SPECIAL ARTICLE

Multifactorial Inheritance Hypothesis for the Etiology of Congenital Heart Diseases

The Genetic-Environmental Interaction

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SUMMARY

A systematic investigation into the etiology of congenital heart diseases (CHD) has been undertaken through the testing of four alternative hypotheses. Data was obtained for six common congenital heart lesions from the investigation of familial aggregates, twin studies, and chromosomal evaluations. Animal homologies were also considered in the evaluation of the hypotheses. Hypothesis 1—no genetic basis for the etiology of congenital heart diseases—was tested and rejected. Then in an effort to define the possible genetic basis of congenital cardiovascular malformations hypothesis 2—the genetic basis of CHD is determined by gross chromosomal aberrations—was evaluated by reviewing karyotypes of nonsyndrome CHD patients in the literature as well as 104 personal cases. Rejection of hypothesis 2 is required by all available evidence. Hypothesis 3—the genetic basis of common isolated congenital heart lesions is determined by single mutant genes—was also tested and rejected.

Hypothesis 4—congenital heart diseases are a heterogeneous category of developmental anomalies, representing in most cases the multifactorial inheritance of threshold characters, the expression of which is the product of a genetic-environmental interaction—was tested by the same criteria. On the basis of these criteria this hypothesis could not be rejected.

Therefore the multifactorial hypothesis is proposed as a working hypothesis which encompasses both the genetic and environmental factors known to participate in the etiology of congenital heart diseases. It is hoped that the testing of this hypothesis investigation into new areas will be stimulated, which ultimately will lead to active methods of prevention.

Additional Indexing Words:
Heredity  Animal homologies  Familial aggregates  Environment
Twin studies  Chromosomal aberration  Single mutant genes

RECENT ADVANCES in the life sciences provide the opportunity to reconsider the etiology of congenital heart diseases and to conduct new investigations based on these advances. The following report is a review of some of the pertinent literature together with personal research efforts. The

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purpose is to develop a working hypothesis which is readily subject to test. To arrive at
an hypothesis which may carry a high likelihood of approximating the answer regarding
the etiology of congenital heart diseases, alternative hypotheses must also be rigorously
tested.

Genetic and environmental factors are the
two areas which have received attention in
the etiology of congenital cardiac malforma-
tions, but the relationship, if any, between
these areas has not been clearly defined. The
literature is replete with surveys and case
reports documenting the familial nature of
congenital heart diseases.1-3 The influence
on the developing human heart of environ-
mental teratogens, such as rubella,4, 5 thalid-
omide,6 and high altitude7 has also been
documented. The demonstration of terato-
genetic malformation of the heart by many
other environmental agents is quite con-
vincing in experimental animals.8-10 However,
it has been more difficult to demonstrate that
an agent capable of producing cardiac mal-
development in an experimental animal such
as the mouse8 is also responsible for con-
genital heart defects in the human.11

A preliminary step to investigating the
etiology of congenital heart defects is the
isolation and evaluation of the separate poten-
tially contributing factors: heredity and
environment.

Heredity

When considering the genetic basis of dis-
ease, McKusick suggested that there are
three possible categories: (1) single mutant
gene, (2) chromosomal aberration, and (3)
multifactorial causation.12

The first category of genetic disorders, the
single mutant gene diseases, includes the
disorders that conform to Mendelian patterns
of inheritance and that may be analyzed as
autosomal dominants and recessives and X-
linked dominants and recessives. Although
a large number of different diseases are sus-
pected as being caused by single mutant
genes,13 by and large these are the rare
diseases of mankind with population inci-
dence of less than 1/1,000.14 Many of these
single mutant gene syndromes have heart
lesions as part of the clinical picture. Ex-
amples are the autosomal dominant diseases,
Marfan’s syndrome and Ehlers-Danlos syn-
drome; the autosomal recessive diseases,
Hurler’s syndrome and Ellis-van Creveld syn-
drome; and the X-linked recessive disorders,
such as Hunter’s syndrome.12 However, taken
all together, diseases having an established
single mutant gene basis probably account
for less than 1% of patients seen in a typical
cardiology clinic.

