

## Genetics and Congenital Heart Disease: Perspectives and Prospects

REED E. PYERITZ, MD, PhD, EDMOND A. MURPHY, MD, ScD

Baltimore, Maryland

Elucidating the role of genes in the ontogenesis of the cardiovascular system is a task that involves many fields of inquiry. Recent dramatic advances in the molecular biology of transcription and its variations and the prospects for sequencing the entire human genome must not induce complacency; the major task of determining how a one-dimensional code specifies a three-dimensional structure demands an understanding of biologic systems considerably beyond the current level. The study of pathologic cardiovascular ontogeny is equally in need of new insight and

fresh approaches. Although all clinicians might agree that genes are important contributors to both the etiology and the pathogenesis of congenital heart defects, with the exception of a few Mendelian conditions, this knowledge cannot be put to practice beyond crude statements of empirically determined probabilities. In this review, we selectively examine studies that are addressing what we perceive as provocative issues and suggest some areas, such as chaos theory, in which new ideas might be found.

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Congenital anomalies of the structure of the heart have been described since the time of Leonardo da Vinci and Morgagni, but it was Maude Abbott (1,2) early in this century who first investigated systematically and rigorously (from 1,000 personal cases) the pathology of such anomalies. Helen Taussig (3) extended this enquiry to the bedside and showed that much pathology could be deduced by clinical examination and the simple tests then available, the electrocardiogram and chest radiograph. Most clinicians, however, dismissed specific diagnosis because they believed that cardiac anomalies "... were hopeless finalities in which the function of the physician was limited to matters of general advice and prognosis" (4). Angiocardiography, cardiac catheterization and, more importantly, cardiac surgery rapidly and profoundly changed this sense of futility.

Although the diagnosis and management of congenital heart disease have made impressive progress, understanding etiology and pathogenesis has not. This observation is particularly true for the role of the genome in congenital cardiac anomalies, whose study has never engendered any saltatory advances akin to the first repair of a patent ductus arteriosus or the first palliation of tetralogy of Fallot. Indeed, although most embryologists believe that genes are important in the

majority of congenital heart defects, and many pathologists and clinicians might concur, no one has yet developed successful ways to render this belief practical. All who deal with patients give counsel as best they can about current and future affected relatives; but, except for the occasional Mendelian disorder, advice is based on an all-purpose empiric recurrence risk estimate of 3 to 5%. This approach has scarcely changed in 3 decades, an embarrassing contrast with developments in cellular, developmental and molecular biology.

Accurate genetic counseling requires not only precision in diagnosis but knowledge of cause. Effective management of disease requires not only understanding of natural history and therapeutics but knowledge of pathogenesis. In most instances of cardiac anomalies, etiology and pathogenesis are unknown; discovering them is the focus of this article. Our goals are to review in brief the methods used to address these issues and the reasons that, at best, they have led to partial answers, to urge the study of pathophysiologic mechanisms in developmental systems subject to evolutionary constraint and to



*This article is part of a series of articles celebrating the 40th anniversary of the American College of Cardiology. The series attempts to set the stage for the future by describing current state of the art management of selected major cardiovascular problems and the basic knowledge that will provide directions for advances in diagnosis and therapy.*

From the Center for Medical Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland. This study was supported in part by Grant R01-HL35877 from the National Institutes of Health, Bethesda, Maryland.

Address for reprints: Reed E. Pyeritz, MD, Blalock 1012, Johns Hopkins Hospital, Baltimore, Maryland 21205.

identify directions of research likely to promote clinical effectiveness. We limit ourselves to structural anomalies of the heart evident during embryogenesis and, if compatible with survival, evident at birth. Excluded are disorders of electrophysiology, some of which are clearly genetic, and the many age-dependent heritable disorders that are not congenital, for example, the autosomal dominant hypertrophic and dilated cardiomyopathies, mitral valve prolapse and the Marfan syndrome.

### Preliminary Considerations

We well understand the risk of compressing the massive information bearing on the genetics of congenital heart disease. But, because our chief purpose is to place what is known in the perspective of where future investigation might be directed, we state our beliefs with brief support.

*First, the embryology of the heart is unquestionably under genetic control.* For example, some disorders of ontogeny segregate in families as Mendelian traits. About 11% of autosomal and X-linked disorders that are compatible with survival into adulthood affect the cardiovascular system; the figure is 17% for those Mendelian disorders lethal in the neonatal period (5). Moreover, most phenotypes caused by aneuploidy, including common ones such as the Down and Turner syndromes, lead to major cardiovascular malformations.

*Second, how genes exert their effects on organogenesis is not at all clear from simple inspection of the clinical phenotype.* In some Mendelian disorders, the cardiovascular manifestations of the phenotype tend to breed true, as in the common atrium of the Ellis-van Creveld syndrome. In the Noonan syndrome, the malformation is quite diverse in detail (septal hypertrophy, pulmonary valve stenosis, pulmonary artery stenosis, aortic valve stenosis), but perhaps somewhat more unified in general (all defects are obstructive). In lesions less clearly Mendelian, in which multiple genes and environmental factors are usually incriminated, relatives of probands, although at increased risk of cardiovascular malformations, often have anatomically disparate lesions. If what is encoded by the genome is the specific *anatomic* structure, these empiric facts are difficult to understand. On the other hand, ready interpretation may prove possible if the genome encodes *mechanisms of development*. For instance, we surmise that genes control both the amounts of factors that govern growth and development of a particular organ as well as whether they are active or inactive. A defect in one developmental gene might have pleiotropic effects not in one organ only but in distant parts of the body as well (6).

