CONGENITAL HEART DEFECTS-PROGRESS AND PROSPECTS

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Congenital heart defects (CHD) are a leading cause of mortality in infants. Management of patients with congenital heart diseases has been greatly transformed during the past six decades; nevertheless the underlying causes for the defects and pathogenic mechanisms remain enigmatic. Tools of molecular biology and human genetics have provided avenues for recognizing disease genes causing congenital heart defects. A small portion of all cases of congenital heart defects can be attributed to Mendelian inheritance, chromosomal anomalies or the action of known environmental teratogenic agents. There is seldom any evidence of the causative agents in the common forms of CHD. A better understanding of the molecular and genetic mechanisms of heart development is expected to help in delineating the morphogenesis of cardiac anomalies and in defining ways of effective prevention or in utero repair of the defects at embryonic or fetal stage.

The beginning of a systematic approach to the study of congenital anomalies can be traced to Sir William Osler. Osler, when at McGill University initiated a collection of specimens of malformed hearts, which was referred to as the Osler collection. Osler encouraged Maude Abbott, who was curator of the medical museums at Montreal, to work at his museum and in 1936 they published the first atlas of congenital heart diseases. Some years earlier in his Hunterian lectures, published in The Lancet, Sir Arthur Keith had also chronicled a full account of cardiac malformations. These writings are considered to have spurred the development of pediatric cardiology and cardiac surgery.

Not much changed in the field of congenital heart disease until the middle of the 19th century. Congenital heart disease grew from a field of limited clinical interest into one of considerable practical importance in the 1940s.

Despite the early recognition of cardiac malformations, William Osler in his book The Principles and Practice of Medicine published in 1892, commenced the chapter on congenital heart disease, thus: “These (congenital affections of the heart) have only limited clinical interest, as in a large proportion of the cases, the anomaly is not compatible with life and in others nothing can be done to remedy the defect or even to relieve the symptoms”. Angiocardiography, cardiac catheterization and cardiac surgery rapidly changed this sense of futility. Surgical pioneers like Robert Gross, Alfred Blalock, William Rashkind and Aldo Casteneda and pediatric cardiologists like Helen Taussig paved the way for successful therapy in congenital heart disease as well as the growth of interest of researchers in elucidating the causes and morphogenesis of cardiac anomalies. Methods for diagnosis and defining the structural and functional defects associated with congenital cardiac anomalies have improved thanks to the advent of techniques such as echocardiography, angiocardiography, magnetic resonance imaging and multi slice computed tomography. Progress in pediatric catheterization, neonatal cardiac surgery and development of various interventional devices contributed to effective treatment of infants and children with congenital heart defects and prolonging their life span.

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Table 1. Classification of congenital heart diseases

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<thead>
<tr>
<th>Structural defects</th>
<th>Conduction defects</th>
<th>Functional defects</th>
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<tr>
<td>a) Simple</td>
<td>• Progressive cardiac conduction defect (PCCD) also called Lenegre or Lev disease</td>
<td>• Inherited primary cardiomyopathies</td>
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<tr>
<td>i) Simple</td>
<td>• Long Q/T syndrome</td>
<td>• Dilated</td>
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<tr>
<td>ii) Complex</td>
<td>• Restrictive</td>
<td>• Right Ventricular</td>
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<tr>
<td>iii) Abnormalities of valves</td>
<td>• Arhythmogenic</td>
<td>• Hypertrophic</td>
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<td>iv) Ebstein’s anomaly</td>
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<td></td>
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<tr>
<td>v) Tetralogy of Fallot</td>
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<tr>
<td>v) d,l-Transposition of Great Arteries</td>
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<td>v) Total Anomalous Pulmonary Venous Connection</td>
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Although diagnosis and management of CHD have made impressive progress, understanding of causes and pathogenesis has not. This is particularly true with respect to the role of the genome in congenital cardiac anomalies. Accurate genetic counseling requires not only precision in diagnosis but knowledge of the cause. Effective management of disease requires not only understanding of natural history and therapeutics, but also knowledge of pathogenesis. In most instances of cardiac anomalies causes and pathogenesis are unknown.

Studies on the causation and morphogenesis of congenital heart disease gained momentum only towards the later part of the 20th century. Several approaches have helped our understanding of the nature and origin of CHD. These comprise of careful inspection of the phenotypes, clinopathological and embryological correlation, genetic analysis, theoretical modeling, embryology, teratology and investigations of molecular and cellular dysfunction. These approaches have often been complementary. During the last two decades there have been major advances in understanding the cellular and molecular basis of development of the cardiac and vascular systems.