The next category of genetic disorders is
the group of chromosomal aberrations, of
which mongolism is the prototype. The num-
ber of different disease entities caused by
identifiable chromosomal aberrations is small;
Turner’s syndrome and the D and E trisomies
being the most important after mongolism.
However, this category is responsible for a
larger number of patients with congenital
heart defects than are found in the single
mutant gene category. The overall signifi-
cance of these chromosomal aberrations in
the etiology of congenital heart diseases re-
mains small, with probably less than 5% of
patients with congenital heart defects having
an identifiable chromosomal anomaly.

The third category of genetic disorders,
multifactorial inheritance, is a specific entity
which has received much recent emphasis,
but historically it may be the oldest category.
Probably this is what Hippocrates was sug-
gest in his “Doctrine of Diathesis,” the
tendency of diseases to run in families. Our
present understanding of this mode of in-
heritance has been developed through the
basic work of Wright15 and Gruneberg16 and
the applications to human genetics by Pen-
rose,17 Edwards,14 and Carter.18 Multifac-
torial inheritance implies that many genes
interact with environmental influences to re-
sult in an observed trait or disease. Traits,
such as height and intelligence, are not the
product of one gene but of many genes inter-
acting with environmental factors (nutrition).
Similarly, many common malformations have
now been demonstrated to conform to a mul-
tifactorial pattern of inheritance.18

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Multifactorial inheritance of a disease meets the following criteria: There are familial aggregates; the disease is common; the recurrence rate in sibs is 1 to 5%; there is evidence of response to environmental influences, such as variation in frequency with seasons of birth, birth rank, sex of the patient, parental age, socioeconomic class, and geographical distribution. Further confirmation is obtained through twin studies in which usually 25 to 50% of identical twins have both members of the twin pair affected, whereas both members of nonidentical twin pairs are affected no more frequently than the recurrence risk for sibs (1 to 5%).

**Alternative Hypotheses**

With the preceding background of information there are at least four hypotheses relevant to the genetic basis of congenital heart diseases which are appropriate for testing.

1. There is no genetic basis for congenital heart diseases.
2. Gross chromosomal aberrations cause most congenital heart lesions.
3. Single mutant genes are responsible for most congenital heart lesions.
4. Congenital heart diseases are a heterogeneous category of developmental anomalies, representing in most cases the multifactorial inheritance of threshold characters, the expression of which is the product of a genetic-environmental interaction.

How may the hypotheses be tested: McKusick suggested several approaches for evaluating the presence of genetic factors. Among these are: (1) demonstration of familial aggregation, (2) twin studies, and (3) animal homologies.

This presentation will review personal studies and those of other investigators which test the alternative hypotheses.

**Hypothesis 1. No Genetic Basis**

**Familial Aggregates**

The *sine qua non* for any genetic hypothesis is familial aggregation. Several large surveys in the past have shown a clear tendency for congenital heart disease to run in families. Differences in frequencies and interpretations prompted us to conduct our own survey which differed from some previous studies in that our protocol called for personal examination of all first degree relatives and as many distant relatives as possible, rather than relying on questionnaire studies conducted through the mail.

We found that 34% of 517 randomly selected patients presenting with congenital heart lesions had one or more relatives with unequivocal evidence of congenital heart disease. In a matched control group, using 100 probands, each without a congenital heart defect, there was a positive family history in only 9% of the families. Equally striking was the finding that 53 of 1,602 (3.4%) in the congenital heart disease group and none of 227 siblings in the control group had congenital heart defects. A statistically significant difference is demonstrable (\(P < 0.01\) for positive family histories and \(P < 0.001\) for affected first degree relatives).

The results of this test require us to reject the hypothesis of no genetic basis.

**Twin Studies**

Twin studies offer another means of testing for the presence or absence of genetic factors in common diseases. In diseases which have a genetic basis, both members of identical twin pairs are affected more often than both members of nonidentical twin pairs. Furthermore, if both members of identical twin pairs are not affected with the same condition 100% of the time, then the environment must be recognized as playing a role in the disease.

Several conflicting twin studies of congenital heart anomalies have been conducted in the past decade. Some excellent, and frequently quoted, twin studies do not approach the problem in the same way. Therefore, we have elected to summarize the data from only those twin studies which specify the number of cases, the number of discordant pairs, and the basis for the diagnosis of the heart defect. For present purposes these studies are divided into those in which the diagnosis was based on symptoms and physical examination or on cardiac catheterization. The results from these studies are given in Table 1.

