GENETIC DISEASES AND ANTENATAL DIAGNOSIS*

WONG HOCK BOON

Wong Hock Boon
MBBS, FRCP (Edin), FRCP (Glas),
FRACP, DCH (Lond), PPA, PJG

Head
University Department of Paediatrics
National University of Singapore
Singapore General Hospital

Director
School of Postgraduate Medical Studies
National University of Singapore
College Road
Singapore 0316

Head
WHO Collaborating Centre for Research and
Training in Human Genetics
Singapore

Address for correspondence:

Professor Wong Hock Boon
Head, Dept of Paediatrics
National University of Singapore
Singapore General Hospital
Singapore 0316

SUMMARY

The different methods of prenatal diagnosis are discussed with special reference to ultrasound scan, amniocentesis for cell culture with processing for chromosome study, biochemical analysis and DNA recombination analysis. Chorionic villi aspiration and fetoscopy are new methods which will enhance considerably the methods for prenatal diagnosis.

With regard to chromosome study of amniotic cells, experience with 623 cases is reviewed. 2.7% demonstrated chromosome anomalies and of these Downs anomaly was the commonest. A large proportion of cases requesting for amniocentesis are Caucasians who represent only 2% of the population in Singapore, but 25% of the 440 requests were from Caucasions. The various problems associated with the different methods for prenatal deafness are discussed.

INTRODUCTION

All diseases have a certain genetic load, some more than others.[1,2] There are two groups of diseases with a high genetic load and they include Mendelian disorders and the chromosome diseases.

The Mendelian disorders are due to mutations of single genes which normally have critical

**The I.S. Puvan Memorial Lecture, delivered at the Inaugural Meeting – MASEAN Chapter of Obstetrics and Gynaecology, Kuala Trengganu, Malaysia on 15 April 1985.*

functions, and as a result such mutations result in disease. If the mutation occurs in one of the two allelic genes, the disease is passed on in an autosomal dominant manner, and if both alleles need to be mutated before the disease state is manifested, the disorder is termed autosomal recessive; if the mutation occurs in a critical gene resident in the X-sex chromosome, the disease is termed Mendelian sex-linked. Because of the very nature of these critical genes, the risk of offspring inheriting the disease from their parents can be worked out clearly in a strict mathematical manner.

The second group of diseases are the chromosome diseases[3] where usually there is loss or gain of chromosome material, and because chromosomes carry many genes, in such states, large numbers of genes are lost or gained. In this manner, it is almost certain that some critical genes are definitely involved, and we are not surprised that abnormalities result.

Although chromosomal diseases are strongly genetic, yet only a few are inherited. For example, Down anomaly (D) or Mongolism is the result of the presence of an extra chromosome, No. 21, Most DAs are produced randomly by non-disjunction in meiosis of the parental gametes or non-disjunction during mitosis of the zygotes. Such non-disjunctional events are often environmentally triggered e.g., old maternal age, drugs, infections, etc. The parents' chromosomes and genes are themselves normal. However, a small percentage of DA are the result of abnormalities in chromosomes in one of the parents, e.g., DA can be the result of abnormal translocated chromosomes, and in such instances, the risk of future offspring being DA reaches Mendelian orders of risk.

But both these diseases, i.e. Mendelian and chromosomal, only account for 5% of all human diseases, at the most. The bulk of human diseases depend on both genes and the environment. The genes involved, each by each, is not 'critical', but because many of the genes are involved, they interact with each other, and produce a milleau whereby with the presence of unfavourable environmental factors, a disease state is produced. Hence, such diseases are inherited in a **multifactorial** manner, and is the resultant of polygenes and the environment (Fig. 1). Therefore, 95% of all diseases are determined in this manner, and nearly all congenital malformations are found in this way together with common diseases afflicting man, such as thyrotoxicosis, cardiac infarct, hypertension, bronchial asthma, cancer, schizophrenia, etc.

In conclusion, whether the disease is Mendelian, chromosomal or multifactorial, as obstetricians, we should try and prevent the birth of such foetuses, if this is possible in the particular diseases. This is the essence of prenatal diagnosis.

The foetus had, for millions of years, been shielded from the eyes of man and it was only when he is born could man discover any abnormalities present. But doctors had always hoped that they could diagnose these inherited diseases before the foetus is born, in the hope of providing "treatment" it before birth, or abort it if unable to treat. We have arrived at the era when we can do the latter but are still very far from the former. It is the purpose to discuss the capability and accuracy of modern methods in the diagnosis of genetic diseases *in utero.*

All the three types of genetic disease – Mendelian, chromosomal and multifactorial – are yielding to the probing methods of the medical scientist, e.g., prenatal diagnosis of haemoglobinopathies, Down's anomaly and neural tube defects respectively, have been achieved in many instances. The methods available have been non-invasive as well as invasive (Table I).

