THE CONTRIBUTION OF GENETICS

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I am going to discuss four ways in which genetics can clarify the aetiology and prevention of congenital abnormalities. The first is by a knowledge of gross (i.e. visible under the microscope) chromosomal abnormalities. Though these are responsible for only a very small proportion of abnormalities they are important to recognize. Secondly, a knowledge of ordinary Mendelian genetics (the meaning of dominance, recessivity and so on) can occasionally help in deciding what proportion of individuals are ' at risk ' in those few conditions which obey the simple Mendelian laws. Thirdly, genetics can help to assess whether or not there is an inherited component to an abnormality. In common diseases, we recognize more and more that there are both environmental and genetic aspects, and we need to know the criteria by which we can tell whether or not an inherited component is present. Fourthly, I wish to take a specific entity, namely Rh. embryopathy, and show how a knowledge of simple immunogenetics has helped to design a method of treatment which seems promising as a preventive measure.

Chromosomal abnormalities

In a discussion of these, there are seven conditions which we must understand:

1. Non-disjunction, and its corollaries of trisomy and monosomy in the sex chromosomes.
2. Chromatin-positive and chromatin-negative and the idea behind the Lyon hypothesis.
3. Isochromosome.
4. Non-disjunction in the autosomes.
5. Translocation.
6. Insertion.
7. Mosaicism.

1. *Non-disjunction*

In all the somatic tissues of the body, and here we include the precursors of the spermatozoa and ova respectively, there are 22 pairs of autosomes and a pair of sex chromosomes, two X's in the female and an X and a Y in the male. The end result is that each spermatozoon or ovum contains one of each pair of autosomes and one of each pair of sex chromosomes—therefore there will be two sorts of sperm, X and Y, but all the ova will be X. If a pair of autosomes

or the two sex chromosomes 'stick together' instead of going into separate gametes, non-disjunction is said to have taken place. If this happens with regard to the sex chromosomes in a male, one spermatozoon will have an X and a Y, and another no sex chromosome at all (though the autosomes will be present in the normal number of 22). If either of these abnormal types of spermatozoa fertilizes a normal egg, the result may be an abnormal XXY male with Klinefelter's syndrome, or an abnormal XO female with Turner's syndrome; the former is said to show trisomy and the latter monosomy for the sex chromosomes (see figure 1a).

		Abnormal male gametes	
		XY	O
Normal female gametes	X	XXY	XO
	X	XXY	XO

XXY = Klinefelter's syndrome. XO = Turner's syndrome.

Figure 1a. Non-disjunction in the male parent

If non-disjunction occurs in the ovum, similar situations can arise. One egg can receive XX and the other no sex chromosome, so that on fertilization by a normal male a trisomic Klinefelter or a monosomic Turner's syndrome can arise, *or* an XXX individual. This last used to be called a superfemale, but the term was clearly inappropriate and it is now referred to as a triple-X female. One of the combinations shown in figure Ib (YO) is lethal. In fact monosomics as a general rule never survive, the exception being the XO of Turner's syndrome. Here the individual, although suffering from primary amenorrhoea, is often of fairly normal intellect. The reason for this is connected with the fact that in the cells of the human body two active X chromosomes (or anyhow two complete active X chromosomes) are not necessary.

		Normal male gametes	
		X	Y
Abnormal female gametes	O	XO	YO
	XX	XXX	XXY

Figure 1b. Non-disjunction in the female parent

2. *Chromatin-positive and chromatin-negative*

The Barr body is a densely staining body just inside the nuclear membrane which is present in somatic cells of females (chromatin-

positive), but only rarely in male (chromatin-negative), and it is usually tested for in cells from a buccal smear. It almost certainly represents one of the X chromosomes (the inactive one) since on the Lyon hypothesis only one X in any given female cell is active, the other being inactive or 'non-working', and it is this latter which has the tightly coiled DNA, and therefore stains more deeply and *is* the Barr body. In the male, on the other hand, the X chromosome is always active and therefore does not stain so intensely. In the triple X female mentioned above there are two Barr bodies, and on the very rare occasions where there are four X chromosomes the individual has three Barr bodies—in other words there is always one fewer Barr body than the number of X chromosomes present.

