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GENETIC FACTORS IN CONGENITAL MALFORMATIONS

Report of a WHO Scientific Group

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**WHO SCIENTIFIC GROUP ON GENETIC FACTORS
IN CONGENITAL MALFORMATIONS**

Geneva, 22-28 October 1968

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GENETIC FACTORS IN CONGENITAL MALFORMATIONS

Report of a WHO Scientific Group

A WHO Scientific Group on Genetic Factors in Congenital Malformations met in Geneva from 22 to 28 October 1968. The meeting was opened by Dr A. N. Klimov, Director, Division of Biomedical Sciences, who welcomed the participants on behalf of the Director-General. He pointed out that the purpose of the meeting was to consider the part played by genetic factors in some of the more common birth defects, the etiology of which is still poorly understood. In expressing the hope that the Group would outline methods for differentiating genetic and environmental factors involved in intra-uterine maldevelopment, he noted that it might be possible to prevent many birth defects if their causes were better understood.

Dr A. C. Stevenson was elected Chairman and Dr W. Lenz Vice-Chairman; Dr F. C. Fraser and Dr N. E. Morton served as Rapporteurs.

1. INTRODUCTION

This report examines the present state of understanding of the genetic contribution to common congenital malformations. It also considers a "model", which appears to have considerable utility, for discriminating between genetic and environmental factors in the etiology of such malformations, and proposes methods designed to test such a model.

Throughout the report the term "congenital malformation" is used to describe the conditions listed in categories 740 to 759 of the *International Classification of Diseases*,¹ i.e., the great variety of macroscopic anomalies present at birth even if not immediately detectable. This report will not, however, consider conditions attributable to single gene mutations and chromosomal anomalies.

¹ World Health Organization (1967) *Manual of the international statistical classification of diseases, injuries, and causes of death*, Geneva

The Group noted the report¹ of a WHO Scientific Group on Principles for the Testing of Drugs for Teratogenicity and the findings² of the WHO pilot study of congenital malformations undertaken in 16 different countries, hereinafter referred to as "the WHO study".

The report is concerned with malformations whose etiology is considered to involve both genetic and environmental factors. The nature of the genetic factors is relatively ill-defined, and that of the environmental factors is recognized in only a few instances. The Group decided to discuss in detail the information that is available on the etiology of the following malformations: pyloric stenosis, cleft lip and cleft palate, anencephalus, spina bifida, dislocation of the hip, and talipes equinovarus. In discussing each of these malformations, the following questions were considered:

- (1) The hypotheses that can be tested on the basis of available data on frequency in (a) near relatives of cases, (b) different populations, (c) different geographic areas, and (d) different periods of time.
- (2) The additional data that are needed to permit testing and more precise formulation of etiological hypotheses.
- (3) Methods that are suitable for collecting and analysing data, and additional techniques that are needed.
- (4) Environmental factors that influence the probability that a child will be malformed.

All the malformations noted above have the following characteristics, which make them of particular value for comparative study:

- (a) they do not usually have a known environmental or known simple genetic cause;
- (b) they can be diagnosed with reasonable reliability at birth or in infancy without an autopsy;
- (c) they are frequent, in that they occur at least once per 1 000 births in some populations;
- (d) a high proportion of cases occur as isolated malformations; and
- (e) family studies indicate that they have a specific but complex etiology.

For cleft lip and palate there are unusually good family data. The neural tube malformations — anencephalus and spina bifida — are outstanding as a cause of stillbirth and infant death. Pyloric stenosis shows

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 364

² Stevenson, A. C. et al. (1966) *Bull. Wld Hlth Org.*, 34, Suppl.

the effect on family risks of the sex of the index case (the case or cases through which the family is ascertained) in a condition with a sex ratio strikingly different from unity. Congenital dislocation of the hip provides an instance where some details are known of the genetic and environmental interaction. And talipes equinovarus shows how familial risks may vary with population incidence.

The Group recognized that other malformations showing some, but not all, of these features are also of special interest. For example, congenital heart disease is of great medical importance, but the difficulty of making specific diagnoses in babies without performing an autopsy prevents valid interpopulation comparisons. There is a need for good family and epidemiological studies on this important group of malformations, using accurate methods of diagnosis and considering specific types of cardiac malformations separately.

Throughout this report the term "multifactorial" is applied to the etiology of any defect resulting from the interaction of several genetic and environmental factors, none of which is by itself the cause of the defect. The term "polygenic" is applied to genetic etiological factors that involve several gene loci. The latter term does not imply that the genes have any special properties, but means only that they are multiple and that their effects, in contrast to those of "major" genes, are not great enough to be individually recognized.

Quasi-continuous variants

It is probable that most, if not all, of the selected conditions listed above involve a developmental threshold of some kind that separates a continuously distributed variable into two discontinuous classes, normal and abnormal. Such traits have been termed "quasi-continuous variations". It may be helpful to consider some of their characteristics here, although they are discussed in greater detail in section 3.4.

Cleft palate in experimental animals can be considered as an excellent example of quasi-continuous variation. Here, the continuous variable is the rate at which the palate shelves move from their position lateral to the tongue to the horizontal plane above the tongue. If shelf movement is delayed beyond a certain stage in relation to the growth of the head as a whole, the shelves will be unable to meet and fuse and a cleft palate will result. The degree of delay constitutes a threshold that separates animals with normal from animals with cleft palates.

In the case of malformations such as those considered below, a similar situation exists. The threshold will be near one tail of the distribution, and an affected individual lies beyond it. The distribution for the near relatives will be shifted towards the threshold and the closer the degree of relationship the greater the shift. Thus one would expect a

small increase above the population level in frequency of the malformation in third-degree relatives, a greater increase in second-degree relatives, and a much greater increase in first-degree relatives.

In a malformation that affects males more frequently than females, the distribution of the developmental variable is presumably closer to the threshold in males than in females. Conversely, affected females must be relatively farther out towards the tail of the distribution than affected males, and would therefore be genetically more predisposed. Consequently, a higher proportion of affected individuals is to be expected among the near relatives of affected females than among those of affected males. In principle, therefore, the proportion of near relatives affected by malformations that occur more often in one sex than the other will be greater when the proband is of the less often affected sex.

2. CONDITIONS WHICH EXEMPLIFY APPROACHES TO IDENTIFICATION OF GENETIC COMPONENTS OF ETIOLOGY

2.1 Pyloric stenosis

Pathology

The fibres of the circular muscle of the pylorus are hypertrophied and probably also increased in number. No primary abnormality of the intramural ganglion cells has been demonstrated.

Time of onset

The signs and symptoms of the malformation seldom develop before 7 days after birth, although in premature children they may occur before the expected day of delivery. The tumour has not been seen in a still-born child, but has been observed 3 days after birth in a child dying for other reasons. It seems likely that a certain period of postnatal life is necessary for development of the hypertrophy. The tumour may occur without causing clinical disease. It seldom causes symptoms after the first 4 months of life, although the symptoms occasionally recur in young adult life. "Pylorospasm" probably represents a minor degree of the same condition.

Birth order

The excess of first-born children with pyloric stenosis, described many years ago, is probably only of small degree, and is perhaps no more than the result of the less skilled handling of the child by an inexperienced mother, causing clinical manifestations in mild cases of pyloric stenosis.

Seasonal variations

Some studies have shown a seasonal variation in the incidence of pyloric stenosis, the condition occurring more frequently in children born in the summer. However, other studies have not shown such a variation, and it is not possible to reach a conclusion at present.

Geographical and racial variations

The frequency of the condition probably varies widely from one geographical area to another, although its incidence is difficult to measure in countries where the infantile mortality from gastrointestinal disorders is high and where hospital accommodation is inadequate. The frequency among North-West Europeans is of the order of 1-3 cases per 1 000 total births. Findings in a recent study in Hawaii and the experience of paediatricians in Hong Kong and Singapore suggest that the frequency is much less in Mongolian peoples. Experience in the USA and Africa suggests that it is also lower in people of African descent. In Israel, the frequency is low in Jews, although not as low among Ashkenazim as among other Jews.

Consanguinity

There are indications of a slight association between pyloric stenosis and parental consanguinity. A study in London showed that the parents of 6 patients per 1 000 were first cousins — perhaps 3 times the rate among the general population.

Sex ratio

In most series the sex ratio (male frequency divided by female frequency) is about 5.

Twin studies

The few twin studies reported indicate a higher proportion of monozygotic than of dizygotic co-twins affected, which suggests that the observed familial tendency has a genetic basis.

Family patterns

Family data for first-degree relatives in a London series showing the proportion affected are summarized in Table 1. In this series girl index patients were ascertained over a longer period than boys and so the sex ratio is not that of all patients with pyloric stenosis.

