Congenital heart disease and the role of genetic factors in cardiac morphogenesis

Abstract
Cardiovascular malformations are the most common types of birth malformations, causing a significant increase in mortality worldwide. The etiology of most of these abnormalities remains unknown, but the genetic factors involved are considered to have an increasingly important role. Advances in understanding normal molecular cardiac development have led to the identification of numerous genes required in cardiac morphogenesis and drive to the discovery of a growing number of monogenic causes of human cardiac malformations. Sequencing of the human genome and advances in molecular techniques have led to an increase in proving the crucial role of the genetic factors.

Keywords: congenital malformations, genetic factors, newborn

Introduction
Cardiac malformations are structural or functional diseases of the fetal heart which manifest immediately after birth or later. These anomalies affect the heart and the intrathoracic great vessels and determine poor functioning or predisposition to malfunctioning [1-3]. The clinical signs are:
- cyanosis;
- cardiac murmurs;
- tachypnea;
- respiratory distress syndromes;
- heart failure;
- low pulse, petechiae, hypotension, metabolic acidosis, circulatory failure;
- abnormal heart rhythm (i.e. tachycardia/bradycardia, atrio-ventricular block);
- abnormal location, size and shape [4].

History and general presentation
When a newborn baby presents with cyanosis, respiratory distress syndrome and/or shock, the clinician must choose the right diagnosis, taking into consideration a pulmonary disease, a cardiac disease, a neurological disease or an infection.

Family history and pregnancy medication
a) the existence of a brother or a sister with congenital heart disease triples the risk of recurrence. If the mother suffers from a congenital heart disease, the risk for the child to be affected is higher;
b) alcohol and drugs (i.e. amphetamines, anticonvulsants, lythium, progesterone, estrogen) intake during the first trimester of pregnancy increases the risk of heart malformations. Viral infections (like rubella, enterovirus, coxsakie B), diabetes, systemic lupus erythematosus, maternal age of over 40, phenylketonuria, may be maternal conditions that predispose to heart malformations;
c) Pregnancy, labour and birth complications, may be considered risk factors for congenital heart diseases. For example, intrauterine and perinatal hypoxia is a risk factor for developing myocardial dysfunctioning and persistent pulmonary hypertension;
d) Assisted Reproductive Technologies: the risk of cardiac malformations in babies conceived by in vitro fertilization appears to be a little higher than naturally conceived babies. The most frequent congenital heart diseases are atrial and ventricular septal defects. There is no proven relation between in vitro fertilization and the incidence of cardiac malformations.

Neonatal history: gestational age, onset signs and severity [4,5,6]. If, during the first week of life, the newborn baby presents with general cyanosis, murmurs, heart failure and/or vasculatory collapse, the possibility of a congenital heart disease must be evaluated. Clinical history and detection of onset clinical signs are very important [3].

Prognostical classification of the fetal cardiac diseases
- associated pathology:
  - isolated 50-60%;
  - associated extracardiac malformation 10-20%);
  - genetic syndromes 30%.
- survival:
  - malformations incompatible with life;
  - malformations that can benefit from palliative treatment;
  - malformations that can be entirely treated [7].

On the basis of the prognosis and the possible, genetic or structural, associated pregnancy pathologies may be continued or aborted. Heart defects detected during intrauterine life may be more severe than those detected after birth [4,5].
During the last decades a multitude of studies on the genetic factors and molecular pathways that interfere with the development of the cardiac system have been made. Various structural genes, critical in normal cardiac morphogenesis, have been discovered as the result of these studies and helped the identification of the genetical ethiology of congenital heart diseases\(^\text{10,11}\).

In most of the children born with a congenital heart malformation, their condition is not associated with other inborn errors, but in 25-40% of the cases, heart malformation is associated with another anomaly or is a part of a genetic syndrome\(^\text{12}\). In approximately 30% of the affected children, a cromosomial defect is associated with a heart malformation\(^\text{13}\).

Aneuploidy, abnormal chromosomal number, represents an important proportion of the congenital cardiac malformation (Table 1), so, the most frequent syndromes associated with heart defects are as follows: 50% of the children born with 21 trisomy have a heart defect, 80% of the children with 13 trisomy, and almost all the children born with 18 trisomy have a heart defect, usually the defect being a septal one\(^\text{14}\).

