Cytogenetics in Medical Practice

MATILDA M. NELSON, P. H. BEIGHTON

SUMMARY

A cytogenetics and tissue culture laboratory is an essential component of a medical genetics department. Chromosome analysis provides a definite answer in many cases where clinical diagnosis is in doubt, and supplies information on which counselling may be based. Cultured cells from different body tissues may be the only means by which an inborn error of metabolism may be studied. Information supplied by such a laboratory forms the basis of many of the diagnostic and counselling services offered by the modern human geneticist to his fellow practitioners, either directly or as part of a medical genetics department.

S. Afr. Med. J., **48**, 1577 (1974).

PATIENT REFERRALS

Patients are referred from many sources (Table I), including private practitioners and hospital doctors, and many seen either in the laboratory or in the ward are interviewed at the time of taking blood for culture. Other patients are seen in the Genetics Clinics at Groote Schuur, Princess Alice Orthopaedic or Red Cross War Memorial Children's Hospitals, where full genetic histories are taken, examinations carried out and specimens obtained. In this way the laboratory is accumulating valuable clinical and family data to complement chromosomal findings.

Department of Human Genetics, University of Cape Town
MATILDA M. NELSON, M.A., M.B. CH.B., M.R.C.P., D.C.H.
P. H. BEIGHTON, M.B. B.S., M.D., M.R.C.P., D.C.H.

Date received: 10 April 1974.

Peripheral blood cell cultures and analyses form the bulk of this aspect of the laboratory work. Examination of buccal smear cell nuclei for sex chromatin and fluorescent F bodies is carried out if a sex chromosome abnormality syndrome is suspected.

Amniotic fluid cell cultures form a very important facet of the services which the laboratory offers in connexion with antenatal diagnosis. Amniocentesis is carried out by the obstetrician between the 14th and 16th weeks of pregnancy. The indications for these studies include 'elderly' mothers, where the risk of having a baby with Down's syndrome is reaching a significant level, or women who have had a previous child affected with a chromosomal defect (e.g. Down's, Edwards's or Patau's syndromes), or with a severe central nervous system deformity (e.g. anencephaly or spina bifida).

Uncommon but important reasons for referral include those families where a child has one of the genetic conditions where antenatal diagnosis of an affected fetus is possible (e.g. Tay-Sachs, Gaucher's or Lesch-Nyphan's diseases). Diagnosis of fetal sex only may also be undertaken where the mother is a carrier of a sex-linked recessive condition and the risk of an affected male fetus is high (e.g. Duchenne muscular dystrophy).

The amniotic fluid cell chromosomes are examined in all cases; cultured cells are sent for biochemical studies when necessary. The cell-free amniotic fluid may be passed on to other laboratories in appropriate situations for special studies (e.g. to biochemistry for α-fetoprotein estimations in central nervous system defects).

RESULTS IN 1973

The racial origin of the patients is shown in Table I. The White referrals are somewhat biased, since the

TAELE I. SOURCES OF REFERRALS TO THE CYTOGENETICS LABORATORY 1973

Race	Genetics clinics			Private practitioners	Paediatricians	Haematologists	Alex. Inst.	Endocrinologists	Gynaecologists	Others	Total patients	% of referrals
	GSH	PA	RCH									
White	12	9	17	18	25	28	63	3	11	14	200	57
Coloured	12	11	30	0	39	9	0	7	6	0	114	32,5
Black	2	2	9	0	6	3	0	3	0	11	36	10,5
Total	26	22	56	18	70	40	63	13	17	25	350	

GSH—Groote Schuur Hospital; PA—Princess Alice Orthopaedic Hospital; RCH—Red Cross War Memorial Children's Hospital; Alex. Inst.—Alexandra Institute.

TABLE II. CHROMOSOMAL ABNORMALITIES FOUND IN 350 PATIENTS

	Chromosomes involved							
	Autosomes				Sex			
	G group	E group	D group	Other groups	chromo-some	Total	% referred	% total abnormal
White	13	2	1	6	9	31	16	42
Coloured	24	1	1	4	4	34	30	46
Black	4	0	0	1	4	9	22	12
Total	41	3	2	11	17	74		

Alexandra Institute admits only White patients. If the 63 patients referred from this institution are excluded, the percentage referrals become Whites 47,3; Coloureds 39,7; and Blacks 12,0.

Chromosomal Abnormalities

The abnormalities found and the particular chromosome group involved are shown in Table II. Sixteen per cent of White, 30% of Coloured and 22% of Black patients investigated had a defect of some kind. Forty-six per cent of the total chromosomal aberrations found were in Coloured patients, 42% in Whites and 12% in Blacks.