From Table I, it is seen that the privacy of the foetus is now invaded and we can get an idea of the structure and functions of the foetus as never before. But, of course, this brings with it many problems, some of which are far from being solved and which will be discussed.

ULTRASOUND

At the moment, high resolution ultrasound machines can produce quite detailed foetal images.

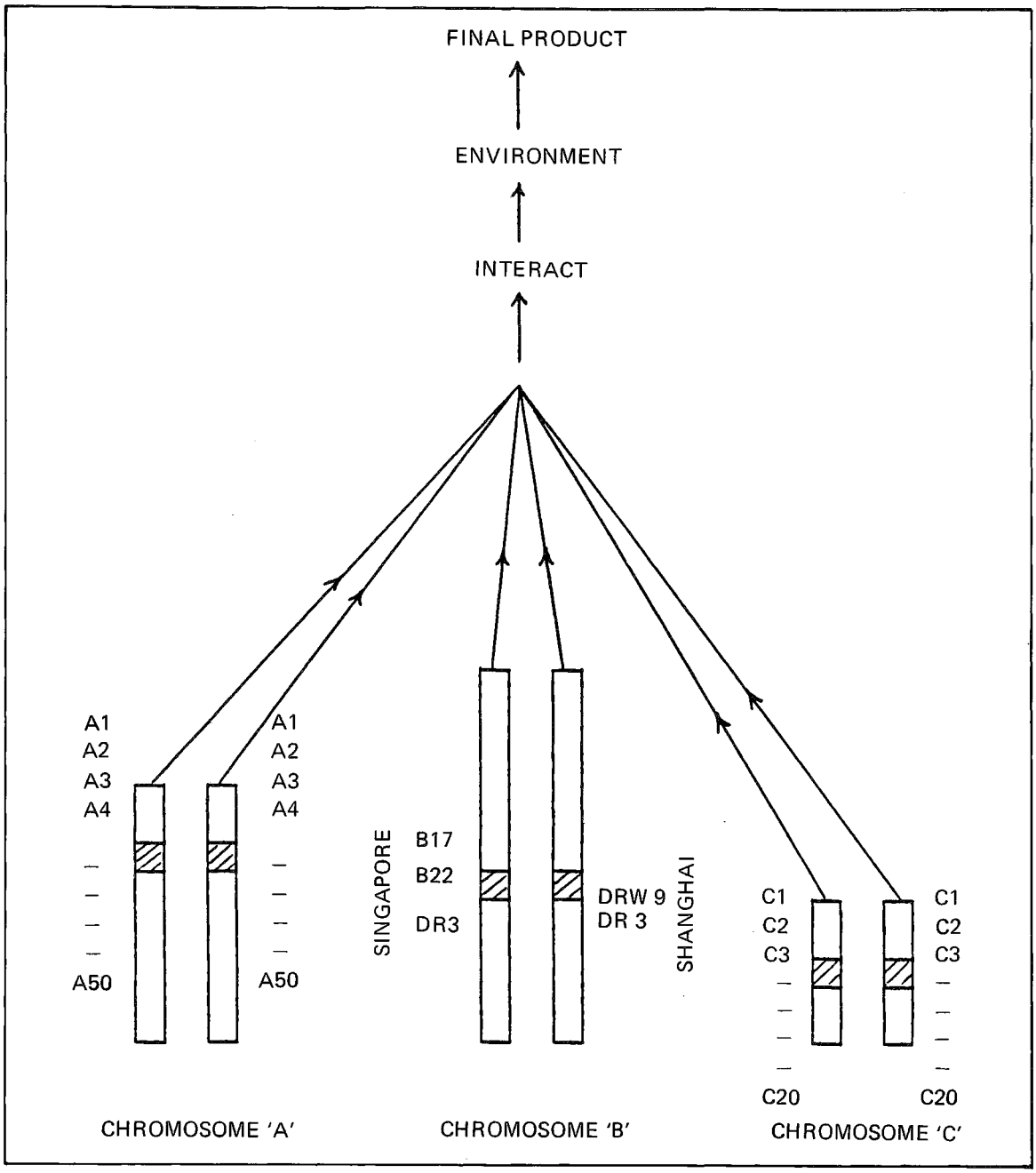


Fig. 1 Showing HLA haplotypes which increase susceptibility to IDDM among Singapore Chinese and Shanghai Chinese. B17, B22 and DR3 are significant in Singapore while DR3 and DRW9 are significant in Shanghai. 'Protective' haplotypes in Shanghai are All, CW4 and DR2.