Most cases of cardiac malformations are present in the Down syndrome, their severity influencing the

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Cardiac malformations</th>
<th>% with MCC</th>
<th>Other clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 trisomy Patau syndrome</td>
<td>DSA, DSV, PCA, SVSH, dextrocardia</td>
<td>80%</td>
<td>microcephaly holoprosencephaly, scalp defects, severe mental retard, polidactilia, cleft palate, urinary and genital malformations, omphalocele, microptalmia</td>
</tr>
<tr>
<td>18 trisomy Edwards syndrome</td>
<td>ASD, VSD, PAC, FT, DORV, ACo, BAV</td>
<td>90-100%</td>
<td>Polyhydramnios, rocky-bottom foot, hypertonia, biliary atresia, severe mental retard, diaphragmatic hernia, omphalocele</td>
</tr>
<tr>
<td>21 trisomy (Down syndrome)</td>
<td>ASD, VSD, SVSD, PAC, FT, HLVS</td>
<td>40-50%</td>
<td>Hypotonia, development retard, characteristic facial appearance</td>
</tr>
<tr>
<td>Turner syndrome</td>
<td>ACo, BAV, AS, HLVS</td>
<td>25-35%</td>
<td>Short stature, primary amenorrhea, flat chest with spaced nipples, short throat, lymphedema</td>
</tr>
<tr>
<td>Klinefelter syndrome</td>
<td>PAC, ASD, mitral valve prolaps</td>
<td>50%</td>
<td>High stature, hypoplastic testicle, delayed puberty</td>
</tr>
<tr>
<td>22q11.2 deletion (DiGeorge syndrome)</td>
<td>AAI Type B, aortic arch anomaly, arterial trunc, FT</td>
<td>75%</td>
<td>Hypoplastic thymus and parathyroid, immunodeficiencies, low implanted ears, hypocalcemia, mental retard, renal anomalies</td>
</tr>
<tr>
<td>7q11.23 deletion (Williams-Beuren syndrome)</td>
<td>Supravalvular AS, SPS</td>
<td>50-85%</td>
<td>Hypercalcemia, characteristic appearance, development retard, deafness</td>
</tr>
</tbody>
</table>

ASD = atrial septal defect; VSD = ventricular septal defect; PAC = persistent arterial canal; HLVS = hypoplastic left ventricular syndrome; FT = Fallot tetralogy; DORV = double outlet right ventricle; ACo = aortic coarctation; BAV = bicuspid aortic valve; IAB = interrupted aortic branch; AS = aortic stenosis; PS = pulmonary stenosis; AVSD = atrioventricular septal defect
Table 2  Genetical syndromes associated with congenital heart malformations

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Cardiac anomaly</th>
<th>Other associated signs</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noonan syndrome</td>
<td>PS, AVSD, HCM, ACo</td>
<td>Short stature, short throat, flat chest, delayed development, cryptorchidism, abnormal facial appearance</td>
<td>PTPN11, KRAS, RAF1, SOS1</td>
</tr>
<tr>
<td>Costello syndrome</td>
<td>PS, HCM</td>
<td>Short stature, delayed development, characteristic facies, high risk of carcinoma</td>
<td>HRAS</td>
</tr>
<tr>
<td>LEOPARD syndrome</td>
<td>PS, cardiac conduction abnormalities</td>
<td>Hypertelorism, genital anomalies, growth retardation, sensory deafness</td>
<td>PTPN11, RAF1</td>
</tr>
<tr>
<td>Alagille syndrome</td>
<td>PS, FT, DSA, PS</td>
<td>Cholestasis, characteristic facies, butterfly vertebra, ocular anomalies, delayed growth, deafness, horseshoe kidney</td>
<td>JAG1, NOTCH2</td>
</tr>
<tr>
<td>Marfan syndrome</td>
<td>Mitral valve prolaps</td>
<td>High stature, finger anomalies, scoliosis, skeletal anomalies, spontaneous pneumothorax</td>
<td>FBLN, TGFBR1, TGFBR2</td>
</tr>
<tr>
<td>Holt-Oram syndrome</td>
<td>ASD, VSD, AVSD</td>
<td>Limb defects, hematological anomalies</td>
<td>TBX5</td>
</tr>
<tr>
<td>Heterotaxic syndrome</td>
<td>DILV, DORV, TMV, AVSD</td>
<td>Intestinal malrotation</td>
<td>ZIC3, CFC1</td>
</tr>
<tr>
<td>Char syndrome</td>
<td>PCA</td>
<td>Dismorphic figures and digital anomalies</td>
<td>TFAP2b</td>
</tr>
<tr>
<td>CHARGE syndrome</td>
<td>ASD, VSD, valve defects</td>
<td>Coloboma, canal atresia, delayed development, genitourinary anomalies</td>
<td>CHD7, SEMA3E</td>
</tr>
</tbody>
</table>

*PS* = pulmonary stenosis; *AVSD* = atrioventricular septal defect; *HCM* = hypertrophic cardiomyopathy; *CoA* = aortic coartation; *FT* = Fallot tetralogy; *DSA* = atrial septal defect; *DSV* = ventricular septal defect; *DILV* = double inlet left ventricle; *DORV* = double outlet right ventricle; *TMV* = transposition of great vessels; *PAC* = persistent arterial canal