<table>
<thead>
<tr>
<th>Zygosity</th>
<th>Concordant Pairs</th>
<th>Discordant Pairs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monozygotic</td>
<td>6 (46%)</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Dizygotic</td>
<td>1 (4.2%)</td>
<td>23</td>
<td>24</td>
</tr>
</tbody>
</table>

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support a genetic hypothesis. Other twin studies which support a genetic hypothesis are not frequently quoted. In an effort to clarify for ourselves this conflict (which could easily be related to the small sample size) we undertook our own twin study (table 1), and then combined our twins with all the precisely diagnosed, randomly ascertained twins we could find in the world literature.

In our series, 46% of identical twins had co-twins with congenital heart defects, and in the world literature, 25% of precisely diagnosed identical twins had affected co-twins. On the other hand, 4.2% of nonidentical twins, in our series, and 4.9% of nonidentical twins in the world literature had both members of the twin pair affected. The nonidentical twins were thus not affected with a significantly higher frequency than would be predicted from the empiric risk figures for sibs. The difference between affected identical and nonidentical twins is significant ($P < 0.01$).

From the results of twin studies we must again reject the hypothesis of no genetic basis of congenital heart diseases.

Animal Homologies

Another area of investigation which may be used to test genetic hypotheses is that of animal homologies. The excellent review of Detweiler provides a variety of good animal models of “families predisposed to congenital heart disease.” Dogs, swine, cattle, rats, pigeons, and turkeys are among the many animal species to demonstrate familial cardiovascular disease.

Our investigations in this area have been with strains of mice. We have found that the A/Jax mouse spontaneously has atrial septal defect (first observed by Rosen, personal communication) in about 1% of the fetuses and may thus be viewed as being predisposed to atrial septal defect. This predisposition may be accentuated by the administration of a teratogen, such as dextroamphetamine, which results in 12% of the fetuses having congenital heart lesions. Although several different heart anomalies may result from exposure to this teratogen at the vulnerable period of cardiac development, the predominating lesion is atrial septal defect, which is the cardiac malformation “running in the family.”

We have now accumulated enough data on the C57BL/6 strain of mice to propose that this strain has a familial predisposition to ventricular septal defect, which occurs spontaneously in less than 1% of fetuses, but which is produced in more than 10% of fetuses exposed to dextroamphetamine.

The demonstration of familial occurrence of congenital heart defects in animal homologies is acceptable evidence for the rejection of the hypothesis of no genetic basis of congenital heart disease.

Hypothesis 2. Chromosomal Aberration

If there is a genetic basis for congenital heart diseases, what is its nature? The three possibilities have already been proposed. The first of these are gross chromosomal aberrations. Testing this hypothesis is relatively simple. One needs only to make chromosomal karyotypes on patients with congenital heart defects.

Although everyone is familiar with the existence of heart lesions in patients with the few established syndromes caused by chromosomal anomalies, such as mongolism, the D and E trisomies, and Turner’s syndrome, it should be re-emphasized that these syndromes probably account for less than 5% of patients with congenital heart lesions.

Efforts have proved fruitless to demonstrate that patients with congenital heart lesions (who do not also have multiple stigmata of chromosomal anomalies) have consistently demonstrable chromosomal aberrations. In the early stages of chromosomal analysis, a scattering of reports, such as that of Böök and associates appeared to offer promise that nonsyndrome, isolated congenital heart lesions might have a gross chromosomal basis, but subsequent investigations have failed to show consistent or reproducible changes.

Our own cytogenetic evaluation of patients with cardiac anomalies consists of 104 individuals in 46 unrelated families, most of
which had more than one family member with a congenital heart disease. These patients had most of the common congenital heart defects, but did not have clinical evidence of a gross chromosomal aberration such as mongolism. We were unable to demonstrate a consistent gross chromosomal anomaly in these patients.

Although submicroscopic chromosomal anomalies have not been ruled out, the specific hypothesis under consideration is that gross chromosomal aberrations are responsible for most congenital heart lesions. All available evidence from chromosomal analyses leads us to reject this hypothesis.

**Hypothesis 3. Single Mutant Genes**

That a single mutant gene can be responsible for congenital heart disease as part of a syndrome, such as the Ellis-van Creveld syndrome, has already been mentioned. In these single mutant gene conditions the cardiovascular involvement is only part of a syndrome, which may affect many systems. This is the pleiotropic effect of a mutant gene in a key synthetic or energy pathway. We have mentioned that single gene inheritance has been useful in explaining many of the rare diseases in humans (that is, incidence <1:1,000), but it has become obvious from recent studies that the majority of illnesses, particularly the common diseases (that is, incidence >1:1,000), do not conform to simple Mendelian patterns.