TABLE I. PROPORTION AND PERCENTAGE AFFECTED OF FIRST-DEGREE RELATIVES OF MALE AND FEMALE PATIENTS WITH PYLORIC STENOSIS

Index patients	Sons	Daughters	Brothers	Sisters
Male 640	19/347 5.5 %	8/337 2.4 %	21/546 3.8 %	15/565 2.7 %
Female 352	20/106 18.9 %	7/100 7.0 %	25/273 9.2 %	10/263 3.8 %

The proportion of relatives affected is about three times greater for female than male index patients. This phenomenon is explicable on a multifactorial hypothesis if the genetic predisposition is assumed to be wholly or partly of the additive polygenic type, since female patients will be more extreme deviants from their population mean (page 8). Relatively to the incidence of pyloric stenosis in the same sex — estimated to be 1 in 200 of total male births and 1 in 1 000 of total female births in the London area, — the increase in risk to relatives is about 10-fold for male relatives of male index patients, about 25-fold for female relatives of male index patients, about 30-fold for male relatives of female index patients, and about 50-fold for female relatives of female index patients. This gradation is also readily explicable if the genetic predisposition is polygenic but not on any other genetic hypothesis. No convincing explanation is available for the apparently higher risk to the offspring than to the sibs of index patients. Lower but significant increases relative to the general population are also seen in the proportion of affected nephews and nieces and first cousins of index patients, and there is no suggestion that the maternal genotype is of any importance. It is interesting that affected individuals later tend to be athletic.

The incidence of offspring born after an index patient has already had one affected child shows the expected increase in the proportion affected; 3 out of 17 where the father was the index patient and 5 out of 13 where the mother was the index patient.

In sum, family data strongly support the view that pyloric stenosis is polygenic.

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2.2 Cleft lip and cleft palate

Throughout the report the following terminology will be used :

Cleft lip :	a cleft involving the primary palate, including the lip
Cleft lip (+ cleft palate) :	a cleft of the primary and secondary palate
Cleft lip (\pm cleft palate) :	the above two groups combined
Cleft palate :	cleft of the secondary palate without cleft lip

Except when otherwise stated the discussion will be limited as far as possible to cleft lip (\pm cleft palate) and cleft palate without other major malformations.

There is good embryological evidence that cleft lip and cleft lip (\pm cleft palate) are varying degrees of the same embryological defect, and that cleft palate is a separate entity. The cleft palate that often occurs with cleft lip appears, in the mouse at least, to be a secondary developmental consequence of the defect in the lip, and not a result of any primary abnormality in the palate.

Family studies lead to the same conclusion. If the proband has a cleft lip or cleft lip (\pm cleft palate) there is an increased frequency of cleft lip (\pm cleft palate) but not of cleft palate in the near relatives; if the proband has a cleft palate there is an increased frequency of cleft palate in the near relatives, but not of cleft lip (\pm cleft palate).

Admittedly there are certain exceptions to the rule. In Tasmania and at least one American Indian tribe cleft lip (\pm cleft palate) and cleft palate both show an increased frequency in the near relatives of probands with either defect. This would suggest that there may be a genetic system that predisposes the embryo to develop either cleft lip (\pm cleft palate) or cleft palate in certain unusual families or populations. There is also at least one mutant gene which can cause either cleft lip (\pm cleft palate) or cleft palate, or neither defect, along with lip pits. Nevertheless, in most populations, and excluding rare syndromes, cleft lip and cleft lip (\pm cleft palate) appear to be etiologically distinct from cleft palate. It is most important that they be recorded and reported separately. Much of the published data suffers from the failure to do this.

2.2.1 Cleft lip (\pm cleft palate)

Development

Little information exists in man on the development of cleft lip (\pm cleft palate). In a Japanese series the lip is completed before Streeter Horizon XIX, or a postconceptional age of about 7 weeks. In the mouse the first visible abnormality (at the equivalent of about 4 weeks of gestation) is failure of fusion between the epithelia of the medial nasal and

lateral nasal processes at the posterior end of the nasal pit, followed by breakdown of the bridge joining the medial nasal, lateral nasal, and maxillary processes.

The excessively large prolabium that develops in association with cleft lip interferes with the normal forward movement of the tongue when the palatal shelves begin to close, at the equivalent of 6-7 weeks, and the tongue tends to remain between the shelves. The resulting delay in shelf movement is a reasonable explanation for the cleft palate that often accompanies cleft lip.

Frequency

Estimates of frequency range from 0.6 to 1.6 per 1 000 births in various reasonably large series, roughly 1/3 being cleft lip and 2/3 cleft lip (\pm cleft palate). The frequency is said to be increasing in Denmark, but it is difficult to rule out the possibility of previous under-reporting.

Sex ratio

There is usually an excess of males, the excess being greater when the palate is involved than when it is not and when the lip defect is bilateral rather than unilateral — i.e., the more severe the defect the higher the excess. It would be useful to know whether there are differences in face shape or rate of development between male and female embryos; a study of abortuses is needed.

Laterality

In unilateral clefts there is a preference for the left side (about 60 % are left-sided). The embryological basis for this is unknown. In a series of abortuses observed in Japan there has been no evidence so far that asymmetries of facial development in the embryo account for cleft laterality.

Parental characteristics

The effect of parental age is difficult to evaluate because of the difficulty of getting good control data. In some series, but not in others, the incidence somewhat increases with advancing parental age, particularly the father's. No consistent variations have been found in relation to the parity or social class of the parents.

Race

The frequency per 1000 total births of cleft lip (\pm cleft palate) is low in American Negroes (0.4), higher in Caucasians (1.3), and higher still in Japanese (2.1). Certain North American Indian tribes also have a high frequency. It would be valuable to correlate racial differences in

cleft lip frequency with differences in face shape. American Negroes appear to have more median clefts of the gum than Caucasians.

Environmental causes

Exogenous factors suspected of causing cleft lip (\pm cleft palate) include thalidomide, rubella, meclizine, and maternal diabetes, but in none of these is the association well established, and they could account for only a small proportion of cases.

With respect of the possible relation of other unusual events during pregnancy to cleft lip (\pm cleft palate), information collected retrospectively is notoriously unreliable, but with careful attention to possible memory biases and comparison with suitable controls — such as albinos, phenylketonurics, or persons with other conditions obviously not caused by the prenatal environment, — evidence may be acquired that can at least suggest more specific studies. One such study has shown that maternal emotional stress is recalled more frequently by mothers in pregnancies resulting in a child with cleft lip (\pm cleft palate) than in pregnancies resulting in unaffected sibs, but that the same difference occurs between control probands (with clearly inherited diseases) and their sibs. Thus a maternal memory bias is clearly indicated, and there is no evidence that maternal stress is teratogenic. There is, however, some tentative evidence that maternal bleeding in the first trimester and toxæmia of pregnancy may be increased in pregnancies resulting in a child with cleft lip (\pm cleft palate), but other unusual events in pregnancy have not shown an association with the malformation. There is also an indication that pathological conditions of the reproductive tract occur more frequently in mothers of cleft lip (\pm cleft palate) children than in control mothers. But other factors show no difference between the mothers of malformed children and controls.

Secular trends may also contribute information about environmental factors. For instance, the absence of changes in cleft lip (\pm cleft palate) frequency in Germany before, during, and after the Second World War indicates that maternal stress or nutritional restriction is not relevant.

The frequency of cleft lip (\pm cleft palate) does not seem to be increased in twins, suggesting that intra-uterine crowding or other uterine differences resulting from twinning are not causal factors. However, a case is known of discordant monozygotic twins in which the affected twin had an abnormal placenta. Further intensive study of placental structure and function in babies with the malformation is required.

Genetic factors

(a) *Mutant genes.* A number of rare mutant genes cause cleft lip (\pm cleft palate) as one manifestation of a syndrome. They include a number

of autosomal dominant genes causing such syndromes as the basal naevoid cell carcinoma syndrome, cleft lip and palate with split hand and foot, cleft lip and/or palate with cysts of the lower lip, mandibulofacial dysostosis, and the popliteal webbing syndrome, and an autosomal recessive gene causing the syndrome of tetraphocomelia with clitoral enlargement. Such syndromes account for only a small proportion, perhaps 5%, of cases of the malformation. It is important that they should be excluded from population or family studies of cleft lip (\pm cleft palate) of the usual type, as their inclusion can produce misleading results. They may, however, provide valuable opportunities for the study of developmental mechanisms.