*Third, 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.* That such

requirements are reliably met is unequivocal, but details and the way that specialized nuances, such as asymmetries either of structure (for example, the rotation of the heart) or of function (for example, the cerebral hemispheres) are accommodated, remain obscure. Selection pressures require that these developmental demands be met with high reliability; one result is the precise conservation of the d-cardiac loop across diverse taxonomic classes. Reliability would presumably be favored by simple processes rather than complex ones, by properties that are intrinsic (such as the geometric consequences of physical relations) rather than arbitrary (as the genetic codes) and by redundancy rather than uniqueness. We thus begin with strong presumptions in favor of robust processes with rich properties, rather than complicated, fragile and narrowly specific devices.

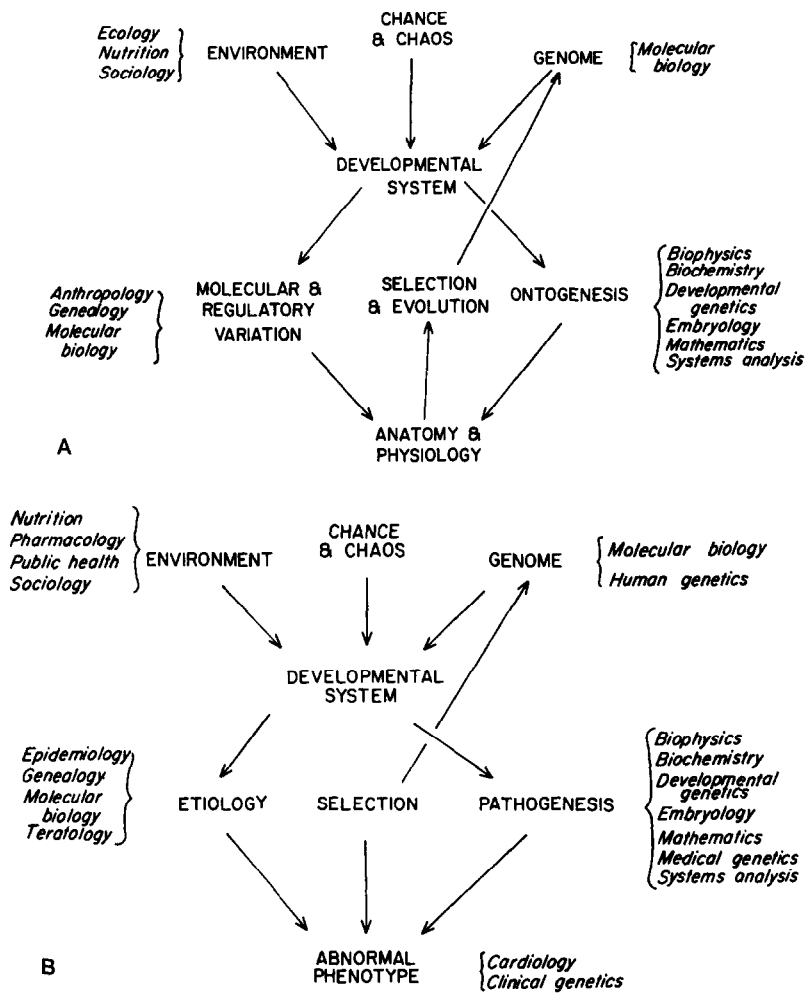
*Fourth, the heart has a vital role at every stage of evolution of higher organisms.* Mutation is rare and not at once relevant unless it alters the overt phenotype. In contrast, reproduction, by which mutation is integrated and perpetuated in the human gene pool, is common and efficient. As a result, cardiac defects are heavily penalized in the genetic sense that selection impedes reproduction. For our purposes here the most important implication is that ontogenic mechanisms need not be complex.

*Fifth, ontogeny is regulated to some extent by homeostasis or "feedback" (7).* Common experience, such as navigating across large distances, shows that provision for reducing cumulative effects of small *initial* errors and their propagation vastly reduces the demands of accuracy of the preliminary course. Hence, there is powerful selective value to feedback control in developmental mechanisms, just as there is for physiologic ones (8,9). However, feedback control is necessarily unbiased and *any such process with a mean effect of zero would not ordinarily be detected by classic quantitative genetics.*

### Fundamental Approaches to Congenital Heart Disease

Several academic approaches have helped our understanding of the nature and origin of congenital heart disease: high resolution investigations of molecular and cellular dysfunction, embryology and teratology, careful inspection of clinical phenotypes, formal genetic analysis of empiric data and theoretic modeling. They are most usefully seen as complements rather than as rivals (Fig. 1). The genetics of cardiac anomalies cannot be fully understood until the diverse demands of every approach are satisfied. Another, generally tacit, principle is that congenital heart disease will not be grasped until "normal" cardiac development is understood, and vice versa.