The question now is: Could single mutant genes be responsible for such common malformations as isolated congenital heart defects which are not part of a syndrome? To answer this question, we must stop mixing congenital heart lesions together as if they were all one disease (the reason for preferring the term "congenital heart diseases"). While some cardiac anomalies are developmentally related, others are not. Perhaps no single misconception has more delayed an etiological understanding of congenital heart diseases than this; namely the assumption that a heterogeneous category of anomalies could be approached as a single disease.

**Familial Aggregates**

Meaningful and valid data can be derived from the intensive study of specific heart lesions, and then, and only then, does the developmental relationship or lack of relationship become apparent. We have studied six of the most common congenital heart anomalies in depth: (1) atrial septal defect, (2) ventricular septal defect, (3) patent ductus arteriosus, (4) valvular pulmonic stenosis, (5) valvular aortic stenosis, and (6) tetralogy of Fallot. The findings for all six lesions are similar; so for ease of exposition, only the first lesion that we studied, atrial septal defect, ostium secundum, will be discussed in depth. What is said about atrial septal defect applies equally to the other five common heart malformations.

The reason for studying atrial septal defect first was that it is the most common congenital heart anomaly seen in adults. We felt that suitable defects for intensive study should be compatible with life into the reproductive age, should be common enough to provide an adequate number of patients, and should be diagnosed with reasonable ease.

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**Figures 1 and 2**

Pedigrees showing direct transmission of ASD, simulating autosomal dominant inheritance. (From Nora, J. J. and associates. Circulation 35: 448, 1967.)

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and accuracy. All the diseases we have studied fulfill these criteria except tetralogy of Fallot, which, prior to the surgical era, did not commonly permit survival into the reproductive age.

Genetic factors in atrial septal defect have received attention in the past. Howitt reported a family manifesting direct transmission and concluded that an autosomal dominant mode best explained this pedigree. Carleton and associates found affected sibs without affected parents, which they interpreted as being consistent with autosomal recessive inheritance. We studied 100 families in which the index case had atrial septal defect and found that 32 families had at least one other individual with acceptable evidence of a congenital heart lesion. In some we found direct transmission as seen in autosomal dominant inheritance (figs. 1 and 2).

In others we found affected sibs in a ratio which approached one in four, without affected parents, the typical finding in autosomal recessive inheritance (figs. 3 and 4). What could be wrong with these two interpretations other than the fact that they disagree with each other? What is wrong is that we have chosen to present only selected pedigrees from the 100 families.

If, in the usual random mating situation, single gene autosomal dominant inheritance (with full penetrance) were operating, the expectation would be that one parent should transmit the disease to each of the probands, and that about one in two of the offspring would be affected. However, only seven parents in 100 families (not 100 parents) and only 10 of 279 sibs (3.7% not 50%) were affected. As for autosomal recessive inheritance, the parents should not be affected, and about one in four offspring should have the disease, but seven parents were affected and the 3.7% affected siblings falls far short of a one in four expectation.

Therefore, when one stops selecting pedigrees to support either a single gene autosomal dominant or recessive mode of inheritance and looks at a sizable sample of 100 randomly ascertained families with atrial septal defect, it is clear that no single mutant gene hypothesis is tenable. Although 32% of the families have positive histories for congenital heart disease, only 7% have affected parents and only 9% have affected sibs. The data are not consistent with autosomal dominant (P < 0.001) or autosomal recessive (P < 0.001) inheritance in atrial septal defect or in the other five lesions studied. Table 2 gives the results of an ongoing survey to present time.

The hypothesis that a single mutant gene is responsible for most congenital heart defects must be rejected on the basis of familial aggregate data.

Twin Studies

In single mutant gene inheritance both members of an identical twin pair will have the disease caused by the single mutant gene. Whether or not the gene is dominant or

**Figures 3 and 4**

Pedigrees showing siblings affected with ASD in approximately a one in four ratio, without affected parents. Autosomal recessive inheritance is simulated in these families. (From Nora J. J. and associates. Circulation 35: 448, 1967.)

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recessive, 100% of identical twin pairs will have both twins affected. In nonidentical twins the co-twin will be affected with the same frequency as other sibs. Therefore, in autosomal dominant inheritance (usual random mating), 50% of co-twins of affected nonidentical twins will also have the disease, and in autosomal recessive inheritance, 25% of co-twins will be concordant with affected dizygotic twins.