TABLE I

METHODS OF PRENATAL DIAGNOSIS

Non-Invasive:

1. Ultrasound

Invasive:

1. Amniocentesis and examination of amniotic fluid and/or culture of amniotic cells for chromosome processing biochemical and other tests.

2. Chorionic villi aspiration and examination of the cells in the same way as amniotic cells.

3. Foetoscopy allows of direct visualisation of parts of the foetus as well as allowing of foetal blood aspiration and other tissue biopsies.

As a result, it has been used for conditions mentioned in Table II.

The problems which we have to resolve include those in Table III.

There have been several series[4, 5] of babies who had been scanned by ultrasound as a foetus, with follow-up not revealing any defects. However, these follow-up series are for short periods of time only, certainly only during childhood; longer periods of follow-up are needed till adulthood and ideally for a whole generation. This cautious attitude is necessary because of the discovery of certain chromosome changes which may occur after ultrasound in the young, viz. the presence of sister chromatid exchanges[6, 7] discovered *in vitro*. What the relevance is to the human foetus is not certain. It is wise to heed these findings and a healthy attitude has developed among workers in that most have agreed that **routine** ultrasound in foetuses should be deplored,[8] it should be carried out only in the presence of certain specific criteria or needs.

The three main side-effects found, *in vitro*, and on animals *in vivo*, have been thermal effects, cavitation and other direct side-effects. A summary of such experimental effects with comparable 'doses' necessary to produce these effects is in Table IV.

TABLE II

USES OF ULTRASOUND IN PRENATAL DIAGNOSIS

CNS:

Neural tube defects (spina bifida/anencephaly), hydrocephalus, microcephaly, porenchephaly.

Alimentary Tract:

Obstructive Lesions, Omphalocele/Gastrochisis, diaphragmatic hernia.

Renal System:

Renal Aplasia, Dysplastic kidney, obstructive uropathy.

Skeletal System:

Dwarfish and other malformations.

Tissue Masses:

Congenital tumours, cystic hygroma.

Others:

Foetal maturity, maniotic fluid volume, placental site, hydrops foetalis, congenital heart, sex determination.

TABLE III

ULTRASOUND : PROBLEMS

Possible Foetal Tissue Damage : Sister Chromatid Exchange (SCE).

Possible Diagnosis only at certain gestational ages.

Role of intra-uterine treatment of detected anatomical defects.

TABLE IV

EXPERIMENTAL EFFECTS OF ULTRASOUND

Tissue	The lesion	Doses (X Normal)	Reference
Animal and human brain	Necrotic lesions	10,000 X	9
Rat spinal cord	Paraplegia	100 X	10
Cord made hypoxic	Paraplegia	40 X	10
Mouse cells	Unwinding of DNA	Normal but long periods	11
Human lymphocytes	Increased frequency of sister chromatid exchange	Normal	12
Human cells in culture	Decreased ability to attach to plastic	Normal	13

So far no obvious damage has been observed after 5-10 years observation of children who had ultrasound examination as foetuses. However, the possible side-effects of any newly-introduced diagnostic technologies take many more years of observation before side-effects can be seen. For instance, X-rays and their side-effects in producing thyroid cancer many years after they were used in infancy and childhood for thymic enlargement, came to light only in the last two decades.[8,9] I need not remind obstetricians about the use of di-ethylstilboesterol and their effects on both female and male children only after they have reached puberty. Thus, recently, in UK, the same advice, viz. foetal ultrasound should be carried out only with valid indications, was given again to all doctors there.

The second problem is that certain anomalies can only be detected at a relatively late period of gestation so that should therapeutic abortion be decided on, it may be to late, e.g. ectrodactyly, syndactyly, polydactyly may be difficult to demonstrate till late pregnancy.

The third problem is what should be done with certain abnormalities detected, usually unexpectedly, when scanning for other lesions, For example, when hydrocephalus is seen or when renal obstructive lesions are demonstrated, should foetal surgery be carried out or should the foetuses be left alone and only deal with the lesions postnatally, when further damage would have accrued, or should therapeutic abortion be carried out. Some of these lesions have been found to be transient [10] with spontaneous intra-uterine resolution.

In conclusion, with the advent of new medical technologies, we must be prepared to deal with problems arising from such advances. We have learned that medical advances always bring in their train certain problems as a cost we have to pay.

AMNIOCENTESIS

I shall deal mainly with chromosome analysis of amniotic cells for the diagnosis of foetuses with abnormal chromosome karyotypes or for sexing

in dealing with sex-linked diseases. To date, our Human Genetics Division has dealt with 623 requests for amniotic cell chromosome culture and successful cultures were obtained in 97% of cases. The chromosome failures were due to bacterial contamination, too few cells, heavy blood contamination, etc. Some of the date on the first 440 cases are shown in Tables V-VIII.