Autosomal abnormalities: These were classified as follows:

G group — abnormalities in the chromosomes of this group were the most numerous, and Down's syndrome was the most frequent clinical diagnosis. Three of the possible chromosomal variations in Down's syndrome (trisomy 21, trisomy 21 with a normal cell line—mosaicism, and translocation) were encountered (Table III). The two translocations were both G/G and were both sporadic, the chromosomes of the parents being normal in each case.

TABLE III. CHROMOSOME ABNORMALITIES IN PATIENTS WITH DOWN'S SYNDROME

	Trisomy 21	Trisomy 21 mosaicism	Trans-location	Total
White	6	5	0	11
Coloured	16	3	2	21
Black	2	0	0	2
Total	24	8	2	34

In an additional patient, trisomy 22 was suspected. This cytogenetic diagnosis has not yet been completely confirmed by banding. However, the patient's clinical features were consistent with previous descriptions of this entity, and certainly did not resemble those of Down's syndrome.

E group — few patients were referred with specific clinical diagnoses involving chromosomes of the D or E groups (8 cases only). The cytogenetic diagnosis of Edwards's syndrome (E trisomy or trisomy 18) was made in 2 patients who had the characteristic clinical appearance of this condition. Conversely, in one patient where the clinical diagnosis of Edwards's syndrome was made with confidence by two experienced paediatricians, the chromosomes were normal. The remaining E group chromosome abnormality was a ring formation in a chromosome 18, found in the blood of a White male infant referred because of physical and mental retardation associated with a peculiar facies. The ring varied considerably in size but was present in all cells examined.

D group — only one case of Patau's syndrome or trisomy D was found. The extra chromosome was present in 93% of the cells examined. The other D group anomaly was found in a White patient with ataxia telangiectasia. Almost the entire length of the long arms of a D chromosome (No. 14) was translocated on to a C group chromosome in this individual.

Others — 11 other instances of autosomal abnormalities were encountered (Table II). Three patients with Fanconi's anaemia had an excessive number of breaks in their chromosomes. Another patient who had a consistent break in the long arms of an A2 chromosome was the brother of an individual with mosaicism for Down's syndrome.

There were 4 instances of translocations involving C group chromosomes; one of these involving chromosomes 7 and 10 (46,XX, t (7q −, 10q+)) was found in the mother of a child who proved to have Down's syndrome. Two of the individuals with translocations were mother and son; in the case of the phenotypically normal mother, the anomaly was presumably balanced, but it was present in an unbalanced state in the son (46,XY, t (2q +, 7q −)). The last translocation was present in 6% of cells examined from a mentally retarded White female: one of the D and one of the C group chromosomes were missing and an extra chromosome the size of an A3 was present; this was the result of the translocation.

An additional C group chromosome was present in one mentally retarded patient where 10% of cells examined carried the extra chromosome. Another severely mentally retarded individual had lost the short arms of a C group chromosome in 20% of her cells. Finally, an extra chromosome, smaller than those of the G group, was present in 35% of cells examined from a male referred for evaluation of hypogonadism.

Sex chromosomes: Forty-eight patients were referred specifically for sex chromosome examination, and of

TABLE IV. SEX CHROMOSOME ABNORMALITIES

	Turner's syndrome		Klinefelter's syndrome		Extra Y	'Intersex'		
	45,X	45,X mosaic	47,XXY	47,XXY mosaic	syndrome (48,XXYY)	Testicular feminisation	Other	Total
White	2	6	0	1	0	0	0	9
Coloured	0	0	1	1*	1*	3	0	5
Black	0	1	0	0	0	0	2	3
Total	2	7	2	2	1*	3	2	17

* Same patient.

these, 35% were found to have abnormal sex chromosome constitutions (Table IV). There were 9 patients with Turner's syndrome, 7 of whom were mosaics with, in addition to the line of cells with only one X chromosome, either a line of normal cells (4 patients), or one with an isochromosome X (2 patients), or a ring chromosome (1 patient). Eight of these 9 individuals were Whites.

Three patients had Klinefelter's syndrome, 2 of whom had additional cell lines. One of these men had a normal 46,XY line in addition to the 47,XXY line, while the other had a 48,XXYY chromosome pattern in 5% of the cells examined and 47,XXY in 95%. There were 3 proven cases of testicular feminisation (female phenotype with XY chromosomes), and 2 cases of male phenotype with XX chromosomes. In this group of 8 patients, only 1 was White.

Buccal Smear Studies

In addition to the peripheral blood culture studies, interphase nuclei, either in peripheral blood or buccal smears, were examined in patients where the clinical stigmata indicated that the sex chromosomes might be involved.

