THE RELATIONSHIP BETWEEN SATELLITE ASSOCIATION AND TRISOMY 'G'

A Thesis Submitted to

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by

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SUMMARY

A hypothesis was put forward, based on a personal observation, that there appeared to be an increased frequency of satellite associations between the acrocentric chromosomes in a young parent of a child with trisomy 'G'. It was therefore considered that there might be a predisposition towards trisomy in some families, and that satellite association was a possible factor in the production of such.

The literature covering the cytogenetics and technical significance of satellite association, together with the clinical, cytogenetical and parental age involvement in trisomy 'G', has been reviewed.

The design of this investigation was divided into two parts, namely methodological and clinical.

In the first part of the study a standardised technique was developed to provide the lowest frequency of satellite associations and accurate identification of participating chromosomes. Blood samples from thirty-five normal controls were used. A standard cultural method was employed to compare the satellite association frequencies observed in potassium chloride, sodium citrate and Hanks/water used as hypotonic solutions. Two further experiments were used to evaluate the effect of time and molarity of the hypotonic solutions on satellite association frequency. Hanks/water was used as the hypotonic solution of choice giving fewer satellite associations per cell.

The second part of this investigation dealt with the cytogenetic analysis of eighteen parents of regular Down's infants matched for age with control parents of normal children. The specific identification of the individual chromosomes involved, and the category of associations was recorded in both groups.

Application of the results to statistical analysis showed that in certain specific configurations, there was a significant increase in satellite association frequency in parents of Down's infants.

The possible role and causes of satellite association are discussed.

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I N D E X

THE RELATIONSHIP BETWEEN SATELLITE ASSOCIATION AND TRISOMY 'G'

I. INTRODUCTION

I.i Hypothesis

It is known that in families having one child with an autosomal trisomic anomaly, the chances of producing another child with a trisomy, even of a different type, are greater than average (Carter 1970). There seems therefore to be a predisposition towards trisomy in some families.

The formulation of the present hypothesis was stimulated by the personal observation that, in a young parent of a child with trisomy 'G' there was an unusually high number of D/G satellite associations in the metaphase spreads. The finding was sufficiently striking to suggest that it did not represent merely a chance exaggeration of the normal, but reflected an actual anomaly present in the chromosomes. It is suggested that this could be a mechanism which could account for the apparent familiar predisposition to aneuploidy.

The most frequent cause of anueploidy in man is non-disjunction where there is a failure in the separation of the chromosomes in the dividing cell. The most common of the autosomal trisomies is trisomy 'G' (Down's Syndrome or Mongolism) which has an incidence of one in seven hundred live births, and trisomy 'D' (Patau's Syndrome), which is seen in one in five thousand live births. Both of these anomalies stem from non-disjunction and both occur in the

chromosomes which show satellite association.

There are therefore, reasons for studying the phenomenon of satellite association as a possible factor in the production of trisomy in groups 'D' and 'G'. As satellite association is seen in normal metaphase spreads, the hypothesis requires that in the parents of children with these trisomies, a different degree of the normal affinity between associated chromosomes or a different quality of affinity possibly due to structural factors, is present in these chromosomes.

I.ii Review of Literature

a) Cytogenetics - General

In the human karyotype there are two groups of chromosomes which possess satellites. These so-called satellites appear on the short arms of the acrocentric chromosomes of the 'D' and 'G' groups (see Figure 1). It is known that these satellites are not artefacts of preparation but are structural features of these chromosomes, and as such are inherited. All of the ten acrocentric chromosomes probably bear satellites on their short arms. Howell *et al*., (1975) found that in apparently normal individuals, the number of acrocentric chromosomes which exhibited satellites, varied from person to person. However, each individual tended to have a consistent specific number of satellites, and it is thought that this constitutes a true variation.

It was in 1961 that the phenomenon of satellite association was first described in human mitotic metaphase spreads (Ferguson-Smith and Handmaker, 1961, Harnden, 1961, Ohno *et al*. 1961). It was observed that the satellited chromosomes assumed specific positions in the metaphase spreads showing an apparent affinity between the satellites (Figure 2). It was seen that any number of associations could occur involving all the satellited chromosomes. The possibility of this being a random non-specific artefact was eventually disproved: finally, Ferguson-Smith described it in meiotic chromosomes in 1964, where the association of bivalents of the acrocentric chromosomes was observed in

Figure 1

a) Normal female karyotype - giemsa stain.

b) Karyogram of the 'D' and 'G' groups of chromosomes.

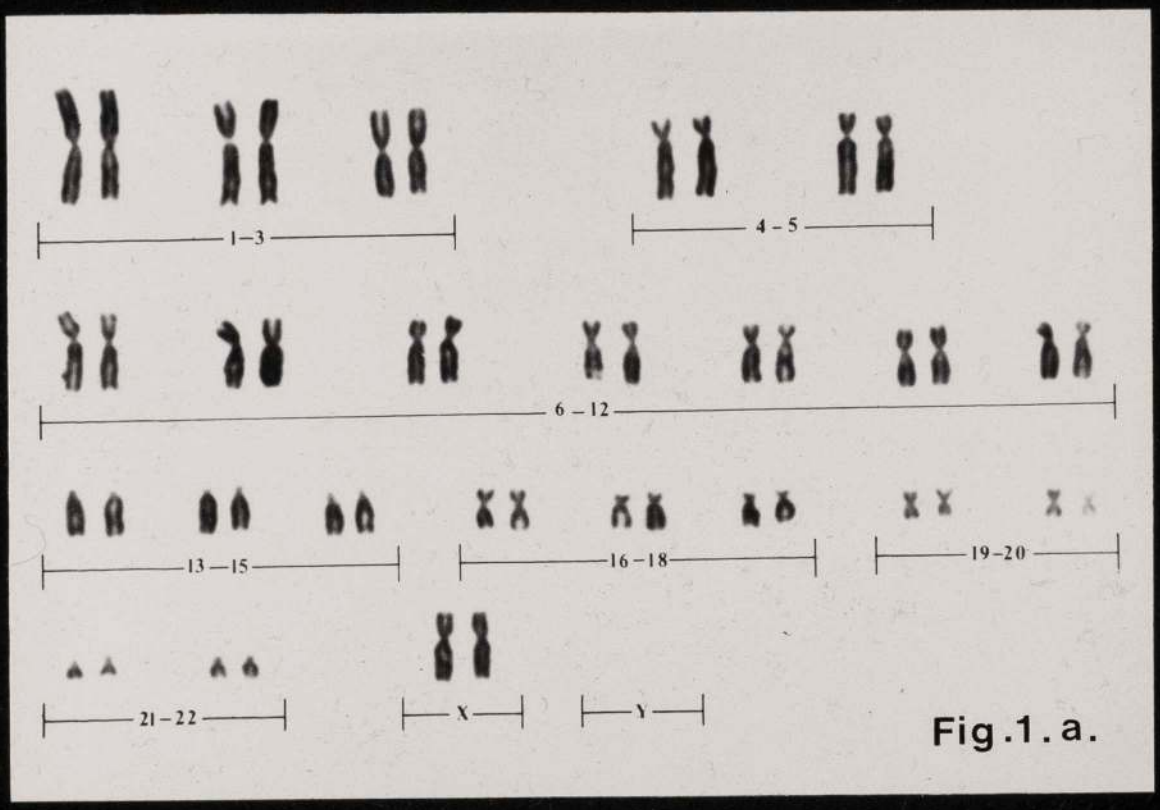


Fig.1.a.

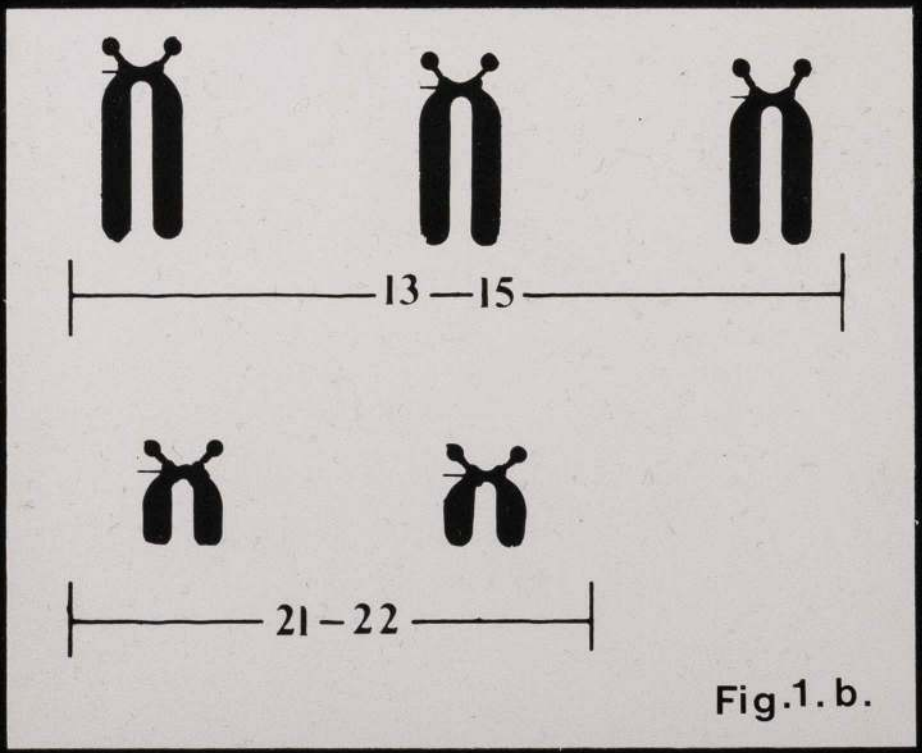


Fig.1.b.

Figure 2

Normal female metaphase spread - giemsa stain, showing apparent affinity between the acrocentric chromosomes.

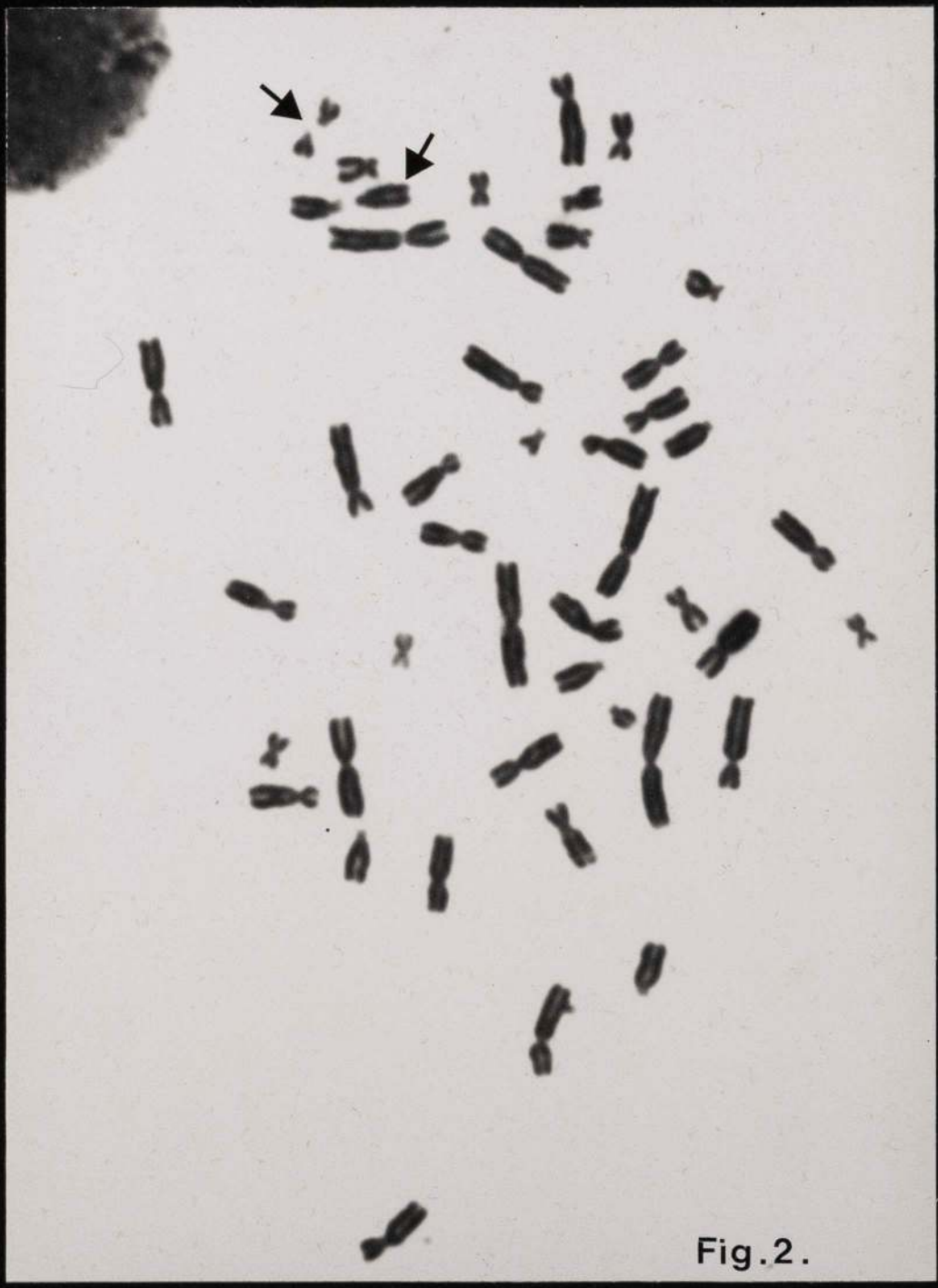


Fig.2.

human pachytene chromosomes, obtained from direct squash preparations.

The formation of satellite associations has often been attributed to the involvement of the satellited chromosomes in the nucleolar formation of the cell. It was postulated that the satellites were an integral part of the nucleolus and this involvement produced the adhesion needed to hold the satellites together during mitosis. If two or more nucleoli fuse to form one larger nucleolus this then would increase the chances of damage to the satellite segment with obvious risk of breakage (Ohno et al. 1961). Thus, if the breaks occur in more than one of the segments involved, the proximity of the broken ends might predispose to the translocation of material. This possibility might be the answer to the increased tendency of non-disjunction in the satellited chromosomes, e.g. trisomy 'G'. This is particularly relevant as autoradiographic studies have shown (Mikkelsen 1969) that chromosome D13, E18 and G21 are late in DNA replication as compared with the other autosomes.

The most common form of satellite association seen is the di-association where two satellited chromosomes are involved (see Figure 3). Less common is the tri-association, which, as the name implies, shows three satellited chromosomes involved (see Figure 4). In fact all combinations can occur until all of the ten acrocentrics are in association (see Figure 5). Occasionally, a non-satellited chromosome may be involved in an association. Perhaps the most common is A-1, where the centrimeric area appears to be the part to

Figure 3

Satellite association - Di-association - giemsa stain.

Figure 4

Satellite association - Tri-association - giemsa stain.

Figure 5

Satellite association - Multi-association - giemsa stain.

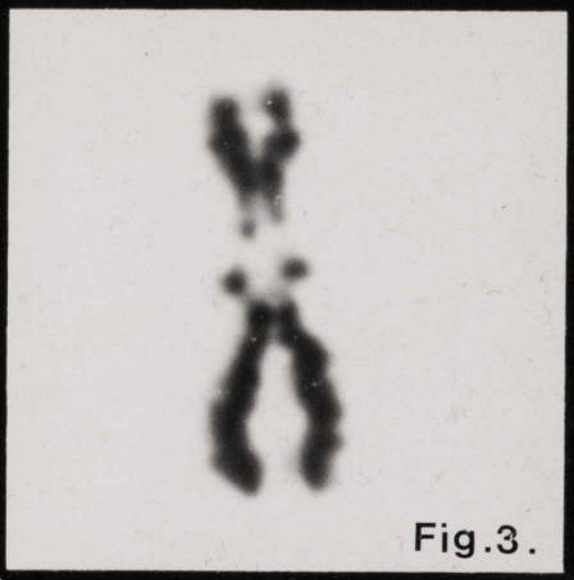


Fig.3.

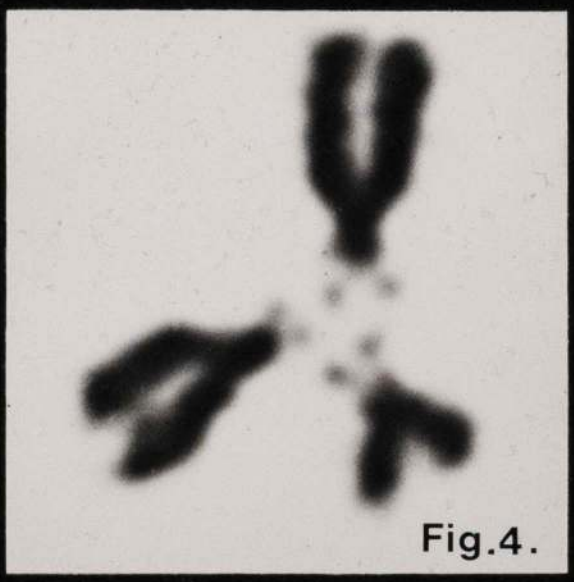


Fig.4.