It is noted that 64% of the requests came from relatively older mothers 35 years and beyond, and those 41 years and older comprised nearly 10%. This confirms the fear that older mothers are more prone to produce foetuses with abnormal chromosomes than younger mothers. This is seen in Fig. 2 from the European Collaborative Study[11] of 52,965 pregnancies dealing with mothers 35 years and over.

Among Caucasians, foetal trisomy-21 at maternal ages of 35 years and over is shown. The rate peaks at a maternal age of 46 years and then declines significantly thereafter, showing very celarly that foetuses of older mothers are more prone to chromosomal abnormalities. If all chromosomal abnormalities are considered, 1,200 foetal chromo-

TABLE V

AMNIOCENTESIS AND CHROMOSOME CULTURE

Maternal Age (Years)	No.
< 35 years	158
35 – 37	158
38 – 40	83
41 and above	41
Total	440

TABLE VI

CHROMOSOME ANOMALIES DETECTED ON AMNIOCENTESIS

Anomalies	No.
Downs Anomaly	7
DA Translocation Carrier	2
Sex Chromosome Abnormality	3
E Trisomy	3
16/20 Translocation	1
C-Trisomy	1

TABLE VII

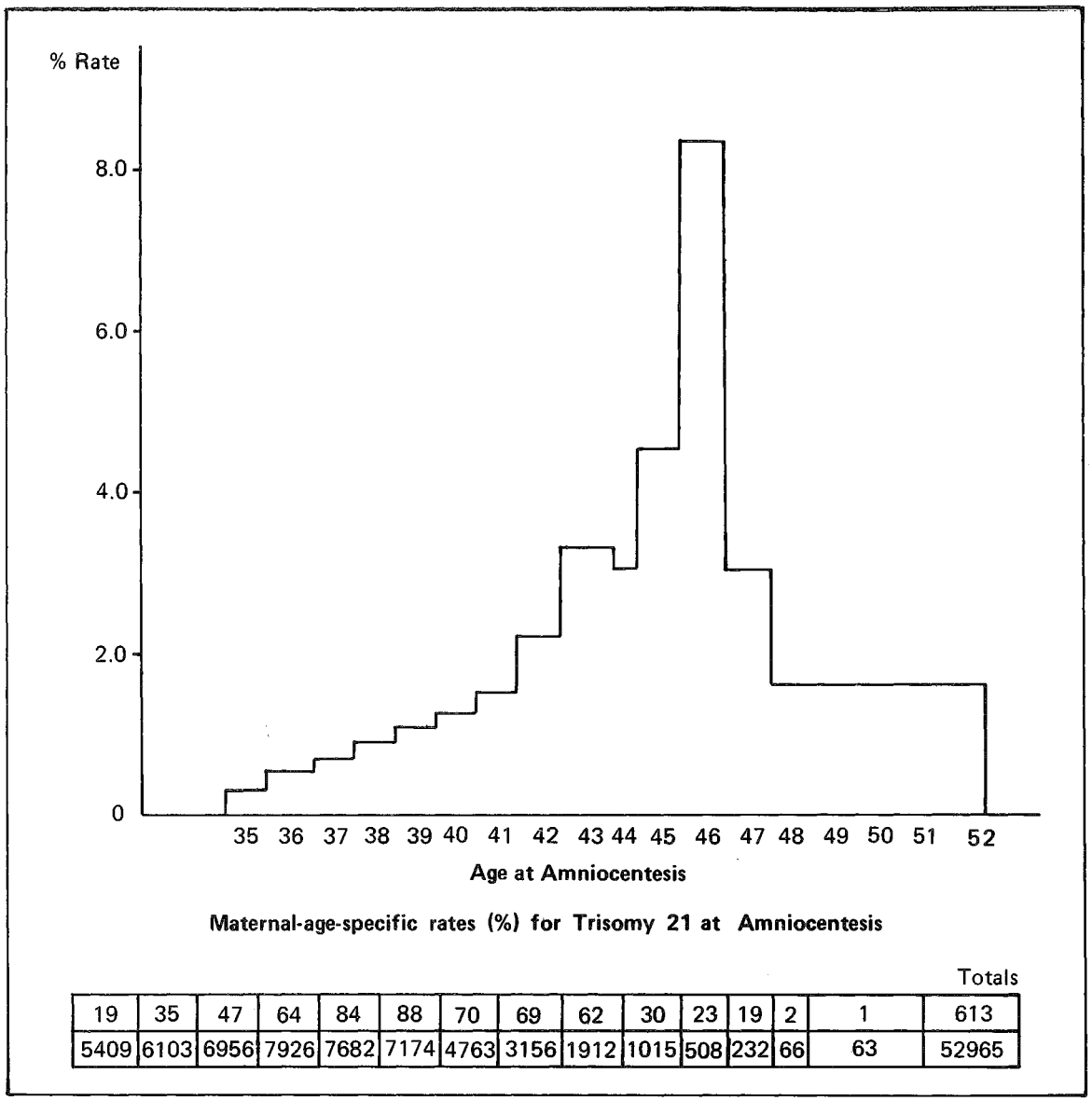
ETHNIC GROUP OF MOTHERS WITH AMNIOCENTESIS

Group	No.	%
Chinese	263	59.9
Caucasian	115	26.1
Indian	29	6.6
Malay	13	2.9
Others	20	44.5
Total	440	100.0

