Familial Congenital Heart Disease

II. Chromosomai Studies

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Genetic imbalance, the result of aneuploidy or chromosomal structural rearrangements, is now recognized as a cause of anomalous embryogenesis. In general, the anomalies have been extensive and severe when the chromosomal abnormalities have affected segments lengthy enough to be detected by use of currently available techniques. The purposes of the present study were: (1) to determine the incidence of detectable chromosomal aberrations in familial congenital heart disease, and (2) to consider the role of detectable chromosomal morphological variation, known to exist in healthy individuals in the general population, in the causation of anomalous development of a single organ, in this case the heart. Analysis of the chromosomal complements of 35 patients with congenital heart disease forms the basis of this report. The patients were highly selected, each being a member of a family in which multiple instances of cardiac anomalies had been documented.

Methods

Each of the 35 patients whose chromosomal complements were analyzed represented the index case of one of 35 families in each of which multiple members had cardiac anomalies. The first patient from a family whose blood became available to the cytogenetics laboratory became the index case. In most families, the chromosomes of the other affected relatives, as well as some unaffected, were also examined. The patients and those relatives investigated were diagnosed and described by Ehlers and Engle in Part I of these papers, and the same numbering of families and individuals is used here. The total of 85 persons whose chromosomes have been analyzed are indicated by asterisks on the pedigrees in figure 1 of their paper, and the 35 arrows point to the index cases (see also figure 1 of the present paper). The total number of individuals, affected and unaffected, who were analyzed is tabulated in table 1.

Phytohemagglutinin-stimulated dividing blood cells from 3-day-old cultures were studied for chromosomal number, and at least two good cells...
from each individual were photographed for analysis of the karyotype. If a chromosomal aberration was suspected in a given patient, additional cells were photographed, the complements of additional suitable cells were analyzed directly through the microscope, and the culture was repeated. The chromosomes of fibroblasts from a skin biopsy of patient A 3a were examined. Autoradiographic studies were performed in patient B 1a, and the terminal pattern of DNA synthesis in his chromosomes of group 16-18 was compared with the pattern found in normal individuals.  

Results and Comments

The modal number of chromosomes per cell was 46 in every case, and no evidence of numerical mosaicism was found. Except for the morphological autosomal variations found in certain individuals from four different families to be described below (fig. 1, double asterisks), there were no consistently detectable chromosomal aberrations. Slight differences in length observed in the Y sex chromosomes were attributed to its polymorphism in the general population and, therefore, were not included in the tabulations presented.

In family A 2 there were five children: three with atrial septal defects and two with normal hearts. The three children with the cardiac lesions (A 2a, A 2b, and A 2c) and the unaffected father had a prominently enlarged short arm on a chromosome of group 13-15 (fig. 2). The unaffected mother and the two unaffected children had no detectable chromosomal aberration.

In cells from the index case of family A 3, a child (A 3a) with a ventricular septal defect and mitral stenosis, the two nos. 16 differed strikingly in appearance, owing to increased length and also to what seemed, after prolonged study with various optics and filters, to be excessive mass of the long arm of one of the pair (fig. 3). This impressive variation was observed in almost all blood cells with a suitable display of chromosomal morphology and also in fibroblasts from the skin. The variation exceeded that difference in length of the two nos. 16 which is not uncommon in an occasional cell from apparently normal individuals, and at times the longer no. 16 resembled members of group 6-X-12. This same morphological difference of the nos. 16 was found consistently in cells from the unaffected mother and the unaffected maternal grandmother. It was not present, however, in cells from the single culture from the propisita's affected sib (A 3c), although each of the two sibs had the same type of cardiac anomaly. The third sib (A 3b) could not be studied because she had died from her severe cardiac anomaly.

In the affected father (B 1a) in family B 1, prominent satellites were present on one no. 17 chromosome (fig. 4). The fact that these were easily detected in most good cells from cultures prepared on 6 different days but were not seen in cultures prepared on two other occasions is an indication of the capricious nature of this variant. Autoradiograph-
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revealed no phic comparison of individuals, since the no. 17's are distinctively early in cessation of synthesis, a late-replicating region on the short arm of one of the pair, if present, could have been detected. This man and his two children (B 1b and c) had atrial septal defects. Two successful cultures were obtained from each child. In the daughter, no satellites were detectable on the no. 17's, whereas in the son an occasional cell had one no. 17 with satellites, a finding which is not recorded, however, as a variant in the tables. The no. 17 in cells from the index patient's mother and from one of his two unaffected sisters showed no satellites; however, they were clearly visible on one no. 17 in 10% of the cells from the other unaffected sister, and their appearance was quite similar to that of the propositus.

In family D 4b, patient D 4b had a distinctive enlargement of the short arm of a chromosome of group 13-15 (fig. 5). This chromosome resembled the one seen in family 2. His unaffected father had this same chromosomal variant, but not his affected cousin (D 4a) who had the same cardiac anomaly as D 4b, tetralogy of Fallot.