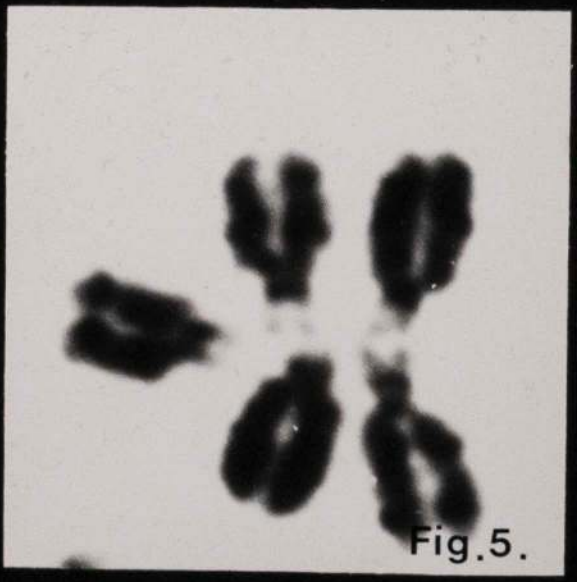


Fig.5.

which the satellites are most attracted.

b) Cytogenetics - Techniques

According to Ferguson-Smith and Handmaker (1961), satellite associations can be seen in up to 60% of normal mitotic metaphase spreads. Hansson (1970) found that the frequency varied between 62% to 80%, the mean value being 70.5% of normal cells had at least one satellite association present. However, Sasaki and Makino (1963) observed an increase in the number of secondary constrictions after culturing in a calcium free media, and this suggests that the cultural methods are likely to affect the frequency of the observed associations. Zang and Back (1967) confirmed that the frequency of association is influenced by the conditions of culture and the technique of preparation. Recently, Zhdanova and Deryagin (1975) have shown that all of the acrocentric chromosomes take part in the formation of satellite associations at the 52nd, 72nd and 90th hours of culture. They found that this occurs in the first mitosis with equal frequency, but in further mitoses, different association capacities appear. Zang and Back (1968) reported that the incidence of satellite association differed significantly between two of their experimental series. They compared the micro-method of Arakaki and Sparkes (1963) with the macro-method of Moorhead et al. (1960). In the micro-method, heparinized whole blood is inoculated into the basic culture medium, whereas in the macro-method, sedimented leucocyte rich plasma is used. Using TC. 199 as the basic culture media, 1,300 metaphases were examined in

the macro-method. In the micro-method, 1550 metaphases were analysed, these were grown in McCoys 5A media. It was found that in the latter series (the micro-method), all absolute counts of satellite associations were significantly lower; 37.8 $\pm$ 6.44 percent of available acrocentric chromosomes were involved in satellite association complexes in the macro-method, compared with 23.9 $\pm$ 4.09 percent in the micro-method. In their later paper (Back and Zang 1969) they compared several variants of the macro and micro-methods and their effect on the frequency and pattern of the satellite associations. These experiments included the same types of culture media as above, (macro-method using TC 199 and McCoys 5A and the micro-method again using McCoys 5A as the basic media). This time, their results gave the same satellite association frequency for both types of culture. Interesting differences were found, however, in the various qualitative properties and it was concluded that the ratio of the associating 'D' and 'G' groups were significantly influenced by the methodology of their experiments.

In a similar series of experiments, Hansson (1970 a,b) used TC 199 as the basic culture media for both micro and macro-methods. His results, however, were completely opposite to Zang and Back (1968,1969), showing an absolute count of satellite associations of 38.0 $\pm$ 3.21 percent in the macro-methods compared with 61.8 $\pm$ 3.12 percent in the micro-methods: 700 metaphases were analysed in each of the methods. He considered that the cultural factors influenced the outcome of the satellite association patterns significantly and that the discrepancy in the results of

Zang and Back (1969) with his own, might well be explained by the differences in the basic media used for the cultures. Hoehn, Nagel and Krone (1971) found that there was a decrease in the satellite association frequencies when the glucose concentration of the media was doubled.

Hansson (1970a) also questioned the addition of the nutrient serum or plasma to the basic medium. He used both human serum and calf serum in his experiments. However, no difference in satellite association patterns was observed. Back and Zang (1969), also searched for any small methodological differences in both of their series. The only difference noted was in the origin of the mitogenic agent, Phytohaemagglutinin (PHA). In their first series, the PHA was obtained from Difco whilst in their second series, Burroughs Wellcome were the suppliers. However, they considered that neither the PHA or the exposure of 5% CO_2 in air to the cultures to be as influential on the satellite association pattern as the cleanliness of the glass slides for the final preparation!

Both Zang and Back (1968), Back and Zang (1969) and Hansson (1970a,b) did not consider the subsequent hypotonic treatment in their experiments as having any influence on the satellite association pattern. However, the choice of hypotonic solutions was considered to be very important by Pyatkin et al. (1969, 1972) who found greater frequencies of aberrations in cells treated with potassium chloride than with Hanks solution in distilled water. In their work on the cytogenetics of bone marrow after gamma-radiation, they

found that at the lower level of radiation and in unexposed samples, the number of aberrant metaphases was 36% with 0.5% potassium chloride, compared with 22% with 25% Hanks solution in distilled water. The hypotonic solution used by Zang and Back (1968) and Back and Zang (1969) in all of their series was sodium citrate, the concentrations varying between 0.7% and 1%. Hansson (1970a,b, 1976b) used distilled water, added to three times the volume of the culture medium.

The effect of fixation upon the cells has not been thought to contribute to any significant changes in the association patterns. Zang and Back (1968), Back and Zang (1969) and Hansson (1970a,b, 1975a,b) in all of their experiments have used glacial actic acid as the main constituent of the fixative. Zang and Back (1968) and Back and Zang (1969) used the conventional glacial acetic acid/methyl alcohol (3:1) solution whilst Hansson (1970a,b, 1975a, b) used a glacial acetic acid/1N hydrochloric acid (9:1) mixture.

However, Back and Zang (1969) did find an increase in the satellite association patterns when the fixed cells were resuspended in a 70% glacial acetic acid, rather than the glacial acetic acid/methyl alcohol mixture. No explanation was given for this finding other than to endorse their original statement that the actual mechanical procedure in producing the slides was critical. Flame drying of the smear, differences in the temperature and thickness of the water film on the slide and blowing on the slides to help spread the cells were some examples given as

possible influences affecting the number of associations seen (Back and Zang, 1967, Hoehn, Nagel and Krone, 1971). It was considered that preparation of the smears by one individual would help minimise some of the inevitable sources of error in the preparative technique.

It can be seen from these results that the cause of satellite association patterns is a complex phenomenon the nature of which is still largely unknown, but that the technical factors on culturing the cells can influence the outcome significantly.

c) Cytogenetics - Nuclear Fine Structure

Many authors have thought there might be some correlation between satellite association and chromosomal non-disjunction. (Ferguson-Smith and Handmaker 1961a,b, 1963, Harnden 1961, Ohno et al. 1961, Ferguson-Smith 1964, Kiossoglou et al. 1964, Tips et al. 1964, Lyons et al. 1965, Zellweger and Abbo 1965, Abbo et al. 1966, Mertz and Prempree 1966, Zellweger et al. 1966, Evans 1967). The most frequent cause of aneuploidy in man is non-disjunction and this phenomenon might provide one possible explanation for the translocation and non-disjunction in mitosis and meiosis. Hansson (1975a) showed a significantly increased number of satellite associations between the number 14 and 21 chromosomes in cases of hypothyroidism. This is particularly relevant as there appears to be an increased frequency of hypothyroidism in mothers of mongol children, which would greatly increase the risk of non-disjunction occurring.

It is known that the satellited chromosomes are involved in the formation of the nucleolus (see Figure 6) (Hsu et al. 1965) and that the regions below the satellites are the loci of ribosomal DNA, synthesising nucleolar material (Henderson et al. 1972). Indeed, Ferguson-Smith and Handmaker in 1961 used the term "nucleolar chromosomes" when describing the acrocentric 'D' and 'G' groups of chromosomes. It was noted that the satellites on the short arms were in frequent association with the nucleoli during mitotic prophase (Ohno et al. 1961). Furthermore, the association of their bivalents with nucleoli in the pachytene of spermatogenesis (Ferguson-Smith 1963, Hungerford 1971) and oogenesis (Stahl and Luciani 1972) has been reported. This would clearly assign the function of nucleolus formation to the satellites. However, correlation between biochemical and cytogenetical findings of r-DNA in nucleolus organising regions, (Dittes et al. 1975), shows that the amount of r-DNA in the human genome is not primarily a function of the number of acrocentric chromosomes. More recently Zankl and Zang (1972) proved that the loss of two or more acrocentric chromosomes significantly reduced the number of nucleoli present.

At the fine structural level, Kasten and Strasser (1966) observed that perinucleolar chromatin often intrudes directly into the nucleolar mass and by in-situ DNA-RNA hybridization techniques, the ribosomal cistrons have been assigned to the satellites (Henderson et al. 1972, Bross and Krone 1972). This has been demonstrated by differential staining of the

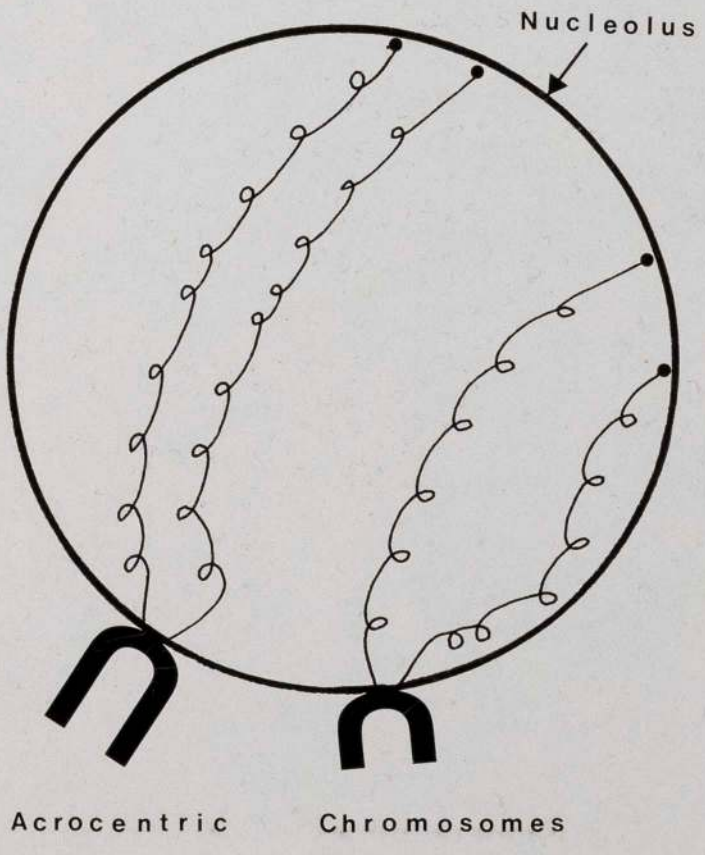


Figure 6 Diagram showing the acrocentric chromosomes attached to a nucleoli as they appear in early prophase. The nucleolus-organising regions are widely stretched. (After Ohno et al, 1961).

satellite regions by Matsui and Sasaki (1973), Howell et al. (1975), Denton et al. (1976) and Goodpasture and Bloom (1976) (Figure 7).

Threads connecting satellites are occasionally visible, for example, Zang and Back (1968) reported them in 10% of associations. Lampert et al. (1969) and Du Praw (1969) showed the presence of inter-satellite fibres by electron microscopy. These consisted of a type 'B' nucleoprotein. In order for these fibres to become visible to the light microscope, a large bundle of such fibres would be needed, hence, any connecting threads between associating chromosomes are probably present more often than actually seen. Henderson et al. (1973) using in-situ hybridization techniques, found that in 18 out of 105 metaphases examined, there appeared to be connections between the satellite regions of different acrocentric chromosomes.

Altered association frequencies of individual acrocentric marker chromosomes having enlarged, tandem (duplicate) satellites or elongated short arms (stalk), have also been reported. Bauchinger and Schmid, 1969, Rocchi et al. 1971, Gigliani et al. 1972, De Capoa et al. 1973, Schmid and Krone 1974) compared the relationship between these polymorphic variants and the satellite association patterns. Their results confirmed that acrocentric chromosomes possessing two satellites (tandem) and those having elongated short arms (stalk or nucleolar constriction) associated more frequently. However, those with short arm deficiencies associated less (or not at all)

Figure 7

Differential staining of satellite regions by the silver method of Goodpasture and Bloom (1976).

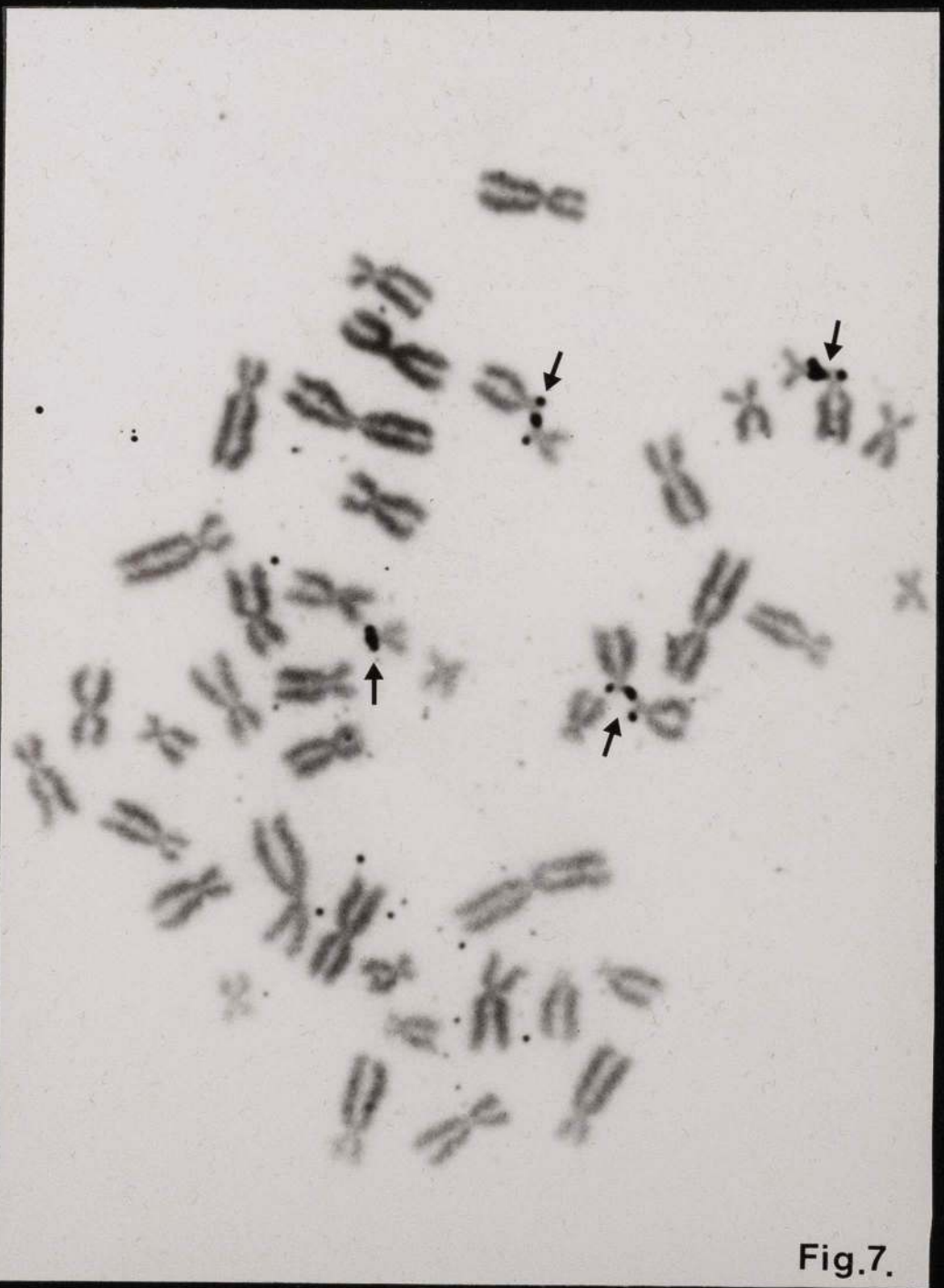


Fig.7.

than the "normal" acrocentrics. They concluded by assuming that an acrocentric chromosome participates in the formation of a nucleolus more often if it has a longer nucleolar constriction with satellites, than those without.

Contrary to this, Zankl and Zang (1974) observed a decreased frequency of association in their series of 130 acrocentric marker chromosomes (Dp+, Gp+). However, with the enlarged satellited markers (DS+, GS+), they too found increased association patterns but only with the DS+ type. They considered that the differences in the satellite association patterns of the marker chromosomes may indicate an involvement of the nucleolus-organising regions into some structural re-arrangement of the short arm or satellite regions. In fact recent work by Henderson and Attwood (1976) show a definite correlation between the amount of r-DNA and the frequency of participation in satellite associations seen in double satellited acrocentric chromosomes.

When nucleoli fuse, the mechanical stretching might lead to the breakage of the nucleolus forming segment of the chromosome (Ohno et al. 1961). It has been demonstrated that an increase of nucleolar area can be produced by the addition of thioacetamide to human embryonic fibroblast cultures (Zhdanova 1974). Observations on the subsequent metaphase spreads revealed a significant increase in the satellite association patterns. Hence, when the nucleoli amalgamate at the beginning of G1 and are isolated during cell division, non-disjunction could occur, especially if

these fused nucleoli persist throughout. Thus, if the nucleolar organising region is damaged, and breakage occurs, the proximity of the broken ends would predispose to the increased tendency of non-disjunction involving the satellited 'D' and 'G' groups of chromosomes. In fact, an increased percentage of acrocentric chromosomes with satellite variants have been observed in mongols and mothers of mongols (Zankl and Zang 1974). This is particularly relevant, as trisomy 'G' or Down's Syndrome (Mongolism) is the commonest autosomal trisomy in man with an overall incidence of one in seven hundred live births.

I.iii Down's Syndrome (Mongolism)

a) General

Down's Syndrome or Mongolism, was first described by John Langford Down in 1866 who called the condition "mongolian idiocy". However, Seguin in 1846 appears to have referred to the syndrome under the description of "furfuraceous cretins", and as such, was not recognised as distinct from other forms of mental subnormality. Almost certainly, however, it had been known to exist long before then, possibly as far back as the seventh century (Brothwell 1960), while some sixteenth and seventeenth century paintings have depicted infants with mongoloid features. There has been much speculation as to its aetiology, and studies in different parts of the world show that the condition occurs in all national and racial groups.

