Genes in Cardiovascular Malformations: A Review

PRIYA RANGANATH[†]

Department of Anatomy, Bangalore Medical College & Research Institute, Bangalore 560002, Karnataka
E-mail: priya_ranganath@rediffmail.com

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ABSTRACT: Heart defects have an incidence of about 1 in100 live births. They are one of the common classes of congenital malformations. In the present review, different defects in the heart produced by different genes are enumerated. Different syndromes with genes involved in each of them are discussed. They may also have an environmental basis, as considered by many authors. The greater sophistication of cytogenetic and molecular genetic techniques and an increased willingness among geneticists to address complex traits has resulted in a similar growth in interest among geneticists.

INTRODUCTION

With an incidence of almost 1 per 100 live births, heart defects represent the most common class of congenital malformations. Because of the close physiological balance of the circulation, most malformations produce symptoms. Cardiovascular embryology involves a large number of genes.

GENES IN VARIOUS DEFECTS OF HEART

Septal Defects: Individuals with autosomal dominant mutations of Nkx2-5 gene have a high incidence of abnormalities of septum secundum, resulting in atrial septal defects. Associated with ASD is an equally high incidence of AV block, which can lead to sudden death in affected individuals whose hearts are not assisted by pacemakers. Another condition that is strongly associated with atrial (and ventricular) septal defects, as well as limb anomalies, is the Holt-Oram syndrome. This syndrome is caused by a mutation in the T-box gene Tbx-5, the gene that is expressed in the upper limb, but not the lower limb.

Persistent AV Canal: A number of specific causes of AV canal defects may exist, ranging from inappropriate expression of Msx-1 to defects in the production or reception of inductively active extracellular matrix components (e.g., subunits of adherons) by the endocardial cells.

Malformations of Outflow Tract: All malformations of this area cannot be attributed to defective neural crest development. A number of defects of the outflow tract are associated with translocations or deletions in chromosome 22, and most of these involve the neural crest. Lesions of the outflow tract can be produced experimentally by interfering with function of specific genes, often genes located on chromosome 22 and genes that affect properties of cranial neural crest cells. For example, outflow tract abnormalities are seen in mice deficient in neurotrophin-3 (NT-3), a member of the nerve growth factor family. In addition, mutations of components of a cascade, starting with endothelin-1, HAND-2, and then neuropilin-1, a receptor for semaphoring in the nervous system and VEGF in the vascular system, all produce varying degrees of outflow-tract anomalies.

[†]Professor & Head

Stenosis: A lesion reminiscent of pulmonary stenosis has been produced in mice bearing a null mutant of the gene for connexin-43, which encodes a protein component of the gap junction channel. Why such a genetic lesion would affect principally the pulmonary outlet of the heart is not yet known. Pulmonary stenosis or tetralogy of Fallot is one of the conditions that characterizes the Alagille syndrome. This condition is caused by a mutation in Jagged-1, a ligand of the Notch receptor.

Interruption of the Left Aortic Arch: To be compatible with life, this lesion is usually accompanied by a patent ductus arteriosus, which allows blood flow to the lower part of the body. This lesion has been produced in mice that are lacking in the winged helix transcription factor MFH-1 (mesenchyme fork head-1).

Coarctation of Aorta: In preductal coarctation, which may be related to inadequate expression of MFH-1, the ductus arteriosus typically remains patent after birth.

Systemic Venous Drainage: It is rarely of major genetic importance, although absence of the last segment of the inferior vena cava and its replacement by an azygos connection to the superior vena cava is a valuable diagnostic sign of lack of a morphologic right atrium in left isomerism sequence. Abnormality of pulmonary venous drainage is of much greater significance. If one or more of the pulmonary vein orifices are displaced, the term anomalous pulmonary venous drainage is applied, which may be partial, involving up to three vessels, or complete, when it is known as total anomalous venous drainage (TAPVD). Displacement to the right on the posterior atrial wall is one cause of a sinus venosus ASD. In the absence of a morphologic left atrium in cases of disturbed laterality, the pulmonary veins find an outlet to one of the neighboring venous structures, such as superior vena cava or to the hepatic veins below the diaphragm.