In our study only 46%, not 100%, of identical twins were concordant for congenital heart diseases. Further, the concordance in nonidentical twins was 4.2%, far below the 50% expectation for dominant inheritance or the 25% expectation for recessive inheritance. \( P \) (probability) is less than 0.01 for the expectation of single gene inheritance in these twin studies.

Therefore, on the basis of twin studies, the hypothesis that a single gene is responsible for most congenital heart defects must be rejected.

**Animal Homologies**

If single mutant genes are responsible for congenital heart defects, one would expect to find among the many well-studied animals in which cardiac anomalies have been reported, some evidence of Mendelian inheritance. In the extensive review of Detweiler\(^2\) of many different animal models, no clear-cut

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**Figure 5**

Display of approximation of multifactorial inheritance found in present population study of ASD, VSD, patent ductus arteriosus, pulmonic stenosis, aortic stenosis, and tetralogy of Fallot.

(From Newcombe\(^3\)).

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pattern of Mendelian inheritance emerged. Although many workers have been able to increase the incidence of cardiac anomalies through inbreeding, and some have tentatively suggested single gene inheritance, analysis of available data fails to support Mendelian modes.

Our own investigation of two strains of mice predisposed to congenital heart diseases has also failed to support any Mendelian pattern. In the A/Jax mouse, 1% of the offspring have ASD spontaneously and 12% have ASD or other heart lesions produced by teratogens. Assuming the strains are truly isogenic, one would expect that a defect produced by a single mutant gene should be found in all offspring. Further, this exquisite sensitivity to environmental teratogens is not characteristic of Mendelian inheritance, in which the expression of the disease is determined by the presence of the mutant gene and is relatively independent of environmental influences.

Available evidence from animal homologies requires rejection of the hypothesis that single mutant genes are responsible for most congenital heart lesions.

**Hypothesis 4. Multifactorial Hypothesis**

We have earlier defined what the characteristics are of diseases inherited through a multifactorial mode. Such diseases are common, show familial aggregates, have a recurrence rate in sibs of from 1 to 5%, are susceptible to environmental influences, and are found much more commonly in both of identical twins than in nonidentical twins.

To these we would like to add mathematical approximations suggested by Edwards and Newcombe. Edwards has calculated that threshold traits inherited through multifactorial inheritance appear in the first degree relatives (that is, sibs and parents) of affected individuals with a frequency approximating the square root of the population frequency; that is, if the frequency of a congenital anomaly is 1/100 (1%) in the population, it should be found in 1/10 (10%) of sibs.

Newcombe has devised a graph which may be used to distinguish simple dominant, simple recessive, and multifactorial inheritance (fig. 5). On the abscissa is disease incidence in the population, and on the ordinate the ratio of incidence in sibs to incidence in the population. This offers one more way of testing a multifactorial hypothesis.

**Familial Aggregates**

That there are significant familial aggregates which are non-Mendelian in a population study are supported by the testing and rejection of hypotheses 1 and 3.

The paradox of the same congenital defect (that is, ASD), appearing as an apparent recessive in one family, as an apparent dominant in another family, and proving to be neither recessive nor dominant in a population study was first appreciated by Wright, in 1934. This simulation of Mendelism in multifactorial inheritance has been recently elucidated by Edwards. Briefly, Mendelian inheritance can be simulated by a system in

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**Table 2**

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Probands</th>
<th>Affected sibs</th>
<th></th>
<th></th>
<th>Affected parents</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Exp. ( \sqrt{P} )</td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>VSD</td>
<td>207</td>
<td>23/536</td>
<td>4.3</td>
<td>4.2</td>
<td>8/414</td>
<td>1.9</td>
</tr>
<tr>
<td>PDA</td>
<td>131</td>
<td>12/364</td>
<td>3.2</td>
<td>2.9</td>
<td>6/262</td>
<td>2.5</td>
</tr>
<tr>
<td>Tetralogy</td>
<td>118</td>
<td>6/273</td>
<td>2.2</td>
<td>2.6</td>
<td>1/236</td>
<td>0.4</td>
</tr>
<tr>
<td>ASD</td>
<td>117</td>
<td>10/308</td>
<td>3.2</td>
<td>2.6</td>
<td>7/234</td>
<td>3.0</td>
</tr>
<tr>
<td>PS</td>
<td>114</td>
<td>9/310</td>
<td>2.9</td>
<td>2.6</td>
<td>5/228</td>
<td>2.2</td>
</tr>
<tr>
<td>AS</td>
<td>101</td>
<td>7/269</td>
<td>2.6</td>
<td>2.1</td>
<td>4/202</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>788</td>
<td>67/2060</td>
<td>3.2</td>
<td></td>
<td>31/1576</td>
<td>1.9</td>
</tr>
</tbody>
</table>
which an underlying process, controlled by a large number of genes, each with a relatively small effect, is related to a biological threshold so that all individuals on one side of the threshold are unaffected and all on the other side are affected. The threshold is related to many genes, not to a single gene, and whether or not a dominant or recessive pattern is simulated depends on all of the factors determining the threshold in the given individual.