In 1932 Waardenburg and then Penrose in 1939, suggested that mongolism might be caused by a chromosome abnormality. This was not validated until 1959 when Lejeune, Gautier and Turpin demonstrated in nine affected children, the presence of 47 chromosomes in every cell. Down's Syndrome was thus shown to be associated with an additional chromosome. This extra chromosome was found to be always identical with the 'G' group of chromosomes. Modern banding techniques have proved that this extra chromosome is in fact a number 21, (Figures 8 and 9). Down's Syndrome is now described as Trisomy 21 and in this form of the condition is found in approximately 90% of all cases, these are described as "regular mongols". In the other 10% of cases, the extra

Figure 8

Giemsa banded karyotype of a regular trisomy 21 female.

Figure 9

Quinacrine fluorescent banded karyotype of a regular trisomy 21 female.

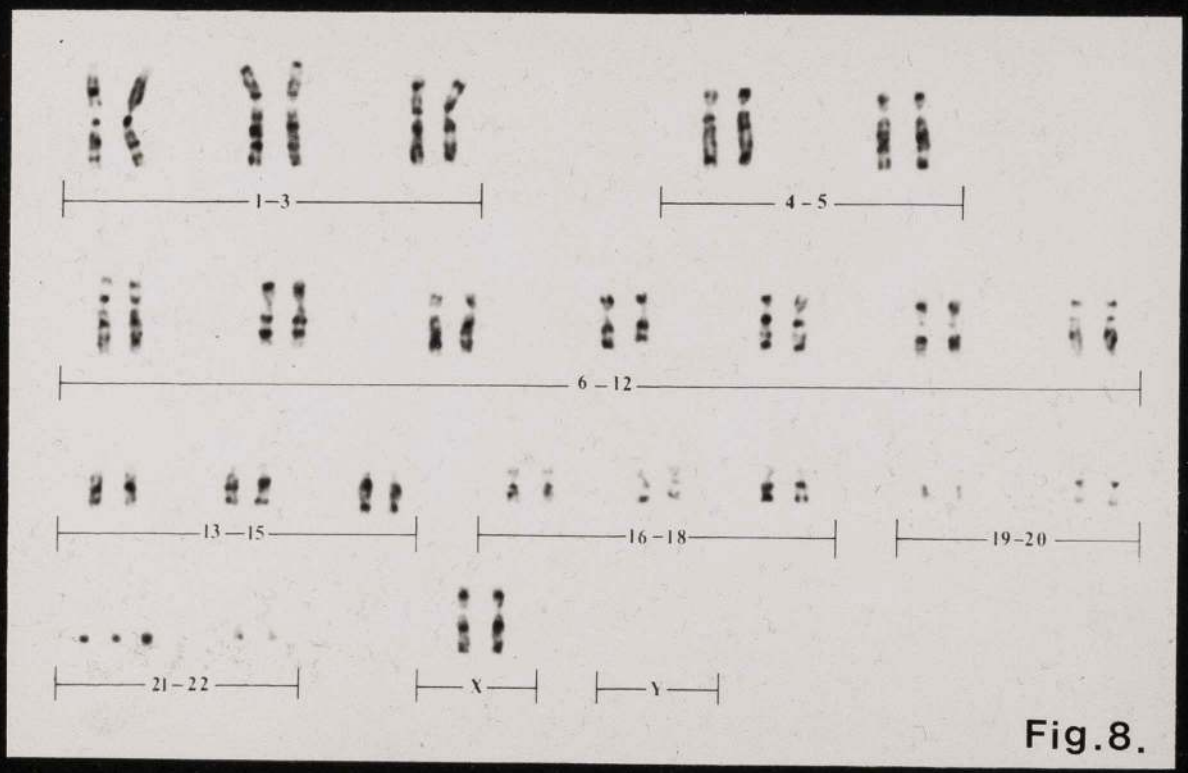


Fig.8.

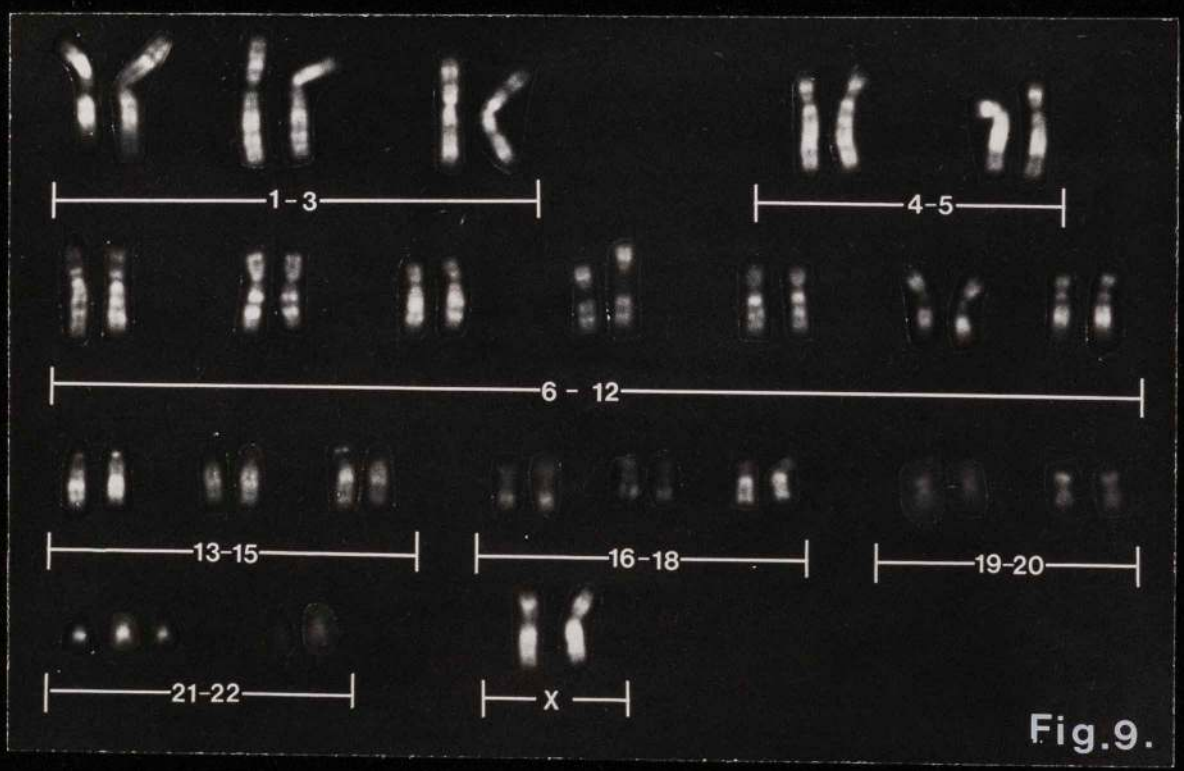


Fig.9.

chromosome is fused to another chromosome belonging to either the 'D' or 'G' groups (Figures 10 and 11). The term used for this group is called "translocation mongols", which can, though not always, be familial in origin.

Children suffering from Down's Syndrome are unique in many ways, and as such, few remain undiagnosed. The affected children look alike and are markedly different from both normal and other retarded children (Figure 12). Penrose and Smith (1966) have compared the frequency of physical signs quoted by different authors for Down's Syndrome in the newborn. The ten most characteristic signs are given in Table I. The diagnosis in the newborn is more difficult than in later life, and as expected with an abnormality present in every cell nucleus in the body, the signs and symptoms are widely spread. It has been shown that the complications of Down's Syndrome can sometimes lead to failure to thrive in the neonatal period, the commonest causes being congenital heart lesions or duodenal obstructions. The association of Down's Syndrome with leukaemia has also been described. Holland et al. (1962) found that the death rate from leukaemia in Down's Syndrome to be eighteen times that of the general population. The type of leukaemia found in such cases are predominantly lymphatic or myelogenous. This finding is particularly interesting in the case of chronic myeloid leukaemia, where an abnormal G22 chromosome is seen as a diagnostic feature.

Tan et al. (1973) and Lippitt and Fridowich (1973) established that the locus for the enzyme superoxide dismutase

Figure 10

Giemsa banded karyotype of a G/G (21:21) translocation female.

Figure 11

Giemsa banded karyotype of a D/G (14:21) translocation female.

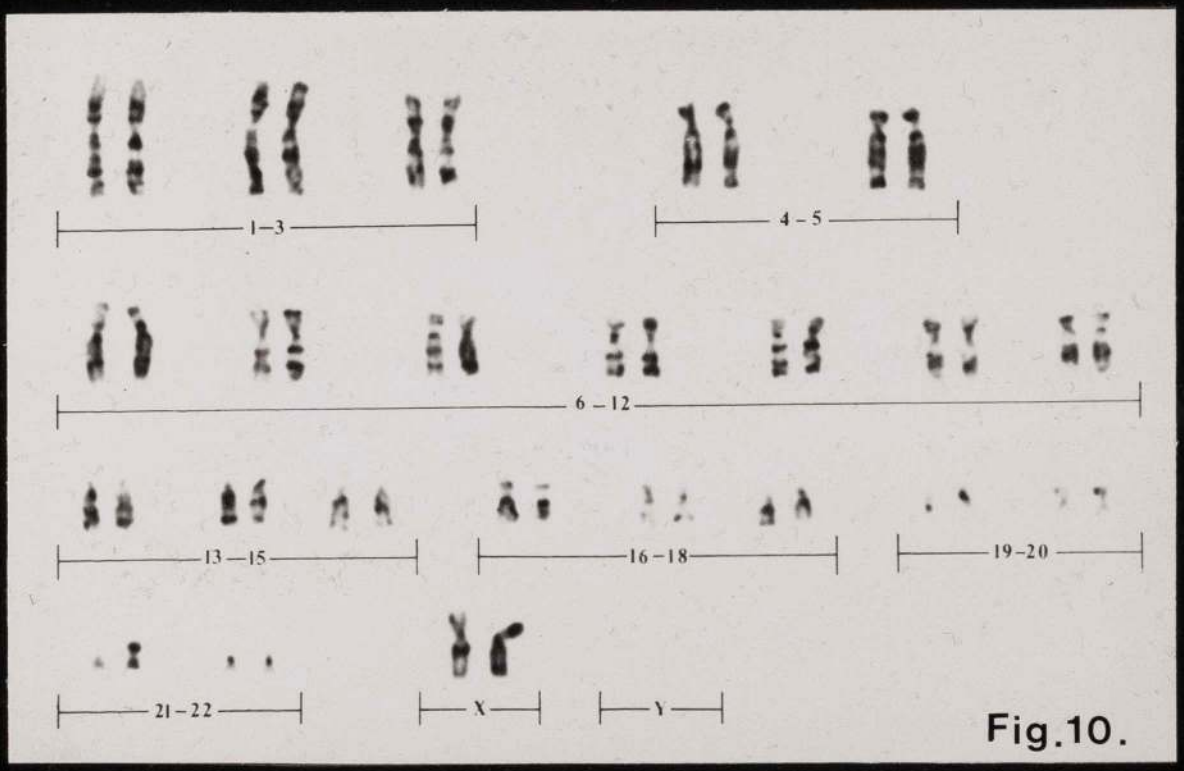

1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

Fig.10.

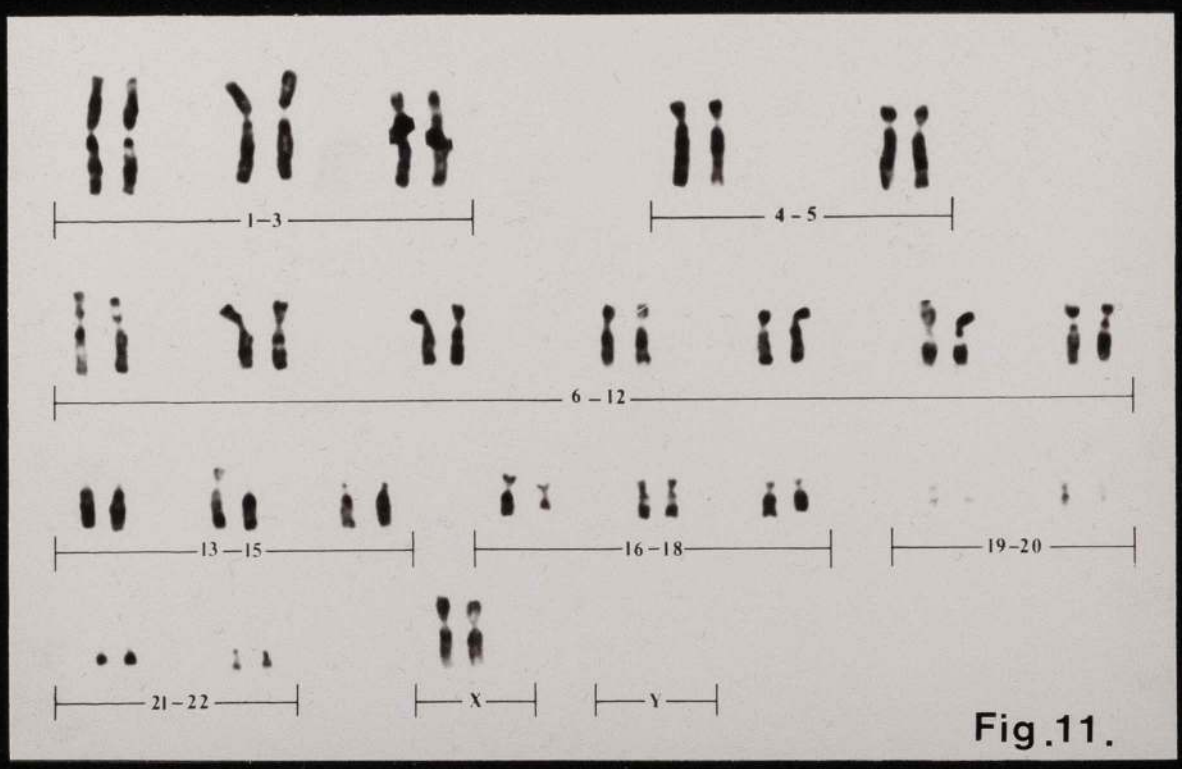

1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

Fig.11.

Figure 12

Typical physical appearance of a Down's Syndrome child.

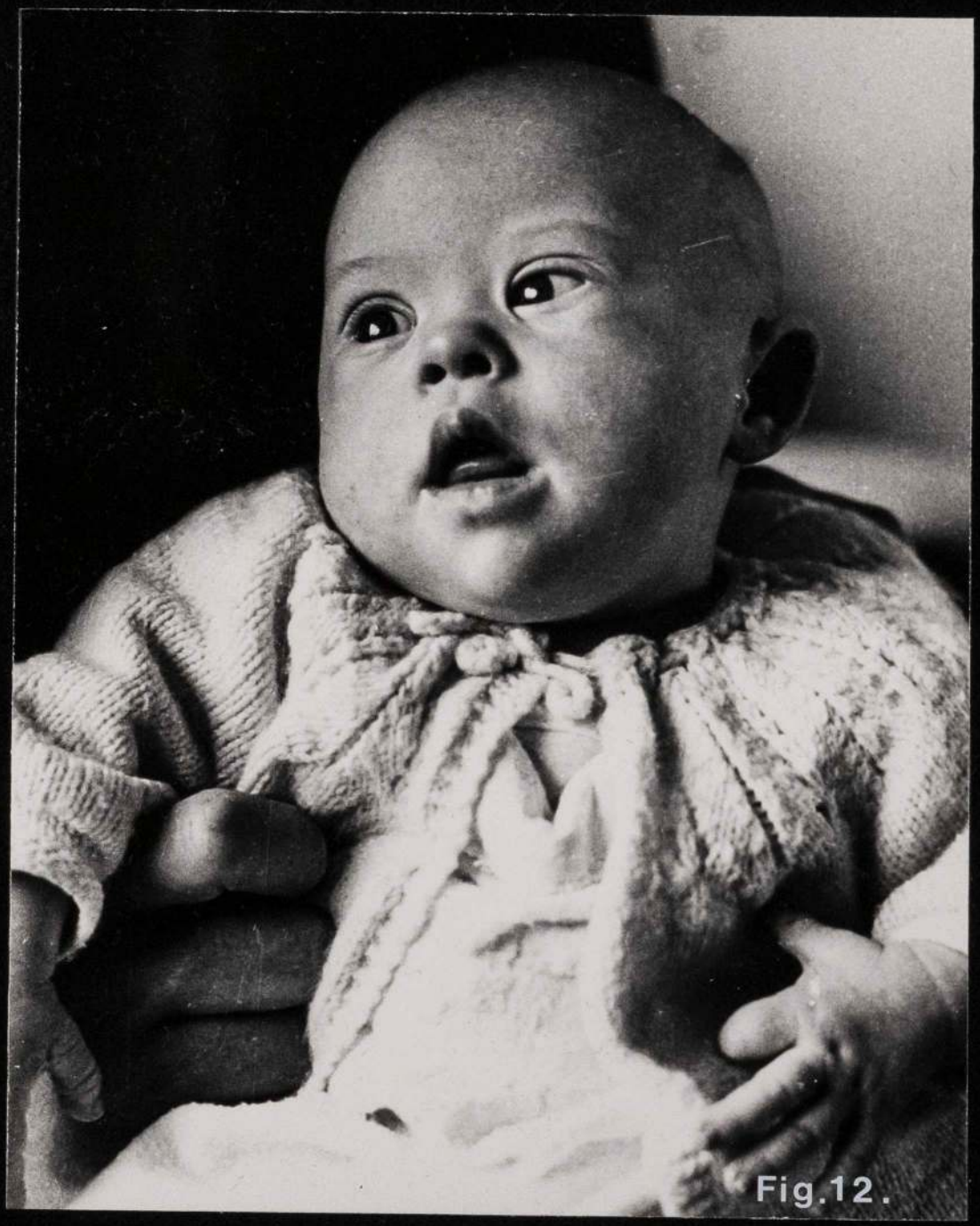


Fig.12.