Each approach must accommodate certain demands. The functioning heart of a progenitor must be perpetuated by reliable mechanisms in the offspring. These mechanisms



**Figure 1.** Epistemologic linkages in normal and pathologic development. **A**, In normal development, the genome is the primary component of the system that directs development; the macroenvironment (for example, the mother) and the microenvironment (for example, local oxygen tension) as well as stochastic (for example, chance and chaos) factors influence the system but not to the point of disruption. The arrows indicate the directions of information flow. Alongside the components of this schema (italics) are the fields of study especially devoted to them. **B**, In pathologic development, the schema is identical to that of **A**, but the components reflect subsets of the former and the fields of study have been modified. Medical genetics subsumes cytogenetics, immunogenetics and biochemical genetics.

must prescribe, at a minimum, a means of encoding in deoxyribonucleic acid (DNA) both the chemical constituents of the cardiovascular system and the ways the chemical messages are to be translated into the phenomena of embryology—how a one-dimensional genetic instruction specifies a three-dimensional structure. It is not enough that the message instructs cells to form diverse tissues: a teratoma does that. There must also exist methods of ensuring both topologic and geometric organization of these tissues to form the heart itself and the spatial orientation of the heart within the body. When the mechanism goes awry, it is not enough to document that the same cardiac lesion recurs in the relatives of probands; one must also explain why there are both a higher risk of disparate cardiovascular lesions in the relatives of probands and structural changes at distant sites, especially the craniofacies and the hand. Moreover, a reliable ontogenic mechanism alone is insufficient; the phylogenetic path by which the mechanism evolved must be deduced and satisfy evolutionary dynamics and conservatism. The latter is exemplified by the use of the same parts and mechanisms repeatedly.

Finally, it is not enough to know in exquisite detail the biochemistry of development, because genetic (and hence evolutionary) selection operates not on these basic mechanisms but on the remote, coarse-grained clinical phenotype, which governs such crass matters as survival, reproduction and death. The demands made by the various constituents of Figure 1 may even be in open conflict. The physiologist and embryologist perhaps suppose that mechanisms do not have to be simple, and indeed there is much biochemistry to support grand multiplicity. On the other hand, the evolutionist will seek mechanisms that are simple, robust, pleiotropic and adaptable. We call attention to these clashes, which sober the triumphant accounts of success in many individual, narrow enquires.

### Perspectives on Formal Studies

#### Population Studies

Epidemiologists typically appeal to the notion that things that are independent in cause will have independent effects,

and conversely. This notion is a kind of minimalist logic: it is insensitive, but it is highly secure because the axiom of independence can rarely be challenged. In the present context, this approach has had two areas of application.

First it has shown that certain types of heart defects occur together more often than could be accounted for by chance. Thus arises the notion of the syndrome: the tetralogy of Fallot or the concurrence of lesions of the hand and the heart, as in the Holt-Oram syndrome. Such conjunctions can be studied without any family history at all and do not necessarily imply the interjection of genetic factors.

Second, relatives of probands with congenital heart disease are themselves at higher risk of congenital heart disease than are unselected subjects or their relatives. Many surveys of congenital heart disease have been of this kind and have established beyond doubt that there is "familial aggregation" (10-12). Furthermore, relatives of probands are also at added risk of cardiac lesions other than those in the probands (11,13,14). These findings alone do not prove a genetic cause because relatives tend to share similar habits, diet, social status, infections, any of which might be culpable. Nevertheless, it is at least suggestive evidence. Few epidemiologists go beyond this minimalist logic of independence and, with some justification, need to be taken to task for their neglect of genetic factors (15-17). The impact of the closeness of relation between affected individuals on etiology has been examined to some extent; unfortunately, nebulous terms such as "first degree" relatives have too often been used, ignoring the fact that sibling-sibling, mother-son, mother-daughter, father-son and father-daughter are all first degree relations but with quite different implications under any rational genetic model. There has also been little concern with false paternity, birth order (with the impact of sensitizing pregnancies) and parental age, matters that a geneticist would automatically address. Some recent exploratory studies (12,18) have dealt with these issues much more clearly.

### *Genealogic Studies*

The geneticist generally insists on rather more structure than does the epidemiologist. The most important addition is detailed study of the pedigree. Epidemiologic minimalism would not, for instance, ordinarily distinguish between a population in which all subjects were at 10% risk of a defect and one in which 10% of the population are at 100% risk. To the geneticist, the distinction would be vital.

Five classic approaches may be mentioned.

**Chromosomal approach.** For some 70 years it has been known that genetic information is contained in the chromosomes. Even in disorders that are genetically lethal (that is, preclude reproduction) or nearly so, such as the Turner and Down syndromes, the occurrence of heart defects in the

phenotype is taken to be evidence of a genetic etiology on grounds of karyotype, not of genealogic pattern.