Discussion

Neither the biological significance of these findings nor their relation to the etiology of cardiac malformations can be established at present. This is not only because the number of observations is small but also because the ideal control population is not presently available. The impossibility of being certain that the apparently structural alterations are actually due to extra or missing chromosomal material should also be emphasized. Several other cytogenetic surveys of congenital heart disease from several different countries disclosed variants similar to those found in our survey. In some studies the investigators have viewed the variants as fortuitous or at least insignificant in relation to the etiology of cardiac malformation. Sasaki and associates were less ready to abnegate their findings.

Autosomal variants, often similar to those just described, and also inherited in many cases, were detected by Jacobs and her associates in cells from 23 of 1,020 unrelated individuals chosen from the general Scottish population. In several ways (such as the criteria for selection of patients, their age, and their ethnic and geographic origins) the Edinburgh population study is not completely suitable as the control for our study. However,
Figure 3

Family A 3. Chromosomes of group 16-18 and the shortest member of group 6-X-12 from blood and skin cells of the index patient and from blood cells of her unaffected grandmother. The arrow indicates the variant no. 16. All cells were printed at the same magnification.

Table 2

Comparison of the Incidence of Autosomal Variants in Thirty-five Patients with Familial Congenital Heart Disease with That in the General Scottish Population 4, 5*

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of individuals</th>
<th>Number with detectable autosomal variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index patients in present study</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td>General population, a randomly selected subgroup, age 15-64 5</td>
<td>203</td>
<td>3</td>
</tr>
<tr>
<td>General population, all ages, combined groups 5</td>
<td>1020</td>
<td>23</td>
</tr>
</tbody>
</table>

*These data are suggestive, and when tests of significance are applied (for example, Fisher's exact test), the differences between the index patients in the present study and those listed below indicate statistical significance. However, such tests do not reflect the absence of a suitable control population (see text).
will have some degree of genetic imbalance, and the embryo, as a consequence, will have duplication or deficiency of a chromosomal segment, or both. Depending on the particular segments concerned as well as on other genetic and maternal environmental factors, the imbalance may cause developmental malformation of variable degree, possibly of but a single organ.

The apparently increased frequency of minute chromosomal variations in morphology detected in the present study is a finding of adequate interest to stimulate further investigations in this direction. It focuses attention now on the inheritance of the smallest variations in the human complement in relation to disease. The variations found here were detectable only at the limit of resolution of the cytogenetic techniques used; each was advantageously located in a small chromosome facilitating its microscopic detection. It may
be assumed that others exist, concealed in longer chromosomes until more refined techniques become available.

We are viewing the inherited chromosomal variants in the general population as a **structural load**. (For a more extensive discussion, see reference 9.) The occasional but inevitable imbalance, due to duplication or deficiency of small chromosomal regions, for example, would by this hypothesis result in a disturbance of embryonic development in certain cases. The variants would be transmitted by phenotypically normal, and often genetically balanced, individuals. Such a chromosomal structural load could be responsible for a proportion of birth defects, including malformations of single organs such as the heart. The data presented, although completely compatible with such a reasonable hypothesis, are as yet insufficient to establish the concept.

**Summary**

The chromosomal complement was analyzed in 35 individuals, each selected because of proven cardiac anomaly in him and in one or more of his relatives. The study to date has also been extended to include chromosomal analyses of 29 other affected and 21 unaffected relatives, a total of 85 individuals. In four of the 35 index individuals a small chromosomal variant was found, and in each case it was shown to be familial; in all, variants were detected in 11 of the 85 persons examined.

The various aberrations detected—an enlarged short arm of a member of group 13-15 in two families; heteromorphic nos. 16 in one family; satellites on a no. 17 in one family—are known to exist in apparently normal individuals in the general population. Neither the biological significance of these variants nor their relation to the etiology of cardiac anomalies can be established at present. However, the following working hypothesis has been developed: Small chromosomal variations in structure are transmitted in phenotypically normal individuals in the general population and constitute the chromosomal **structural load**; when the relatively infrequent but inevitable genetic imbalance of an embryo occurs as the consequence of such a chromosomal polymorphism, it may cause embryonic maldevelopment. By this mechanism, the structural load would become responsible for a proportion of birth defects.

**Acknowledgement**

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**Pasteur’s Crystals as Symbols of Pure Science**

... he was already a famous scientist when he began to work on practical problems. From 1847 to 1857 his dominating scientific interests were problems of no apparent practical significance but with large theoretical implications: the relation of molecular structure to optical activity, and the bearing of stereoisomerism on the origin of life; a few years later he became engrossed in other abstract thoughts concerning the biochemical unity of life. As time went on, however, he yielded more and more to the social pressures of his environment, and he spent the largest part of his productive life working on practical problems of fermentation and disease. He became increasingly involved in using science as an instrument of economic conquest rather than as a technique for understanding the universe.

... he often regretted the choice that had been imposed on him by the Zeitgeist. Time and time again he stated that he had been “enchained” by an inescapable forward-moving logic that had led him from the study of crystals... the desire of his early days to work on crystallography and on the nature of life apparently remained with him as a haunting dream. Pasteur’s grandson, Professor L. Pasteur Vallery-Radot, has recently told a moving story... (with Pasteur saying) “Ah! my boy, I wish I had a new life before me! With how much joy I should like to undertake again my studies on crystals!”—RENÉ DUBOS: The Dreams of Reason: Science and Utopias. New York, Columbia University Press, 1961, p. 141.
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