It might be argued that the Turner, Klinefelter and triple-X syndromes are not congenital abnormalities and this would be correct according to the British classification where a congenital abnormality is defined as one which can be recognized macroscopically at birth. In the U.S.A., on the other hand, a congenital abnormality is one which can be detected *by any means* at birth. Thus, although it may not be possible to tell by clinical examination at birth whether a particular individual is suffering from any of the conditions mentioned, yet an apparent girl with no Barr body is probably suffering from Turner's syndrome and an apparent boy with a Barr body is probably a case of Klinefelter's syndrome.

3. *Isochromosome*

The meaning of isochromosome is more difficult. The simplest way to think of it is to remember that not all women who have Turner's syndrome are in fact chromatin-negative. These patients, although usually XO and therefore chromatin-negative, are sometimes chromatin-positive, i.e. a Barr body is present. The reason for this is often as follows. Normally the X chromosome divides straight down the centre so that each daughter cell receives both the long and the short arms of the chromosome with all their genes. If the X chromosome divides *across* instead of down the middle this can result in an isochromosome made up of two long arms of the X. This will contain two sets of the genes on the long arm but none of the genes on the short. Therefore when it pairs with a normal chromosome from the other parent the offspring receives one normal and one abnormal X, and as a result it may exhibit signs of Turner's syndrome and yet be chromatin-positive.

4. *Non-disjunction in the autosomes*

Having mentioned trisomy in relation to the sex chromosomes, we can now consider the results when it occurs in the autosomes.

It is well known that trisomy of chromsome 21 is responsible for the majority of cases of mongolism, one of the germ cells receiving two chromosome 21's at meiosis and the trisomic complement being made up at fertilization. Mongolism, since it can be recognized at birth (anyhow by obstetricians and paediatricians), can be included in the British classification of congenital abnormalities.

Non-disjunction may not only occur with chromsome 21, but with groups 13–15 or 17–18, meaning by this that one cannot be certain whether the chromosomes involved are 13, 14 or 15, or 17 or 18. These other two trisomics have fairly distinct clinical features. In a series of eight patients with 13–15 trisomy and eleven patients with 17–18 trisomy, all had mental retardation, but the presence or absence of eye defects helped to separate the two syndromes. Thus in the 13–15 cases eight out of nine had severe microphthalmia or colobomata, whereas none did of the eleven in the 17–18 group. The low set, malformed, Potter's ears were common to both groups. Paediatricians say that it is possible to distinguish at birth the 13–15 syndrome from the 17–18 one, but in 18 inches of crumpled humanity I personally have found it very difficult.

It should be borne in mind that in any trisomic individual, because there is too much chromatin, the patient is almost always mentally retarded, and this appears to be a non-specific effect of excess chromatin.

5. *Translocation*

In a translocation, two non-homologous chromosomes have joined together. As a result, when the chromosomes are arranged in pairs it is found that one each of two different pairs, say one of the no. 13's and one of no. 15's, is missing, and instead of them there is one abnormally large chromosome. The important thing to remember is that an individual in whom this has occurred is quite normal himself because the correct amount of chromatin is still present. However, he is a carrier of a translocated chromosome which may cause a congenital abnormality in the next generation. For example, a 15–21 translocation is responsible for some cases of mongolism and the chances of transmitting this are much higher than where the mongolism is due to trisomy. In the translocation case, we must consider the precursors of the germ cells (e.g. the spermatocytes) before meiosis has occurred. In these there will be one chromosome 15 (instead of two) and one chromosome 21 (instead of two), and a translocation taking the place of the missing chromosomes.

We must next consider what happens in such an individual when the gametes are formed. The important thing to remember is that

there is independent assortment and therefore any of several possible combinations may arise. Thus the translocation may go into one gamete unaccompanied by either a 15 or a 21; it may be accompanied by the 15 chromosome by an 18, or by both. Lastly, there is the possibility that the sperm would receive none of these chromosomes. Now if a sperm with the translocation and the 15 chromosome fertilizes a normal egg (with a normal 15 and a normal 21), the zygote will then have three pieces of chromosome 15, though one piece will have been translocated on to 21. The individual would then be a mongol, a ' translocation ' mongol instead of a ' regular ' or trisomic mongol, and as would be expected translocation mongols run in families more than trisomic ones. Similar translocation carriers may occur with the 13–15 group, and in a particular family which has been investigated in Liverpool many individuals with 45 chromosomes were found (i.e. the translocation carriers), but surprisingly enough there were no affected individuals with extra chromatin (the ' effective trisomics ').