**Mendelian approach.** More than a dozen congenital disorders of cardiac structure show evidence of being caused by a defect at a single genetic locus and occur often enough to be familiar to most clinicians. A far greater number of "private" syndromes, occurring in apparent Mendelian pattern in one or a few families, have been described; however, few meet the most stringent standards of proof. Many pseudomendelian disorders are known in animals in which extended breeding studies have shown that a trait is due to a tight cluster of genes rather than one locus. In humans, such deception would show up in an extensive pedigree, as genes in a cluster eventually segregate; however, because of a trend to smaller families and of reproductive disability, a given rare phenotype commonly is not transmitted, or, if it is, rapidly dies out and so deprives us of the critical evidence. For this reason, Mendelian inheritance is, paradoxically, more readily demonstrated for recessive traits, such as the Ellis-van Creveld syndrome, than for the more conspicuous dominant disorders.

**Galtonian approach.** Certain traits are evidently under the control of multiple loci, all more or less equally important and with additive effects. Fisher (19) showed that, when measurable, such traits tend to follow the Gaussian ("normal") distribution, and the degree of similarity among relatives can be adequately expressed—as Galton had expressed it 40 years previously—by regression and correlation coefficients. A thriving field of quantitative genetics has resulted. We mention four shortcomings of this model (20). First, the "democratic" assumption that the genes are many and have comparable impacts on the phenotype. The model does not, for instance, account for a phenotype *partly* determined by a major single locus. Second, to require additivity limits the area of applicability. Many well established examples violate this assumption, for example, chemical interactions subject to the law of mass action. Third, the model is not concerned with what the components are and hence (especially in view of the "democratic" assumption) has no heuristic value; indeed, it is a dead end inquiry. Fourth, in the present context, congenital cardiovascular defects are not matters of measurable changes but *quantal* phenotypes; that is, the person concerned either exhibits the trait or not—there is no intermediate state. (To anticipate, we may point out that modern trends in noninvasive high resolution phenotyping will shift inquiry toward quantitation of structural distortions, such as that which occurs in the Ebstein heart, the size of septal defects and subclinical manifestations of any malformation.)

**Threshold approach.** Although widely used in analysis of hypercholesterolemias, blood pressure and allied topics, classic Galton-Fisher analysis has shed little light on cardiac malformations. However, a modification derived from the work of Pearson (21) is the threshold model. It is based on

the idea that a measurable trait may itself exhibit continuous variation, but only those values above a threshold lead to overt disease. A classic illustration is that blood pressure may vary continuously, but there is an abrupt change in the clinical picture when at some critical pressure a berry aneurysm ruptures. The cardiologist (dealing with the blood pressure) and the neurologist (dealing with subarachnoid hemorrhage) may see the disorder quite differently. Pearson used his threshold model in two distinguishable models: where there is a measurable trait underlying the dichotomy (the analogue of the blood pressure in the hemorrhage) and, somewhat more cautiously, where the measurable trait is unidentified and indeed quite conjectural. The principal proponents of this threshold model in congenital heart disease have been the Noras (22,23) and Sanchez-Cascos (11). There has been neither a claim that the hypothetical "measurable" trait has been identified nor, so far, much attempt to find it. But Nora and Nora (23) have found in extensive family data that the recurrence rate in relatives corresponds to the expected (average) rate predicted from Pearson's method by Edwards' approximation (24). We stress that Edwards predicted the *average* rate, not the *probability* of being affected; and the Noras (22,23) have been in some difficulties in applying their results to the probability of recurrence when more than one relative is already affected. Nor is this surprising, because the mathematics is laborious even with the computer program of Curnow (25) and Smith (26). We may add that as it stands, this model sheds no light on the increased risk of discordant lesions in relatives.

Our intent is not to belittle these studies: in genetic analysis of such traits they represent the highest level of achievement so far, and perhaps nothing more sophisticated is practicable. In the last 15 years, no better analysis has been put forward.

**Logistic regression approaches.** This method of dealing with quantal characters has achieved some popularity in recent years. It has a long history of use in bioassay; and generalization of it to deal with cardiovascular traits due to multiple measurable causal factors has been elaborated (27,28). It is especially valuable in dealing with several causal factors, some discrete and some continuous. It is not expressly Galtonian in logic except that any measurable trait may be seen as the resultant of several additive effects. On the other hand, it much more readily accommodates explicit Mendelian structure than do other quantitative epidemiologic models, and it has been applied recently to familial aggregation of congenital heart disease (12,29).

### *Etiology and Pathogenesis*

The approaches so far have been directed to finding the causes of congenital cardiac malformations. However, it is necessary to make a distinction that to medical readers will be obvious but to many nonmedical geneticists may seem

very subtle. The notion of the cause of a disease comprises two features: etiology and pathogenesis. They deal as it were with the ingredients and the recipe, respectively. To quote a familiar genetic example, the etiology of sickle cell disease is perhaps better known than that for any other disease; yet the pathogenesis of the sickle cell crisis, or growth retardation, or arterial occlusions is still mysterious. Yet it is of these crass complications that the patient dies, and that Darwinian selection takes its toll, rather than the anomalous biochemistry in the beta chain of hemoglobin. In no sense can the genetics of the disorder be said to be understood until all the links from mutation to the "bottom line" phenotype have been made clear. Structural cardiovascular defects are presumably the end point of a disorder in the process by which the heart and vessels develop. To deepen our understanding of it, we must turn to a study of normal development.