Further elaboration of the concept of multifactorial inheritance exceeds the scope of this presentation, but the selected references\textsuperscript{14–18, 31} supplement the preceding statements. Suffice it to say, methods are established for testing multifactorial inheritance, and these methods may be used to test the hypothesis under consideration.

It has been stated that Mendelian inheritance is usually limited to the rarer diseases of mankind, and multifactorial inheritance is found in the more common diseases. As a category, congenital heart diseases are the most common congenital malformations and as individual anomalies, such a specific lesion as isolated ventricular septal defect is seen more frequently than such common malformations as cleft lip or congenital dislocation of the hip. We use the findings of Richards and associates\textsuperscript{32} that 0.7\% of liveborn infants have congenital heart defects, although we feel that it is quite conservative.

In retrospect, multifactorial inheritance should have been the first consideration because this is what is found in common diseases, and the multifactorial pattern has already been demonstrated in other common malformations, such as cleft lip and palate, pyloric stenosis, congenital dislocation of the hip, spina bifida, and club foot.\textsuperscript{18}

We have studied at least 100 families for each of the cardiac malformations under discussion (table 2). The incidence of the lesion in the population has been crudely approximated by multiplying 0.7\% by the proportion represented by the specific cardiac anomaly in pediatric congenital heart registries, such as the Toronto Heart Registry.\textsuperscript{33} For example, ASD is found in 10\% of pediatric patients with congenital heart diseases, and 0.7\% of a North American population has congenital cardiac anomalies; therefore, an approximation of ASD in the North American population is 0.07\%.

Table 2 shows how the observed frequencies of affected sibs of patients with congenital heart lesions correspond to the sib frequencies for multifactorial inheritance as predicted by Edwards' formula ($\sqrt{p}$). The agreement between the expected and the observed for each lesion is very close, with no significant difference being detectable. On the basis of the calculations displayed in table 2, the hypothesis for the multifactorial inheritance of congenital heart disease cannot be rejected.

Plotting these cardiac anomalies on the graph devised by Newcombe (fig. 5), using the sibs in our study, and adding the results of three studies by other investigators again reveals close correlation between the frequencies observed and those expected for multifactorial inheritance. The hypothesis for multifactorial inheritance cannot be rejected on the basis of this test.

To return to table 2, the sib frequencies were closely predicted by Edwards' formula, but not the frequencies of affected parents. One reason for this becomes obvious when noting that there is only one parent with tetralogy of Fallot, but there are the predicted number of parents with ASD. Survival into the reproductive age is certainly a factor. In VSD, spontaneous closure may also be an additional factor. Parents of several patients with VSD insist that they had "serious heart murmurs" as infants and young children which disappeared during childhood.

The preceding analysis of familial aggregates indicated that congenital heart defects meet the criteria for multifactorial inheritance by being common. From the test of hypothesis 1 we reject the no genetic basis. Under hypothesis 3, a Mendelian genetic mode is rejected, but in two tests for multifactorial inheritance (Edwards and Newcombe) it is not possible to disprove that the
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genetic basis conforms to multifactorial inheritance.

Twin Studies
Twin studies support a genetic basis for congenital heart lesions under hypothesis 1 but do not fulfill criteria for a Mendelian inheritance under hypothesis 3.

The next step is to test for multifactorial inheritance. Both identical twins in multifactorial inheritance are not affected 100% of the time as they are in single gene inheritance, because there is an important environmental component to the ultimate expression of the disease of malformation. The usual range of concordance is 25 to 50%. Our twin study showed 46% concordance, while the combined twins from the world literature showed a concordance of 25%.