6. *Insertion*

Insertion is really a special form of translocation, a piece of one chromsome being ' inserted ' into another and therefore producing a bigger one than normal. When a portion of chromosome no. 6 is inserted into no. 1 the result is sometimes the oro-facial-digital syndrome (OFD), characterized by malformations of the face, mouth and hands, a forked tongue being the most striking feature. The condition appears almost always in girls and an interesting point in relation to the increased chromatin is that not all the patients are mentally defective. As in a translocation there may be carriers of the insertion who are clinically normal, but the next generation is ' at risk '. Sometimes the insertion is so small that it cannot be recognized.

7. *Mosaicism*

The last of the chromosomal abnormalities to be discussed is mosaicism, a very important growing point at the present time. Individuals who are mosaics consist of tissues some of which have one chromosomal constitution and some another. Figure 2(a) shows a fertilized egg with the normal complement of 46 chromosomes. After fertilization mitosis has taken place and two daughter cells formed, again each with the normal complement of 46. One of these divides again normally, but in the other mitotic non-disjunction has occurred, so that one of the daughter cells has 45 chromosomes and the other 47 (and these, of course, will continue respectively to produce 45 and 47 chromosomes indefinitely). There are, therefore, three cell lines, normal, 45, and 47, and the individual is a mosaic

for these three types of cell which may affect any tissue or parts of a tissue. Figure 2(b) shows mosaicism with two cell lines. Here the zygote has undergone non-disjunction at the first mitotic division, one cell getting 45 and the other 47 chromosomes, so that here there is no normal cell line. Interest has recently been focused on mosaicism associated with 21 trisomy (mongolism) because numbers of people are being found to have minor features of mongolism, and their chromosomes frequently show mosaicism, some of the cells having the normal 46 and others 47 chromosomes. It is interesting, however, that one cannot infer how mongol-like the patient will appear from the proportion of cells, which are abnormal. If the mosaicism extends to the germ cells, familial mongolism may occur and this is probably the explanation when there is more than one ' regular ' mongol in a family. A sensitive index to mosaicism is the pattern of dermal ridges, but I am not going to say anything more about these and the reader is referred to Penrose's paper " Dermatoglyphs in mosaic mongolism and allied conditions " to be published in the *Proceedings of the Eleventh International Congress of Genetics*, The Hague, 1963.

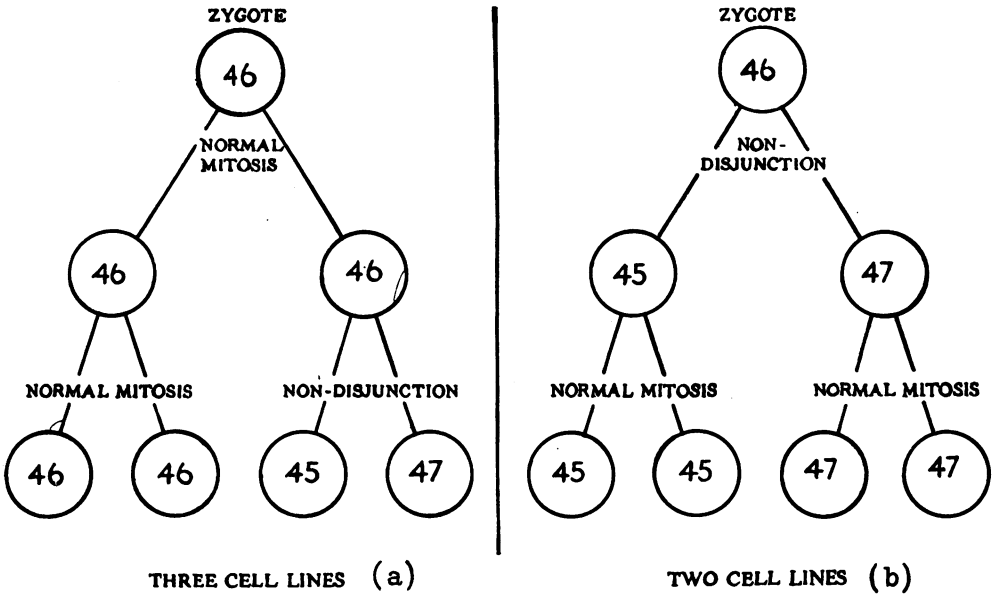

Figure 2. From *Chromosomes in Medicine* (1962), courtesy of J. L. Hamerton, published by Wm. Heinemann Ltd.