(b) *Chromosomal aberrations.* Cleft lip (\pm cleft palate) occurs in association with several kinds of chromosomal aberration, notably in D trisomy, but also in trisomy 18 and deletion of chromosome 18 and 4. In such cases there are always other malformations as well. Most cases of cleft lip (\pm cleft palate) have no demonstrable chromosomal anomaly.

(c) *Multifactorial causes.* This class contains the great majority of cases of cleft lip (\pm cleft palate). There is a strong familial tendency, and the concordance rate is higher in monozygotic pairs (about 40%) than in dizygotic pairs (about 7%).

In relatives of patients with cleft lip (\pm cleft palate) the frequency of cleft lip (\pm cleft palate) is 3-4% in siblings and children; there is a sharp drop to 0.6-1% in second-degree relatives, and a much smaller drop (to 0.4%) in third-degree relatives. This is what one would expect of a multifactorially-determined threshold character. Also consistent with such a character are: (1) an increase in recurrence risk with an increase in the severity of the proband's defect; (2) the higher recurrence risk for female probands; (3) the increase in the risk of recurrence in sibs, from 4% after one affected child to 9% after two affected children; and (4) the higher risk of recurrence in sibs of probands with an affected second- or third-degree relative, as compared with those with a negative family history.

Further comparisons could be made to test the multifactorial hypothesis. Firstly, in populations with a high frequency of cleft lip (\pm cleft palate) the recurrence risk in sibs ought to be correspondingly increased. In a Japanese study this is not borne out, but the numbers are small and further observations are needed. Secondly, in populations with a high frequency of the malformation, the ratio of males to females in affected children might be lower. Comparisons of Japanese and Caucasian figures do not bear this out either. Comparisons of the sex ratio in affected sibs of probands would be interesting.

In mice there is evidence that the maternal genotype predisposes to spontaneous cleft lip, but there is no suggestion from family studies of

such an effect in man, and evidence from inter-racial crosses provides no indication that maternal factors are causally significant.

Microforms

It is not established that any so-called "microforms" of cleft lip (other than minimal degrees of the cleft itself) occur with increased frequency in near relatives. Most studies attempting to identify microforms have suffered from inadequate controls. Missing lateral incisors and nostril asymmetry do not appear to be microforms of the malformation.

Associated anomalies

Other major malformations occur in babies with cleft lip (\pm cleft palate) more often than in the general population, but there is a dearth of large series of cases observed at birth. Some associations may represent specific syndromes. There is some suggestion that they occur more often in cases where the family history is negative for cases of cleft lip (\pm cleft palate) and that the risk of recurrence is lower when the proband has an additional malformation.

2.2.2 *Cleft palate*

Development

Palate closure has been extensively studied in experimental animals. Cleft palate can result from :

- (a) A structurally abnormal shelf.
- (b) A narrow shelf.
- (c) A reduction in shelf force.
- (d) Increased resistance of the tongue, e.g., as a result of the large median process associated with cleft lip; or compression of the embryo from oligohydramnios; or interference with extension of the neck (which in the rabbit, at least, aids in getting the tongue out of the way); or micrognathia.
- (e) A wide head.
- (f) Failure of epithelial fusion.

Both genes and environmental agents can contribute to the occurrence of cleft palate in any one or more of these ways. Experimental models exist for most of these mechanisms.

Frequency

Cleft palate is less common than cleft lip (\pm cleft palate), estimates of the frequency ranging from about 0.4 to 0.5 per 1 000 total births.

More females are affected than males, in a ratio of about 3 to 2. No noteworthy associations have been detected with the season of birth, the social class, parental age, or birth order.

Race

The frequency per 1 000 total births may be somewhat lower in American Negroes (0.41) than in Caucasians (0.48), and somewhat higher than either in Japanese (0.55).

Environmental causes

Though many drugs and other agents cause cleft palate experimentally, few teratogens are known to cause cleft palate in man, and there are enough data on some of these agents (e.g., cortisone and meclizine), to show that if they cause cleft palate they do so very rarely. Rubella and thalidomide and aminopterin cause cleft palate as one manifestation of foetal damage.

Genetic factors

(a) *Mutant genes.* A number of rare syndromes include cleft palate as one manifestation. These include some showing autosomal dominant inheritance (Apert's syndrome, Marfan's syndrome, cleidocranial dysostosis, mandibulofacial dysostosis), a possible sex-linked dominant lethal one in males (the orofacial-digital syndrome), and several autosomal recessive syndromes (chondrodystrophia calcificans congenita, diastrophic dwarfism, the Smith-Lemli-Opitz syndrome). It is important to exclude these from series collected for the study of isolated cleft palate.

(b) *Chromosomal aberrations.* Cleft palate may occur with several chromosomal aberrations, particularly E trisomy and the XXXXY syndrome.

(c) *Multifactorial causes.* Cleft palate shows a familial tendency similar to that of cleft lip (\pm cleft palate). However, there are less data available for analysis, presumably because the condition is rarer. The concordance rate is probably higher in monozygotic than in dizygotic twins. There does not seem to be an increase in the risk of recurrence after two affected (in the small number of families recorded), and the presence of an affected near relative other than a sibling does not seem to increase the recurrence risk. If these two observations were borne out by further studies, they would provide arguments against the multifactorial hypothesis. There is some suggestion of a maternal effect, but more information is needed.

(d) *Microforms.* There is some evidence that bifid uvula may be a microform of cleft palate.

(e) *Associated malformations.* The increase in associated malformations appears to be greater for cleft palate than for cleft lip (\pm cleft palate), but the proportion decreases as the number of recognizable syndromes decreases.

In sum, cleft lip (\pm cleft palate) appears to fit the multifactorial hypothesis quite well, though a few possible discrepancies exist and more data are needed.

Less information is available on cleft palate and the evidence for multifactorial causation is not as convincing. Certain observations at present incompatible with the hypothesis need further study.

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2.3 Anencephalus and spina bifida

2.3.1. Anencephalus

Pathology

The absence of the vault of the skull in anencephalus is characteristic; a mass of disorganized vascular and often haemorrhagic neural tissue, covered by a transparent membrane, forms the top of the head. The eyes are usually prominent and the ears are often deformed. The appearance is so obvious that recognition is invariably assured. Many other malformations may be associated with anencephalus, but complete normality from the head downwards is not uncommon.

Many attempts have been made to separate cases according to specific pathological changes and to relate the groups to differing epidemiologies. However, epidemiological characteristics have not been as yet associated with specific morphological variants.

Embryology and time factors

The defects in anencephalus represent disturbances of structures derived from the anterior end of the neural tube, which normally closes

in the fourth week after fertilization. It is not at all clear whether anencephalus represents a defect in the primary closure of the neural tube or whether the anterior neuropore closes, perhaps even prematurely, with a subsequent defect in differentiation followed by degeneration. It is likely that there is heterogeneity in the timing and mechanisms involved, as there is in experimentally induced neural tube defects. However, if it is true that the defect can be brought about after closure of the neural tube, it is not safe to assume that the abnormality must be determined before the end of the fifth week of gestation.

Associations with disturbances of pregnancy or abnormalities in mothers

A high proportion of cases of anencephalus are associated with hydramnios. Precise figures are difficult to collect owing to the difficulty of detecting and measuring hydramnios, but there can be no doubt that it occurs in well over half the cases of anencephalus. The hydramnios sometimes seems to be of the very early acute type, in which case the foetus is often born before 28 weeks. It is doubtful if toxæmia of pregnancy is especially associated with anencephalus.

Late in pregnancy the abnormal head shape probably predisposes to a high frequency of late fixation of the head in primiparae and of other than vertex presentation. A high proportion of the foetuses are premature and at least 90 % are stillborn, often macerated.

Early losses of anencephalic foetuses

Anencephalic foetuses are found in abortions at all periods of gestation. In a Japanese series of induced abortions, both anencephalus (2.2 per 1 000) and myeloschisis (2.2 per 1 000) appear to occur more frequently than they do at birth (0.6 and 0.3 per 1 000 total births respectively).

Twinning and anencephalus

Anencephalus is no more common in twins than in single births. It has been described in both members of pairs of dizygotic twins, in both of monozygotic twins, and in one instance in all three of identical triplets. Two instances are on record of only one of conjoined twins being affected. Many instances have been reported where only one of dizygotic and of monozygotic twin pairs has been affected. The data available do not provide reliable estimates of concordance in monozygotic as opposed to dizygotic pairs, as cases in unselected series are few and reporting of individual pairs not in series is likely to be selective. Unbiased twin studies, with a reliable determination of the zygosity and a careful examination of the placentæ, would be valuable.

Effects of maternal age and birth order

Birth order and maternal age are probably both associated with variation in the frequency of anencephalus, though which is the more important is not clearly established.