Physical Signs	Frequency (%)
Flat facial profile	89
Lack of Moro reflex	82
Abundant skin (neck)	81
Oblique palpebral fissures	80
Hyperextensibility	77
Hypotonia	77
Flat occiput	74
Small teeth	71
Short broad hands	69
High arched palate	67
Dysplastic pelvis	67
Dysplastic ears	62
Furrowed tongue	59
Dysplastic fifth middle phalanx	58
Four-finger crease	54
Curved fifth finger	48
Epicanthic folds	28

Table I The most common physical characteristics of Down's Syndrome

(SOD-A) is carried on chromosome 21. Further work on this enzyme (Sinet et al. 1974, Sichitiu 1974 and Crosti et al. 1976) has demonstrated increased activity in trisomic 21 cells, to show the presence of a simple gene dosage effect. The exact location of this gene on chromosome 21 will be of value in cases of partial trisomy 21. Lejeune (1976) considers that the loci for the clinical appearance of Down's Syndrome is situated on the long arm of chromsome 21. Recently, Poissonnier et al. (1976) reported a case of an abnormal chromosome 21 (in which a duplication of segment 21q21→ 21q22.2 occurred) which showed all of the signs of Down's Syndrome. When the phenotype was compared with that of other partial and total trisomy 21 cases, it was postulated that the characteristic features of mongolism and in particular mental retardation, is due to trisomy 21q22.1 and perhaps 21q22.2 (Figure 13).

b) Satellite Association Involvement

Several workers have noticed cases of increased satellite association in families in which trisomy 'G' occurs. Zellweger et al. (1966) found in four families of translocation mongolism that both of the affected children and their parents had a raised satellite association pattern. A statistically significant increase in the satellite association patterns was noted between normal and mongol children by Rosenkranz et al. (1969). However, neither Froland and Mikkelsen, (1964) nor Zang and Back (1966) could show any significant increase in the frequency of

Figure 13

a) Diagrammatic representation of the banding patterns on the X, Y, D, E, F and G groups of chromosomes.

b) Enlargement of the G21 and G22 banding patterns to show regions and bands.

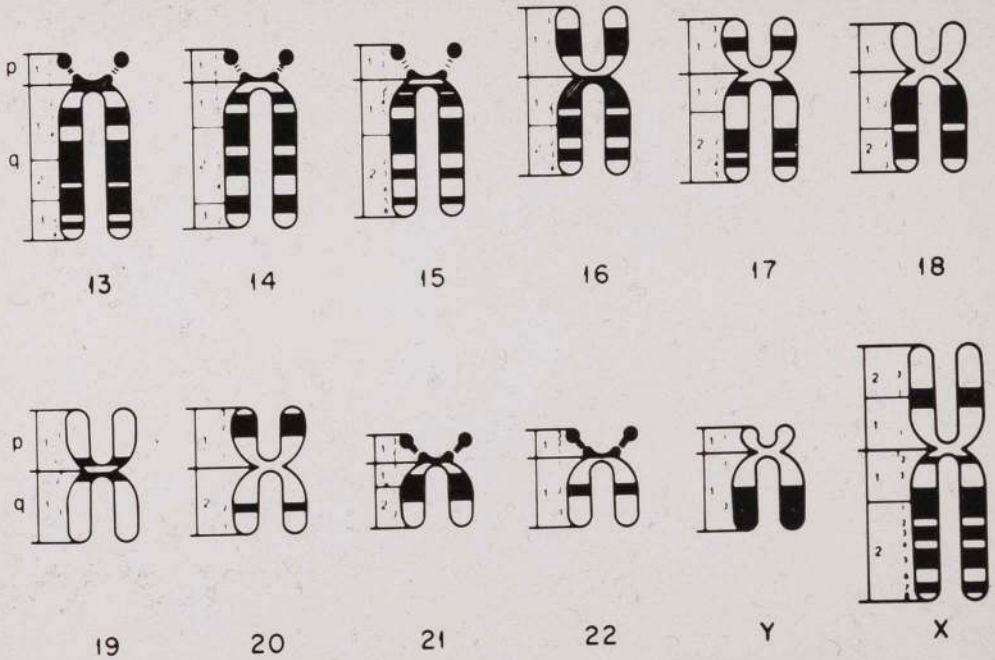


Fig.13 a.

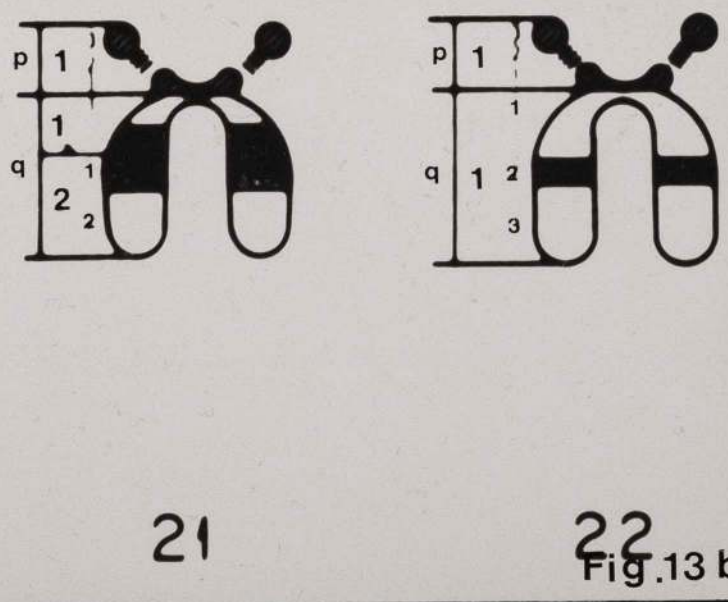


Fig.13 b.

satellite association in mothers of mongols or children, compared with the normal population. More recently, Cooke and Curtis (1974) were not able to establish any definite pattern although a number of significant differences emerged between parents and controls which needed further study. Taysi (1975) studied the association patterns using Giemsa banding techniques from chromosomally normal parents and parents of regular mongol children. His results showed that chromosomes 21 and 22 were involved in satellite associations more frequently than any of the other acrocentric chromosomes. However, he did not find any difference between the parental and normal control groups in relation to these frequencies.

However, Hansson and Mikkelsen (1974) found a significantly increased satellite association pattern involving the 21 chromosome in mothers of regular mongol children as compared with mothers of chromosomally normal children. This tendency was also observed in mothers of children with Robertsonian translocation children by Hansson, (1975) and Mikkelsen et al. (1975). Broustet et al. noted a higher than average incidence of satellite association in the parents of a sibship with trisomy 21 and monosomy X. Mattei et al. (1974) found in parents of trisomies, lowered associations involving chromosome number 15 and increased associations with number 22.

c) Parental Age

The association between Down's Syndrome and parental age

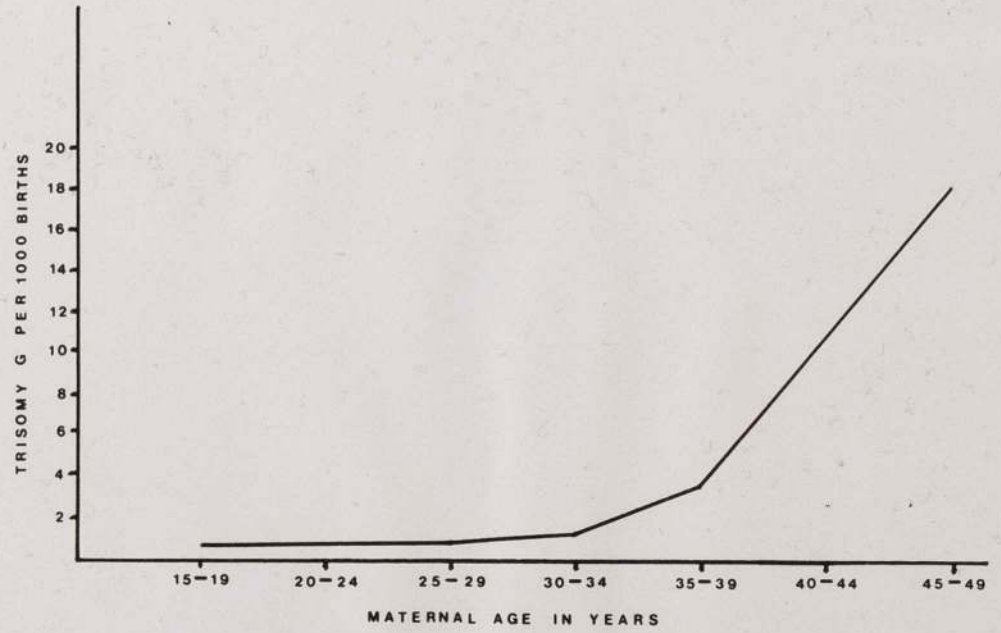


Figure 14 Graph showing the relationship between the incidence of Down's Syndrome (mongolism) and the maternal age (after Levine, 1971).

has been known for some time, with Frazer and Mitchell (1876) first producing evidence of a later maternal age effect. Subsequently, Penrose (1932) and Jenkins (1932) confirmed this finding showing that the paternal age was irrelevant (but see page). Penrose (1934) was then able to show that the late maternal age effect was independent of parity (Figure 14). Other than this, no significant etiological factor has so far been confirmed and the significance of this relationship is unknown, but it might represent a decrease in meiotic efficiency with the increasing age of the oocyte. German (1968), postulated that this relationship might be explained by a delayed fertilization in older women due to spasmodic or decreased frequency of coitus. However, Penrose and Berg (1968), Cannings and Cannings (1968) and Matsunaga and Maruyama (1969) have shown by their results that German's hypothesis cannot account for this relationship unless some other factors are related to age, other than coital intervals.

Viral infection (Coleman and Stoller 1962), fluoride concentration (Rappaport 1963) and atmospheric pollution (Greenberg 1964) have all been reported as a possible contributary factor in the aetiology of Down's Syndrome. In recent years, radiation (Schuman and Gullen 1970, Wald and Turner, 1970) and hepatitis (McDonald 1972) have been suggested. Carr (1967, 1970) reported an increased frequency of triploidy among abortions in mothers who had ceased to take oral contraceptives less than 6 months before conception. The number of trisomies was not increased, but McQuarrie (1970)

found an increased incidence of chromosome breakage and satellite associations in 23 female patients using oral contraceptives.

The paternal role in the aetiology of Down's Syndrome has also been questioned. Sasaki and Hara (1973) and Uchida (1973) have both reported cases in which it was demonstrated that the extra G21 chromosome was of paternal origin. In both cases, a brilliantly fluorescent satellite was used as a marker to determine the origin of the extra chromosome. Paternal mosaicism is discounted as no metaphase spreads with the extra G21 chromosome were observed in 1,000 cells examined. In two recent surveys using fluorescent G21 marker chromosomes, Wagenblicher et al. (1976) and Mikkelsen et al. (1976) found that in 34 parents of mongol children, the extra G21 chromosome was of maternal origin in 21 cases and of paternal origin in 13.

It is known that certain families are more prone to the occurrence of chromosome aneuploidy and that the recurrence risk figures for Down's Syndrome (mongolism) appear to be greater than chance alone (Table II), especially when the maternal age is under 35 years at the birth of the first trisomy. From the graph showing age distribution of mothers of children with Down's Syndrome (mongolism) in Sweden, Australia and England, this consistency can be seen (Figure 15). The term "non-age related mongolism" can be used to describe such a group of cases. Indeed, recent epidemiological evidence has shown (Lindsjo 1974) that a statistically constant number of cases

MATERNAL AGE GROUPS	INCIDENCE OF MONGOLISM	
	IN POPULATION	IN FAMILIES AFTER ONE TRISOMY-21 CHILD
15 - 19	1/2,400	1/800
20 - 24	1/1,500	1/500
25 - 29	1/1,200	1/400
30 - 34	1/900	1/300
35 - 39	1/300	1/100
40 - 44	1/100	1/30
45 - 49	1/40	1/10

Table II Calculated risk values for Down's Syndrome (mongolism) in the general population and in families after one affected child. (From Allen et al. 1974)

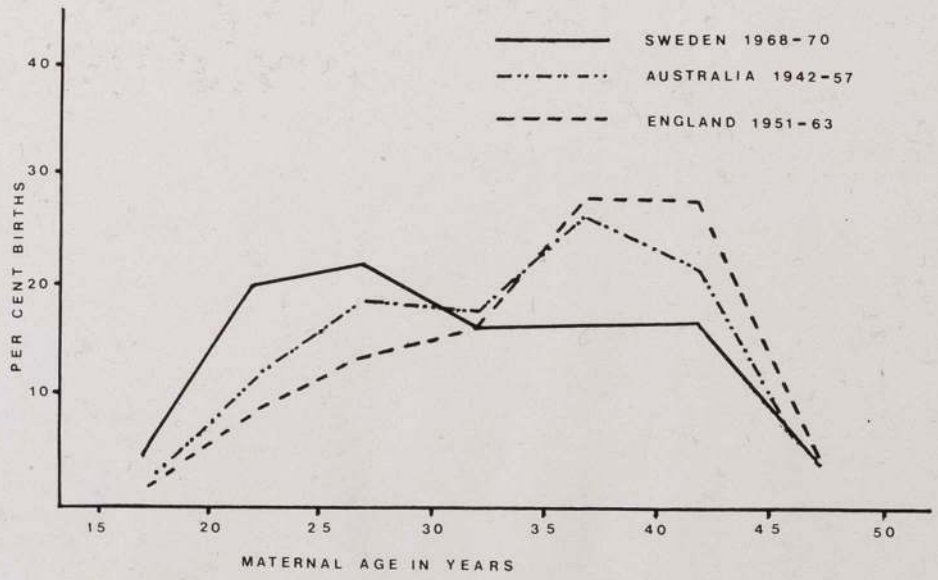


Figure 15 Graph showing the age distribution of mothers of children with Down's Syndrome (mongolism). (After Linsjo 1974).

were born to parents up to 35 years of age.

The figures relating to the overall incidence of Down's Syndrome, have fallen from a 1:650 (Carter 1951, Penrose 1954, Hall 1964) to a 1:755 in the latest Swedish figures (Lindsjo 1974). A meaningful comparison between these figures and the ages of all mothers must be taken into account (Figure 16). The shift towards a lower maternal age in the Swedish material from a median of 27 years to a median of 25 years over the past 10 years is interesting. This is clearly reflected in the distribution of mongol children in relation to maternal age graph (Figure 14). A minor peak occurs between 25 and 29 years of age for "non-age related mongolism", in the British and Australian figures. However, with the Swedish data, the trend is reversed, showing the relevant peak between 25 and 29 years, which cannot be described as age-related. In their series, Penrose and Smith (1966) divided mothers of mongol infants into two classes; those showing no shift to maternal age and those who do not. Moran (1974) questions these classes statistically, but does confirm a small group of mothers not showing a maternal age shift.

In a recent survey, Goad et al. (1976) examined 40,371 newborn infants and found a seasonal variation in the incidence of mongol children born to mothers under 35 years of age. In any one year, it was found that there were three times as many mongol children born between the months of May and October than in the rest of the year. However,

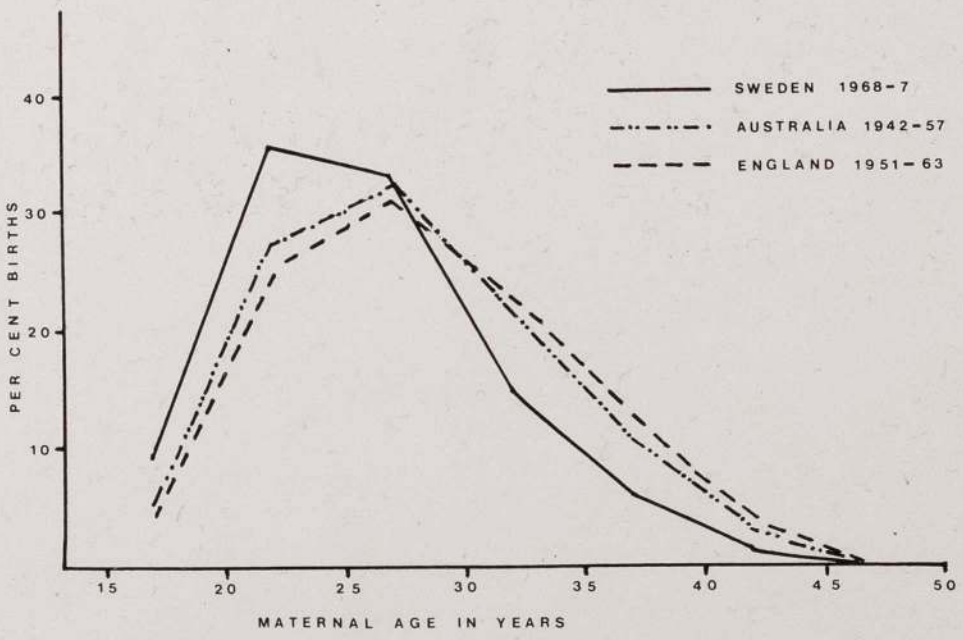


Figure 16 Graph showing the age distribution of mothers of all liveborn children. (After Linsjo, 1974).

no seasonal difference was observed in mongol children born to mothers aged 35 and over. They concluded that three distinct components can be statistically identified regarding the incidence of trisomy 21: 1) a steady endemic component in mothers under 35 years, 2) an epidemic component in mothers under 35 years and 3) a stable, larger component for mothers aged 35 and over.

I.iv The Design and Objectives of this Investigation

This investigation can be divided broadly into two sections: methodological and clinical.

The distribution pattern of acrocentric chromosomes *in vivo* remains unknown. In the first part, therefore, the object was to look closely at the effect that a hypotonic solution had on cultured cells, with regard to their satellite association patterns. Three hypotonic solutions were compared so as to obtain the lowest number of satellite associations observed in thirty-five normal males and females. From the results, a standardised technique was evolved to produce a base line from which any significant increase in association patterns could be detected.

Before any conclusions could be drawn about the effects of the various hypotonic solutions, specific criteria as to the exact definition of a satellite association had to be formulated. These criteria had to incorporate the distance, number and arrangements of the association chromosomes, so that they could then be used as a standard for all of the observations throughout the investigation.

In order to identify accurately the chromosomes involved in satellite associations, a specific form of staining is required. There are basically two techniques which can precisely identify individual chromosomes, these are fluorescence microscopy or light microscopy with critical staining. These methods are far from being infallable, and some modification was found to be necessary in order to give reproducible results for accurate

standardisation. Both of these techniques were assessed for consistency and the most suitable were selected.