### *Embryology*

We consider this vast subject not only in the terms in which it has been formulated by most embryologists but also in its relation to fields to which it is ordinarily a stranger. What are the genetic factors involved in embryology, and are the appropriate methods qualitative or quantitative? How far are the studies directed to grappling with, not anatomy alone or biochemistry alone, but with the *explicit* operation of the mechanism connecting the two? Can the data of experimental and descriptive embryology on genesis and pathogenesis be cast in terms that can be usefully explored in humans?

In the century since Born's pioneering studies (30) on human cardiogenesis, much descriptive research has appeared and been critically reviewed (31-33). Not surprisingly, controversy persists about aspects of normal human cardiac development, and the major impediment of access to specimens cannot be totally circumvented by examination of animals, even other mammals. Extrapolations to human organogenesis from structures observed in other higher organisms are hazardous and not supportable solely on the basis of striking similarities of macromolecules across species. Species should be expected to differ in how they regulate complex processes such as embryogenesis, and little variation in control is required to produce major alterations in the finished product. As Jacob (34) has put it, evolution "... is always a matter of tinkering."

**Morphogenetic processes in embryogenesis.** Impressive gains in the understanding of the cellular and molecular biology of human fetal development in general (35-37) and of the human heart specifically (38,39) have far outstripped more classic embryologic morphology. One widely held schema explains embryogenesis by the six morphogenetic processes listed in Table 1. Much current basic research focuses on the molecular bases for these processes, their regulation and their coordination (36,40-42).

**Table 1.** Fundamental Processes of Morphogenesis

Cell proliferation
Migration of cells
Migration of sheets of cells
Cell death
Cell differentiation
Pattern formation

### The Role of Classification

In clinical medicine as in all other fields, the prime requisite for any classification is utility. Inevitably, a variety of classifications for the same group of disorders emerges and reflects a diversity of interests. For congenital heart disease, a clinical classification based on the presence or absence of cyanosis, shunts, abnormal pulmonary blood flow and so forth has ready application in bedside diagnosis, but with no evident or accepted relevance to etiology or pathogenesis. This common, if unobtrusive, clash between the goal of the clinician to cater to individual needs and that of the scientist to seek sound generalities (43) exists in medical genetics as well. Classic human genetics appealed to the existence of homogeneous classes, so that the findings in unrelated families could be generalized. Only thus has it been possible to understand concepts like mutation rate, paternal age effect and selection. In major ways, modern molecular biology has undermined this premise, and for the population survey, we find substituted exquisitely detailed studies on one family, one individual or perhaps a single cell line.

**Classification of congenital malfunctions based on pathogenetic mechanisms.** It is unclear how best to adapt the burgeoning molecular understanding of normal and abnormal development for the epidemiologist, the genetic statistician or the clinician interested in malformations of the cardiovascular system. A key issue is whether the classification of cardiovascular malformations is cast in the right terms. Utility of the threshold model, beyond simple predictions of

**Table 2.** Classification of Congenital Cardiovascular Malformations Based on Pathogenetic Mechanism\*

Mechanistic Group	Example of Malformation
Mesenchymal tissue migration errors	Tetralogy of Fallot
Intracardiac blood flow defects	Coarctation of the aorta
Extracellular matrix abnormalities	Endocardial cushion defect
Abnormal cellular death	Ebstein anomaly
Looping and situs abnormalities <sup>†</sup>	L-transposition of the great arteries
Abnormal targeted growth <sup>†</sup>	Total anomalous pulmonary venous return

\*Classification scheme after Clark (44,45); <sup>†</sup>these groups are hypothesized and not yet substantiated by experiment.

recurrence risks in populations, has not blossomed because it has been used without any attempt to identify the characteristic on which the threshold was predicated.

If, as we surmised in the previous section, the real importance of genes in congenital heart disease resides in perturbations of mechanisms, then both theoretic modeling and a search for empiric validation must be based on a classification of defects rooted in ontogeny. We find the scheme proposed by Clark [(44) and personal communication 1989] appealing (Table 2). The first four of his six pathogenetic classes are supported by experimental evidence, mostly in animals. An ongoing, prospective investigation of congenital heart disease, with reasonable attention to both genetics and epidemiology, has utilized this classification to stratify data analysis (12,45,46), with results somewhat different from other recent work (13,23,47).