It would seem that the frequency is slightly higher in first than in second and third births, but in subsequent births the frequency rises sharply to levels higher than that in first births. There is almost certainly an association of anencephalus with an increased frequency of miscarriages in other pregnancies of the same mother. Probably 25% of other such pregnancies end in abortion, as compared to about 15% in the general population.

Association with blood groups

The finding of an apparent association with blood group O in one series has not been confirmed by several other studies.

Frequency

There is considerable variation in frequency between different countries and in different areas within countries. The highest recorded frequencies, of over 5 per 1 000 total births, have been found in cities in Northern Ireland and the Republic of Ireland, while the condition appears to occur in certain African and South American countries and in Japan in frequencies of less than 1 per 1 000. However, within France, the United Kingdom, and the USA, there is much to suggest that frequencies vary by as much as a factor of 3. In North America there appears to be an East-West cline with frequencies tending to decrease towards the West.

Figures for all births are often erroneously compared with those for hospital births, and it has been noted in one city that the rate in hospital births in one particular year was 6.78 per 1 000, whereas the rate in births at home was only 4.58 per 1 000. It has been repeatedly shown that within given countries frequencies are about five times as high in the poorer socio-economic groups (represented, for example, in the United Kingdom by those where the fathers were unskilled labourers) as in the highest (professional or managerial classes).

Studies of migrant populations are useful in pointing to the presence or absence of possible environmental factors. For instance, anencephalus appears to be rather high among Punjabi Sikhs and also (on the basis of the small sample available) among migrants from the Punjab to the United Kingdom.

There is suggestive evidence from some series of a seasonal variation in the frequency of anencephalus in children. Thus in Scotland a high proportion of cases appeared to be born in November, December, January,

and February. Some other series do not appear to show such a phenomenon or show a concentration at a different time of the year. There is some evidence of steady rises and falls in frequencies over years in certain areas.

A relationship between anencephalus and maternal influenza has been claimed but has not been substantiated by further studies.

Sex ratio

In all large series of anencephalics reported there has been an excess of females, there being on average a sex ratio of about 0.3. However, there is a considerable variation in different series. The significance of this is not understood and these differences have not been related to any other variable. There is apparently no difference in the sex ratio of cases which are and are not complicated by the presence of spina bifida.

Consanguinity

A slight increase of consanguineous marriages has been reported in parents of children with neural tube defects over the low prevailing rates of consanguineous marriages in the United Kingdom, but the numbers are small and a specific association with anencephalus cannot be demonstrated. In one series from Pakistan where the consanguinity rate was about 30 %, 15 of 17 children with neural tube defects had consanguineous parents.

The WHO study shows highly significant associations between neural tube defect and consanguinity in Alexandria and in Bombay. In the remaining countries (where either consanguinity was uncommon or the frequency of neural tube defects was low) marriages of the parents of children with neural tube defects were also more often consanguineous than those with normal children, although the difference was not significant at a 5 % level. The same remarks apply to anencephalus as to the total of neural tube defects. The association however is at least as strong in respect of hydrocephalus with associated spina bifida which, being predominantly due to the Arnold-Chiari malformation, is likely to have many etiological factors in common with anencephalus. Subsequent to the WHO study, a follow-up over another 10 000 births in Alexandria has revealed essentially the same association.

Familial incidence

There can be no doubt that the frequency of anencephalus is greater in offspring born to the same parents subsequent to the birth of one anencephalic child. Estimates of the frequency of anencephalus or spina bifida in subsequent sibs vary between about 1 in 20 and 1 in 50. The

frequency of sibs after a second anencephalic child is born is still higher, estimates being of the order of 1 in 10.

There is much to suggest that in effect there are "high-risk families", but these can only be identified when more than one case has occurred. Instances of 4 or 5 anencephalics in a sibship have been reported. A remarkable isolated report is of considerable interest. A woman had had 3 anencephalics, and her fourth pregnancy, which resulted from an artificial insemination from a donor other than her husband, yielded yet another anencephalic. It is not yet clear whether the risk to a subsequent child differs after the birth of a male or of a female anencephalic.

It is extremely difficult to collect sufficient information to show whether the frequency is higher in maternal or paternal aunts, uncles, or cousins. There is a small increase in frequency in second- or third-degree relatives of index cases.

Experimental teratology

Studies on embryos with the exencephaly and pseudencephaly that occur spontaneously in some strains of experimental animal or can be induced in them by various teratogenic agents should help clarify the nature of the primary developmental deviation, but it is difficult to be sure how similar these anomalies are to anencephalus in human fetuses.

Unbalanced translocations induced by parental radiation were shown over 30 years ago to cause pseudencephaly in mice. Similar conditions are readily produced in mice and rats by exposure of embryos *in utero* to radiation and a wide variety of insults at appropriate times, among these starvation of the mothers, vitamin E deficiency, excess of vitamin A, folic acid antagonists, and a large number of cytotoxic drugs and chemicals whose mode of action is ill understood. The time of exposure to these toxic agents is specific, from the 7th to 9th day in rats, and about the same or somewhat earlier in mice. In pseudencephaly there is good evidence that the anterior neuropore does not close and the anterior end of the tube does not separate from the epidermis. The developmental anomalies are too complex to discuss here, but an important point is that the anomalous development of the forebrain is followed by superficial necrosis around the 14th to 16th day, which progresses until all the forebrain is destroyed. There is usually an excess of amniotic fluid with pseudencephaly, but it is invariably bloodstained — a finding not paralleled by anencephalus in man. There appears to be no departure from a sex ratio of 1 in spontaneous or induced pseudencephaly.

Evidence bearing on etiology

The picture that emerges from the foregoing suggests very strongly that environmental factors are of great importance. Indeed it could

plausibly be argued that the preponderance of cases in sibships is mostly determined by a common intra-uterine environment. As there are so many teratogenic agents that could probably produce anencephalus, it is not unlikely that many sporadic cases of the condition are determined by a wide variety of non-recurring insults.

Nevertheless, there are a number of indications to suggest a genetic contribution to the etiology, not determined at a single locus. They include the association with consanguinity and probably the different frequencies in ethnic groups, which are independent of environmental factors — although the persistence of dietary habits after migration makes it difficult to be confident about this.

The etiologies of anencephalus and of spina bifida are considered further in the next section, since they have much in common.

2.3.2 *Spina bifida*

Pathology

The essential lesion in spina bifida is failure of the primitive neuro-ectodermal groove to close so that, at its slightest manifestation, in spina bifida occulta, there is only a gap in one or two vertebral arches, the meninges and the cord remaining in the vertebral canal. However, that ectodermal as well as mesodermal elements are involved is often indicated by dimples, haemartomata, moles, or a growth of hair over the defect.

Conventionally, when there is protrusion of the meninges only the condition is termed meningocele; and when the cord or the cauda equina is attached to the protruded meninges the condition is called meningo-myelocele. The term myelocele is used in various ways, but the essential feature is that the neural plate is exposed. The term spina bifida cystica is often applied to all degrees more severe than spina bifida occulta: This may be confusing, in particular when describing a condition of rachischisis with an abnormal canal or neural plate exposed over the whole length of several vertebrae and no evidence of a "cyst" (spina bifida aperta). Many cases may be classed as hydrocephalus and spina bifida (including the Arnold-Chiari malformation) or just spina bifida, depending on whether an autopsy has been carried out or whether or not there is obvious head enlargement, or according to the preference of the classifier who is using the datum for statistical purposes.

Nevertheless, it is clear that not all cases of hydrocephalus with spina bifida are due to the Arnold-Chiari malformation. Occasional cases of stenosis or forking and effective obliteration of the aqueduct of Sylvius are encountered, and cases where there are ill-defined gliomatous overgrowths pressing on the aqueduct. In other cases there is obstruction of the foramina of Luschka and Magendie leading from the fourth to the

lateral ventricles. Finally, there are cases where the mechanism of the hydrocephalus is not understood. Cases are excluded where hydrocephalus is secondary to inflammatory changes or ill-advised surgical interference with spina bifida.

Frequency of spina bifida

There is little doubt that there are real variations in the frequencies of spina bifida in different communities. These differences are both absolute and relative to those of anencephalus. However, great care must be taken in comparing frequencies, as with anencephalus, because some series are derived from hospital births and others from all births in the community and there may be differences in the criteria employed for calling the condition spina bifida. Spina bifida occulta is usually, but not always, excluded and there must be some difference of opinion as to where spina bifida occulta ends and spina bifida aperta begins. More serious, however, is the difference in practice over whether hydrocephalus associated with spina bifida and spina bifida as an apparently isolated phenomenon are classed separately or whether both conditions are simply termed spina bifida.