Several recent reviews offer a comprehensive coverage of selected aspects of cardiac development. A number of noteworthy books are also available on the subject.

About 8% of patients with CHD have a primary genetic basis for their heart defects. Almost this entire group has a CHD in association with a syndrome, either chromosomal or Mendelian. A chromosomal basis is seen in 5% and 3% have a mutant gene. Chromosomal abnormality such as Turner’s syndrome, trisomy 13, trisomy 18 and trisomy 21 are associated with CHD. Single gene abnormality associated with CHD is seen in Marfan’s syndrome, Holt-Oram syndrome and Alagille syndrome.

Following major advances in human karyotyping the association of cardiovascular maldevelopment with gross chromosomal errors is strikingly evident. In the past, normal chromosomal variations were suggested as causes of CHD. Ultimately it turns out that majority of cases of familial CHD cannot be explained by Mendelian modes or chromosomal aberrations, but are rather most consistent with the mechanism of multifactorial inheritance, in which a genetic – environmental interaction underlies the process of mal development. Generally it is believed that genetic and environmental influences are fairly well balanced in CHDs with a slight predominance of environmental influences in the ultimate expression of the mal development.

Chromosome banding techniques have allowed correlation of abnormal chromosomal segments with cardiac development in certain syndromes. Almost all the autosomal anomalies carry with them a high risk of cardiovascular maldevelopment. Chromosome studies have not demonstrated a relationship between chromosomal anomalies and discrete familial cardiovascular malformations unaccompanied by mal development of other systems and structures. Be that as it may, high resolution methodologies may prove informative and eventually provide a basis for prenatal diagnosis. It would be of interest to locate chromosomal regions having very specific influences on the development of the heart. It may also be possible to correlate congenital anomalies of discrete structures with minor chromosomal abnormalities.

The essential components of cardiovascular maldevelopment are the following:
(i) a genetic predisposition to react adversely to one or more environmental triggers, (ii) a genetic predisposition to one or more forms of mal development and (iii) exposure to an environmental influence at a vulnerable period of embryogenesis.

It is generally accepted that CHD will not be grasped until normal cardiac development is understood. A major area of uncertainty relevant to both normal and defective ontogeny is how genetically encoded chemical messages are transformed into processes with spatial orientation, handedness and highly organized timing.

Applications of new molecular techniques and model systems have provided insights into many of the very basic questions relevant to the control of cardiac morphogenesis. The new knowledge gained is
expected to throw light on the molecular basis of many forms of congenital cardiac defects.

New insights into the mechanistic basis that underlie controlled gene expression in the developing heart have been obtained from studies carried out in both non-mammalian and mammalian systems. They include:

(i) The molecular basis for positional information in the temporal path of gestation, (ii) differentiation of the mesoderm via the combination of positional information and inductive influences, (iii) restriction of a mesoderm subset to the cardiac muscle cell lineage and (iv) the activation of those genes which encode the proteins whose functions are responsible for the heart’s action as a pump.

The observation, that mice deficient for endothelin-1 have aortic arch and craniofacial anomalies along with VSD, provided one of the first cues that the endodermal signaling cascade might be involved in defining neural crest migration to the heart and pharyngeal arches. Recently, detailed examination of the developmental expression of endothelins-1&3 as well as endothelin receptors A&B during cranial and cardiac development in the human embryo has uncovered a very complicated pattern of expression. It is not surprising that defects in ET-1 lead to dramatic cardiovascular defects.

Insights into factors regulating delamination from the neural tube and migration to the conotruncal region of the heart are also beginning to appear. In an effort to study the role of gap junction proteins, Reaume and colleagues created a targeted null mutation of the connexin 43-(Cx43) gene. Mice carrying a homozygous null mutation died at birth not from a primary conduction abnormality (which may be anticipated) but from severe pulmonary outflow tract obstruction and right ventricular hypertrophy. Transgenic mice over-expressing Cx43 in the cardiac neural crest cell population also developed conotruncal defects involving the right outflow tract. These data strongly suggest that the level of Cx43 mediated intercellular communicaton is critical for normal cardiac neural crest migration to the outflow tract of the heart and thus is essential for normal conotruncal septation. A subsequent study using high resolution magnetic resonance microscopy and in utero ultrasound further detailed the abnormalities in conotruncal development that result from altered levels of Cx43 expression.