Though mosaicism is a very interesting phenomenon it should be invoked with some caution because with it one can get out of any

difficult situation, and in this it resembles a " dominant gene with incomplete penetrance ", which can explain almost anything in human genetics.

The inherited component in congenital abnormalities

Although aggregation of cases within families is a *sine qua non* for a genetic aetiology, yet sometimes environmental factors produce a similar picture, for example, thalidomide or rubella might account for multiple cases in a pedigree. In this connection it is interesting in the older literature to see that in 1916 several pedigrees were published showing that pellagra was clearly inherited as a Medelian dominant. Therefore, in looking for a genetic component it is essential when examining pedigrees to see if Mendelian ratios are present, either obviously, or in the correct proportions according to the different mating types. Consanguinity is also important; if it occurs frequently in a pedigree then a recessive gene is likely to be operative. Twin studies also have their uses, but they are now somewhat out of fashion and it must be remembered that one-egg twins are not identical as regards the cytoplasm, and it may well be that geneticists neglect this part of the cell which is so important in the organization of growth.

In assessing a genetic component we also have to ask whether people with particular genetic constitutions are ' at risk ' for producing congenitally deformed babies. For example, prediabetes and latent hypothyroidism have both recently been suspect. It is said that if a woman has had several stillbirths or congenital malformations she should be suspect for prediabetes and if an abnormal glucose tolerance test can be demonstrated she should be given insulin with her next pregnancy and the infant is then likely to be normal. Similarly it is advised that thyroid should be given if radio-active iodine studies reveal mild hypothyroidism. If both these claims are true it represents a very important advance, but the evidence seems to me to be incomplete and further work is needed on the matter.

Crosses between races might clarify the nature-nurture problem in congenital abnormalities, though it would be difficult to get a large enough series. It would be interesting, however, to know the pattern of abnormalities in Caucasian X Negro crosses living (a) in West Africa and (b) in Europe. Some work has been done on these lines by Morton (1962) in Hawaii, where there are considerable mixtures of Caucasian, Mongoloid and Polynesian populations. In some preliminary observations, Morton found that mothers with a high proportion of Caucasian genes had a higher twinning rate than those whose genetic constitution was largely Mongolian. Anencephaly is known to be commoner in Caucasians than in

Mongolians and talipes *vice versa*. In the crosses the situation was somewhat inexplicable. If the father had many Caucasian genes and the mother was Mongoloid, the incidence of anencephaly was higher than if the woman was a Caucasian. In other words it looks as though the father's race was having something to do with the type of abnormality produced. Since there is no known genetic mechanism which operates only through the father (other than Y linkage), the problem at present remains unsolved.

If one includes chorion carcinoma as a congenital abnormality, geographical considerations might also be helpful. The tumour is rare in Europe, but common in Hong Kong. What is the incidence of the disease in Europeans living in Hong Kong or in Chinese living in England? Furthermore, what happens in hybrids living in either locality?

Rhesus embryopathy

A knowledge of simple immunogenetics may be leading to a method of preventing Rhesus haemolytic disease of the newborn and this is a problem in which I have been particularly interested. The stimulus derived from the fact that Rh. negative women married to Rh. positive husbands are almost always protected from having ' Rhesus ' babies if they are ABO incompatible, i.e. mother group O, foetus group A. We have tried to mimic the destruction of the ABO incompatible cells (by the naturally occurring anti-A and anti-B in group O women) by giving anti-D in the Rh. situation. The work can be summarized as follows. Rh. negative women are sensitized by Rh. positive cells crossing the placental barrier, and it seems likely that most (but not all) sensitization occurs as a result of ' bleeds ' which take place at delivery. The giving of high titre incomplete antibody (or better still anti-D gamma globulin) to the mother immediately after the delivery of an Rh. positive child clears the foetal cells very rapidly from the maternal circulation and experimentally, anyhow using men, sensitization is mostly prevented. The treament envisaged would be preventive only and of no use once a woman had become sensitized. It is a good example of how a knowledge of genetics is not merely an academic acquisition but may have important practical thereapeutic applications.

REFERENCE

Morton, N. E. (1962) Genetics of inter-racial crosses in Hawaii. *Eugen. Quart.*, **9**, 23-24.