When the first part of this investigation had been completed, a standard technique was developed to provide the lowest frequency of satellite associations in normal metaphase spreads, together with the accurate identification of the chromosomes involved. Only in this way was it possible to detect any significant differences in the individual patterns in the second part of the study.

The second part of the investigation deals with the clinical aspects involving parents of regular mongol children and normal controls. Blood samples from both parents of mongol children and parents of normal children were taken, with their consent. The parents of normal children were, as near as possible, matched for age with the parents of the mongol children. Confirmation of the diagnosis of mongolism of the children of the parents in this survey was carried out by the same cultural method as standard.

The specific identification of the individual chromosomes involved and the category of associations was recorded in both groups. Thus, if any significant differences in satellite association patterns were likely to be present between parents of mongol children and parents of normal children in this sample, they should be easily detected by these procedures.

II. EXPERIMENTAL PROCEDURES

II.i Experimental Design and Methods

a) Satellite Association Patterns - The Effect of Hypotonic Solutions

The first part of this study is to determine the effect of hypotonic solutions on satellite association patterns. Normally in metaphase spreads, there are always random associations, or chance proximities of the 'D' and 'G' chromosomes. The cultural technique was controlled so as to give the minimum number of chance associations. This was necessary before true, non-random associations could be evaluated. As previously reported, the basic type of culture method did not appear to influence the satellite association patterns (Zang and Back 1968), Back and Zang 1969, Hansson 1970, Curtis and Cooke, 1974) so the subsequent hypotonic solutions were examined.

The three hypotonic solutions used were potassium chloride, trisodium citrate and 25% Hanks solution, diluted with distilled water. The results from each of these procedures would indicate whether the hypotonic treatment on the cells has a major influence on the number of satellite associations per cell. It would also demonstrate which solution gave the minimum number of associations per cell. This hypotonic solution would then be used for all cultures in the second part of the study.

In order to define what constitutes a true association, of standard criteria are needed. It was therefore, decided

that two criteria would be used:

a) the satellited ends of the associating acrocentric chromosomes had to be directed towards each other, and

b) the distance between one or both of the satellites of the associating acrocentric chromosomes should not exceed the width of 1 chromatid. If the distance was greater (even if they were directed towards each other) they were excluded (see Figure 17).

Chromosome preparations were obtained from short term cultures according to the method of Arakaki and Sparkes (1963) with some modifications. TC.199 (Burroughs Wellcome) was used as the culture medium, with 15% calf serum together with Penicillin B.P. and Streptomycin B.P. (Flow Laboratories). Phytohaemagglutinin (Burroughs Wellcome) was used as the mitogenic agent. Whole blood was inoculated with aseptic technique into sterile plastic cultures tubes, each sample having three tubes (A.B. and C.). The cultures were then incubated for 70 hours at a temperature of 37°C. They were agitated at least twice per day. Colcimid (CIBA) (Colchicine 0.02%) was then added and further incubation at 37°C for 1½ hours was continued. The cultures were then transferred to a glass centrifuge tube and centrifuged at 1,000 r.p.m. for 5 minutes. After centrifugation, the supernate was discarded and the cell deposit subjected to the varying hypotonic treatments.

Cells from the first culture (A) were treated with a

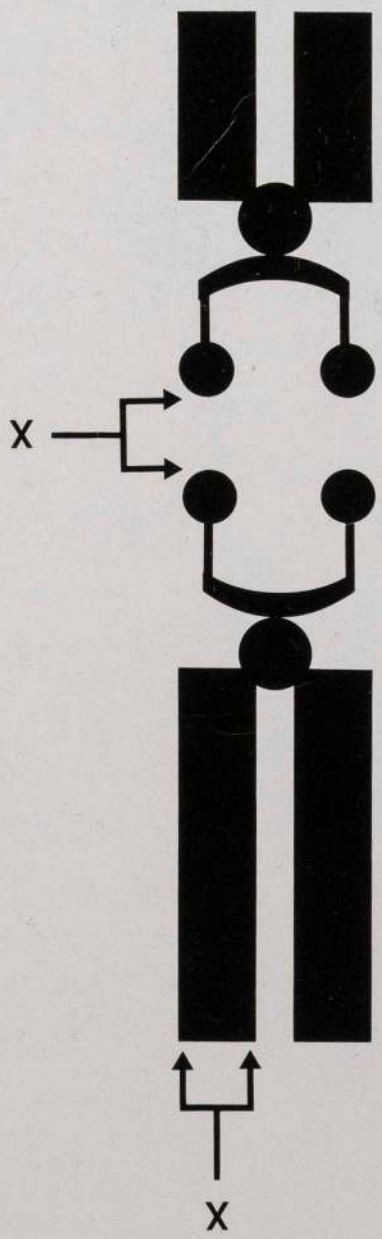


Figure 17 Satellite association criteria - the distance between one or both of the satellites should not exceed the width of one chromatid (X).

pre-warmed solution of potassium chloride (0.075M.aq.) for 8 minutes at 37°C with continuous agitation.

Cells from the second culture (B) were treated with a pre-warmed solution of sodium citrate (1% aq.) for 10 minutes at 37°C with continuous agitation.

Cells from the third culture (C) were treated with a pre-warmed Hanks distilled water solution (1:3) for 20 minutes at 37°C with continuous agitation.

All the cultures were then centrifuged at 1,000 r.p.m. for 5 minutes, the supernate discarded, and the cells fixed by adding a freshly prepared ice-cold acetic acid/methanol (1:3) fixative, drop by drop from a Pasteur pipette on to the cell deposit whilst shaking carefully. When approximately 5ml. of fixative had been added, the cell suspension was left at room temperature for ½ hour. They were then centrifuged for 5 minutes at 1,000 r.p.m., the supernate discarded and the cell deposit resuspended in fresh fixative. This washing procedure was continued twice more until the supernate was clear and the deposit of cells clean, finally resuspending in approximately 0.5ml. fixative to produce a slightly opaque cell suspension. Slides were then prepared by adding one drop of this cell suspension from a Pasteur pipette on to an inclined pre-cooled clean, glass slide, and blown gently to obtain even cell distribution until dry. Six slides per culture were made. (A more detailed account of the whole technique can be found in Appendix I).

One slide per culture (total 105 slides) was stained with Giemsa (1% aq.) for 5 minutes, rinsed in distilled water, blotted dry and mounted in DPX.

Full microscopic analysis on 15 metaphase spreads was performed on each slide, one representative cell was photographed and a chromosome karyotype constructed. This was to confirm that all of the controls had a normal chromosome complement. A further 10 metaphase spreads from each slide were then analysed for their satellite association frequencies. The criteria previously described was used and the types of satellite associations found were categorized in this order: D-G; D-D; G-G; D-D-G; D-D-D; D-G-G; G-G-G; Others; and Total. The results of the frequencies found in each of the three hypotonic solutions were recorded.

From the results of the above experiment (see page 60) it was decided to look at the effect of potassium chloride further. Two further experiments were devised to see:

a) if the molarity of the solution had any effect upon the satellite association frequency, and

b) if the time of exposure also affected the satellite association frequencies.

In the first experiment, five cultures were set up from a single blood sample from a known chromosomally normal male control, and cultured as in the standard technique used in the first experiment. For the hypotonic stage, however, each culture was treated with a different solution of potassium chloride of the molarities outlined in Table III.

	Culture A	Culture B	Culture C	Culture D	Culture E
Molarity of KCl	0.02M	0.04M	0.06M	0.08M	0.10M
pH of KCl	7.10	6.75	6.60	6.50	6.45
Chloride Ions	20	40	75	90	101

Table III Composition of solutions of varying molarities of potassium chloride

	Culture X	Culture Y	Culture Z
Time in 0.075M KCl	4 mins.	8 mins.	16 mins.

Table IV Varying incubation times of the cultures in 0.075M potassium chloride

The pH and chloride ions of the solutions were determined in the laboratory using a Corning-Eel pH meter (model 109) and by a Corning-Eel chloride meter (model 920).

All of the cultures were incubated at 37°C for 8 minutes with continuous agitation. The fixation, staining and microscopic analysis of the satellite association frequencies were carried out exactly as in the previous experiment, and the results recorded.

In the second experiment, using the same male control, three cultures were set up and cultured as in the standard technique as in the previous experiments. For the hypotonic stage, each culture was treated with pre-warmed 0.075M KCl and incubated at 37°C for different times, as outlined in Table IV.

The fixation, staining and microscopic analysis of the satellite association frequencies was carried out exactly as in the previous experiments and the results obtained recorded.

b) Satellite Association Patterns - Parents and Controls

For the second part of the study, further criteria has to be laid down so that a more specific assessment of the participating acrocentric chromosomes involved in satellite associations can be established. The concept of what actually constitutes a satellite association is the same as that specified in the first section (see page 39), but with a more critical observation on the positions of the

associating chromosomes. It was decided to group the types of associations into two categories:

Type A (Figure 18) - where both satellites and chromatids are positioned directly towards each other. i.e. the angle between chromatids is 180^{o}.

Type B (Figure 19) - where both satellites and chromatids are positioned obliquely to each other. i.e. the angle between chromatids is less than 180^{o}.

Thus, the positional category will be recorded as either type A or type B for each satellite association counted.

The second important criteria required is to identify each individual chromosome involved in a satellite association. This is achieved by staining the chromosomes so that each homologous pair is sufficiently different from each other so as to be accurately identified. It was at first thought that the identification would have to be by autoradiography, but this has been superseded by the use of specialised banding methods.

Caspersson(1970) was the first to use a fluorescent dye (Quinacrine dihydrochloride) for accurately identifying each chromosome by a definite banding pattern which was characteristic for each homologous pair. The banding

Figure 18

Satellite association criteria: Type A a) as represented in a di-association, b) as represented in a tri-association.

Figure 19

Satellite association criteria: Type B a) as represented in a di-association, b) as represented in a tri-association.

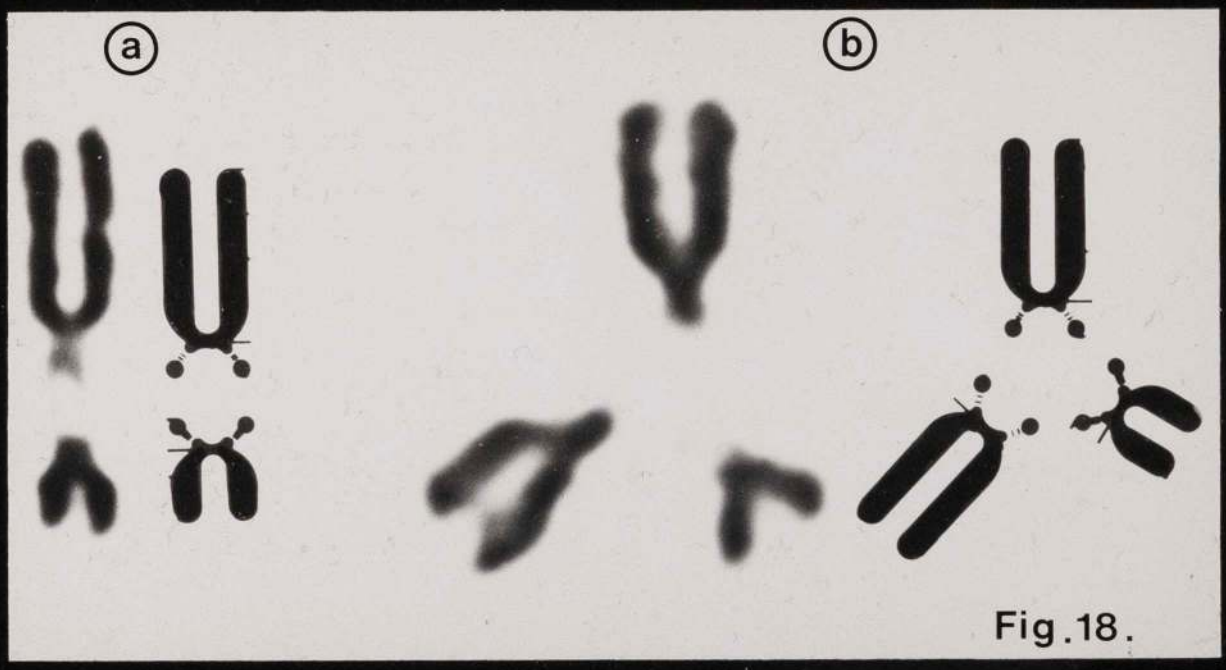


Fig.18.

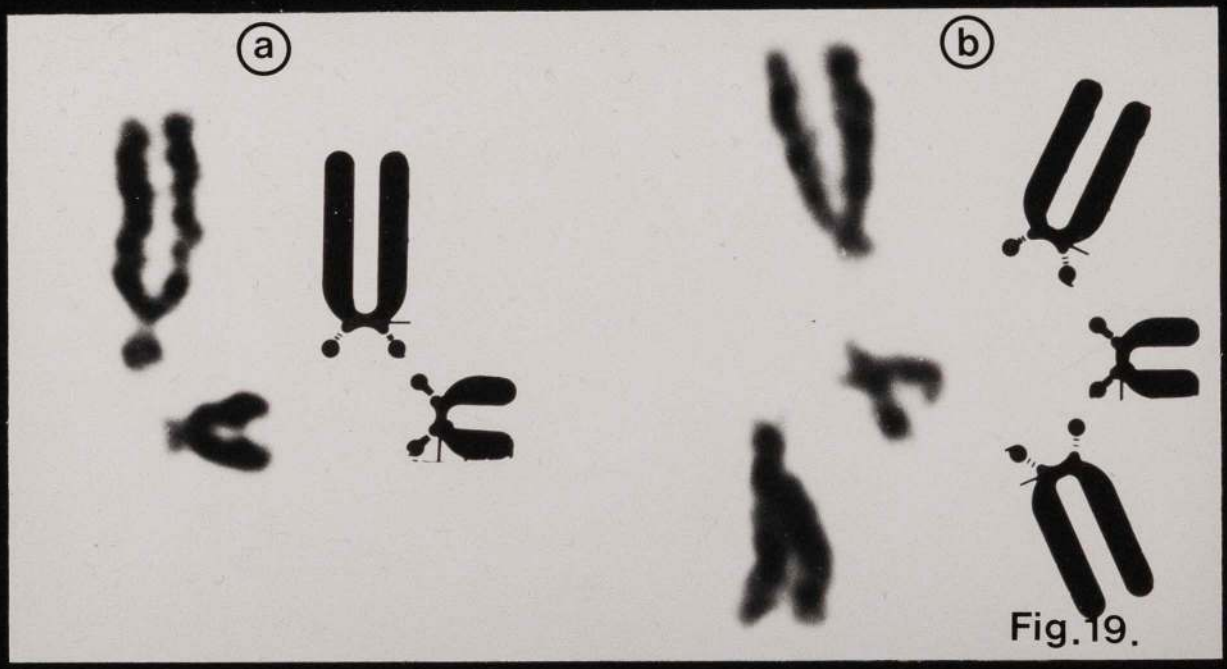


Fig.19.

pattern for the 'D' and 'G' groups are shown in Figure 20. It was thought that this method would be used and was carefully considered. However, it has the disadvantage that the dye is susceptible to fading and that a high quality fluorescence microscope is required to produce acceptable results.

It was, therefore, decided to try another banding method using Giemsa as the staining agent. This technique was first described by Sumner et al. (1971) and consisted of a pre-treatment of the slide in a salt solution (Acetic-Saline), followed by staining in a Giemsa solution (G.T.Gurr). This method failed to produce consistent results and so a modified technique (Seabright, 1971) was tried. In this method the slides were pre-treated with a trypsin solution before the Giemsa stain. However, after further experiments with this method it was found that although it gave consistently precise identification of individual chromosomes, the morphology was grossly changed. This made it difficult to see the satellite associations, and impossible to categorize them (Figure 21).

An improved quinacrine derivative (quinacrine mustard) became available in this country in 1973, and also access to a good fluorescence microscope became possible. More experiments continued in parallel with a method which combined the acetic-saline-giemsa with the trypsin-giemsa methods (Richardson and Gallimore 1973). Comparison between the two methods showed that both gave consistently good identification of the individual chromosomes without any

Figure 20

The 'D' and 'G' groups of chromosomes showing the individual giemsa banding patterns as observed microscopically and drawn diagrammatically.

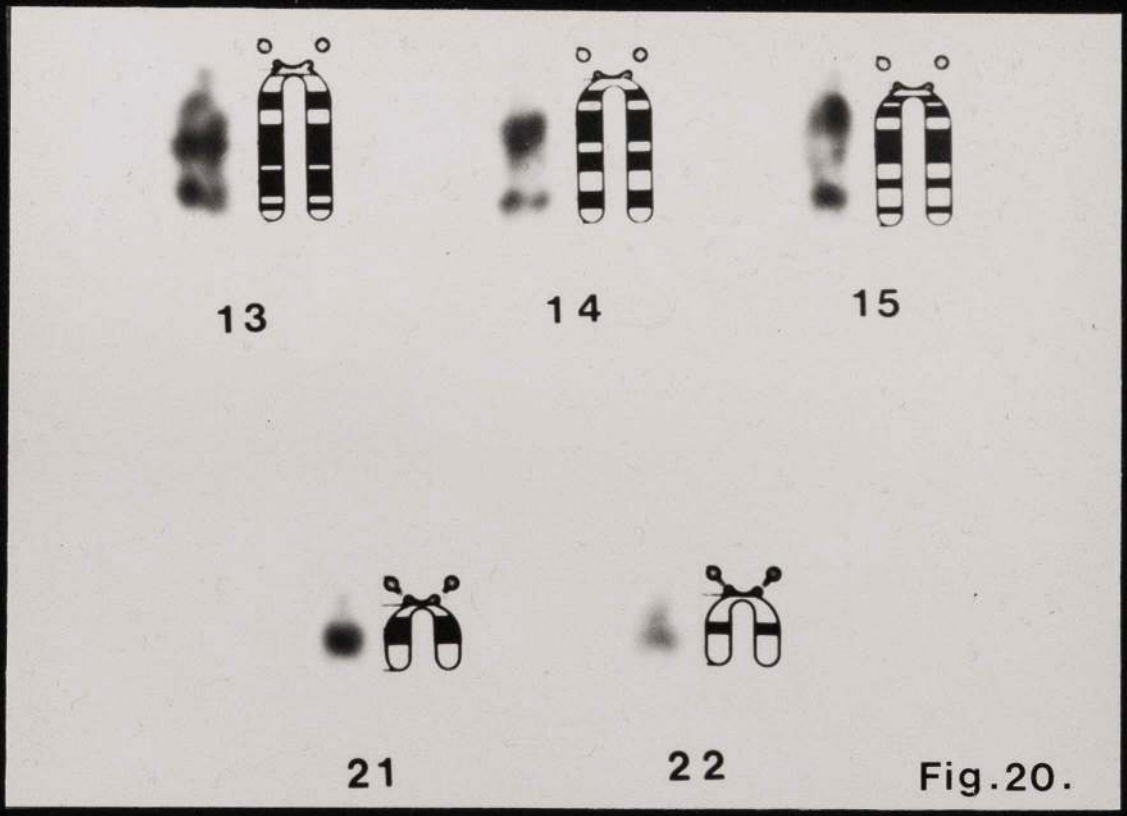


Fig.20.