Vascular Malformations and Hemangiomas: Localized abnormalities of the vasculature exist in a number of forms. One of the most dramatic is a hemangioma, which is actually a vascular tumor that typically appears within a few weeks of birth, rapidly expands, and then spontaneously regresses, usually before age 10. The endothelium of a hemangioma is mitotically very active. In contrast, vascular malformations are typically purplish, with a somewhat raised and irregular surface. Malformations of larger vessels consist of tangles of vessels that are relatively inactive mitotically, and their growth typically keeps pace with that of the rest of the body. They do not regress. Some familial cases occur because of mutations of Tie-2, the angiopoietin receptor. These mutations paradoxically result in a more active sprouting stimulus. Capillary malformations, often called port wine stains, constitute the most common vascular anomaly of the skin. Typically reddish and later changing to purple, these are harmless, but do not disappear.

Certain Hox genes, expecially those of the Hoxa and Hoxb families, play an important role in some aspects of hematopoiesis. Exposure to bone marrow to antisense oligonucleotides against specific Hox genes results in the suppression of specific lines of differentiation of blood cells. Conversely, engineered over expression of genes, such as Hoxb-8, Hoxa-9, and Hoxa-10, causes leukemia in mice. Evidence is increasing for the involvement of Hox genes in the pathogenesis of human leukemias. One important function of the Hox genes in hematopoiesis is the regulation of proliferation.

GENES IN DIFFERENT SYNDROMES WITH HEART DEFECTS

Trisomy 21 (Down syndrome): Trisomy 21 remains the most important association between major chromosome defects and heart malformation. Before the introduction of widespread maternal serum screening for Down syndrome, 5% of heart defects in newborn infants were accounted for by trisomy 21 cases. In addition to this numerical importance, there is a specific association of great interest in the molecular studies of heart defects; about 40% of children with Down syndrome have heart defects, and of these, almost half have the otherwise rare AVSD. On the basis of quantitative Southern blot analysis and FISH studies in cases of partial duplication, the critical region for the Down phenotype to the telomeric segment of the chromosome beyond 21q22.12 has been localized. Mouse chromosome 16 has a large segment of homology with human trisomy 21; the trisomy 16 mouse has heart defects that closely resemble those seen in trisomy 21. The most telomeric

segment, including most of band 22.3, does not share homology with the mouse chromosome 16. The gene or genes responsible for the AVSD in Down syndrome likely reside in a 6.63 Mb segment of which 21q22.2 forms the middle third (Korenberg and Kurnit, '95). A gene which continues to attract great interest is the col6Al gene (Davies *et al*., '95). Haplotype analysis within this gene in Down syndrome families revealed a nonrandom distribution, suggesting that the contribution from the disjoining parent may be a determinant of CHD risk in Down syndrome babies.

Collagen VI is a candidate gene because it maps to human chromosome 21, but a major argument against this gene is that it does not map to chromosome 16 in the mouse (MacDonald *et al*., '91). The growth and/or adhesion of the endocardial cushions or the cellular structures in their immediate vicinity, is highly likely to be implicated in the disease process. Identification of genes expressed in the cushion region that are chromosome 21-specific should greatly reduce the number of genes of interest. Elaborate analysis of heart cDNA libraries may be necessary, since the relevant gene may be more widely expressed. Kurnit and colleagues ('85) put forward the hypothesis that there was a failure to form the atrioventricular septum because the over expression of a gene involved in cell adhesion led to premature cessation of growth.

Trisomy 18 (Edwards Syndrome): Heart defects are a recognized association and are often quoted as the cause of early demise. Embleton and colleagues ('96) reviewed all cases of trisomy 18 over a six-year period in northern England and identified 66 babies and fetuses among 282,583 births. Postmortem examination, echocardiography, or both was carried out in 25 babies of which 21 had a heart defect. Of the remaining nine live born neonates, six had no heart murmur or heart defect, and two had murmurs without failure but died of apnea. Of the 21 heart defects, 7 were VSDs, 5 were AVSDs, 2 were double outlet right ventricles, and 3 were hypoplastic left hearts. In only 5 was the heart defect considered the cause of death, yet all babies in the series had died by the end of the first year.

Trisomy 13 (Patau Syndrome): There is a high incidence of cardiac defects, in particular ASDs and VSDs. Disturbance of cardiac position, including dextrocardia, is common (Schinzel, '83), suggesting a role for a gene or genes on chromosome 13 in laterality development.

Tetrasomy 22p (Cat Eye Syndrome): Tetrasomy 22p individuals typically show a marker chromosome, an isochromosome of 22p. The marker may be lost from the blood and other tissues with age (McDermid *et al*., '86). Clinical features of heart failure were noted when seen in the genetic clinic despite an absence of heart murmurs. The strong association of tetrasomy 22p with total anomalous pulmonary venous drainage prompted referral for echocardiography. TAPVD was found and treated, but not before irreversible lung damage due to pulmonary hypertension had resulted. The strong association of TAPVD with tetrasomy 22p is another clue to the location of key genes in heart development.