For both nonidentical twins and sibs, the concordance should be 1 to 5% in multifactorial inheritance, rather than the 25% concordance in recessive and the 50% in dominant inheritance. Our nonidentical twins showed 4.2% concordance, and the combined twins from the literature, 4.9% concordance.

The findings in twins are precisely what would be predicted in multifactorial inheritance. Or to put it in terms of the null hypothesis, we are not able to reject the hypothesis that there is no difference between what we found in twins and what would be expected in these twins under multifactorial inheritance.

Animal Homologies
Under hypotheses 1 and 3, evidence was presented in animal models supporting a genetic transmission of congenital heart defects which is non-Mendelian. The previously discussed low frequency of spontaneous anomalies and the sensitivity to environmental agents are precisely what would be expected in multifactorial inheritance.

It is, therefore, not possible to reject a multifactorial hypothesis on the basis of animal homologies.

Conclusions from Tests of Genetic Hypotheses
Through the use of some generally accepted methods for testing for the presence or absence of genetic factors, four alternative hypotheses have been evaluated.

Available evidence led us to reject hypothesis 1 of no genetic basis. Then, in an effort to define what the nature of the genetic basis could be, we successively rejected hypothesis 2 of gross chromosomal etiology and hypothesis 3 of single mutant gene etiology. The remaining genetic mechanism, hypothesis 4, multifactorial inheritance, was tested by available methods and could not be rejected.

This leads to the proposal of multifactorial inheritance as a suitable working hypothesis justifying further testing and investigation.

Environment
In the past, the influences of heredity and environment in the etiology of congenital heart diseases have been separated as if they represented conflicting, if not irreconcilable, points of view. The multifactorial hypothesis proposes that the two possible etiologies of congenital heart anomalies are interrelated. That the environment plays an established role as evidenced by congenital heart defects following exposure to rubella and thalidomide has already been mentioned. That environmental effects in human malformations take place in the context of genetic predisposition has been suggested by studies of animal models but has been difficult to document in human beings. (We have accumulated data of a very preliminary nature that there may be a demonstrable familial predisposition to intracardiac defects and hearing loss in patients affected with these lesions in such an obviously environmentally determined condition as the rubella syndrome.)

An interaction of heredity with environment may be suspected from the finding that in only 46% of identical twins were both members of the twin pair affected, and in some identical twin pairs, in which both twins had congenital heart defects, there were some differences in the malformation. For example, in a set of our identical twins, both having atrial septal defect, one twin
had the additional lesions, coarctation of the aorta and patent ductus arteriosus. In another set of identical twins one patient had a large ventricular septal defect requiring closure with a Dacron patch and the other a small ventricular septal defect requiring only direct suture closure. Ross described a set of monozygotic mongoloid twins, one of whom had an atrial septal defect and the other a ventricular septal defect. This demonstrates (as do the animal homologies of isogenic strains, such as A/Jax mouse) that, even with the identical genetic background, differences in expression of congenital heart defects may be produced by the environment.

The environmental exposure may be very subtle and difficult to define in multifactorial inheritance. Even the difference in the sex of the patient may sufficiently alter thresholds to allow expression of malformation. Some examples of this are congenital anomalies most commonly found in boys, such as pyloric stenosis, transposition of the great vessels, and coarctation of the aorta; and malformations most frequently encountered in girls, such as congenital dislocation of the hip, atrial septal defect, and patent ductus arteriosus.

Many different viruses and drugs, radiation, and hypoxemia may be suspected of playing a role in the etiology of congenital heart disease, but not a hit-or-miss role. A pattern appears to be emerging. Environmental agents act on individuals predisposed to the malformation and to the agent, and the exposure must occur at a vulnerable period of cardiac development. To isolate and document these elements in animal models is relatively easy. To isolate these variables in a human population is an undertaking of such magnitude that documentation has yet to be obtained. Although very large cooperative studies may implicate some of the many environmental agents potentially capable of causing cardiac malformations in predisposed individuals, the more appropriate focus should be on the genetic-environmental interaction.

Genetic-Environmental Interaction

What are the pathogenetic components of cardiac maldevelopment? In what ways do individuals predisposed to anomalies differ from those not predisposed? Then one may ask what genetic-environmental interactions could predictably be responsible for malformation in the predisposed individual.