In Belfast the population frequency in the WHO study per 1 000 total births, including occipital meningoceles and encephaloceles, was 4.5 and in South Wales 4.3. Relatively high incidences were seen in Alexandria (2.1), but the overall frequency was a little under 1 per 1 000.

Maternal age and birth order

Maternal age and parity appear both to have an effect on the frequency of spina bifida and, as in the case of anencephalus, there is much to suggest that the condition is more frequent in first than in second and third births. There appears to be also a social class gradient of frequency, as with anencephalus, but it is perhaps not so marked. As regards seasonal variation, again there is some difference of opinion. If there is any time of the year in the United Kingdom at which spina bifida births are more common than at other times, it is probably at the end of the first quarter.

Embryology and time factors

Most extensive dorsal spina bifidas must have had their origin in failure of the neuro-ectodermal tube to close properly at a very early stage in embryogenesis, as closure begins in the future dorsal area and is complete in the fourth week after fertilization. However, there is good evidence that the condition can follow rupture of a hydromyelia at a later stage in embryogenesis.

Association with disturbances of pregnancy

As in the case of anencephalus, there is an association with hydramnios. The proportion of cases associated is probably not as high as in anencephalus, and it is not clear whether particular degrees of spina bifida or the types associated with the Arnold-Chiari malformation are particularly closely associated. It may be that severe rachischisis is particularly associated with early acute hydramnios.

Twinning and spina bifida

The available data are insufficient in precision and in amount to permit any conclusions to be drawn.

Sex ratio

The sex ratio is higher than in anencephalus and appears to average about 0.85, but there is considerable variation in the different series reported. The ratio will depend on what is included in the term spina bifida (see page 22).

Consanguinity

There is some suggestion of an association with consanguinity in a number of series of cases published over the years. In the WHO study, if hydrocephalus with spina bifida and spina bifida alone are considered together, out of 214 affected children 20 (9.3%) had consanguineous parents, as compared with a frequency in parents of children who were not malformed of 13 763 out of 369 567 (3.7%) ($P < 0.001$).

Familial incidence

About 1/15 to 1/30 of the sibs of index cases of spina bifida also have spina bifida and/or anencephalus. There appear to be no significant differences in the overall frequencies that depend on whether the first case in the family is a male or a female. There is not so much evidence for specific high-risk families in respect of spina bifida as of anencephalus. It may be that children with spina bifida are more often born alive and many survive for long periods, and there is therefore a greater likelihood that family limitation will be practised subsequent to the birth of an affected child.

As in the case of anencephalus, insufficient good twin data are available for any conclusions to be drawn.

Experimental teratology

There is very little on the experimental production of spina bifida in mammals. The condition appears to be produced relatively infrequently,

or the teratogens at a given time produce pseudencephaly with or without spina bifida rather than spina bifida alone. An autosomal recessive form of spina bifida occurs in rabbits and in mice.

Evidence regarding the etiology of spina bifida

The remarks in section 2.3.1 on anencephalus are essentially applicable to spina bifida.

Etiological factors common to different types of neural tube defect

It is well known that in sibships where an index case has one type of neural tube defect other sibs have another type more frequently than would be expected by chance. This has repeatedly been demonstrated, although the frequency of the same or of different defects varies from series to series of sibs. However, in sibships with anencephalus or spina bifida as index cases there is no convincing evidence that hydrocephalus without spina bifida occurs more frequently than would be expected by chance. In these cases of hydrocephalus the sex ratio is high in many series. For example, in the WHO study the sex ratio was 1.0 or more in 18 of the 24 centres and the mean for all hydrocephalics in all countries was 1.33. This is accounted for in part at least by a substantial contribution of cases due to X-linked genes, but the size of the contribution is not known.

It has been shown that in populations where the rate for one type of neural tube defect was high the rates for all types tended to be high. In the WHO study the frequencies of the 7 groups of neural tube defects were all positively correlated in the 24 centres. Further, all the correlations were significant at a 5% level, except some of those involving categories 5 (occipital meningocele) and 7 (other neural tube defects), in which the numbers were very small.

Genetic hypotheses

Much of the variation in the population frequencies of the neural tube defects must be attributed to environmental causes, although the evidence from associations with parental consanguinity and from increased frequencies in second- and third-degree relatives indicate genetically determined predisposition. It is clear that no monofactorial hypothesis can adequately explain the data in respect of anencephalus and spina bifida, and though X-linked hydrocephalus occurs it is numerically unimportant. Most of these conditions are lethal so there are no data for two generations. This imposes some limitations when considering the appropriateness of multifactorial hypotheses. Nevertheless, the serial non-linear increase in risk to sibs born after one or more have been affected and the relationship with consanguinity are compatible with a multifactorial

hypothesis. If data are obtained to show an appropriate relationship between intrafamilial risks and population frequencies, a multifactorial hypothesis would be the most appropriate one.

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2.4 Congenital dislocation of the hip

Incidence

Estimates of the frequency when the diagnosis was made around the first birthday were about 1 per 1 000 total births in the United Kingdom and Sweden. The condition appears relatively uncommon in Africa and in some Mongolian populations. High frequencies have been reported in Lapp and in certain American Indian tribes where it is the practice to swaddle infants tightly with hips extended and adducted.

The recent improvements in methods of diagnosis of hips that are dislocated or capable of dislocation have led to estimates of frequencies of 4 per 1 000 or more. The lower frequency of clinical dislocation at one year of age must mean that the majority of these cases do not progress to permanent dislocation.

The sex ratio is in most series about 0.13, i.e., 1 boy to 7 or 8 girls. It has been suggested that this is due to the female foetus developing a hormonally induced joint laxity late in pregnancy.

Environmental factors

It has long been known that the condition is associated with breech birth and in particular, in primiparous mothers, with breech births associated with hip flexion and knee extension (frank breech presentation). This position has been shown to cause dislocation in the newborn rabbit.

Thus at least two predisposing environmental factors are known: the intra-uterine position of the hips and the postnatal position of the hips, the latter perhaps preventing a natural recovery from congenital dislocation or subluxation.

Family studies

A large-scale twin study in Germany indicates that the monozygotic twin concordance is about 40% and the dizygotic about 3%.

Family studies show an increased risk of the condition in sibs and offspring, which is more marked for the relatives of male index patients. For female index patients the risk to sibs is less than 1% for brothers and about 5% for sisters; for male index patients about 4% for brothers and 7% for sisters.

Mechanisms of gene action

Two genetic factors have been recognized :

(1) A generalized joint laxity, found in the majority of affected male patients and usually present in one parent, which suggests that it is an autosomal dominant condition. This persistent generalized joint laxity is observed in a smaller proportion of female patients.

(2) A shallow acetabulum (resulting in an inadequate roof to the acetabulum), seen on the unaffected side in unilateral cases and detected in minor degree in some of the unaffected parents and sibs of index patients. The genetic predisposition here is likely to be polygenic.

In individual families with affected members the interaction may be seen of familial generalized joint laxity, shallow acetabula, and breech birth.

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2.5 Talipes equinovarus

Club foot is a condition of varied causation and therefore studies are best made after subdivision into specific types. The best defined entity and that which causes the greatest handicap is talipes equinovarus, and here the type that is persistent and resistant to treatment must be distinguished from postural talipes, which disappears without treatment. The latter does not seem to be a minor manifestation of the former.

Development

The malformation appears to represent a persistence of the talipes equinovarus normally present in the 7th week of foetal life, which should disappear in the 8th week.

Frequency

The frequency at birth of the severe form is probably a little over 1 per 1 000 total births, with a sex ratio of about 2 in many different series.

The condition appears to have a high incidence in Polynesians, as exemplified by the Maoris of New Zealand, and the native Hawaiians.

Family studies

Twin studies in Germany indicate a monozygotic twin concordance of about 30 % and a dizygotic concordance of about 3 %.

Two family studies, one in Devon, England, the other among the Maoris of New Zealand, illustrate the effect of population incidence on the proportion of relatives affected. In Devon, with a population incidence of about 1 per 1 000, about 2 % of first-degree, 0.6 % of second-degree, and 0.2 % of third-degree relatives were also affected. In the Maoris, with a population incidence of about 6 per 1 000, about 6 % of first-degree, 2 % of second-degree, and 1.5 % of third-degree relatives were also affected. This relationship is that which would be expected if the genetic predisposition was polygenic.

Mechanisms of gene action

There is evidence that familial joint laxity may play a part in predisposing to talipes equinovarus, though less markedly so than in congenital dislocation of the hip.