Tetrasomy 12p (Pallister-Killian Syndrome): About a quarter of children with tetrasomy 12p have heart defects, VSD being the most common. Other common defects include coarctation of the aorta, PDA, ASD, and aortic stenosis (Reynolds *et al*., '87).

Based on studies of aborted fetuses, it is estimated that up to 99% of 45,X fetuses are lost as early miscarriages. At least some of these losses have a cardiovascular basis, particularly hypoplastic left heart. The birth incidence is at least 1 in 1850 live births (Nielsen and Wohlert, '91). Simpson ('76) found that among 228 pooled cases, 22 (10%) of heart defects were listed, though details of cardiovascular status were provided in only 119, for a potential prevalence of 16%. Miller and colleagues (Miller *et al*., '83) identified 80 girls with 45,X in a pediatric endocrinology clinic, 13 of whom had aortic coarctation. In 35 girls not known to have a heart defect, echocardiography revealed bicuspid aortic valve in 12, mitral valve prolapse in 2, and idiopathic aortic root dilatation in 2. Conversely, heart defects are rarer in women presenting with Turner syndrome in gynecology clinics. There is likely to be an association between dysmorphic features such as neck webbing and heart defects, which would result in a greater prevalence of heart defects in cases diagnosed earlier. As a general guide it would be reasonable to deduce that 10% of all girls with Turner syndrome have a clinically evident heart defect and that a further 10% will display pathology at echocardiography.

Studies of parental origin of chromosomal loss reveal evidence of imprinting. Chu and colleagues (1994) found 9 out of 43 girls who had retained the maternal X had heart defects as opposed to only 1 out of 20 girls with a retained paternal X. With pooled data from 3 previous studies, there were 34 of 90 cases of heart malformation where the mother's X chromosome was retained and only 4 of 38 where the father's X chromosome was retained. Unrecognized mosaicism could explain the results, but it seems more likely that a gene or genes on the paternal X are important in normal development of the aorta or its surrounding structures. There was a similar parent of origin effect on the presence of neck webbing, supporting the belief that these malformations may have a common pathogenesis (Clark, '84).

Wolf-Hirshhorn Syndrome: Heart malformations are frequent but not specific to the syndrome. Prognosis is poor, although cardiovascular problems are rarely a major factor in survival.

Williams Syndrome: The cardinal cardiovascular malformation is supravalvular aortic stenosis (SVAS). Less well recognized and more difficult to detect are peripheral pulmonary artery stenoses, which produce murmurs over the lung fields. The striking association with SVAS, which had been described as an isolated dominant trait in many families, prompted the speculation that Williams syndrome would turn out to be a deletion involving the gene responsible for SVAS (Burn, '86). The identification of a balanced translocation associated with SVAS provided the clue. Curran and associates ('93) showed first that dominant SVAS mapped to chromosome 7 at a translocation break point (Ewart *et al.*, '93a) and subsequently that most Williams syndrome cases could be shown to have a deletion of this region of chromosome 7 (Ewart *et al*., '93b). Elastin was shown to be the causative gene in isolated SVAS by the demonstration of loss of function mutations (Ewart *et al.*, '94). A 500 kb region in 7q11.23 deleted in a series of 30 patients with Williams syndrome was characterized. A total of nine transcribed segments were identified including the previously recognized genes ELN, for tropoelastin, LIMKI and RFC2.

Alagille Syndrome: Up to 90% of affected individuals have single or multiple areas of peripheral pulmonary artery stenosis. In about one-third, a variety of other intra and extra-cardiac malformations are seen (Mueller and Emslie, '95). There was a case of mother and child with a deletion (Anad *et al*., '90) involving 20p11-2. This deletion is demonstrable in only about 10% of cases. The critical region was found to be at 20p11.2-20p12 (Hol *et al*., '95). Subsequent studies have shown that loss of the JAGGED1 gene is responsible for the phenotype.

The 22q11 Phenotype: 22q11 deletion is associated with a wide spectrum of heart malformations and is the most common autosomal deletion known.

DiGeorge syndrome and the immunology perspective: Cardiac malformation, particularly defects of the outflow tract, were a key feature of he syndrome (Freedom *et al*., '72).