TABLE VIII

REASONS FOR AMNIOCENTESIS AND CHROMOSOME CULTURE

To exclude Downs	210
Previous Downs	96
Previous child with Chromosome Abnormality	8
Previous child with MD	4
Other reasons	122
Total	440



Maternal-age-specific rates (%) for Trisomy 21 at Amniocentesis

														Totals
19	35	47	64	84	88	70	69	62	30	23	19	2	1	613
5409	6103	6956	7926	7682	7174	4763	3156	1912	1015	508	232	66	63	52965

Fig. 2 Showing incidence of Down Anomaly infants in 52,965 pregnancies in the European Collaborative Study. It is seen that Down Anomaly is commoner among mothers with advanced maternal age at pregnancies.

some aberrations (with 613 trisomy 21 cases) were detected, i.e. an incidence of 2.3% of all pregnancies 35 years and over. The same increase in incidence with maternal age was seen with XXY Klinefelters and triplo-X females, trisomy 13 (Patau's Syndrome) and trisomy 18 (Edward's Syndrome). No significant increases with maternal age was seen in XYY and XO Turners Syndrome. It must be realised that these rates are not equivalent to the rates found at delivery for many of the most severely affected infants will be lost by spontaneous abortion or stillbirth between the

time of amniocentesis and delivery. It has been estimated that for trisomy 21, the rate at birth is likely to be 30% less than at amniocentesis and the reduction is even greater for the other autosomal trisomies and for 45 XO Turners Syndrome.[12] On the other hand, few of the pregnancies with XXY, XXX and XYY Syndrome will be lost in this way, and amniocentesis rates are likely to be similar to postnatal rates in these conditions.

In our series of 440 and lately up to 623 cases stretching over the whole range of maternal ages, there were 17 chromosome abnormalities detected as in Table VI.

This gives a 2.7% of positive rates for amniocentesis in the Department and considering the maternal ages requesting for amniocentesis (Table V), the incidence of chromosomal aberrations is higher than encountered in the European study.

The ethnic groups requesting amniocentesis are shown in Table VII.

It is thus seen that the Caucasians are significantly over-represented considering the ethnic representation in Singapore. Among the three main ethnic groups in Singapore, it is seen that: requests from Chinese are under-represented ($p < 0.0001$); requests from Malays are also under-represented ($p < 0.0001$); requests from Indians are appropriate ($p < 0.05$); requests from Caucasians are over-represented ($p < 0.0001$); requests from other ethnic groups are over-represented ($p < 0.05$).

The reasons given (logical or not) for seeking chromosome culture were listed in Table VIII. The largest fear is Downs anomaly which is logical as it is the commonest serious chromosomal disorder. But what are the problems in chromosomal culture of amniotic cells? There are many besides the odd case where culture (Table IX) fails.

Maternal cell contamination is a real problem though fortunately it is rare. In this instance, the maternal cells grow instead for the foetal cells.

TABLE IX

TECHNICAL PROBLEMS OF CHROMOSOME CULTURE

Maternal cell contamination
Laboratory errors
Mosaicism

This is more likely to occur when there is scanty fluid obtained, when there is heavy contamination with maternal blood, when there is more than one attempt at amniocentesis and when the cell cultures have taken longer than usual to grow. As a precaution, it is wise to use a stillette and discard the first few drops of amniotic fluid withdrawn.

Another precaution is to examine the amniotic cells for Barr bodies. If there are chromatin negative cells and the subsequent culture shows a female cell line, maternal contamination is likely. If there are chromatin positive cells, it may mean a correct female fetus or maternal cells are being grown, and it is important to analyse cells from at least two primary cultures especially if factors predisposing to maternal cell contamination (as described above) had been present before. Under these circumstances, besides more than one primary culture, study of the parental karyotypes may assist in clarification. In spite of all these precautions, once in a while, maternal cell growth does occur and it must be accepted that this is one of the hazards of amniotic chromosome culture.