Table 3  Non-syndromic cardiac anomalies

<table>
<thead>
<tr>
<th>Cardiac anomalies</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD, FT, tricuspid valve anomalies, conduction anomalies</td>
<td>NKX2.5</td>
</tr>
<tr>
<td>ASD, VSD</td>
<td>GATA4</td>
</tr>
<tr>
<td>ASD, HCM</td>
<td>MYH6</td>
</tr>
<tr>
<td>Cardiac anomalies associated with pulmonary hypertension</td>
<td>BMPR2</td>
</tr>
<tr>
<td>BAV</td>
<td>NOTCH1</td>
</tr>
<tr>
<td>TGV</td>
<td>PROSIT-240</td>
</tr>
</tbody>
</table>

*ASD* = atrial septal defect; *VSD* = ventricular septal defect; *FT* = Fallot tetralogy; *TGV* = transposition of great vessels; *BAV* = bicuspid aortic valve; *HCM* = hypertrophic cardiomyopathy
Congenital heart disease and the role of genetic factors in cardiac morphogenesis

Roşca et al.

The development of fluorescence in situ hybridization technique (FISH), a procedure in which marked fluorescent probes are hybridized to metaphase chromosomes, detecting small submicroscopic chromosomal deletions, various syndromes caused by chromosomal defects have been elucidated (Table 1), such as 22q11deletion known as DiGeorge syndrome and William-beuren syndrome. DiGeorge syndrome is caused by the microscopic deletion on chromosome 22q11.2 and leads to defects of the heart, thymus and parathyroid glands, dysmorphic facies due to the abnormal development of the pharyngeal arch. The most frequent cardiac malformations are common arterial trunk and Fallot tetralogy. Genetic tests are required in patients with cardiac defects, William-Beuren syndrome caused by the microdeletions on the 7p11.23 chromosome, is characterized by cardiac defects, associates typical elf facial appearance, hypercalcemia, renal impairment and cognitive disability. Loss of elastin is thought to be the cause of cardiac defects in this syndrome.

Along with the progress in genetic technology and the discovery of the human genome, singular genetic defects that lead to syndromes associated with congenital cardiac malformations have been elucidated (Table 2). Most recent studies have made possible the discovery of the mutation of fibrillin 1, the cause of Marfan syndrome characterized by the progressive dilatation of the aortic root with a high risk of dissection, pathology of the lens and skeletal anomalies. Holt-Oram syndrome characterized by atrial and ventricular septal defects, a progressive disease of the atrioventricular conduction system and limb anomalies, is associated with mutations of the transcription factor Tbx5. Alagille syndrome, that encodes a ligand in signaling pathway Notch is characterized by intrahepatic biliary atresia and cardiac malformations (pulmonary stenosis, mpulmonary valve stenosis and Fallot tetralogy).

In Noonan syndrome phenotyp that includes cardiac defects, pulmonary stenosis and hypertrophic cardiomyopathy as well as mental retardation, characteristic figure, hemorrhagic disorders, mutations of protein tyrosine phosphatase non-receptor type 11 are involved in 50% of the cases. Heterotaxic syndrome that associates cardiac, pulmonary and gastrointestinal positional defects is commonly complicated with congenital heart diseases (atrioventricular septal defects, great vessels transposition).

Singular genetic defects associated with isolated/non-syndromic congenital heart malformations (Table 3) have also been discovered.

The progress in this field also proves that singular genic defects can lead to congenital heart diseases and reveal more information about the molecular pathways in cardiac morphogenesis.

In spite of the ultimate discoveries, the great majority of patients with mutated in colorectal cancers do not have singular genic defects. Along with the sequencing of the human genome, new information about the genetic variety has been reported.

One type of genetic variation, changes in children number, leads to a change in the gene dose and affects approximately 12% of the human genome. These variations are considered to be polymorphisms when present in more than 1% of population and are more susceptible in presenting associated diseases when present in less than 1% of the patients.

The variation in the number of copies is associated with cardiac malformations.

CHARGE syndrome, a constellation of anomalies that includes coloboma, cardiac defects, coan atresia, growth retard and anomalies of the ear, has been re-
cently associated with a microdeletion on the 8p21 chromosome\(^{(32)}\). Later sequencing of the critical region showed heterozygous mutations in chromodomain helicase deoxyribonucleic acid (DNA)-binding 7 (CHD7), a gene that encodes a binding protein in DNA\(^{(32)}\). Further studies have identified pathogenic mutations in CHD7 in over 50% of the patients with CHARGE syndrome\(^{(33)}\). Array and contributors helped with detecting the gene responsible for the great majority of cases of this syndrome, proving the importance of this method in discovering the gene, especially when studying rare diseases\(^{(33)}\).