Takao (conotruncal anomaly face) syndrome and pediatric cardiology perspective: The Japanese group led by Takao ('80) was the first to recognize the major contribution of the phenotye ('conotruncal anomaly face') to the patient population with outflow tract defects.

Radford ('85; '88) also recognized the association of dysmorphic facies with outflow tract defects (Burn, 1989) particularly truncus arteriosus and tetralogy of Fallot.

Strong syndrome and familial perspective: Strong ('68) reported on a family with right-sided aortic arch.

The 22q11 Genotype: There are about 20 genes in the deleted segment most commonly lost. A number of these have been implicated in the neural crest migration defect implicated in the phenotypes resulting from 22q11 deletion. An early candidate was TUPLEI (Halford *et al*., 1993), later renamed HIRA, which has a role in development of the outflow tract of the heart (Farrell *et al*., '99). It shows an expression pattern which predicts a neural crest role. Most recently, mice homozygous for a deletion o fTBX1, a transcription factor, have been shown to manifest the CATCH phenotype (Jerome and Papaioannou, 2001). Lindsay *et al.* (2001) in parallel experiments also demonstrated the pivotal role of TBX1 using an elegant transgenic mouse approach. Having engineered a deletion (df1) of the mouse chromosome 16 area syntenic to 22q11.2, they narrowed the critical area to 200kb, including Tbx1. Gene targeting of Tbx1

reproduced the aortic arch abnormalities in a Tbx1/df1 compound heterozygote. Heterozygous mice were normal. This may reflect a greater developmental sensitivity in humans or, more likely, that several of the genes in the critical region including TBX1, HIRA, UFDIL (Yamagishi *et al*., '99) and CRKL (Guris *et al*., 2001) are implicated. It remains unclear whether several genes must be haploinsufficient to cause a clinical phenotype or whether a single locus predominates. Of the14 coding sequences identified in the critical region to date, the most interesting remains Tuple 1 (Halford *et al*., '93).

There was a family comprising of four sibs, two of whom had undergone surgical correction of heart defects before the birth of the girl with DiGeorge syndrome. One had had a coarctation of aorta and the other a VSD. All three were found to have a deletion inherited from the mother (Wilson *et al*., '91). This family lent support to the hypothesis that familial outflow tract defects might result from 22q11 deletion in the absence of other features of the syndromes.

Deletion analysis was carried out in a series of families where there had been more than one case of heart malformation with at least one case involving the outflow. In five of the nine families, 22q11 deletion was apparent (Wilson *et al*., '92). This finding made 22q11 deletion of primary importance in the genetic management of families wit children affected by heart defects; any child with an outflow tract defect and dysmorphic features became eligible for deletion analysis.

Goldmutz and colleagues ('93) found five cases among 17 patients with isolated outflow tract defects. In their series, deletion is particularly likely in type B interrupted aortic arch and truncus arteriosus but is rarely associated with transposition of the great arteries. In the northern region of England, detailed analysis of all significant heart defects over five years revealed a steady annual rate of around 200 cases among 40,000 births. Among 1993 births, 10 affected children had been fund by spring 1994. This suggests that 22q11 deletion can account for 5% of all heart defects and has a minimum prevalence of around 1 in 4000. One of the affected infants born in 1993 died at the time of catheterization. The father was found to carry the deletion. His heart was normal on echocardiography. His wife was pregnant at the time of diagnosis. The couple chose prenatal diagnosis, which revealed a deletion in the fetus. Subsequent fetal echocardiography revealed a complex outflow tract malformation with pulmonary atresia, and the parents decided to terminate the pregnancy.

In an assembled data on 558 cases of 22q11 deletion (Ryan *et al*., '97), 28% of the cases where parents had been tested had inherited deletions with a marked excess of maternally inherited deletions. 8% had died, over half within a month of birth primarily due to severe congenital heart defects, though a contribution from immune deficiency is likely in many. Clinically diagnosed immune deficiency was uncommon. When measured, calcium was low in 60% of cases, and 75% had a clinically significant heart defect. Among 409 patients with a heart defect, 355 fell into the five diagnostic categories tetralogy of Fallot, VSD, interrupted aortic arch, pulmonary atresia VSD and truncus arteriosus.

Phenotype/Genotype Correlation: The CATCH Phenotype: CATCH 22 (cardiac defect, abnormal facies, T-cell deficit, cleft palate, hypocalcemia due to 22q11 deletion) embraces several phenotypes. Those presenting with a predominantly cardiac phenotype may be called Takao syndrome in preference to conotruncal anomaly face syndrome.