During the year, as part of an ongoing study by Dr D. H. G. Close, 1 180 buccal smears were examined for F bodies (indicating the presence of a Y chromosome), and for sex chromatin bodies (indicating the presence of two X chromosomes). No Y abnormalities were detected, though in one instance no F body was found in a phenotypic male. The peripheral blood investigations of this individual showed him to have a small Y chromosome, a normal variant which does not fluoresce in interphase nuclei. Three X chromosome abnormalities were predicted by buccal smear studies and subsequently confirmed by karyotype analyses.

Amniotic Fluid Studies

During 1973, 8 patients were referred for amniotic fluid investigations. The reasons for referral are given in Table V. In 2 instances the referral was made because a previous child had been affected by either anencephaly or spina bifida, and the amniocentesis was carried out in order to obtain liquor for α-fetoprotein estimations. In both patients the level was within normal limits. As a matter of routine, the cells from these two amniotic fluids were cultured, and one fetus was shown to carry

TABLE V. DIAGNOSTIC AMNIOTIC FLUID CULTURES

Reason for referral	
Advanced maternal age	4
Advanced maternal age and a previous child with Down's syndrome	2
A previous child with severe CNS abnormality	2
Predicted outcome	
Normal	7
Abnormal	1
Outcome known	
Normal	4
Abnormal	1
Not yet delivered	3

a G/G translocation (Down's syndrome). Four of the 7 patients where a normal outcome was predicted, have delivered normal healthy infants; as yet no information is available on the remaining 3.

During the first 2 weeks of 1974, predictions of the intra-uterine presence of one fetus affected with anencephaly and another with Down's syndrome, were confirmed. This situation will be discussed elsewhere.

Other Investigations

Skin biopsy specimens from 6 patients with various metabolic disorders were cultured. Since cells were obtained for histochemical investigations rather than cytogenetic studies, they are not discussed further in this article.

A wide variety of chromosome defects occur in haematological conditions. Five instances of the classical Philadelphia (22q −) were found, and 1 patient with polycythaemia had a G ring chromosome. These cases also fall outside the scope of this article.

DISCUSSION

This analysis of the results of cytogenetic investigations reflects the wide range of patients who were referred. Though the number of abnormalities detected was small in the particular subgroups examined, the trends are consistent with those of other large published series. The proportions of patients referred from particular

sources probably reflect the interest and awareness of the practitioners concerned.

The bulk of autosomal chromosome abnormalities are concerned with the G group, especially No. 21, which is responsible for Down's syndrome. This disorder is the commonest clinical condition due to a chromosome defect. Hamerton[1] reviewed the miscellaneous chromosome anomalies which were present in the families of probands with Down's syndrome. The majority of these changes were other aneuploidies or minor autosomal anomalies. The two cases we report here (46,XY with a consistent A2 break and 46,XX, t (7q −, 10q+)) are not of the types previously described and their significance is not clear; they may be chance findings.

Severe mental retardation is a part of recognised chromosomal syndromes such as Down's, Edwards's, Patau's and the *cri-du-chat* syndromes. Moderate retardation may be a feature of the sex chromosome aneuploidies such as Klinefelter's or Turner's syndromes. Three of the patients reported in this article with moderate mental retardation had chromosomal defects (46,XX/45,XX, −D, −C, +t (D,C); 46,XX/47,XX, +C and 46,XX/46,XX, Cp−,), none of which are common. It is not certain that the cytogenetic abnormalities can be blamed for the mental retardation, but it is highly likely that there is an association.

It is impossible to make any definite statement on the racial distribution of sex chromosome aberrations, since the total numbers are too low, but the fact that 8 out of 9 patients with Turner's syndrome were White, and that all the 'intersex' individuals were non-White, may be significant.

The amniotic fluid cell series is small, but it reflects the increasing interest in the possibilities of antenatal diagnosis. As yet, the definitive indications for such investigations are limited, but there are reports of successful predictions in a rapidly increasing number of conditions. Antenatal diagnosis by amniocentesis is one of the most fruitful fields of research at the present moment.

The value of cytogenetic studies in clinical medicine is already obvious. Diagnostic precision based upon chromosome investigation permits accurate prognostication for many children with unusual stigmata. The carrier status of parents of children with chromosome defects can be evaluated, and counselling given on the likelihood of recurrence. Infertility may be explained. Repeated abortion may be explained by the demonstration of the presence of chromosomal abnormality. Amniotic fluid cell investigations have considerable potential.

It can be confidently foreseen that cytogenetic investigations will play an increasingly important part in medical genetics.

The research activities of the Department of Human Genetics are supported by grants from the University of Cape Town's Staff Research Fund and the South African Medical Research Council.

REFERENCE

1. Hamerton, J. L. (1971): *Human Cytogenetics*, vol. II, pp. 257 - 260. New York: Academic Press.