Figure 21

Normal male metaphase spread after treatment with the trypsin-giemsa method.

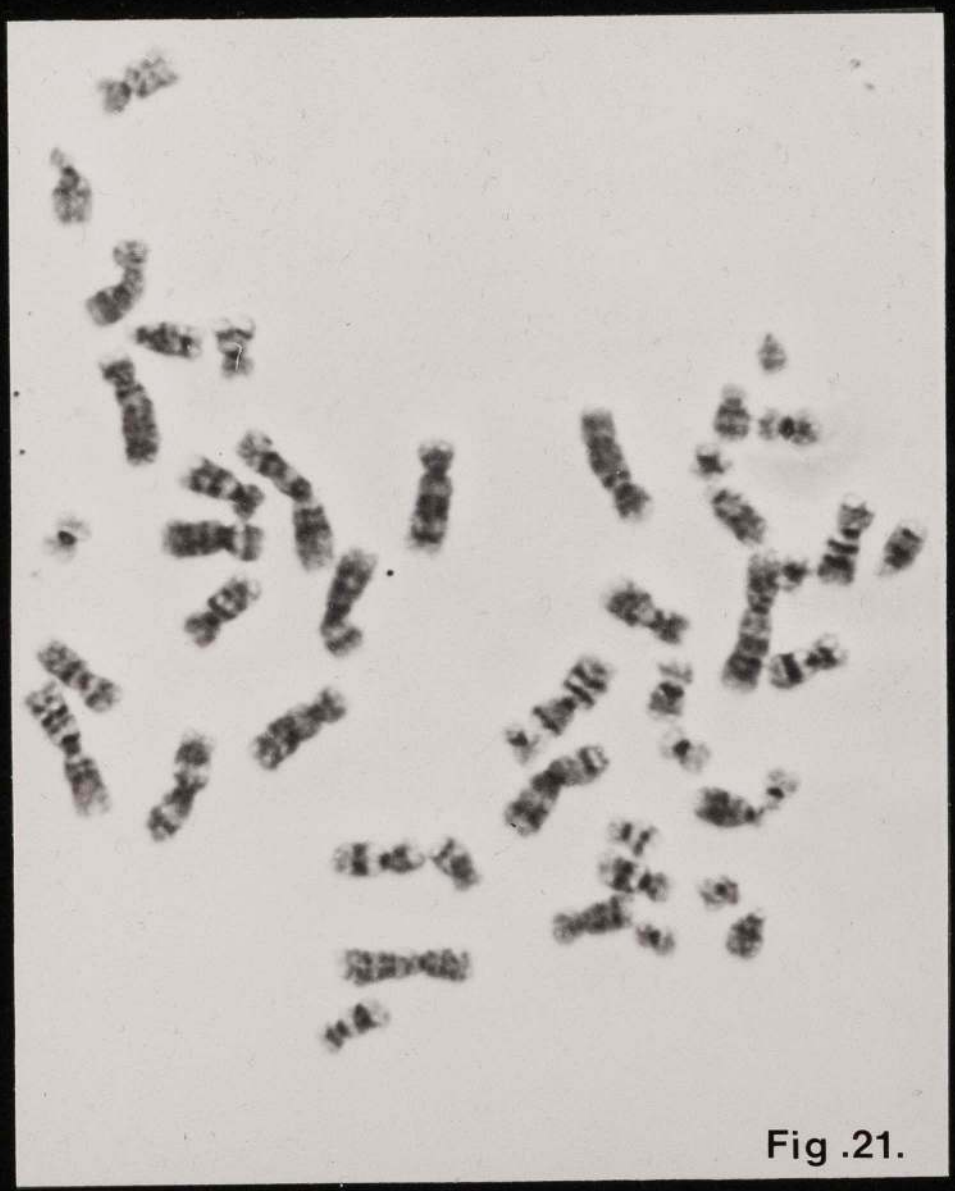


Fig .21.

alteration to their morphology. However, the modified Giemsa technique gave better photographic reproduction, together with a more permanent preparation than that of the fluorescence method (Figure 22).

It was, therefore, decided to use the modified Giemsa method for staining all of the material in this investigation. Further slight modifications were made, and the reproduction improved. Basically, the method consisted of first pre-treating the slides at 60°C with sodium chloride/sodium citrate solution (pH 6.8). This was then followed by a trypsin-saline solution at 10°C, and finally stained in Giemsa (10% aq.) stain, they were then blotted dry and mounted. (A more detailed account of the method is presented in Appendix I).

As in the first part of the study, the chromosomes were studied in short-term cultures to a modified method of Arakaki and Sparkes (1963). TC.199 was used as the basic culture medium, with 15% calf serum together with Penicillin and Streptomycin. Phytohaemagglutinin was used as the mitogenic stimulant. Whole blood was innoculated with aseptic technique into 10ml. sterile plastic culture tubes. Each sample was innoculated into two tubes. The cultures were then incubated for 70 hours at a temperature of 37°C. They were agitated at least twice per day. Colcimid (Colchicine) was then added and further incubation at 37°C for 1½ hours was continued. The cultures were then transferred to a glass centrifuge tube and centrifuged at 1,000 r.p.m. for 5 minutes. After centrifugation, the supernate

Figure 22

The 'D' and 'G' groups of chromosomes showing the individual fluorescent banding patterns as observed microscopically and drawn diagrammatically.

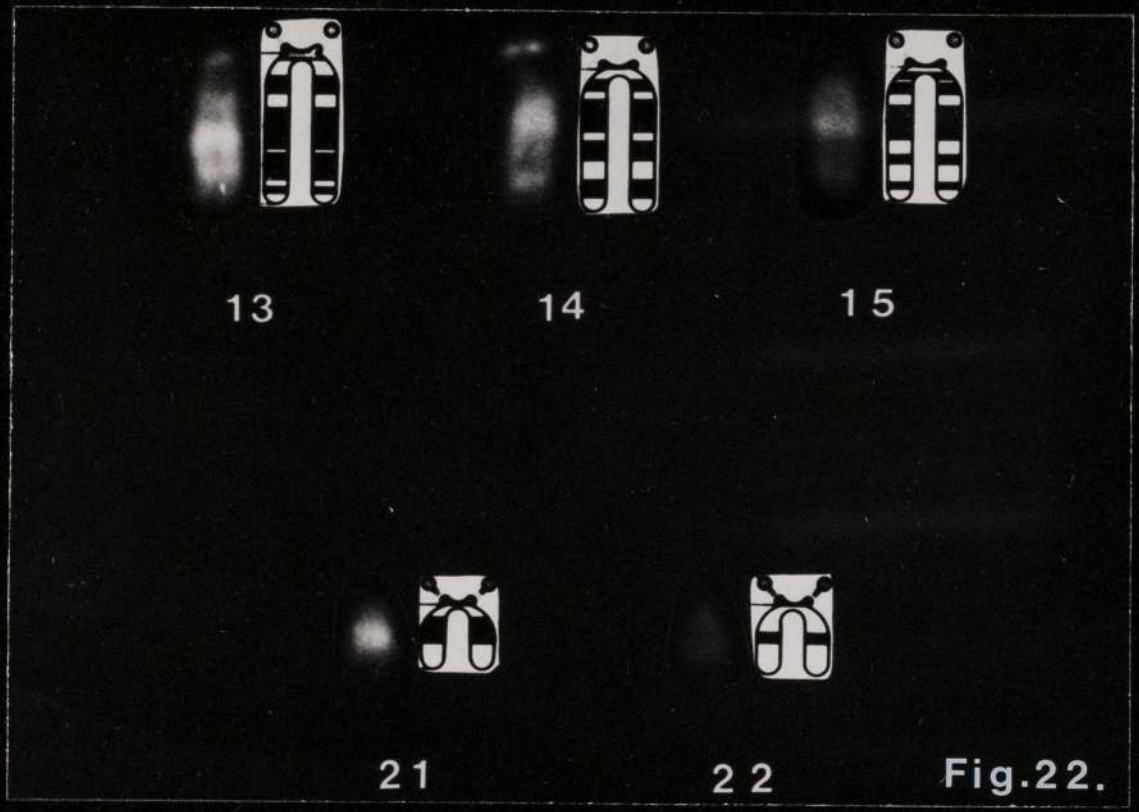


Fig.22.

was discarded and to the cell deposit, the hypotonic solution added.

The hypotonic solution used and corresponding exposure times will be those that in the first part of this study gave the lowest overall number of satellite associations per metaphase spread. After this treatment, fixation and slide preparation was completed in exactly the same manner as previously described in the earlier experiments (see Appendix I). Six slides per culture were made.

The unstained slides were screened using phase-contrast microscopy to determine whether the cultures had grown satisfactorily enough so as to provide sufficient metaphase spreads for examination. If of poor quality and insufficient, a repeat culture from the original blood sample was again set up. If this failed, no further examination on this sample was done.

Four slides from each case were then stained with the Giemsa banding technique (Richardson and Gallimore 1973) as described earlier (see page 50). Full microscopic analysis on 15 metaphase spreads was routinely performed on each case. One representative cell was photographed and a chromosome karyotype constructed. All of the microscopy and photomicroscopy was carried out on a Zeiss Photomicroscope III using the X6.3 Apochromat for screening, and the X100 Planapochromat for the final analysis. The film used for the photomicroscopy was Kodak 'Recordak Microfile'; this was developed in Kodak D-11 developer and fixed in Kodafix. The negatives were enlarged to whole plate size (16cm. x 22cm.)

printed on Kodak WSG paper, developed in Kodak D163 and fixed in Kodafix.

When all of the cells from the parents of mongol children and the control parents were confirmed chromosomally normal, the actual analysis of the satellite association patterns could begin. From each case, 100 normal metaphase spreads of good quality were examined, and the following observations recorded:

a) the number of associations seen in each cell.

b) specific identification of each individual acrocentric chromosome participating in an association.

c) the category of the association.

Strict adherence to the criteria previously described was followed in all observations.

II.ii Experimental Sample

a) The Effect of Satellite Association Patterns - Hypotonic Solutions

A total number of 35 normal controls were used for this part of the experiment. These consisted of 21 male and 14 female volunteers, ages ranging from 19 to 25 years with a mean age of 20.4 $\pm$ 0.27 years; all were chromosomally normal (see Table V). A sample of 5ml. venous blood was taken into a lithium heparin tube from each individual. This was given a consecutive number to be used throughout the experiment as the only means of identification.

For the molarity and pH experiments, a 5ml. venous blood sample was taken in a lithium heparin tube from a chromosomally normal male aged 34 years with no family history of chromosome abnormalities.

b) Satellite Association Patterns - Parents and Controls

Blood samples were obtained from parents of mongol children and normal control parents. A 5ml. venous blood sample was taken into a lithium heparin tube from each individual, and was treated identically, and issued with a consecutive number. This number was the only means of identification throughout the procedure, thus giving no opportunity for bias during the analysis of the chromosome spreads. This type of blind trial is extremely important in that no preconceived ideas on the satellite association patterns can be seen until all of the experimental data is

complete.

A form was designed and issued, so as to obtain as much information as possible, regarding the history of both parents of mongol children, and for the normal controls. The following details were provided in confidence:

surname, forename, address, date of birth, nationality, general practitioner, children (d-o-b, sex, details), pregnancy (drugs, accidents, radiation etc.) and family history (illnesses, affected siblings etc.)

This information was recorded on one form for both husband and wife (full sample details are recorded in Appendix II).

The co-operation of consultant paediatricians, clinical geneticists, obstetricians and gynaecologists in the Birmingham and Solihull Hospitals was secured. The Down's Babies Association was also approached, and kindly assisted in providing volunteer parents willing to donate a blood sample for this investigation. For the purpose of this study, parents up to the age of 35 years were used, having a mean age of 26.7 $\pm$ 1.10 years (see Table VI). This enables the "non-age related" groups of parents only (see page 30) to be evaluated.

The controls, namely parents of chromosomally normal children, were matched for age and parity as near as possible. Blood samples were obtained from married colleagues, friends and parents visiting children on the surgical wards at East Birmingham Hospital. All of the normal controls were volunteers and at no time was any

coercion practiced in order to obtain the specimens. The average age for the control group was 27.6 $\pm$ 0.79 years (see Table VII).

Difficulty in obtaining specimens was at first encountered. One problem was that samples received sometimes only came from one parent, usually the mother. This situation improved, and throughout the time of the study, 56 parents of mongol children (i.e. 28 couples) were received. Where possible, samples from the mongol children of the families concerned were also forwarded.

Control samples from parents of normal children were also difficult to obtain. It was first thought that volunteers from the family planning clinic might be a source of such material. However, this proved to be a very unsatisfactory source and was abandoned. Finally, a total number of twenty-four parents (i.e. twelve couples) were obtained.

Number	Sex	Age	Karyotype
1	F	21	46,XX
2	M	22	46,XY
3	F	21	46,XX
4	M	22	46,XY
5	M	20	46,XY
6	M	23	46,XY
7	F	21	46,XX
8	F	23	46,XX
9	F	23	46,XX
10	F	22	46,XX
11	M	19	46,XY
12	M	19	46,XY
14	M	20	46,XY
15	M	19	46,XY
16	M	20	46,XY
17	M	21	46,XY
18	M	19	46,XY
19	M	19	46,XY
20	M	20	46,XY
21	M	19	46,XY
22	M	20	46,XY
23	F	19	46,XX
24	M	19	46,XY
25	M	19	46,XY
26	F	19	46,XX
27	F	21	46,XX
28	F	19	46,XX
29	M	19	46,XY
30	F	19	46,XX
31	F	21	46,XX
32	F	25	46,XX
33	M	20	46,XY
34	M	19	46,XY
35	F	23	46,XX
Mean age: 20.4 ± 0.27 years			

Table V Composition of sample - Control group for hypotonic solutions experiment

Number	Sex	Age	Karyotype	Infant
5	F	22	46,XX	47,XX,G21+
6	M	23	46,XY	
15	M	34	46,XY	47,XY,G21+
16	F	32	46,XX	
19	F	20	46,XX	47,XX,G21+
20	M	22	46,XY	
21	F	21	46,XX	47,XX,G21+
22	M	23	46,XY	
23	F	22	46,XX	47,XX,G21+
24	M	24	46,XY	
25	M	29	46,XY	47,XY,G21+
26	F	26	46,XX	
27	F	29	46,XX	47,XY,G21+
28	M	29	46,XY	
31	F	28	46,XX	47,XY,G21+
32	M	30	46,XY	
39	F	34	46,XX	47,XY,G21+
40	M	34	46,XY	
Mean age: 26.7 ± 1.10 years				

Samples used in the experiment from parents of children with Down's Syndrome

Reason	No.	Comment
Failed cultures	16	(either one or both failed)
Maternal/paternal age 35 years or above	20	(either one or both older)
Abnormal maternal karyotype	1	(46,XXt (1:17) (q21:q21)

Samples not included in the experiment from parents of children with Down's Syndrome

Table VI Composition of sample - parents of children with Down's Syndrome (mongolism)

Number	Sex	Age	Karyotype
13	F	26	46,XX
14	M	26	46,XY
33	F	26	46,XX
34	M	28	46,XY
41	F	29	46,XX
42	M	27	46,XY
43	F	21	46,XX
44	M	23	46,XY
45	M	24	46,XY
46	F	24	46,XX
47	M	34	46,XY
48	F	33	46,XX
51	M	28	46,XY
52	F	27	46,XX
59	M	30	46,XY
60	F	29	46,XX
61	M	32	46,XY
62	F	30	46,XX
Mean age: 27.6 $\pm$ 0.79 years			

Samples used in the experiment from control parents of normal children

Reason	No.	Comment
Failed cultures	4	(either one or both failed)
Maternal/paternal age 35 years or above	2	(either one or both failed)

Samples not included in the experiment from control parents of normal children

Table VII Composition of sample - control parents of normal children

III. RESULTS

III.i) Satellite Association Patterns - The Effect of Hypotonic Solutions

The results obtained from this series of experiments should indicate whether the hypotonic treatment has any effect on the satellite association patterns.

It can be seen that the number of satellite associations observed in metaphase spreads after treatment with the three hypotonic solutions, vary considerably (Appendix II, Table XXI) When potassium chloride was used as the hypotonic solution, the frequency histogram (Figure 23) of satellite associations showing one or more associations, ranged from 50% to 100% in each cell examined, with a modal value of 80-90% of cells. With sodium citrate (Figure 24) the values were lower, ranging from 40% to 100%, this time with a mode of 60-69% of cells.

The shape of the frequency histogram when water/Hanks solution was used shows a nominal modal value of 40-49% (Figure 25). If all the observations using the three hypotonic solutions are combined, the frequency histogram (Figure 26) clearly demonstrates a mode of 60-69% of cells that are involved in satellite associations.