Disruptions of left-right patterning can result in catastrophic cardiovascular defects. A large number of molecules have been identified that have asymmetric expression in the early embryo and are potentially involved in defining left-right body plan. An exciting discovery is the demonstration that the iv (inversus viscerum) in the mouse results from a mutation in a gene encoding an axonemal dynein heavy chain. This mutation causes left-right inversion or situs inversus in half of live born homozygotes. The left-right dynein (lrd) expression can be detected by hybridization, well before the markers of asymmetry, in ventral cells of the node during gastrulation, but is not detected in the heart at any stage suggesting that it is involved in the very earliest stages of left-right determination.

Experiments of D.Srivastava and colleagues suggest that two complementary basic helix-loop-helix transcription factors dHAND and eHAND, restricted in expression to the right and left ventricles respectively, might play a critical role in determining left-right asymmetry in the developing embryo. Targeted disruption of dHAND resulted in marked attenuation of the primitive right ventricle and right ventricular outflow tract. Genetic crosses of dHAND and eHAND mutant mice with the inv/inv situs inversus mice showed that dHAND was expressed in the pulmonary ventricle and eHAND in systemic ventricle whether the ventricles were in their normal or reversed orientation. These studies provide opportunities to begin investigation of downstream targets of HAND gene activation and molecular characterization of ventricular specification.

It has generally been assumed that cardiac pump dysfunction observed in humans with CHDs or in animal models of CHD is secondary to the abnormal haemodynamics resulting from the structural anomalies. However, fundamental abnormalities in myocardial $\text{Ca}^{2+}$ regulation have been demonstrated in animal models of neural crest-related cardiac malformations. T.M.Nosek and colleagues physiologically ablated neural crest in chick embryos in order to generate embryos with persistent truncus arteriosus. Marked reduction in $\text{Ca}^{2+}$ transients were observed in the animals, which appeared to be because of impaired $\text{Ca}^{2+}$ induced $\text{Ca}^{2+}$ release from the Sarcoplasmic Reticulum and diminished SR reuptake of $\text{Ca}^{2+}$. The mechanisms remain unclear. Myocytes from embryonic hearts with persistent truncus arteriosus exhibit very small $\text{Ca}^{2+}$ transients and significantly diminished L-type $\text{Ca}^{2+}$ current. The implications of these findings for human cardiac anomalies are unclear.

In embryos with null mutations of the transcription factor NFATc or NFAT 2 (preferentially expressed by endocardial cells of the developing heart) there is complete absence of aortic and pulmonary valve formation as well as ventricular septal defects. While the embryonic stimuli for activation of NFATc and subsequent downstream targets have not been identified, further elucidation of major components in this signaling cascade may provide important insights into endocardial differentiation and semilunar valve formation.
Significant advances have been made in identifying congenital heart defect genes for specific lesions and in elucidating how mutations in the genes affect pathogenesis of lesions. Mutations in three transcription factor genes TBX5, NXX2.5 and GATA4 cause secundum atrial septal defects. The protein products of these genes interact and interfere in the interaction aids pathogenesis of the lesion. Mutations in CRELD1, which encodes a cell adhesion molecule can result in atrioventricular septal defects. A genetic locus for an autosomal recessive form of patent ductus arteriosus has also been identified.

Interestingly, familial bicuspid aortic valve is now recognized to be a channelopathy, resulting from mutations in the potassium channel gene KCNJ2. PTPN11 mutations, known to be a Noonan syndrome gene causes pulmonary stenosis and this results from gain of function effects on the protein product SHP-2. JAG1 mutations, which cause Alagille syndrome give rise to left side heart lesions and tetralogy of Fallot in addition to pulmonary branch stenosis.

Table 2. Genes associated with certain subtypes of congenital heart defects

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<tr>
<th>Cardiac phenotype</th>
<th>Genes affected</th>
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<tr>
<td>VSD</td>
<td>TBX1, TBX5, NXX2.5</td>
</tr>
<tr>
<td>ASD</td>
<td>TBX5, NXX2.5</td>
</tr>
<tr>
<td>TOF</td>
<td>JAG1, TBX1, NXX2.5</td>
</tr>
<tr>
<td>PDA</td>
<td>TFAP2B</td>
</tr>
<tr>
<td>Ebstein’s anomaly</td>
<td>NXX2.5</td>
</tr>
<tr>
<td>Pulmonary atresia</td>
<td>PTPN11</td>
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Availability of a genetic blue print for the developing heart symbolizes the progress in biology of congenital heart diseases. Genetic or epigenetic alterations of cardiac transcription factors and proteins that functions as signaling molecules during early stages of heart development can be considered as important factors in the causation of CHD. By knowing the genetic cause of CHD it may be feasible to correct the defects at the embryonic stage.