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2.6 Other congenital malformations

In the preceding sections the conditions chosen illustrated particular points of interest, had individual frequencies of over 1 per 1 000 total births, and were readily recognizable at birth. A very large number of different types of malformations occur, but relatively few have such high frequencies and many that affect the internal organs may not be recognizable at birth or may be missed by any but the most careful examination of stillborn and newborn infants.

Congenital malformations of the heart are very common, probably having in total a frequency of between 5 and 7 per 1 000 liveborn children in Caucasian populations. The percentages of the commoner types are approximately : ventricular septal defect, 20 %; 10 % each for atrial septal defect, coarctation of the aorta, patent ductus arteriosus, the tetralogy of Fallot, and transposition of the great vessels; aortic stenosis, 5 %; and miscellaneous, 15 %. Although morphologically different heart malformations are sometimes reported in sibs, this is probably due mainly

to the high overall frequency of heart defects. More studies are needed to clarify the concordance and discordance in affected close relatives. It seems likely however that a corresponding genotype predisposes to each specific anomaly of development, so that cardiac malformations in close relatives of index cases, even if not identical in morphology, are similar and traceable to the same initial errors in development. For atrial septal defects the proportion of sibs and offspring affected is 3-4%, and for patent ductus arteriosus 1-2%.

Some cardiac malformations have such severe effects or determine such characteristic physical signs that they are easily recognized at birth. It may however be impossible to be sure whether or not a child is affected at birth and, even if it seems certain that it is, to identify precisely the nature of the defect. Even after extensive radiology and sophisticated investigations, the precise anatomical diagnosis may only be established at operation or at autopsy. Finally, many children in whom congenital heart disease is strongly suspected at birth may subsequently prove to have normal hearts.

Congenital abnormalities of the urogenital tract present just as great difficulties. Although gross cystic kidneys, renal agenesis, or lesions affecting the bladder and urethra and the external genitalia may be identifiable at birth, many urogenital defects are only manifest in later childhood. This is characteristic of most types of cystic kidney (including some monofactorial in origin), horseshoe kidney, and various duplications, ectopias, and hypoplasias of the kidney and ureter. Further severe anomalies are often found at autopsies in patients of all ages who have never had symptoms attributable to the malformations. All these malformations of structures developed from the urogenital ridge may be single or at least confined to that system; however, they are also extremely common in subjects with malformations elsewhere. They also occur as part of well-recognized recessive and other syndromes. Clearly, investigation of these conditions is beset with difficulties.

There are many foetuses with multiple anomalies of every degree in every part of the body. Various attempts have been made to group cases with combinations of specific anomalies, particularly when there is some evidence of recurrence in sibships, and to identify them as syndromes. However, no systematic attempt appears to have been made to relate the different anomalies in the same individual to times of presumptive origin in intra-uterine life. Any work that could resolve heterogeneity in these cases would be valuable.

Many anomalies such as oblique facial cleft are individually extremely uncommon. It follows that frequency estimates are often extremely sensitive to chance variation; although it is assumed that frequencies in sibs are higher than those prevailing in the population, in many cases evidence of this is also scanty. It seems unlikely that sufficiently extensive

studies could be undertaken to provide much material to test genetic hypotheses for these rare conditions. The basic problem however is whether to attribute them to rare recessive genes or to multifactorial predisposition; if they are recessive, this will be detected by a relatively small series of families.

3. GENETIC HYPOTHESES

3.1 Regular phenotype systems

Geneticists are most familiar with phenotype systems that are regular, in the sense that each genotype manifests only one phenotype (complete penetrance). The responsible alleles and loci may differ among families. Powerful tests have been developed for this hypothesis, based largely on relations between the population incidence, recurrence risks (called segregation frequencies) in various mating types, and the effects of inbreeding. These tests have shown that none of the congenital malformations is a regular phenotype system, although containing such systems as minor components (for example, the lip-pit syndrome). As far as possible such simple genetic entities should be separated in analysis and genetic counselling from the large residual group of more complex etiology.

3.2 Sporadic cases

One extension of the above hypothesis considers a mixture of a regular phenotype system with sporadic cases of obscure etiology, few effects of inbreeding, and low recurrence risks in close relatives. This model is convincing only if segregation frequencies in multiplex families (with two or more affected sibs) are compatible with a regular phenotype system. In addition, there may be a relatively large increase in the frequency of multiplex families with inbreeding, in the absence of a corresponding increase of simplex families (with only an isolated affected member) that include sporadic cases as well as chance-isolated cases arising from the accidents of segregation in small families.

The hypothesis of sporadic cases has been validated for certain diseases (limb-girdle muscular dystrophy, deaf-mutism and low-grade mental defect). Sporadic cases may be associated with mutations, illegitimacy, chromosomal anomalies, rare instances of penetrance of a usually recessive gene in heterozygotes, environmental insults, and more complex etiologies. Applied to congenital malformations, the low segregation frequency even in families with two or more affected sibs makes this mechanism not entirely convincing, even though it appears to fit the available data as well as other hypotheses.

3.3 Incomplete penetrance

An obvious extension of regular phenotype systems is to allow some genotype to manifest more than one phenotype. Such incomplete penetrance may be demonstrated for rare dominant genes whose manifestation occasionally skips a generation, but penetrance (the proportion of susceptible genotypes recognizably affected) must be low to account for relatively small recurrence risks. Major genetic factors with low penetrance cannot be demonstrated in non-experimental material except by the development of methods that detect non-affected carriers.

3.4 Quasi-continuity

Many malformations appear to represent the cumulative effect of two or more genetic and environmental factors. As a model for this, one may postulate a continuous variable called the liability x , with a mean value of zero and a variance of unity in the general population. For example, if there are n equal and additive factors contributing to liability, the liability of an individual experiencing n of these factors is :

$$x_n = \frac{n - \bar{n}}{\sigma_n}$$

where \bar{n} and σ_n are the mean and standard deviation of n in the population. In general, however, the effects of different factors need not be equal, nor their frequencies the same.

Associated with x is a risk function $g(x)$, which gives the probability of an individual with liability x being affected. Two variations of this model have been studied. In one, x is the phenotype liability, including both genetic and non-genetic factors, and the risk function is discontinuous :

$$\begin{aligned} g(x) &= 0 \text{ for } x < a \\ g(x) &= 1 \text{ for } x > a, \end{aligned}$$

where "a" is the threshold for affection (this is the same model as is commonly used for artificial selection on a quantitative trait x). As shown by Falconer, this formulation leads to estimation of the heritability (h^2), i.e., the proportion of underlying continuous variation that is genetic, on the assumption that the causal factors are multiple and additive and therefore normally distributed. Support for additivity is provided by studies of small viability effects in *Drosophila*. A disadvantage of this model is that, while supposing a normal distribution of liability in the general population, it predicts a skewed distribution with an altered variance for liability in relatives, and the recurrence risks must therefore be approximate.

Recently Edwards has developed a different model in which x represents the genetic component of liability and the risk function is taken to be exponential :

$$g(x) = e^{a+bx},$$

where a is the parameter related to the environmental contribution and b the parameter related to the genetic contribution, $-b^2/a$ being analogous to heritability (h^2) in the previous model. This risk is an approximation to a sigmoidal function, which seems more plausible but mathematically intractable. Edwards's model has the remarkable property that the distribution of liability is normal, has the same variance in relatives of probands as in the general population, and is normal, but with a greater variance, for children of consanguineous marriages. Consequently the predicted risks are simple functions of the two parameters a and b .

Several types of morbidity, including the specific congenital malformations considered in this report, appear to fit this model well. Other traits (noted in section 3.2) deviate significantly, apparently because of a substantial admixture of recessive entities.

Models of quasi-continuous variation are compared in Fig. 1 and 2.

The hypothesis of quasi-continuity leads to some important consequences :

(1) Since the recurrence risk is variable among families, the conditional probability that the next child will be affected is a known function that increases rapidly with the number of affected sibs and decreases slowly with the number of normal sibs, providing a basis for genetic counselling which takes into account the condition of previous siblings.

(2) Given limited data on recurrence risks in relatives of index cases, the risks for other relatives may be predicted.

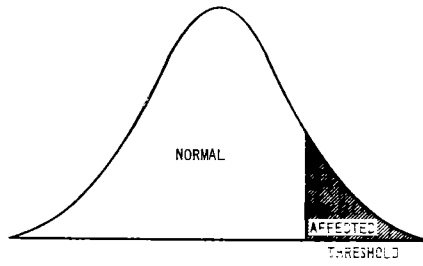
(3) Given limited data on recurrence risks in relatives and/or children of consanguineous marriages, the risks for other inbreeding levels may be predicted from the close relationship between the inbreeding coefficient F and additive genetic variance which increases in proportion to F .