Laboratory errors are usually of two types in the first type, administrative errors such as wrong labelling of specimens. This has happened once with us and now we do not accept more than one specimen at the same time from the same obstetrician. Secondly, consider interpretation errors. This can happen with inexperience, poor preparations and poor growth, e.g. a female 47 trisomic 21 Downs anomaly foetus may have poor preparations where 46 cells are counted and the presence

of five small acrocentrics, one of which is wrongly interpreted as the Y chromosome will lead to a wrong diagnosis of a normal male foetal.

More than one cell line may be grown and it is not always indicative of a true mosaic, e.g., if there is maternal cell contamination. When more than one primary culture is carried out, these may show its absence in the other cultures and its presence only in one. Occasionally such pseudomosaicims may occur during cell culture itself. All so-called mosaics should have a repetition of culture of the cells of the baby after he is born. The other side of the coin is also possible, viz. true mosaics fail to demonstrate two cell lines because of failure of growth of one of the cell lines.

In our series of 440 amniocentesis, there were two errors in sex identification; in both cases they were reported as females, and male babies were born instead. Most probably, in both cases, maternal cells were grown instead of foetal cells and in both cases, the growth was scanty.

THALASSAEMIA PRENATAL DIAGNOSIS

The next topic which is relevant in this part of the world in prenatal diagnosis is the foetus diagnosis of β-thalassaemia major, a potentially lethal genetic disease. There is no problem in the technology which consists of Hb chain separation and depending on the β/γ chain ratio (whether it is β° or γ^{+}—thalassaemia major), a firm diagnosis can be made. The problem is the aspiration of relatively pure fetal blood not contaminated by maternal blood, a problem which is universal when blind needle probe of the placenta is carried out to obtain blood. Methods are available for separating out the foetus cells from the maternal cells but unless these methods are successful, diagnosis may not be accurate.

Such haemoglobin chain separation carried out on a foetal at 16 weeks gestation (Fig. 3), where the foetal was not affected.

However, two alternative methods are available.

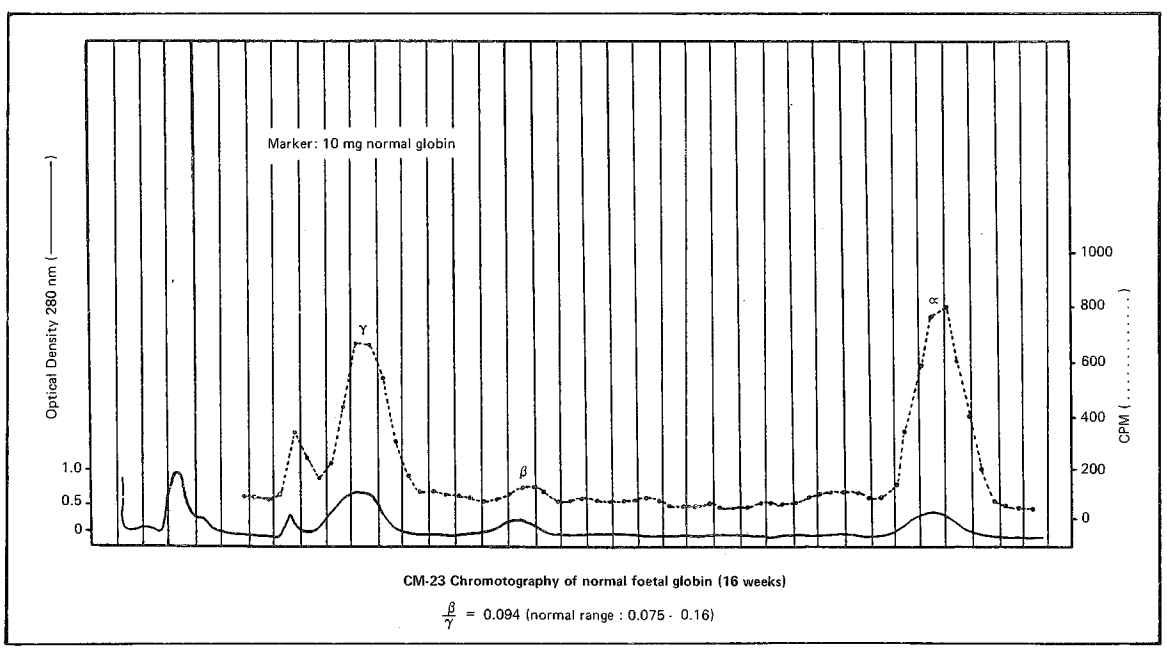


Fig. 3 Showing haemoglobin chain separation in a normal foetus at 16 weeks.

Fetoscopy

Use of the fetoscope in competent hands has allowed needle entry into foetal vessels in the cord so that almost pure foetal blood can be obtained. However, this expertise seems to reside only in a few centres in the world but with practice, it is hoped that more obstetricians may be competent enought with this procedure. Even in competent hands there is a 5% incidence of foetal mortality and a 2% perinatal mortality.

