4 gene (605206).

### *Genetics of dilated cardiomyopathy (DCM)*

About 20%–35% of DCM patients have the familial form, although with incomplete penetrance [8]. Although genetically heterogeneous, the predominant inheritance is autosomal dominant. However, some cases are X-linked autosomal recessive and mitochondrially inherited (from the mother). Non-genetic cases like inflammation of myocarditis due to viral infection are also common.

Numerous genes have been correlated with DCM. Mutations in genes that code for cytoskeleton (delta-sarcoglycan, metavinculin, desmin, lamin A/C), Z-disk (ZASP, alpha-actinin-2, MLP, titin) and sarcomere (beta-myosin, alpha-tropomyosin, myosin binding protein-C, troponin-T) have been linked to DCM. Furthermore, X-linked genes, e.g. dystrophin, which causes Duchenne and Becker muscular dystrophies, have also been linked to DCM. Barth syndrome is another X-linked cardiomyopathy, caused by mutations in the G4.5/tafazzin gene. Like HCM, mutations in many of the sarcomeric genes (beta-myosin, alpha-tropomyosin, myosin binding protein-C, troponin T) can also cause DCM and are autosomal dominantly transmitted.

Other causes of DCM have been characterized. Abnormalities of mitochondrial function due to mutations in the mitochondrial DNA and inborn errors of metabolism related to the acyldehydrogenase genes can be causes of DCM in infants. Severe infantile cardiomyopathy is the most common clinical phenotype of VLCAD, very long chain acyldehydrogenase deficiency, and is found in about 67% of cases, often resulting in sudden death [23]. Majority of inborn errors of metabolism are inherited in autosomal recessive fashion and the diagnosis is commonly made by a blood test followed by an enzymatic test performed from a skin biopsy. The diagnoses of fatty acid oxidation disorders are important because the heart function can be improved by dietary manipulation with carnitine [23].

## Methods in genetic testing

There are several genetic tests that can help the clinician in diagnosing genetic changes in the child with heart defects. Most commonly used tests are cytogenetic analysis (karyogram or karyotype), fluorescence in situ hybridization (FISH), SNP array (or oligo array), and DNA mutation analysis (DNA sequencing or PCR).

### *Karyotype—chromosome analysis*

Karyogram is the typical complement of chromosomes [48]. Cytogenetics deals with karyotype analysis. The human karyotype has 46 chromosomes – 22 pairs of homologous chromosomes and one pair of sex chromosomes. Karyogram can be done in various cells and tissues, such as blood, bone marrow, amniotic cells, and tumour tissue. The sample is set up in a liquid nutrient medium under sterile conditions which allows cells to multiply, from 1 to 10 days, depending on the sample type [48, 49]. The culture is harvested when cells are in metaphase of cell division, placed on glass slides and stained. The chromosomes of one cell are analyzed in a karyogram to detect changes such as deletions, insertions, duplications and translocations. About 20 cells are analyzed for one sample (Figure 1) [50].

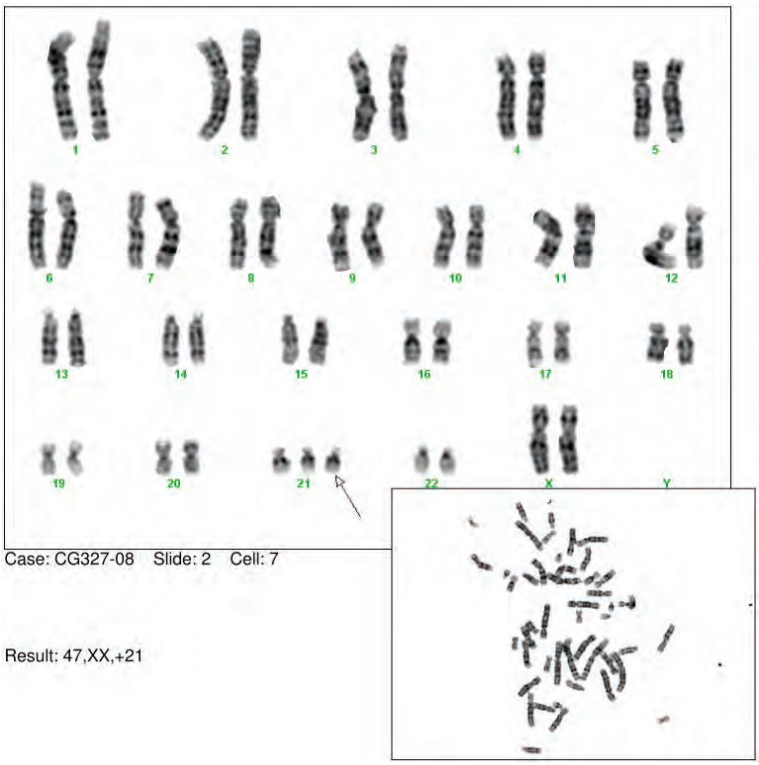


*Figure 1. Female karyotype with 47 chromosomes – trisomy of chromosome 21 which indicates Down syndrome.*

A standard chromosome analysis has a low pick-up rate – about 8% to 13% of neonates with congenital heart defect have detectable karyotypic aberrations [51].