**Classification based on embryonic hemodynamics.** Table 3 expands on the class of anomalies due to abnormal embryonic blood flow patterns; some defects are caused by decreased flow in the right heart chambers and others caused by decreased flow in the left. The probands in the Baltimore-Washington Infant Study (12) were partitioned by this classification and the familial prevalence of cardiovascular mal-

**Table 3.** Congenital Cardiovascular Defects Associated With Altered Embryonic Hemodynamics

Timing of Alteration	Type of Alteration	Resultant Defect	Percent of Flow Defects*
Before ventricular septation	Increased left heart flow	Perimembranous ventricular septal defect	30.8
After ventricular septation	Decreased left heart flow	Hypoplastic left heart, mitral or aortic atresia	10.7
		Aortic valve stenosis	13.5
		Bicuspid aortic valve	n.d.
	Increased right heart flow	Patent ductus arteriosus	5.5
		Hypoplastic right heart, tricuspid/pulmonary atresia	4.4
		Pulmonary valve stenosis	14.9
		Secundum atrial septal defect	11.0
Independent of ventricular septation	Abnormal direction of ductus arteriosus blood flow	Interruption of the aortic arch, type A and aortic coarctation	9.1

\*Adapted from ref. 48 on the basis of 363 probands with a congenital cardiovascular malformation classified as due to abnormalities of hemodynamics out of a total of 570 cases ascertained. n.d. = not determined.

formations was determined by history. In relatives of the 363 patients with flow lesions (29% of all probands), 37 first degree relatives were affected (45,48). Further partitioning probands by defect and race and employing regressive logistic models produced strong evidence for etiologic heterogeneity in familial aggregation. Relatives of black probands with perimembranous ventricular septal defects and relatives of white probands with right heart defects were both at increased risk of some cardiovascular malformation. The data are still insufficient to ask whether the heterogeneity is due solely to race (an imprecise descriptor in genetic terms) or to additional genetic factors. For one flow-associated defect, hypoplastic left heart, the collaborators in this study performed echocardiography on the first degree relatives of probands in 14 families (49), five relatives with previously undetected and one with known bicuspid aortic valve (another flow-associated defect) were found, a frequency of 12.5% which was considerably greater than the estimated population prevalence of about 1%. Affected relatives clustered in families in which probands had no extracardiac malformations.

### Prospects for Improved Understanding of the Genetics of Congenital Heart Disease

Little further is to be gained, we believe, by refinements of classification or family studies based on coarse, clinical phenotypes. The mechanistic system proposed by Clark (44) seems useful, at least provisionally, for the immediate future in guiding studies of pathogenesis. A system in generalities and specifics based on etiology will emerge only when a great deal more work is performed, perhaps along the lines we suggest in this section. Descriptions of familial occurrences, even novel ones, of cardiovascular anomalies are unlikely to prove useful beyond counseling of individual families. More bird watching is passé; the ornithologist must reign henceforth.

#### Classic Genetics

One should not infer from the foregoing that family studies will be unprofitable. At least two applications of pedigree analysis need to be capitalized on.

**Linkage analysis.** For cardiovascular malformations that behave as Mendelian traits, their segregation within a pedigree can be compared with segregation of cloned DNA probes that contain restriction fragment length polymorphisms (RFLPs) (50-53). If a given RFLP tends to occur in affected, but not in unaffected, relatives, the DNA probe may be close to a gene that causes the malformation. With a large enough family and a probe sufficiently close to the true mutant locus, the human gene map location of the defect can be narrowed considerably. As the human genome becomes saturated with anonymous DNA probes, mapping a Mende-

lian disorder will become trivial. For the next few years, yield will be highest when a DNA probe from a gene of known function shows total linkage (that is, *no* recombination) with a cardiac phenotype; for the families showing no recombination between a candidate gene and the disorder, the product of that gene, or one very close to it, is part of the cause. This approach has shown the cause of some forms of osteogenesis imperfecta to be mutations in either the pro  $\alpha$  1 or pro  $\alpha$  2 genes of type I collagen (54,55), and the cause of the Stickler syndrome in some families to be the pro  $\alpha$  1 gene of type II collagen (56). Demonstrating nonlinkage is also useful; the cause of the Marfan syndrome is not any of the common fibrillar collagens, elastin or the core protein of proteoglycan (57,58).

**Reverse genetics.** Demonstrating close linkage (that is, occasional recombination) with an anonymous DNA probe only narrows the map location to a few million base pairs. Considerable work remains to "walk" or "jump" along the chromosome to identify the gene sequence in which the mutation resides and then to determine the product of that gene (59). This process, termed "reverse genetics," has demonstrated that, in Duchenne muscular dystrophy, a protein called dystrophin is defective (60) and that, in chronic granulomatous disease, one of the two polypeptides comprising cytochrome b is defective (61). But a great many more apparently Mendelian disorders, including familial polyposis coli, Huntington disease, myotonic dystrophy and cystic fibrosis have been mapped, although their loci remain unidentified or even conjectural (53). Several Mendelian cardiovascular disorders, especially Marfan syndrome and hypertrophic and dilated cardiomyopathies, will likely be mapped and their biochemical defects identified or verified by reverse genetics.

These and other technical capabilities for asking and answering questions engendered by molecular biology will have a major impact on cardiovascular diseases and the practice of cardiology. However, two caveats bear mentioning. *First, at present, gene mapping and reverse genetics are applicable only to those congenital malformations clearly caused by mutation at a single locus.* The next step will be to explain in molecular terms how the presence of one too few or too many alleles at a particular locus, as occurs in chromosome aneuploidy such as the Down syndrome, predisposes to cardiac anomalies. The final stages—explaining the cause and pathogenesis of the majority of congenital heart diseases—is the subject of the latter part of this section.