All children with an outflow tract defect and dysmorphic features should have a deletion analysis. It is highly likely that the prevalence studies now underway will confirm that all children with significant defects of the outflow tract of the heart or the palate should be tested in view of the possibility of recurrence in a more severe form.

OTHER CHROMOSOMAL SEGMENTS ASSOCIATED WITH HEART MALFORMATION

Brewer *et al*. ('98) performed a meta-analysis of reports of chromosome deletion and identified several areas frequently associated with particular heart defects. They include deletion of distal 11q in hypoplastic heart. Such cases are associated with trigonocephaly and attract the eponym Jacobsen syndrome. Another association worthy of note is 8p deletion with AVSD.

Noonan Syndrome: Noonan and Ehmke ('63) reported 9 children with valvular pulmonary stenosis.

2/3 of children with Noonan syndrome have heart defect with valvar pulmonary stenosis in 50%. Often the valve is dysplastic making balloon dilatation more difficult. Among a variety of other defects reported the most frequent are ASD, asymmetric septal hypertrophy, and PDA. VSD occurs in about 5%. The electrocardiograph typically shows left axis deviation with a wide QRS complex, giant Q waves and a negative pattern in the left precordial leads.

Holt-Oram Syndrome: Holt-Oram syndrome is the best recognized of the head hand syndromes. The characteristic anomalies are underdevelopment of the shoulder girdle with triphalangeal thumb and ASD (Newbury-Ecob *et al*., '96). About half of gene carriers have a secundum ASD with occasional reports including VSD, AVSD, and truncus arteriosus. Genetic studies have shown this autosomal dominant trait to be heterogenous. Some families map to 12q21.3-q22 (Bonnet *et al*., '94), but Terrett and associates ('94) found two clinically indistinguishable families which did not show linkage. The causative gene defect involves loss of function of TBX5, another of the 12 or so transcription factors characterized by the presence of the T box domain, a DNA-binding motif.

Ellis Van Creveld Syndrome: About 60% of the affected individuals have a heart malformation (congenital morbus cordis). There is a failure of primum atrial septum. In some cases, this is associated with abnormality of the AV valves, and, rarely, there is a complete AVSD. In some children there is a common atrium, suggesting that the gene plays a key role in formation of the primum septum rather than the endocardial cushions.

The causative gene on chromosome 4p16, designated EVC, encodes a 992 amino acid residue protein of uncertain function (Ruiz-Perez *et al*., 2000). Pathological mutations were identified in 7 pedigrees. On interest in the search for additive genes relevant to non-syndromic heart defects was a missense mutation in another family, a father and daughter, both of whom had the typical heart defect and polydactyly but normal stature.

HEART MALFORMATION AND METABOLIC DISORDERS

Zellweger or Cerebrohepatorenal Syndrome: Thymus hypoplasia and outflow tract malformations occur (Heymanns, '84). There are genetic defects in peroxisomal assembly or the functioning of the single matrix enzymes including PEX1 on chromosome 7q (Fitzpatrick, '96).

Genes Responsible for Congenital Heart Malformations as Monogenic Traits: The investigation of deletion syndromes and traditional mapping has identified the location of genes, and in some cases their identity, which, when defective can produce isolated heart defects. The classic examples are ELN as a cause of supravalvular aortic stenosis and JAG1 leading to peripheral pulmonary artery stenosis. Mapping studies and candidate gene analysis led to the discovery of Zic3 as a cause of X-linked laterality syndrome, and the same approach yielded TFAP2B as the cause of autosomal dominant PDA (Satoda *et al*., 2000). Mapping studies point to a locus at 4q12-13 (Bleyl *et al*., '95) responsible for total anomalous pulmonary venous drainage and another at 1p31-21 (Sheffield *et al.*, '97) causes one form of autosomal dominant AVSD. NKX2.5, a homologue of the Drosophila gene Tinman, a gene so named because without it the fly has no heart, is responsible for an autosomal dominant cardiac syndrome characterized by ASD with atrioventricular conduction defects. About 18% of cases manifest a VSD instead (Schott *et al*., '98). Mutations leading to loss of function and upregulation seem to produce the same phenotype. The propensity to sudden death in these patients makes it of clinical importance to recognize this phenotype. NKX2.5, also known as CSX, is a cardiac transcription factor containing a homeobox domain.