In all of the metaphase spreads examined, those using potassium chloride as the hypotonic solution always showed a higher frequency of satellite associations than with the other two solutions. When a high frequency of associations was recorded with potassium chloride, the corresponding

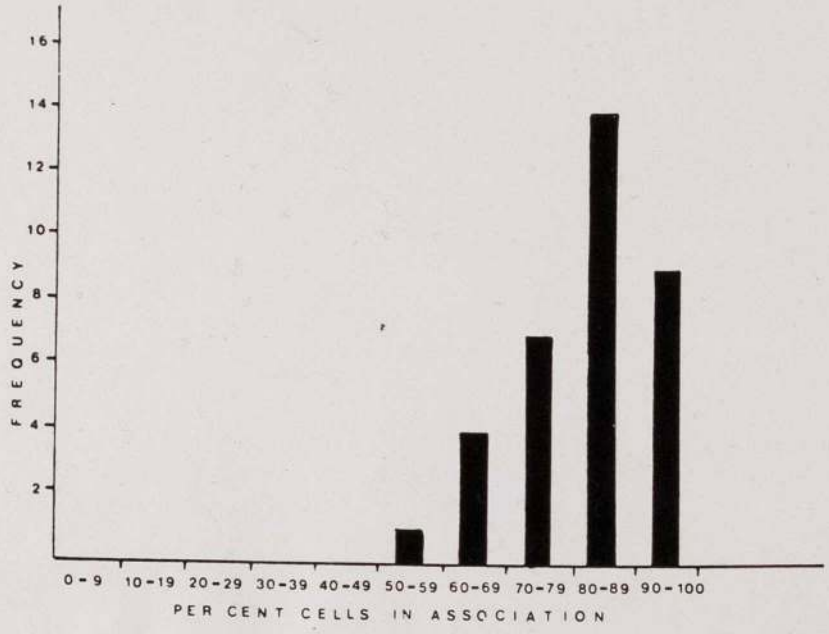


Figure 23

Histogram showing the frequency of one or more satellite associations per cell observed using potassium chloride as the hypotonic solution.

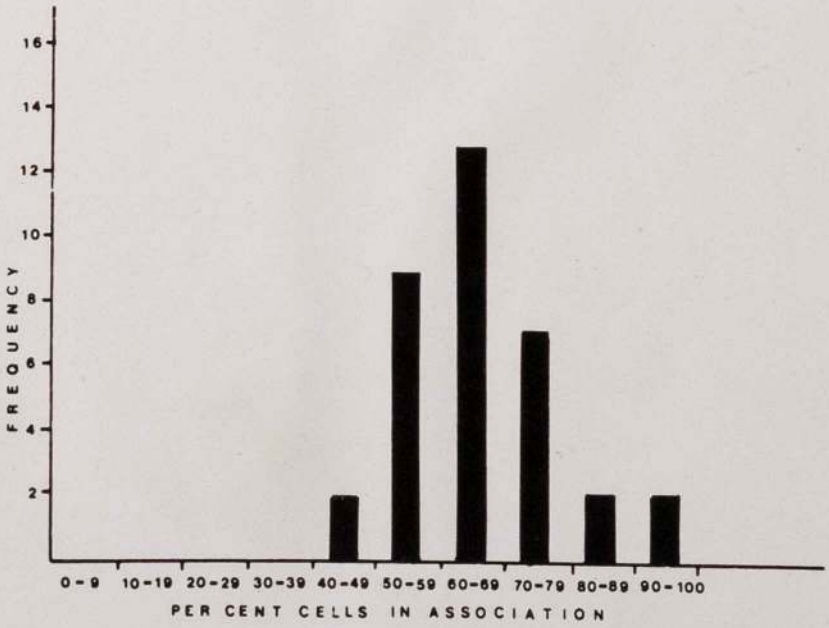


Figure 24

Histogram showing the frequency of one or more satellite associations per cell observed using sodium citrate as the hypotonic solution.

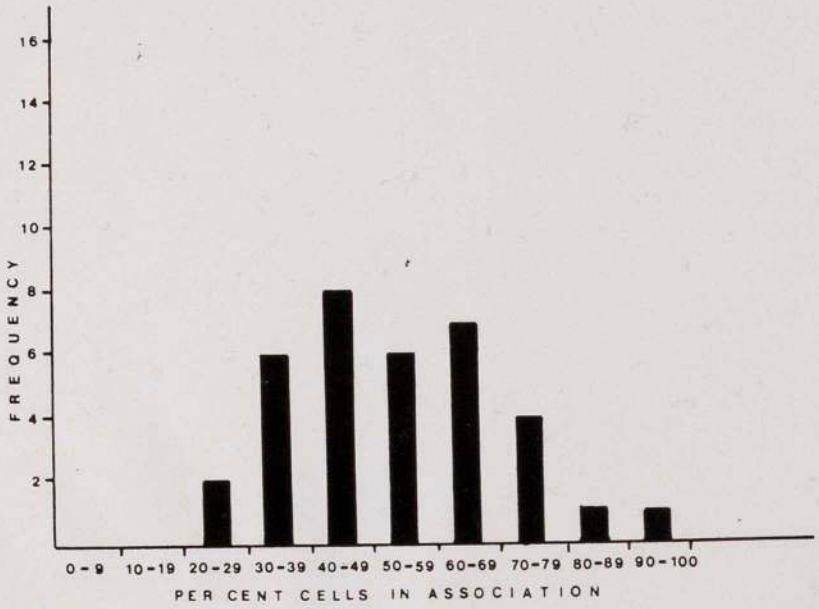


Figure 25

Histogram showing the frequency of one or more satellite associations per cell observed using water/Hanks as the hypotonic solution.

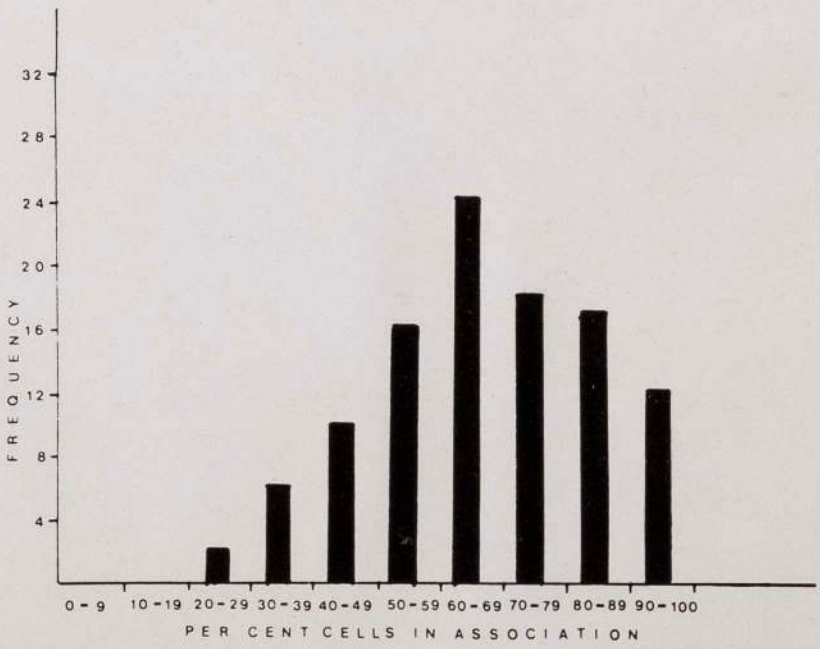


Figure 26

Histogram showing the frequency of one or more satellite associations per cell observed in all of the hypotonic solutions used.

figures with sodium citrate and distilled water, although lower, were also raised.

Comparison of the frequency between the hypotonic solutions (Table VIII) confirms the above finding. This shows that when potassium chloride is used as the hypotonic solution, the frequency of associations observed is consistently higher than with the other two solutions. The standard errors calculated for each of the solutions show a particularly consistent figure considering the sample size and variability in interpretive results.

When the specific group satellite associations are recorded (Appendix II, Table XXII) the same pattern emerges. If frequency histograms are constructed (Figures 27, 28, 29, 30) it can be clearly seen that in all cases, potassium chloride treated cultures have a higher frequency of specific associations than those treated with either sodium citrate or distilled water/Hanks solutions. Di-associations between the 'D' and 'G' groups of chromosomes are far more frequent than those involving the 'D' and 'D' and 'G' and 'G' groups. A greater number of tri-associations between the members of the 'D' and 'G' groups are also seen when compared with those involving the same groups. One interesting result is seen in the groups of di-associations involving the 'G'-'G' group. Here, there is less variability between the solutions, compared with the other groups.

The results from the experiment to determine whether the molarity of the potassium chloride solution affected

Hypotonic Treatment	Number of Controls Examined	Number of Metaphases Examined	Percentage of Metaphases Showing Satellite Associations
Potassium Chloride	35	350	78.28 ± 1.98
Tri-Sodium Citrate	35	350	61.14 ± 2.04
Hanks/ Water	35	350	48.85 ± 2.86

Table VIII Comparison of the frequency of satellite associations observed in the three hypotonic solutions

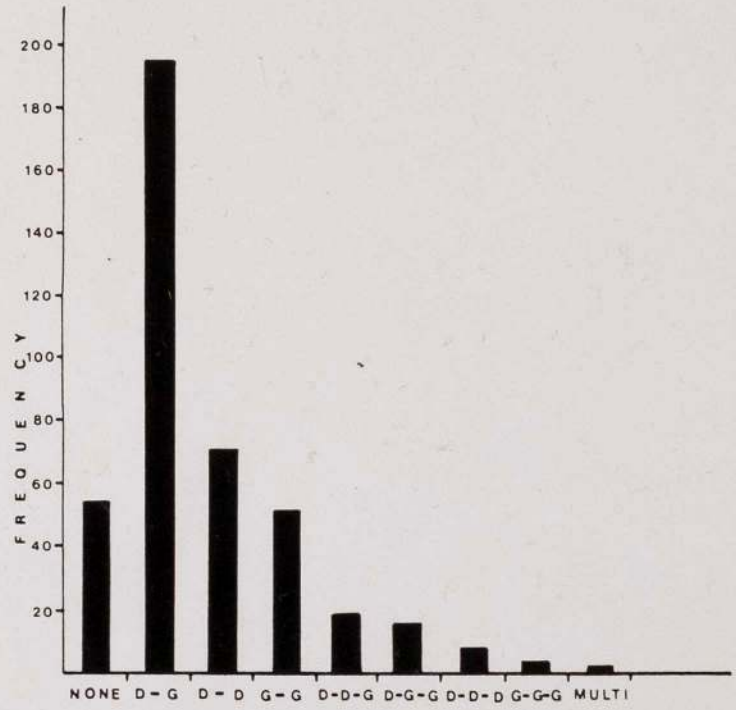


Figure 27

Histogram showing the frequency of the 'D' and 'G' groups in association using potassium chloride as the hypotonic solution.

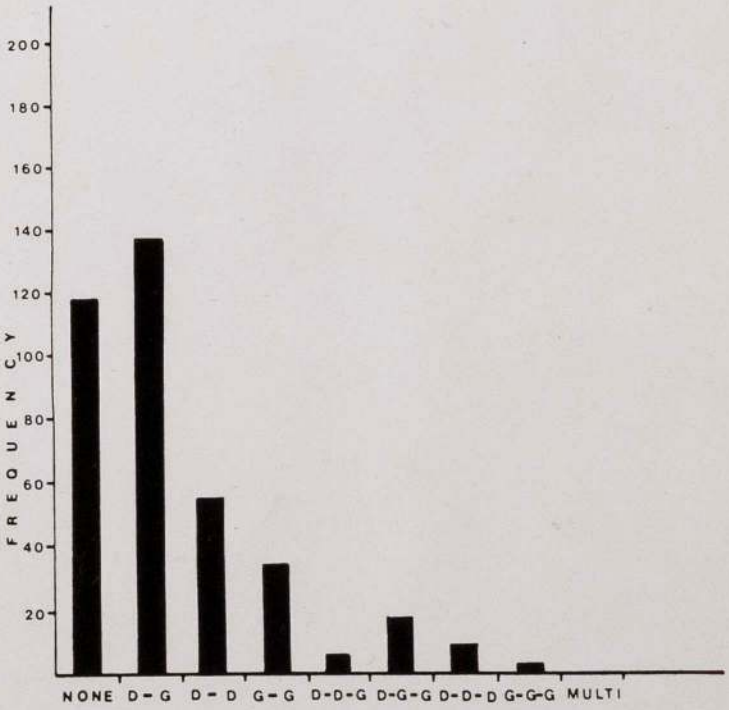


Figure 28

Histogram showing the frequency of the 'D' and 'G' groups in association using sodium citrate as the hypotonic solution.

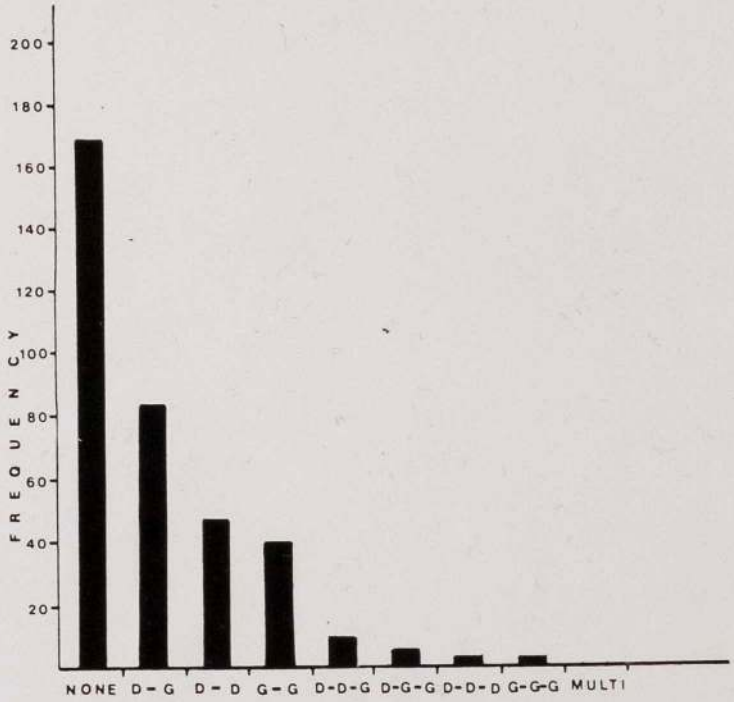


Figure 29

Histogram showing the frequency of the 'D' and 'G' groups in association using water/Hanks as the hypotonic solution.

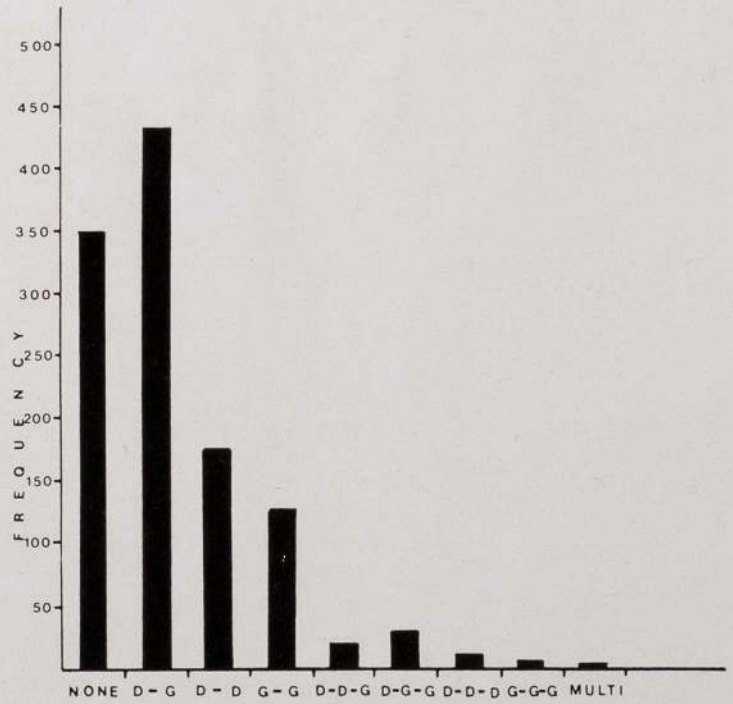


Figure 30

Histogram showing the frequency of the 'D' and 'G' groups in association in all of the solutions used.

the frequency of satellite associations, showed some interesting points (Table IX). The consistent number of associations recorded in 100 cells from each of the 0.04M, 0.06M and 0.08M solutions is remarkable. The trend would appear to show that the frequency of association increases with the molarity of the solution. This is a surprising result, as the opposite effect would usually be expected. Here again, the highest number of specific group associations involved di-associations between the 'D' and 'G' groups.

In the experiment to determine whether the time exposed to potassium chloride (0.075M) had any effect upon the satellite association frequency, it was found that there was no difference in the number of associations seen (Table X). The number of metaphase spreads showing satellite associations after 4, 8 and 16 minutes hypotonic treatment is remarkably constant. The number of specific group associations also show the same consistent numbers. The pattern of associations demonstrate the same trend as in all of the previous experiments, namely that the 'D'/'G' groups associate more frequently than any of the other combinations.

In summarising the results in the first part of this study, it can be seen that the hypotonic solution used to treat the cells, does significantly affect the satellite association frequency. Cells treated with potassium chloride show a higher incidence of associations than with

Molarity of Potassium Chloride	No. of Metaphases Examined	No. of Metaphases Showing Satellite Association	No. of Specific Group Satellite Associations seen in all Metaphase Spreads							
			D/G	D/D	G/G	D/D/G	D/G/G	D/D/D	G/G/G	Others
0.02M	100	Chromosomes too condensed for accurate analysis								
0.04M	100	78	34	13	20	4	3	4	0	0
0.06M	100	80	34	18	9	4	7	3	1	4
0.08M	100	83	29	18	26	1	6	3	0	0
0.10M	100	90	41	20	11	8	9	2	0	1

Table IX Satellite association frequency in relation to the molarity of potassium chloride used as a hypotonic solution

Time	No. of Metaphases Examined	No. of Metaphases Showing Satellite Association	No. of Specific Group Satellite Associations seen in all Metaphase Spreads							
			D/G	D/D	G/G	D/D/G	D/G/G	D/D/D	G/G/G	Others
4 min.	100	80	32	17	16	4	6	0	2	3
8 min.	100	78	31	17	17	4	6	1	1	1
16 min.	100	79	34	13	21	4	4	1	0	2

Table X Satellite association frequency in relation to the time exposed to 0.075M potassium chloride hypotonic solution

those treated with sodium citrate or Hanks/distilled water (1:3). It was, therefore, decided to use Hanks/distilled water (1:3) as the hypotonic solution of choice, for the standard technique. The consistency of the results show that it is possible to evaluate accurately, individual association patterns and so detect any significant changes.