*The second caveat pertains to the highly visible, national and international effort to sequence the entire human genome.* Once this sequence is accomplished, and it surely will be in a decade or two, the job of understanding the molecular basis of common and multifactorial diseases will not be finished—just starting. Indeed, the tasks of deducing how the 3 billion-nucleotide linear sequence is organized into

genes, what these 50,000 or so genes produce and what regulatory elements lie in and around each gene will appear trivial (though they are far from it) compared with the task of deducing how these genes function in systems. An alphabet of 26 letters gives no hint of the richness and complexity of the language. Similarly, to a naive observer enquiring about the diversity of immunoglobulin specificities, being presented with tandem arrays of slightly different nucleotide sequences would provide scant clue; only in light of the system of combinatorial joining, flexible joining and somatic point mutation does the solution emerge.

**Segregation analysis of subclinical phenotypes.** Mendelian disorders, especially dominant ones, show considerable intrafamilial variability. If some aspect of a mechanism of morphogenesis or of a homeostatic system were controlled by a single gene, and that gene were mutant in some members of a family, the phenotype might vary widely among them. If some of these affected relatives had an obvious congenital cardiovascular anomaly, others with the same mutation might have a subclinical anomaly that could remain undetected by insensitive methods of detection such as auscultation, electrocardiogram or chest radiograph. More sensitive, yet noninvasive, techniques are now available for detecting subtle variations in structure; cross-sectional echocardiography, Doppler color echocardiography and, especially, nuclear magnetic resonance imaging may well detect relatives who have a clinically silent cardiovascular anomaly and, hence, are presumptively heterozygous for the same mutant gene as their more flagrantly affected family members. There will be at least two beneficial applications. First, with appropriate hypotheses and controls, segregation analysis and linkage analysis can be improved considerably. Second, pathogenetic mechanisms will be unraveled and major gene effects supported if a spectrum of ontogenetically related defects is found segregating in a family (14,48).

### *Embryologic Modeling*

**Changes in topology and geometry.** In a recent book, Arthur (62) notes that attempts to recast natural selection in genetic terms (known as neo-Darwinism) have been feasible only because genetics had a well established, general abstract theory. In stark contrast, such a *general* and *abstract* theory does not exist for embryology, despite much descriptive and experimental work and even a few attempts at modeling. In the absence of such a theory, entente between embryology and neo-Darwinism is unlikely, and serious study of the genetics of congenital heart disease is seriously impaired. At the same time, it is hard to say what the exact form of this theory of morphogenesis would be. It seems clear that it must be concerned with *topology*, that is, those properties that are unchanged by bending and stretching. For instance, at birth the geometry of the lungs is changed by

aeration, but the topology is unchanged. In distinction, closures of the ductus arteriosus and the foramen ovale change the topology of the cardiovascular system. In addition, cardiac morphogenesis must deal with *geometry*; for instance, a hypoplastic left heart is topologically intact but functionally inadequate. Indeed, because the embryonic heart supports the developing embryo as the heart itself is developing, its geometry, topology and function are intricately related.

**Mathematical models of growth.** A number of topics in pure mathematics—catastrophe theory (63), chaos theory (64,65), fractal sets (66-68)—have been cultivated with some thought of their biologic implications. Progress has languished in part because of their difficulty and chilly abstractness. Nonetheless, some results demonstrate both how worthwhile additional investigations might prove and how difficult it is for intuition, examining the empirical facts alone, to discern the level of complexity of the causal mechanisms. We have found in our own studies in dynamic systems as applied to the regulation of growth that a wide range of apparently quite different and disparate forms—the circle, spiral, dome, membranous sheet, torus and digitations—may result from a single model with only two arbitrary specifications (69,70). In other words, the notion that so complex a task as generating the geometry (as distinct from the fine structure) of the heart requires the instructions from a very large number of genetic loci may be misleading. For instance, a rough approximation to the hand can be achieved by five instructions (Fig. 2); and the same five instructions specify mechanisms that conceivably account for much of the anatomy of the heart.

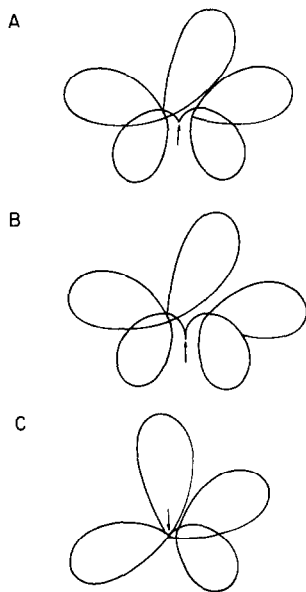
*Another fresh approach is a stochastic (probabilistic) single gene model of mammalian malformations (71).* Introduction of chance in addition to genes and environment has also been suggested as a refinement of multifactorial models (71).