**ETIOLOGY OF CONGENITAL HEART DEFECTS**

**GENETIC FACTORS**

1. **MULTIFACTORIAL INTERACTION**

2. **CHROMOSOMAL**

3. **SINGLE MUTANT GENE**

**Figure 6**

Schema for the etiology of congenital heart diseases, emphasizing major importance of multifactorial inheritance and the genetic-environmental interaction, and the lesser role of chromosomal and single gene inheritance, in which the interaction with the environment is relatively insignificant.
ETIOLOGY OF CONGENITAL HEART DISEASES

Figure 7

The above pedigree demonstrates that in multifactorial inheritance the risk to the unborn increases with number of affected individuals, presumably due to the segregation of a larger number of genes predisposing to the malformation. (From Nora, J. J. and associates. Circulation 35: 448, 1967.)

Figure 6 summarizes the mechanisms involved in the etiology of congenital heart disease derived from the tests of the alternative hypotheses in this study. In each mechanism heredity is an important factor, and environment plays a variable role from relatively minor to major importance.

The least common cause of cardiac malformations, single mutant gene syndromes, probably represents less than 1% of cardiovascular diseases. In this mechanism the genetic basis is of maximal importance, and the interaction with the environment is of minimal consequence. However, the environment still plays a role here. A single gene must act in an environment of 100,000 other genes, as well as an environment of external influences. The phenotypic expression of single mutant gene diseases is determined by the entire genetic and environmental milieu. In Marfan's syndrome, for example, the cardiovascular disease may be primarily aortic or mitral, or there may be no detectable cardiovascular abnormality at all. However, the principle holds that the presence or absence of the disease is essentially determined by genetic rather than environmental factors.

The next most common cause of congenital heart defects, although probably accounting for less than 5% of congenital heart diseases, is the category, gross chromosomal aberrations. Once more the disease is primarily determined by genetic factors, with the environment influencing the expression of the disease only to a small extent.

The last category, which seems to encompass most congenital heart diseases, is multifactorial inheritance. In this concept, the environment plays a very important role, but a role which varies depending on the balance of genes predisposing to the defect. In Mendelian inheritance the risk to the unborn after one affected child is fixed at 1 in 2 for dominants (in the usual random-mating situation), and 1 in 4 for recessives. That is, the risk remains 1 in 4 for phenylketonuria, whether or not the mother has had a viral infection or drug exposure, and remains 1 in 4 for each successive child no matter how many children are affected with the disease.
However, in multifactorial inheritance, the risk increases with the number of affected individuals in the family. The more affected individuals, the more this is an indication of a larger number of genes predisposing to the malformation. If there is only one affected sib with ASD and no affected parents, the average risk predicted for the next unborn child would be the square root of p or 2.6% for ASD. In such a family, exposure at a vulnerable period of cardiac development to an environmental influence or teratogen to which the fetus is sensitive will be all that is necessary to push the patient over the threshold from normal cardiac development to cardiac malformation. This may be the situation in most congenital heart defects.

Yet, even in multifactorial inheritance, genetic factors may be almost entirely responsible for a cardiac anomaly with environment having minimal determining effect as is seen in the pedigree in figure 7. We have several families like this family with atrial septal defect and developmentally related cardiac anomalies, in which our interpretation is that there appears to be such a large number of genes predisposing to cardiac malformation that all, or almost all offspring in a sibship are affected. Such pedigrees are uncommon, but are important to recognize from the genetic counseling point of view. In these families the genetic balance is so unfavorable, that predictions for future risk should be based on the empiric risk already seen in the family. That is, almost all offspring will be affected, and the environment will contribute relatively little to determining the anomaly.

Study of the genetic-environmental interaction offers substantial hope for progress in prevention. This will be achieved not so much by identifying environmental agents which may push the predisposed individual over the threshold from normal to abnormal development, but by studying the families with congenital heart diseases and determining in what parameters these individuals differ from those not predisposed. After identification of pathogenetic components of the disease, it is not unreasonable to consider the possibility of genetic engineering of specific replacement or specific avoidance of factors influencing the threshold of the disease.

The goal in proposing the multifactorial hypothesis for the etiology of congenital heart diseases is to bring together divergent etiological factors and to show that they may be encompassed by a single concept which is subject to test. It is hoped that the multifactorial hypothesis is sufficiently stimulating and provocative to invite attack and attempts at disproof, because research required to test this hypothesis will unavoidably increase our knowledge regarding the etiology of congenital heart diseases.

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