GENES VERSUS ENVIRONMENT

Many authors have favored a major environmental contribution on the basis of twin studies. The 'twin method' provides an estimate of genetic contribution to a disorder by comparing concordance rates in monozygotic (MZ) and dizygotic (DZ) twins; since the former share all their genes, any trait with a genetic component should be more likely to affect both members of the pair than in DZ twins where they share only half their genes. An underlying assumption is that twins are biologically comparable to their singletons. This may be true of DZ twins but cannot be assumed in MZ

twins who are exposed to major and unique intrauterine influences. MZ twins are 'a malformation to whom nature was kind'. Galton in his original work avoided the issue by focusing on disorders perceived to be of postnatal origin. Price ('50) first enumerated the way in which the influences of the twinning process might disturb early development and concluded that application of the twin method to malformations would result in an underestimate of genetic contribution. This did not deter several authors stating that heart defects are not genetic because they usually affect only one member of MZ twin pairs.

McKeown and Record ('60) noted an apparent excess of heart defects in like-sex twins. Leck ('60) and Campbell ('61) raised the possibility that the twinning process itself might result in a heart defect in one of the pair.

Heritability estimates are liable to major error if the twinning process itself is associated with the disorder under study. A variety of mechanisms have been invoked to account for excess malformations among twins. The likelihood is that any of the possible mechanisms would cause a defect in only one of a pair, increasing the number of discordant pairs. The wide variation in heritability estimates emphasizes the unreliability of the twin approach and the likelihood that genetic contribution is greater than twin studies have suggested.

DISTURBANCE OF LATERALITY AND THE HEART

Kartagener syndrome, the association of pulmonary ciliary dyskinesia with situs inversus, nasal polyps and bronchiectasis, is a heterogenous disorder. Histologically cilia typically lack dynein arms. The ciliary axoneme comprises over 100 polypeptides while dynein itself is a complex protein with several subunits. Mutations have been found in axonemal dynein intermediate chain gene 1 (DNAI1) (Guichard *et al*., 2001). The human arthologue of lrd, the gene defective in the iv mouse, is on chromosome 7 and a mutation has been identified in this gene in a child known to have paternal uniparental disomy of chromosome 7 and primary ciliary dyskinesia (Bartoloni *et al*., 2000).

Isomerism Sequence

Only one of the genes in the pathway by which the left-right axis is established was identified through investigation of a family with laterality disturbance, ZIC3 (Gebbia *et al*., '97). Midline defects such as agenesis or neural tube defects often occur along with laterality disturbance in patients with mutations in this X-linked gene. Manifesting females have reported and there has been one case of a phenotypically normal male carrying a mutation associated with congenital heart disease into her family members (Megarbane *et al*., 2001). ZIC3 mutations account for a minority of laterality disturbance cases. There is also evidence of recessive form (Burn *et al*., 1986; Gatrad *et al*., '84; Rimoin *et al*., 2007; Carlson, 2004)

CONCLUSION

As with all birth defects, chromosomal aneuploidy should be suspected in any newborn with congenital heart disease. The association of heart disease with a specific pattern dysmorphic features may suggest a syndrome. Maternal history will identify those associated with the known teratogens. Clinical examination will identify those liable to be associated with a chromosomal deletion not apparent to routine karyotype analysis. A combination of family history and dysmorphic features can identify a further group of heart defects associated with monogenic syndromes. In a small proportion of isolated heart defects, the pedigree is sufficient to recognize monogenic inheritance. The rest must be addressed in the clinic using empirical data from traditional family studies. Chromosome analysis should be performed in any infant presenting with a heart defect associated with other malformations, or dysmorphic features, or both. If the defect is suspected at or before birth, there are usually sufficient mitotic cells in cord blood to allow a rapid analysis. More often, there is time to perform standard analysis, particularly now that widespread use of prostaglandin inhibitors to keep the duct open and maintain circulation has made the need to intervene immediately less common. An exception is that transposition of the great arteries with an intact septum requires immediate action to prevent death. It is rarely associated with chromosomal defects.

Folic acid supplementation reduces the number of conotruncal heart malformations in the offspring. It will be interesting to see if the C677T polymorphism in the methylene tetrahydrofolate reductase gene, which reduces bioavailable folate in homozygotes, is associated with an increased risk of heart malformation.

In the future we may anticipate a greater focus in counseling based on molecular testing for genes that predispose a person to heart defects. The likely success of this approach will depend on the relative contribution of genetic versus non genetic factors and the likely number of genes involved in an individual.

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