### *Embryologic Research*

Further detailed descriptive investigation of human cardiogenesis will be hampered by ethical and political impediments to fetal research. Reexamination of fetal specimens that were preserved years ago will contribute some insight (72,73). Experiments in animals will continue to suggest ontogenetic mechanisms that may be generalizable (74) but difficult to test in humans.

**Molecular biologic approaches to embryogenesis.** We do expect, however, major progress in molecular embryology in the near future. In particular, intriguing results are emerging on the roles of three classes of genes and their products: oncogenes (75) and growth factors (76-78), cell surface molecules such as integrins (79,80) and homeotic genes (37,81). Cardiovascular abnormalities, although not necessarily congenital ones, are well known accompaniments of





**Figure 2.** The *anlage* of a hand generated by a model of a dynamic system. In each diagram, the same two paths are involved, generating the right two digits and the others, respectively. The lobes are unlike adult fingers but resemble their precursors, the primitive buds in the limb disk. The grouping of the fingers corresponds to innervation. Each group is defined by the path of a dynamic process whereby growth is directed by pre-axial and post-axial centers (technically called *targets*). Two variables (measures of the strength and proportions of each adjustment) control each path and a fifth variable specifies the angle between the branches of the cusp (arrow). The tracings show a progressive increase in the lengths of the digits toward the middle. Changes of the cusp angle alone produce patterns characteristic of the hand deformities seen in the polydactylies, Holt-Oram syndrome and various clefting malformations (lobster claw and so forth). The paths start from a common cusp (arrow). A (normal), The thumb lies at the bottom left, the little finger at bottom right. B, The paths start somewhat earlier and a deep cleft forms between the third and fourth fingers as in the so called "absence" deformities. C, The left path starts late and no thumb forms, as in the Holt-Oram syndrome.

heritable defects of the extracellular matrix, such as the mucopolysaccharidoses and the Marfan syndrome. The importance of connective tissue to the structural stability of differentiated tissues is incontrovertable (82-84). Less well appreciated, however, is the crucial role of the extracellular matrix during embryogenesis and organogenesis (85-87). A subtle, genetically specified variation in one macromolecule of connective tissue markedly affects not only that molecule but also the myriad of other components of the matrix (88). These alterations, in turn, can affect any of the fundamental processes of embryogenesis (Table 1). If the genetic change either was inherited from a parent similarly affected or arose in an egg or sperm that underwent a spontaneous mutation, then the entire embryo will be affected and widespread pleiotropic effects may appear (6). Alternatively, the genetic change in connective tissue may arise in a single cell of the early embryo and produce somatic mosaicism in which only

the clonal descendants of the mutant cell will be abnormal. The resultant phenotype and its transmission will depend on where those mutant cells migrate in the embryo, how widely the extracellular matrix they produce encounters nonmutant embryonic cells and other factors yet to be identified (89).

**Somatic mutation: etiology of cardiac malformations.** Somatic mutation is much more common than was thought even 5 years ago, and it is quite obviously not limited to connective tissue. The phenomenon may prove to be extremely important in the etiology of many congenital malformations. One special class of somatic mutation is that which occurs in a gonad and produces germinal mosaicism. The individual who is a germinal mosaic for a mutation that causes a dominant phenotype (as many congenital malformations may be) will be clinically normal, but all offspring will have a risk of being affected in proportion to the number of mutant germ cells. This nuance, among others, will increasingly complicate genetic counseling in the future.

### *Genetic Regulation of Complex Systems*

The molecular biology of gene regulation will hold our interest for years to come. It will become clearer that the same signal (often a polypeptide such as a hormone receptor or a product of a homeotic gene) can induce one gene, repress another and render a third sensitive to regulation by *another* signal such as a hormone. We will begin to understand how environmental factors such as pressure and temperature modulate gene expression and, thereby, developmental and compensatory alterations of cardiovascular structure and function.

*Investigations of the genetic regulation of developmental processes other than organogenesis may well shed light on congenital malformations.* Even a thorough understanding of how genes control physiologic homeostasis, such as blood glucose levels (9), may generate practical solutions to more complex cybernetic systems.

Fields as diverse as fundamental mathematics, fluid dynamics and cellular automata (90,91) may ultimately be applicable to understanding congenital cardiovascular defects. Already the chaos theory is finding wide attention in population dynamics; extending its precepts to cellular dynamics is not unrealistic.

### **Conclusion**

Substantial progress toward understanding how genes are involved in etiology and pathogenesis of congenital heart disease is likely in the next decade. Indeed, this progress will only be part of a much more complete picture of human ontogeny. Achieving this progress will involve vastly more than molecular biology, although that field will have a preeminent technical contribution. The major obstacle will be fundamental investigations of highly complex, interactive

systems; both the theory and empiric studies are in their infancy.

We end with the optimistic projection that before many more years pass, simple reliance on a stock "3% to 5%" recurrence risk for anxious parents will prove not only inappropriate but inaccurate. The burden on the clinician to understand the affected child's congenital defect, its etiology and pathogenesis and the likely contribution of the parents' genes will increase, but the reward in more informed counseling will be even greater.

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