DNA Recombinant Technology

The difficulty in obtaining foetal blood has led to an alternative, viz. culture of amniotic cells, and examining the DNA sequences in these cells to study more closely the DNA comprising the β-globin gene. In essence, the procedure consists of utilising relevant restriction enzymes which would cut the DNA chains in such a way that the DNA of the β-globin genes are separated. The DNA fragments are sized by electrophoresis in agarose gel (Fig. 4) and then transferred to nitrocellulose by a method known as Southern blotting.

The actual DNA comprising the β-gene and the flanking DNA is 'brought out' by hybridisation with a radioactivated normal β-globin probe which will 'seek out' a normal β-gene while it will fail to pair up if the β-gene of the patient is abnormal. The advantages with this method are: it uses the well tried relatively safe and simple procedure of amniocentesis and cell culture; and cells may also be obtained from chorionic villi which allows of earlier sampling than amniocentesis or foetal blood sampling, both of which can be effectively done in the second trimester which does not allow much time for analysis. Chorionic villi aspiration can be carried out in the first trimester and relatively large number of cells can be obtained. However, the technique still has some kinks which will have to be straightened out.

However, the technology of DNA recombinant analysis applied to the prenatal diagnosis of β-thalassaemia major suffers from one disadvantage not inherent in the technology itself. It is inherent in the genetic defect in β-thalassaemia major where in the majority base mutations within the β-globin gene cannot be demonstrated but there may be changes in the flanking genes or polymorphic genes which are closely linked with the β-globin gene.

In other words, these genes are markers for the g-thalassaemia gene. These are termed restriction fragment length polymorphisms (RFLP) and they have to be studied in the parents as well as in the patient. Another way whereby such examination of neighbouring DNA is illustrated in the example of sickle cell haemoglobin.[13] When the restriction enzyme Hpal is used in normal individuals in the region of the β-globin gene, a 7.6 kilobase DNA fragment can be elucidated while in 80% of cases of sickle-cell β-globin on different 13 kilobase length fragment is obtained instead (Fig. 5).

The carrier parents are each heterozygous for the disease allele and thus have one 7.6 kilobase DNA fragment (associated with the normal gene) and one 13 kilobase DNA fragment (containing the sickle-globin gene). This polymorphism is caused by a single-base change 5 kilobase to the 3' sides of the β-globin gene which eliminates the Hpal site.

CONCLUSION

To recapitulate, for the prenatal diagnosis of genetic disorders, ultrasonography is used mainly in delineating multifactorial genetic diseases, while chromosome culture of foetal cells assist in the diagnosis of chromosome diseases, and DNA recombinant technology is solving problems in the diagnosis of Mendelian genetic diseases, a technology which can be applied to all tissues obtained from the foetus.

One group of Mendelian diseases which has not been alluded to include the biochemical inborn errors of metabolism. Many of them can be diagnosed by testing for the enzyme in amniotic cells after culture or, in some cases from foetal blood. However, one has to look at the problem of Mendelian diseases as a whole; there