Thien other authors studied patients with congenital heart malformations and other inborn anomalies and detected a variation in the number of copies in 30%\(^{(34)}\). The relation cause-effect was supported when variation in number of copies include important genes in cardiac development when they appeared by novo.

Variations in the number of copies were also identified in patients with isolated congenital heart malformations. They appear also in parents with no proof of congenital heart malformation, thus showing probably the fact that variation in number of copies increases the sensitivity in developing heart anomalies, also needing another factors.

The decrease in sequencing DNA price, the rate of discovering the gene in cardiac malformations may be higher.

At the same time with the discovery of new genetic anomalies associated with heart malformations, the development of a phenotypic information international database, one can study clinical results and prognosis on the basis of a genetic average\(^{(34)}\).

Yet, the high cost of these genetic tests and the impossibility of performing them in many countries, makes evaluation of children with congenital heart malformations syndromic or non-syndromic almost impossible.

When a new patient is diagnosed with heart pathology, the examination of the relatives to exclude genetic involvement is recommended as well as detailed evaluation of the newborn.

The clinical examination must search for dismorphic appearance, eye and ear anomalies, skeletal and limb anomalies, gastrointestinal and genitourinary pathology, a change in the neurologic status.

Multidisciplinary consults (neurological, ophtalmological, orthopedical, ORL, genetic, imagistic) and adjuvant tests (cardiac ultrasound, abdominal ultrasound, brain imaging) in establishing the diagnosis are recommended when necessary.

Genetic tests should be performed in the following situations:

- every newborn/child with suggestive phenotype
- every newborn with dysmorphic features, multiple anomalies, growth retard without a clear cause
- children with a family history of genetic anomaly
- when anomalies are discovered in fetal ultrasound examination

In case of a normal cariotype, when the child presents with suggestive clinical features for a genetic disorder, detailed genetic tests (i.e. FISH) are recommended for detecting a singular inborn anomaly\(^{(32)}\).

Discovering a genetic anomaly in a child with congenital heart malformation is beneficial for the patient and his/her family, alerting the specialist on the possible associated asymptomatic anomalies, on one hand, and conducting a family investigation which can detect genetic defects in other members, on the other hand, offering important data for genetic counselling of the family\(^{(33)}\).

The most important congenital defects

Congenital heart malformations are the most important congenital defects in a newborn\(^{(34)}\):

1. In Romania there is no efficient functional network for approaching this pathology, there is no national screening program for congenital heart defects to ensure the diagnosis, treatment and follow-up of these children. In our country the number of cardiovascular surgery centers is insufficient to treat all the newborns with cardiac malformations.

2. The obstetrician has an important role in detecting cardiac malformation by fetal 3-dimensional ultrasound. The neonatologist should be informed if there is a high suspicion of a cardiac malformation, as there are many that represent an immediate medical and surgical postnatal emergency.

3. If the diagnosis is made before birth, the pregnant woman can be sent to a high level of neonatal unit or to a specialized local cardio-vascular surgery center or abroad. Thus, mortality decreases and also neurological sequelae and the quality of life increases.

4. The family has an important role in detecting the patients with congenital heart malformations in time, through periodic antenatal and postnatal consults by the paediatrician or family practitioner when discovering certain abnormalities in the infant’s state (growth retardation, central or peripheral cyanosis, feeding difficulties, pale skin, respiratory effort, hypotonia).

5. The newborn should be examined in detail by the neonatologist and if there is suspicion of a heart disease he/she should be monitored in order to establish a rapid diagnosis. Many of the newborn babies with a congenital heart disease may have a real chance if diagnosis is quickly established and treatment is started immediately.

6. Neonatologists should be familiar with cardiac ultrasound as they are the main factor in the precocious detection of congenital heart diseases, thus contributing to a decrease in neonatal mortality and morbidity. If the newborn is in a critical state, neonatal transport to another unit for cardiac ultrasound is made with difficulty and aggravates the state.

7. Unless immediately treated, congenital heart malformations lead to existus or irreversible and severe complications such as persistent pulmonary hypertension, systemic arterial hypertension, hyper-
trophic cardiomiopathy, cardiac failure. All of these create a physical and psychological impairment for the patient. Patients diagnosed at a later time need a more laborious surgical technique or the surgery can not be performed anymore, leading to a decrease of the quality of life.

8. We also need to mention here long and short term cardiac complications of prematurity: persistent arterial canal and later right ventricular hypertrophy.

Conclusions
Genetic factors have a much greater influence on determining various types of cardiac malformations than it was established previous to the multitude of studies in this area. All accomplished knowledge regarding genetic influence over heart malformations can develop early detecting or preventing strategies, of great importance for future generations of affected children with isolated forms or genetic syndromes.

References