(4) In contrast to single gene traits, selection and mutation have quite unpredictable effects on a quasi-continuous trait because the relevant genetic factors may have other and often more important effects on mortality and fertility than their contributions to a rare malformation.

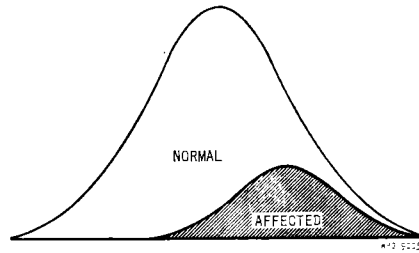
(5) It may be possible to find a phenotype correlated with the liability scale, so that microforms (or other indicators of genetic susceptibility) may be recognized in relatives, perhaps leading to reliable genetic counselling and a better understanding of the etiology.

FIG. 1

TWO MODELS OF QUASI-CONTINUITY



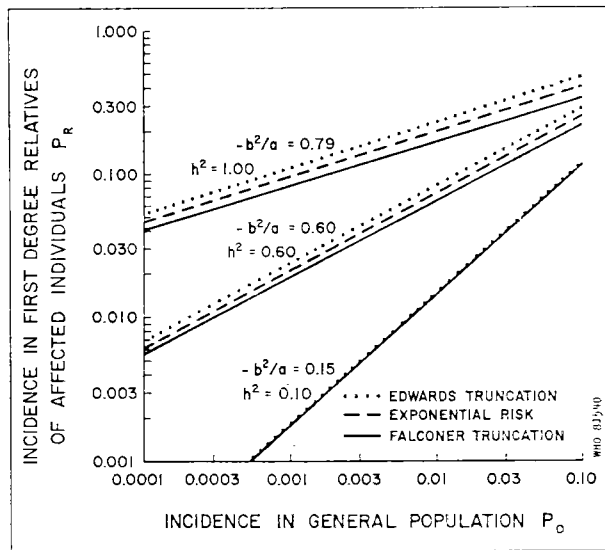
TRUNCATED GENETIC LIABILITY



EXPONENTIAL GENETIC LIABILITY

FIG. 2

COMPARISON OF THREE MODELS OF QUASI-CONTINUITY



(6) If trait severity is determined by the same genetic factors as govern liability, severe malformation will be associated with greater risks of recurrence.

(7) The quasi-continuity hypothesis predicts different recurrence risks for relatives of male and female index cases, if the incidence in the general population varies in the sexes.

(8) In non-lethal traits, the recurrence risks should be the same for sibs and children of probands.

This rich body of consequences makes quasi-continuity an attractive hypothesis, in particular the model of exponential risk because it fits the data better than the model of abrupt truncation and because its predictions are mathematically simpler.

Rigorous testing of the major gene and quasi-continuity hypotheses is important, because reliable genetic counselling and productive research both depend on selection of the genetic hypothesis. Various workers are at present testing the different hypotheses.

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4. METHODOLOGY FOR CLARIFICATION OF ETIOLOGICAL FACTORS

Much of the methodology appropriate for the study of the genetic element in the causation of congenital malformations has been illustrated in the examples given.

4.1 Establishing the frequency at birth

A prerequisite for the interpretation of family studies of malformations is an estimate of the frequency of the malformations among all births in the general population from which the families are drawn. (If births in maternity hospitals are the only births adequately documented, it must be remembered that they are not always representative of all births in the district.) In some areas such estimates will already be available from a continuing register of births, perhaps linked with records of malformations ascertained later. Often however an *ad hoc* study will be needed to ascer-

tain all cases, live and stillborn, in the area under study. The following considerations are important in attempting a complete ascertainment :

(a) The area and time interval of the study must be completely delineated, and care must be taken to exclude children found to be affected in the area but not domiciled in the area at the time of birth.

(b) The criteria for the diagnosis of the malformations should be clearly stated. It may be necessary to classify certain births as "probably" affected and to make alternative estimates which do or do not include such doubtful cases.

(c) Use should be made of all appropriate sources of ascertainment, such as birth and death certificates, maternity hospital, local authority, and school medical records, and registries of handicaps, though care must be taken to see that all such cases are related to the original birth sample.

(d) If it is not feasible to ascertain all cases born in a given region in a given time, an improved estimation of the incidence may be made by careful enumeration of the number of ascertainment per proband and identifications of probands in multiplex families. The assumptions that sources of ascertainment are independent and that probands are independently ascertained within families have been eliminated in recent methods.

(e) Where material from large numbers of induced abortions is available, valuable estimates may be made of the frequency of the malformation in embryos and fetuses. It is also valuable to have information on the frequency of specific malformations in spontaneous abortions.

4.2 Estimating the sex ratio of malformed individuals

The sex ratio of individuals with specific malformations is important in itself, in indicating a genetic factor in causation. Knowledge of the sex ratio is also a prerequisite of family studies. The frequency in, for instance, female relatives of index patients must be compared with that among female births in general, and the family analysis must be made separately for index patients of each sex.

The sex ratio can usually be satisfactorily established by a series drawn from hospital clinics. It is desirable, however, that the findings should be checked by examining the sex ratio in a series ascertained at birth.

4.3 Family studies

Some of the requirements of an adequate family study of congenital malformations are :

(1) It should be based on several hundred index patients, since risks to relatives are usually less than 10 %.

(2) The index patients should be chosen in such a way that any bias in ascertainment is recognized.

(3) The diagnosis in the index patient should be certain, the criteria for diagnosis fully stated, and care taken to exclude all instances of variants of the conditions that have a known etiology.

(4) The study should include any special examinations necessary to diagnose the malformation in living relatives, and include examination of the appropriate records when the relative is dead. For some conditions a history of a near relative being affected may be adequate, for others a full clinical examination may be needed.

(5) The study of the relatives should be extended at least to second-degree or third-degree relatives and specify the exact relationship — distinguishing, for example, between mother's sisters' sons and mother's brothers' sons.

(6) Comparison of the proportion of children and sibs of index patients affected is of special value in relation to the possibility of both recessive inheritance and maternal intra-uterine factors. It is therefore often advantageous (though perhaps more difficult) to take patients born a generation ago as index cases.

(7) Research workers should be alert to the occurrence of related malformations, of microforms, and of other possible indications of genetic predisposition in the relatives studied.

(8) Research workers should be alert to the possible occurrence of affected individuals among abortuses, though it is seldom possible to get reliable data on them.

(9) The family study should include information that may be relevant to environmental factors, including parental age, birth order, socio-economic status, and the extent of family limitation.

(10) Estimates of risks to sibs of index patients may be made by simple enumeration of the children born before and after the index case. Better estimates may, however, be given by segregation analysis, which makes allowance for incomplete ascertainment.

4.4 Parental consanguinity and inbreeding effects

Information on consanguinity should be recorded and, where possible, given in such detail that the inbreeding coefficient of the index case and each parent can be determined. Inbreeding increases the incidence, according to two quite different hypotheses: that of the unrecognized admixture of simple recessive entities and that of the increased variance of liability under quasi-continuity. Both should be tested against the

observations to determine whether one gives a significantly better description than the other and whether the estimates are consistent with other data — e.g., a mixture of simple recessive genes permits estimation of gene frequencies and mutation rates which may be examined for compatibility with regular single-locus systems.

4.5 Twin studies

Information on twins with malformations may indicate the presence of a genetic component in the etiology or, alternatively, provide clues to the mechanism by which environmental factors cause abnormality. Twin data on malformations are, for obvious reasons, scanty. The incidence of twin births in Caucasians is about 1 in 80. A malformation with an incidence of 1 in 1000 births would occur in a twin once in about 40 000 total births. To collect information on 10 twin pairs of which at least one had the malformation, 400 000 births would have to be screened. Since only about one twin in three is monozygotic, for 10 monozygotic twin births information is needed on over a million births. The collection of such twin data would therefore need the combined effort of many hospitals over several years.

The information collected should, in addition to data on the presence or absence of the malformation in the twins, include data by which the zygosity can be determined, among them blood groups and other common genetic markers and a description of the placenta and of the umbilical vessels. Absence of one umbilical artery and marginal insertion of the cord are associated with twin births on the one hand and certain malformations on the other. Placenta and membrane findings in twins discordant for malformations may give a clue to mechanisms of development.

From these studies the proportion of monozygotic and dizygotic co-twins of index patients affected should be determined and compared. The best comparison is usually of monozygotic co-twins with dizygotic co-twins of like sex.