However, with the use of other techniques like FISH and SNP array, the percentage is likely to be much higher. In contrast, of all children with chromosomal abnormalities, about one third have cardiac defects [52]. The standard metaphase karyotype (450 to 550 bands) is diagnostic for many chromosomal disorders, especially those of chromosome number such as trisomy (trisomy 21) or monosomy (45,X or Turner syndrome).

## *FISH*

FISH, fluorescent in situ hybridization, is a molecular cytogenetics technique which can be used together with, or instead of the karyotype, depending on the disease and the extent of the chromosomal changes [48]. FISH utilizes fluorescently labeled DNA molecules as probes for a specific DNA sequence of interest (Figure 2). The advantage of FISH as compared to the conventional karyotype is that small changes cannot be seen on the conventional karyotype, but FISH can detect them. However, FISH can only detect specific changes that the physician is looking for, i.e.

if DiGeorge syndrome specific deletion is found in the sample or not (it cannot detect other changes in the sample). There are several disorders like DiGeorge; Williams-Beuren, Alagille, and Chi-du-chat have been associated with a microdeletion that frequently can be detected only by FISH.

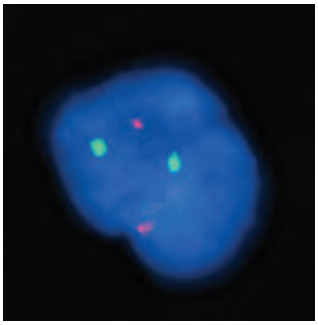

*Figure 2. Example of FISH. The nucleus contains two red and two green signals. The red signal is specific for the microdeletion region characteristic for Williams syndrome – 7q11.23. The green signal is a control region, 7q31. This sample does not show deletion specific for Williams-Beuren syndrome.*

If there is no obvious syndrome, a standard karyotype and FISH 22q11 should be obtained. If the FISH 22q11 is positive, a diagnosis can be established and the pending karyotype is unnecessary. If the FISH result is negative, the karyotype should be checked. If the karyotype is normal, SNP array should be performed. Further testing can be directed by the type of heart defects present or suspected.

### *SNP arrays*

SNP (single nucleotide polymorphism) array is a useful technique for studying slight variations between whole genomes. Another analogous technique called Comparative genomic hybridization (CGH) or Chromosomal Microarray Analysis (CMA) is a molecular-cytogenetic method for the analysis of copy number changes (gains/losses) in the DNA content of a given subject's DNA. CGH detects only unbalanced chromosomal changes. Structural chromosome aberrations such as balanced reciprocal translocations or inversions cannot be detected, because they do not change the copy number. SNP arrays, however, have an additional advantage of being able to detect copy-neutral LOH (loss of heterozygosity, also called uniparental disomy or gene conversion), which is important in syndromes like Prader-Willi/Angelman.

### *DNA sequencing*

The cytogenetic methods described above identify large changes in chromosome number or structure – SNP arrays do detect nucleotide changes, but their genome coverage might not account for the specific changes that are important in patients

with heart defects. However, there are microarrays that are designed to target only the genes specific to heart defects, but they can only be found in several institutions in the world and are not accessible to most physicians. Therefore, in certain disorders, changes occur at the level of a single gene and must be detected by alternative techniques that are more readily available. One of them is gene sequencing.

Genes are complex structures that include not only regions coding for the protein itself, but also other sequences involved in regulation of gene activity. Mutation analysis most often identifies changes in the coding sequence of the gene, including small nucleotide deletions, insertions, or nucleotide substitutions that alter the amino acids and affect the protein structure and function. Most commonly used method for sequencing is Sanger dideoxy method. However, in the last couple of years, another technology called next-generation sequencing, which is the sequencing of whole genomes or exomes, has become readily available.

It is important to emphasize the interpretation of sequencing results, which is not always straight forward. Currently, many mutations have been identified that cause heart defects. In future, the wealth of the sequencing data will have to be correlated with the pathogenesis of the specific aberrations, which would be the leading clues for new drug development.

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