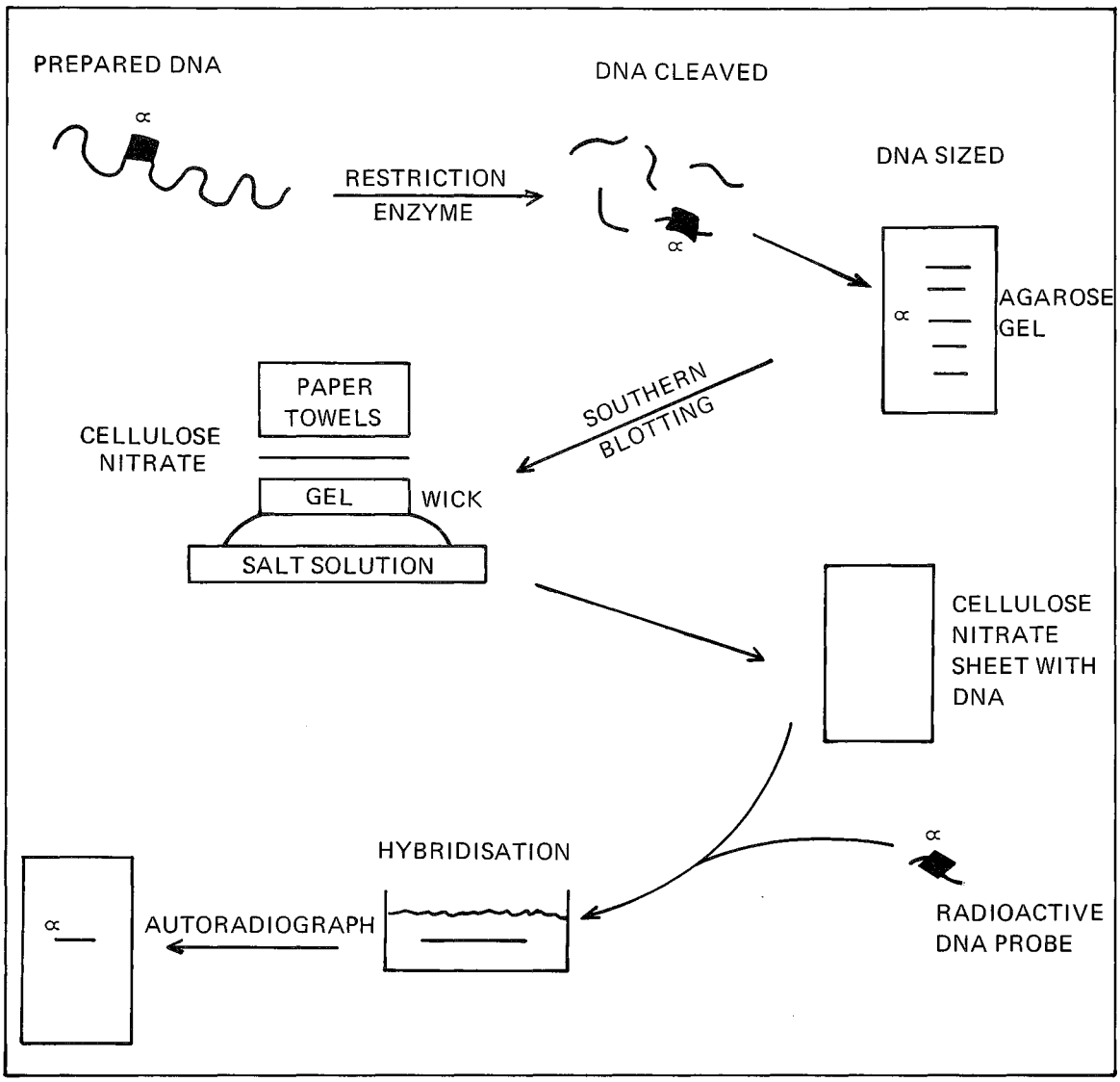


Fig. 4 Principle underline DNA re-combination technique for genetic diagnosis.

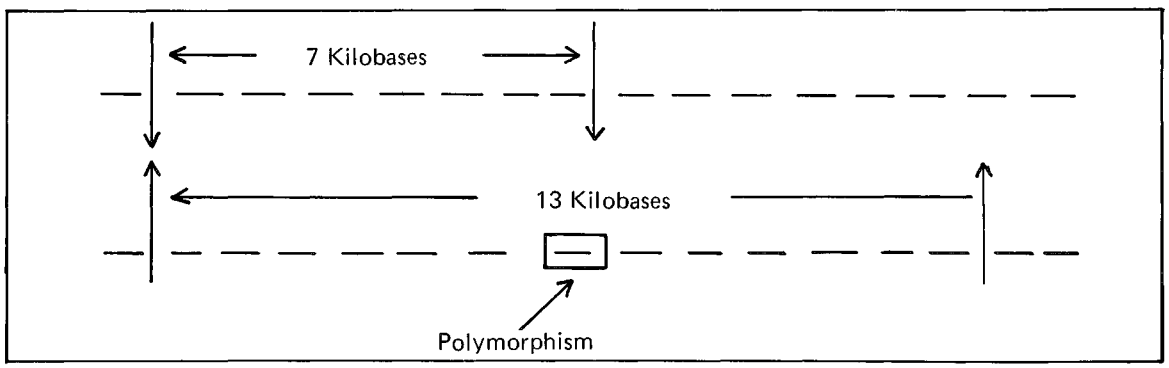


Fig. 5 Showing diagnosis of sickle cell disease of DNA Recombinant technique.

are approximately 1500 known diseases of which only 200 lend themselves to be diagnosed at the molecular level e.g. even cystic fibrosis which is the commonest severe Mendelian gene among Caucasians, we still do not know what the abnormal enzyme or structural protein really is. Thus in 1,300 such diseases due to single gene mutations, no known exact diagnostic methodology is available.

Out of the 200 Mendelian diseases which are capable of molecular diagnosis, only in 70 of these has prenatal diagnosis been reliably achieved; in other words only 5% of all Mendelian disorders lend themselves to exact reliable prenatal diagnosis. We thus have to be aware of the present limitations in prenatal diagnosis but basing in previous experience in this field, many more genetic diseases will be diagnosable in the near future and this opens up a whole field for interesting and useful medical research.

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