If twins are collected from consecutive birth series, as can be done for malformations recognizable within the first few days of life, the problem of whether to choose an index case or a pair does not arise, since both members of a concordantly affected twin pair are index patients. If, however, twins are collected from other clinical series — for instance patients with congenital dislocation of the hip from orthopaedic clinics —, it should be stated whether one or both twins are index patients. The estimate of the frequency with which co-twins of index patients are affected will be much influenced by whether one or both members of a concordant pair are index patients. A malformation occurring in only one of a pair of monozygotic twins cannot be attributed to major environmental factors such as virus disease or a chemical agent, but rather to minor differences

within the common intra-uterine environment. Such minor environmental factors are possibly important in many malformations but are not readily demonstrated by the methods currently available.

Malformations in twins should not be studied without regard to their occurrence in near relatives.

4.6 Geographical and ethnic variations in incidence and comparative family studies

As has been pointed out above, many congenital malformations show striking variations in their frequency at birth. Family studies in populations with very different birth incidences are valuable in testing genetic hypotheses. In addition, migrant populations are helpful in the study of etiology. If due allowance is made for uncontrolled environmental differences and undetected intermarriage, a trend over the years for the specific malformation rates in the migrant population to move towards those of the indigenous population argues for a degree of environmental causation, while persistent differences suggest at least partial genetic determination.

The existence of multiracial populations permits comparison of the incidence, recurrence risk, and symptomatology in different gene pools exposed to similar environments. Even among sympatric populations residual differences in diet and other possibly relevant factors can be reduced by the standard statistical techniques of stratification and covariance analysis. Where differences persist after migration, the frequency of malformation in the offspring of inter-racial crosses provides useful tests of genetic hypotheses, especially the quasi-continuity assumption of additive predisposing factors. Differences between the offspring of reciprocal crosses suggests the existence of specific maternal effects, which may be either genetic or environmental.

4.7 Association with genetic markers

Associations of malformations with polymorphic genetic systems such as the ABO blood group system are likely to be weak and this does not seem to provide a very promising line of research at present. In any large-scale study of specific malformations, however, it may well be desirable to include tests for such systems.

4.8 Genetic linkage

Genetic linkage is detectable only within families. Progress in detecting linkage between regular phenotype systems in man has been so slow that there seems to be no urgent need to extend linkage tests to congenital malformations and other conditions of complex etiology.

4.9 Recognition of cytogenetic anomalies

Multiple malformations associated with mental defect are not infrequently due to chromosomal anomalies; this group should be screened cytogenetically. Few or no isolated malformations are due to chromosomal anomalies, and cytogenetic studies are of doubtful value.

4.10 The identification of individual gene loci in polygenic systems

The identification of individual gene loci in polygenic systems is much to be desired and will come in time, possibly through linkage studies but more probably through the recognition of the specific biochemical effects of individual genes.

4.11 Indications of genetic susceptibility

An important objective of genetic studies, from the point of view both of counselling and of the possible development of preventive measures, is the detection of genetically predisposed individuals. On the multifactorial hypothesis, the indications of genetic susceptibility might be detected in two ways. Firstly, they might appear as microforms of the condition; an individual showing the microform would then be recognized as close to the point of manifestation of the defect (the threshold) and therefore be likely to be genetically predisposed. This raises the question of whether, for instance, a severe spina bifida occulta is a microform of spina bifida aperta, or a high-arched palate a microform of cleft palate.

Secondly, a genetic predisposition might be detected by some more quantitative individual feature. The question then arising, for instance, would be whether the often muscular build of individuals who have had pyloric stenosis is correlated with the degree to which the pylorus hypertrophied in infancy, or whether the shape of the face in the adult is related to a difference in the topography of the embryonic facial processes that would make them more or less likely to fuse normally. Identification of such indicators of genetic predisposition would help to clarify the genetic basis of the defect, improve counselling, and perhaps even indicate possible approaches to prevention.

5. CONCLUSIONS

The Group reviewed present knowledge of the genetic contribution to the etiology of congenital malformations. It considered the information available on a number of conditions that have been inten-

sively studied, and discussed to what extent the pattern that emerges has general application.

It has been recognized for a number of years that about 1 % of all live and stillborn children have or will develop signs of a harmful single gene trait and that a further 1 % have chromosomal abnormalities, many of which determine severe handicaps. In addition, some 3-4 % of all live and stillborn children have congenital malformations, as defined in the introduction, although, as many of these affect internal organs, they may not be recognized until long after birth or at autopsy. These estimates refer to morbidity and are therefore higher than those based on mortality.

The malformations of multifactorial etiology are of great social and biological importance and, where the genetic mechanisms are understood, outnumber defects of the other two types. In a small proportion of these malformations there is a known teratogenic agent, e.g., maternal rubella, toxoplasmosis, exposure in early intra-uterine life to ionizing radiation, and toxic agents or drugs. It must be assumed that there are other cases determined by known or by as yet unidentified teratogenic agents.

Nevertheless all the indications are that the majority of these malformations have a genetic component in their etiology and that that component is polygenic. In almost all there is also evidence, even if indirect, of an environmental component. The evidence further suggests that the environmental influences that act during intra-uterine life are also multiple and probably individually of small effect.

Such a multifactorial hypothesis implies that there is a continuous distribution of liability or predisposition, and that a malformed individual is one whose total liability has placed him beyond a certain point in the distribution — a threshold beyond which orderly development cannot proceed.

The five selected conditions — pyloric stenosis, cleft lip and cleft palate, anencephalus and spina bifida, dislocation of the hip, and talipes equinovarus — have been reviewed with respect to their familial incidence, the effect of the sex of the proband on the recurrence risk, the relation of the recurrence risk to population frequency, the effect of the migration of populations, and inter-racial crossing.

Several hypotheses were considered and it was felt that the one best fitting the data was that which assumed a normally distributed genetic predisposition together with an exponential increase in risk as the genetic predisposition increases.

The Group was of the opinion that this hypothesis has many advantages and could be of value in influencing the direction of future research. As will be clear from the report, it has considerable flexibility. Thus it can be used to test data on population and family frequencies and patterns

of malformations for compatibility, and it can deal with situations where there are differences in frequencies according to sex. It can also be used to predict the relationship between consanguinity and frequency in populations and may thus be of value in affording a logical explanation of the repeated finding of a small association between consanguinity and certain malformations.

The Group called particular attention to one implication of the hypothesis. If the hypothesis is substantially correct, the magnitude and direction of the effect on the incidence of relaxed selection against malformed individuals are difficult to predict, but are probably less than for a single gene trait.

The lines of research most likely to be profitable are indicated in the section on methodology.

6. RECOMMENDATIONS

1. Encouragement should be given to the determination of the frequency of the more common congenital malformations in representative populations.

The WHO study, restricted to maternity units, provides a most useful beginning, which should now be followed by attempts to establish frequencies of specific malformations in total populations of births. Where successful, such studies should either be maintained over many years or at least repeated at suitable intervals of time.

2. Encouragement should be given to the execution of well-planned family studies in populations with varied frequencies of specific malformations — for example, neural tube malformations in Ireland as contrasted with Nigeria, or pyloric stenosis in Sweden as contrasted with Japan.

Such family studies will not only contribute to the understanding of the etiology of the malformation but are also needed to provide correct empirical recurrence risks for genetic counselling in each area.

3. Encouragement should be given to studies of the frequency of specific malformations in reciprocal crosses between members of races differing markedly in the frequency of the malformation. Examples are crosses between Caucasians and West Indians in the United Kingdom for neural tube malformations, or between Caucasians and Japanese in Hawaii for pyloric stenosis, or between Caucasians and Polynesians in New Zealand for talipes equinovarus.

4. The same multiracial populations would also be especially suitable for the study of the effects of migration on the frequency of specific malformations, the frequency being related to that in the population of origin and in the population of the country to which migration has occurred.

5. Such studies require trained personnel, both medical officers trained in genetics and medical auxiliaries trained in the technique of collecting information from families.

The Group endorsed the recommendations of the WHO Expert Committee on Human Genetics for specialized training in human genetics.¹

6. Consideration should be given to the development of new methodologies. This may be illustrated by the valuable information provided by the embryological study of induced abortions in Japan on the frequency and development of specific malformations early in pregnancy. Such studies should be repeated in other countries where the practice of induced abortion is common. Studies on interactions between the gene and the environment should also be carried out. For example, the effect of relatively minor environmental differences could be studied in groups known from the family histories to be genetically predisposed to specific malformations and therefore unusually sensitive to environmental factors. Such groups could also be examined for biochemical or anthropometric indicators of their underlying genetic predisposition.

7. The hypothesis described above and alternative hypotheses should be further developed and tested, their theoretical implications explored, and their predictive value assessed against observational data.

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¹ *Wld Hlth Org. techn. Rep. Ser.*, 1961, No. 238.