Intra uterine interventions for CHD at the gene level [gene therapy] are a future possibility. The most common strategy is exogenous delivery of the gene of interest. Gene therapy could be either for gain of function where the expression of endogenous genes is deficient as a result of mutation or for blockade of the gene in cases where over expression of the endogenous gene is the cause for the disease. The genes are exogenously delivered so as to recreate its function. The choice of the cardiotropic vector systems such as adeno associated virus (AAV) that are capable of continuous expression of the gene of interest, the route for exogenous gene delivery, dosage and volume of genetic material to be delivered are important factors to be considered for effective gene transfer. An important factor to be considered when gene therapy is intended for in utero correction of CHD is the time at which the gene of interest exerts its effect on heart development. Experimental results on animal models of CHD suggest the possibility of gene therapy in some forms of inherited cardiomyopathies. Kawada et al. and Ikeda et al. with their experiments on hamsters have demonstrated that intramyocardial delivery as well as coronary retro infusion of adenosinal vectors coding for δ-sarcoglycan can rescue progression of cardiomyopathy, improve ventricular function and thereby increase life expectancy. Nuss et al. and Donahue et al. in their experiments on rabbits and pigs respectively could demonstrate that myocardial delivery of genes coding for channel proteins or regulatory G-proteins can correct genetic defects associated with long QT syndromes and may help in the treatment of atrial arrhythmias. α-MHC-AAV mediated overexpression of endoglin, NXX 2.5, TBX5, TFAP2B have also been shown to be able to correct septal defects, patent ductus arteriosus, arteriovenous malformations, looping and conductance defects.

As most of the structural and functional defects of the heart resulting from gene mutations are irreversible, the corrective measures should be performed before the developmental programs are affected. Hence diagnostic tools for the detection of gene mutation before the onset of disease are necessary for effective intervention directed at the defective genes. Recent advances in molecular biology which started with the use of chromosomal mapping and identification of mutations and polymorphisms in the causation of CHDs have revolutionized pediatric cardiology. Cell- free fetal (cff) nucleic acids in maternal plasma are now an established diagnostic specimens used in non-invasive prenatal diagnosis of CHD during the second trimester of pregnancy. Functional genomic analysis of cff nucleic acids obtained from amniotic fluid may provide insights to disrupted development as well as disease mechanisms. Recently, Anjali et al. with proteomic protein expression analysis performed on human amniotic fluid demonstrated that a cluster of proteins (WNT16, ST14 and Pcsk1) can function as biomarkers of CHD.

Novel pharmacological strategies and minimally invasive fetal cardiac interventions are available for management of congenital heart diseases. Advances in imaging and instrumentation have facilitated greater precision and effectiveness of fetal cardiac intervention which include sophisticated image-guided or robotic interventional
approaches for complex congenital heart defects. Be that as it may, currently, the treatment options are limited and, long term results are not known. Cell based therapy using stem cells are presently explored as a plausible intervention for pediatric congenital heart disease. The Mayo Clinic last year announced a clinical trial which aims to determine how stem cells from autologous umbilical cord blood can help children with hypoplastic left heart syndrome.

**Conclusions**

Considerable progress has been made in the efforts to understand the incredibly complex processes involved in the formation of the cardiovascular system and the genetic basis of congenital heart disease. Much however, remains to be learned. Current knowledge suggests that hearts have developmental defects when there is an abnormal gene resulting from either a chromosomal anomaly or a mutation in the gene. Qualitative or quantitative defects in the expression of various molecules associated with events in cardiac development such as neural crest migration, cardiac looping, chamber specification and synthesis of contractile proteins or extracellular matrix could lead to cardiac defects. Intricacies of abnormal cardiac development are likely to be unraveled in the coming years. The implications include the ability to perform precise molecular diagnoses, early detection of cardiovascular abnormalities, more rational and specific therapeutic interventions, genetic approaches to treatment and ultimately prevention of many forms of congenital and familial cardiovascular diseases. Alas, research in this domain in India is still in infancy.

**References**