III.ii Satellite Association Patterns - Parents and Controls

The main experimental procedures are those which set out to test whether specific satellite association patterns recorded in cells from parents of mongol children were significantly different to normal control parents. The results were tabulated for distribution, frequency and categorisation of satellite associations and histograms constructed.

In Figure 31 a frequency histogram was constructed to show the range of associations per cell in parents of affected children. When compared with a similar histogram (Figure 32) showing the range of associations per cell in parents of normal control children, it is seen that there are fewer cells without satellite associations in the parents than in the controls. However, this difference is made up by an increase in cells exhibiting two associating chromosomes in the parents than in control samples.

In Table XI the mean frequency of satellite associations per cell can be compared between the parents of infants with Down's Syndrome and control parents of normal children. It can be seen from this that the highest number of associations involve two acrocentric chromosomes. These show a mean frequency of 0.478 in the parents, compared with 0.378 in the controls. There are fewer associations involving three acrocentric chromosomes. The parents show a mean frequency of 0.054 associations per cell compared with 0.037 in the controls. Cells showing four or more

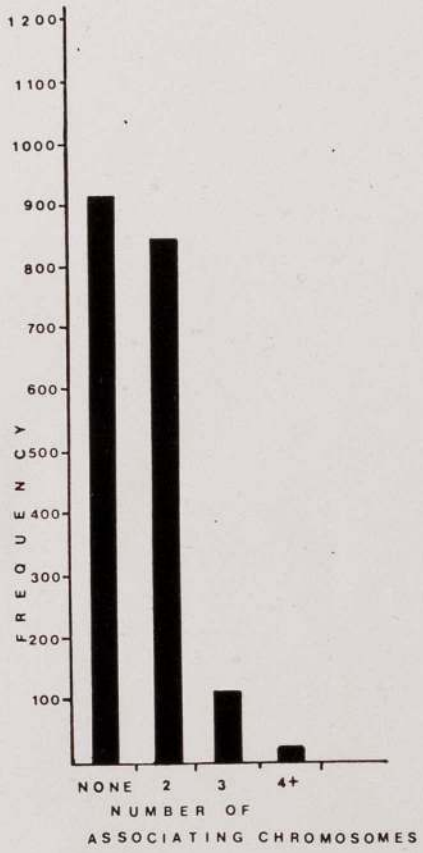


Figure 31 Histogram showing the frequency of association of 'D' and 'G' group chromosomes in parents of Down's children.

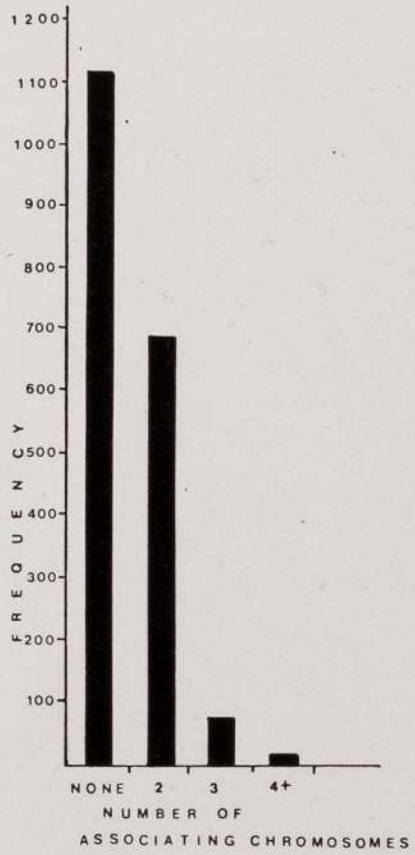


Figure 32 Histogram showing the frequency of association of 'D' and 'G' group chromosomes in parents of normal control children.

	Parents	Controls
Number of cells with 1 satellite association per cell	0.478	0.378
Number of cells with 2 satellite associations per cell	0.054	0.037
Number of cells with 3 or more satellite associations per cell	0.0017	0.0016
Total:	0.534	0.416

Table XI Mean frequency of associations per cell (900 cells per group).

These frequencies are not significant at the P 0.05 level.

(Mann-Whitney test)

association complexes give 0.0017 associations per cell in the parents and 0.0016 per cell in the controls. In all of these results, the overall trend is for the parents of mongol children to show a higher relative figure than those in the control sample. However, when the Mann-Whitney test was applied to test the significance of these results, it was clearly shown that these frequencies were not significant at the $P > 0.05$ level.

The same indication is seen in Tables XII and XIII, where the numbers of satellite associations seen per cell are compared. In 1,800 cells analysed from the parents, there were 861 cells showing one association, 98 showing two associations and 3 with three or more associations per cell. This can be compared with 680 cells with one association, 67 with two and 1 with three or more associations per cell in the control group.

If the above results are divided into maternal and paternal groups (Tables XIV and XV) any potential sex involvement will be demonstrated. In Table XIV mothers of mongol children are compared with mothers of normal controls in relation to the number of chromosomes associating per cell. Here again, the trend is for the mothers of affected children to show a higher number of associations per cell than mothers of normal children. This is clearly seen in all sections of the Table.

Table XV shows the same type of comparison between the fathers of mongol children and fathers of normal children. A similar pattern is observed as above, namely that the

Numbers of Satellite Associations Found in a Total of 1,800 Cells	
Number of cells with 1 satellite association per cell	861
Number of cells with 2 satellite associations per cell	98
Number of cells with 3 + satellite associations per cell	3

Table XII Summary of the frequency of satellite associations seen in 1,800 cells from the parents of children with Down's Syndrome (mongolism)

Numbers of Satellite Associations Found in a Total of 1,800 Cells	
Number of cells with 1 satellite association per cell	680
Number of cells with 2 satellite associations per cell	67
Number of cells with 3 + satellite associations per cell	1

Table XIII Summary of the frequency of satellite associations seen in 1,800 cells from the control parents of normal children

	No.of Acrocentric Chromosomes Associating per Cell				
	None	2	3	4+	Total
Fathers of children with Down's Syndrome	471	412	52	4	939
Control fathers of normal children	557	337	40	4	938
Total:	1028	749	92	8	1877

Table XIV Comparison between fathers of Down's infants and normal controls

	No.of Acrocentric Chromosomes Associating per Cell				
	None	2	3	4+	Total
Mothers of children with Down's Syndrome	457	424	64	7	952
Control mothers of normal children	541	336	21	1	899
Total:	998	760	85	8	1851

Table XV Comparison between mothers of Down's infants and normal controls

fathers of affected infants show a marked increase in association patterns compared with the fathers of normal infants.

If the two sets of results (Tables XIV and XV) are compared with each other, it can be seen that in all associations involving two chromosomes, the figures obtained show a striking similarity between mothers of affected children and controls and fathers of affected children and controls.

Frequency histograms were constructed from the above figures for both mothers of affected children and mothers of normal control children (Figures 33 and 34). Other histograms were drawn for the fathers of these children and for the fathers of normal control children (Figures 35 and 36). From these histograms it can be seen that both sets of parents and controls show an almost identical shape, and that in each case the differences in shape are wholly as a result of the increased number of di-associations in both parents.

Table XVI gives a summary of the di-associations seen in parents and controls, with special regard to the category of association. It can be seen that the total number of di-associations counted in 100 cells vary in individual parents from 34 to 74. The control parents show a range from 33 to 46 in 100 cells counted. If these di-associations are categorised into two types - A and B, it can be seen that in the parents, type A associations varied between 13 and 37 of the di-associations, whilst in

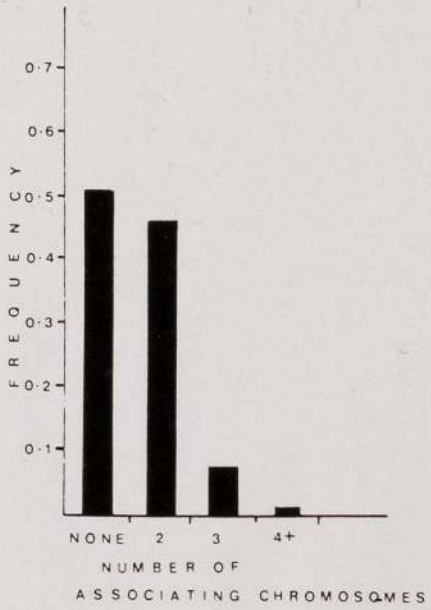


Figure 33

Histogram showing the mean frequency per cell of acrocentric chromosomes in association in mothers of children with Down's Syndrome.

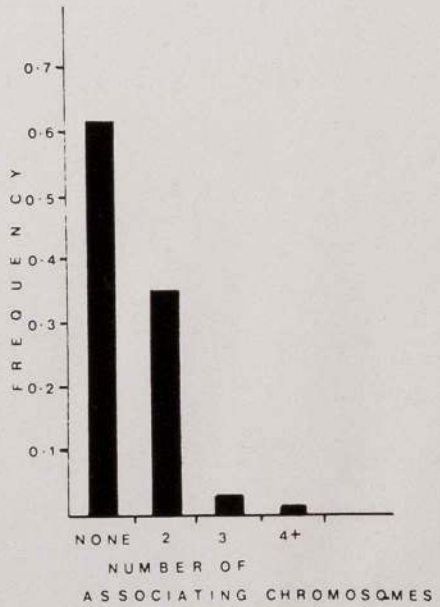


Figure 34

Histogram showing the mean frequency per cell of acrocentric chromosomes in association in mothers of normal control children.

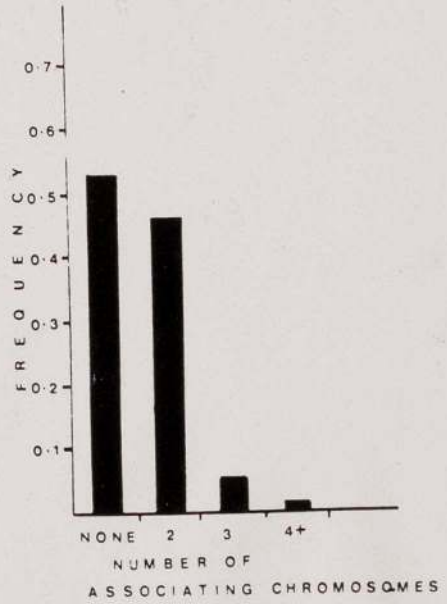


Figure 35

Histogram showing the mean frequency per cell of acrocentric chromosomes in association in fathers of children with Down's Syndrome.

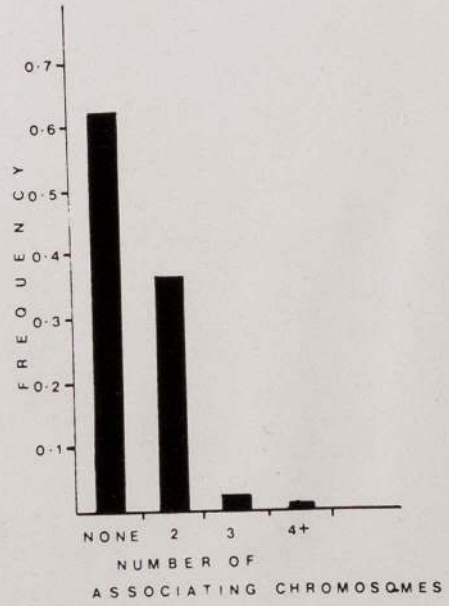


Figure 36

Histogram showing the mean frequency per cell of acrocentric chromosomes in association in fathers of normal control children.

PARENTS					CONTROLS				
Subject No.	Sex	Total	Type A	Type B	Subject No.	Sex	Total	Type A	Type B
5	F	54	19	35	13	F	42	9	33
6	M	51	20	31	14	M	39	12	27
15	M	74	37	37	33	F	38	14	24
16	F	41	16	25	34	M	35	15	20
19	F	47	16	31	41	F	37	9	28
20	M	48	22	26	42	M	37	14	23
21	M	53	29	24	43	F	34	4	30
22	F	38	15	23	44	M	35	8	27
23	F	37	22	15	45	M	34	5	29
24	M	46	15	31	46	F	39	15	24
25	F	46	23	23	47	M	42	13	29
26	M	48	15	33	48	F	40	14	26
27	F	57	30	27	51	M	34	4	30
28	M	41	14	27	52	F	38	14	24
31	F	41	23	18	59	M	33	10	23
32	M	36	15	21	60	F	36	8	28
39	F	46	16	30	61	M	46	10	36
40	M	34	13	21	62	F	34	5	29

Table XVI Summary of the total number of di-associations observed in both parents and controls (100 cells per subject)

the controls, type A associations ranged from 4 to 15 of the di-associations examined. Type B associations varied between 15 and 37 in the parents, and between 23 and 36 in the controls.

In Table XVII, the above di-association figures are broken down into percentages of the total number of satellite associations observed in each case. In the parents, the number of di-associations ranged from 77.94% to 96.00% of the total number counted. In the controls, the number of di-associations ranged from 82.35% to 100.00% of the total number counted. Here again, when these di-associations are categorised into types A and B, the percentage of type A seen in the parents varied from 31.25% to 59.45% whilst in the controls between 11.76% and 42.85% of the di-associations were of the category.

If frequency histograms of total di-associations seen in parents and controls are constructed (Figures 37 and 38), it is seen that the shape of these histograms show normal distribution in both individual groups. This finding is verified when both results are combined (Figure 39).

From the above figures it can be seen that the same trend continues as in the other tabulations, in that parents of mongol children show a higher incidence in the total number of di-associations than those observed in the control sample. However, if these associations are broken down into the two types of association, it is seen that only in the type A category of di-associations is there any significant increase. The type B category is identical for

PARENTS					CONTROLS				
Subject No.	Sex	Total %	Type A %	Type B %	Subject No.	Sex	Total %	Type A %	Type B %
5	F	90.00	35.18	64.81	13	F	89.36	21.42	78.57
6	M	94.44	39.21	60.78	14	M	84.78	30.76	69.23
15	M	94.48	50.00	50.00	33	F	97.43	36.84	63.15
16	F	89.13	39.02	60.97	34	M	97.22	42.85	57.14
19	F	92.15	34.04	65.95	41	F	86.24	24.32	75.67
20	M	96.00	45.83	54.16	42	M	84.09	37.83	62.16
21	M	77.94	54.71	45.28	43	F	89.47	11.76	88.23
22	F	88.37	39.47	47.91	44	M	87.50	22.85	77.14
23	F	84.09	59.45	40.54	45	M	85.00	14.70	85.29
24	M	83.63	32.60	67.39	46	F	92.85	38.46	61.53
25	F	79.31	50.00	50.00	47	M	82.35	30.95	69.04
26	M	82.75	31.25	68.75	48	F	100.00	35.00	65.00
27	F	90.47	52.63	47.36	51	M	91.89	11.76	88.23
28	M	89.13	34.14	65.85	52	F	95.00	36.84	63.15
31	F	80.39	56.09	43.90	59	M	89.18	30.30	69.69
32	M	78.26	41.66	58.33	60	F	100.00	22.22	77.77
39	F	85.18	34.78	65.21	61	M	95.83	21.73	78.26
40	M	89.47	38.23	61.76	62	F	97.14	14.70	85.29

Table XVII Summary of the di-associations observed in both parents and controls (100 cells per subject) expressed as a percentage of the total number of associations counted

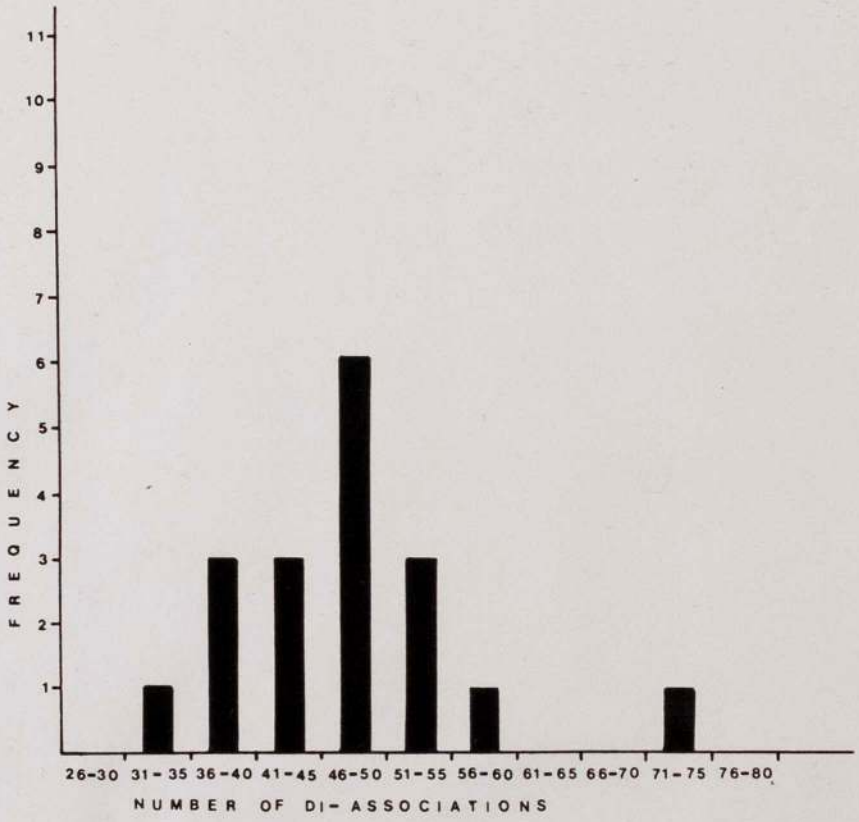


Figure 37 Histogram showing the frequency of the total number of diassociations in parents of children with Down's Syndrome.

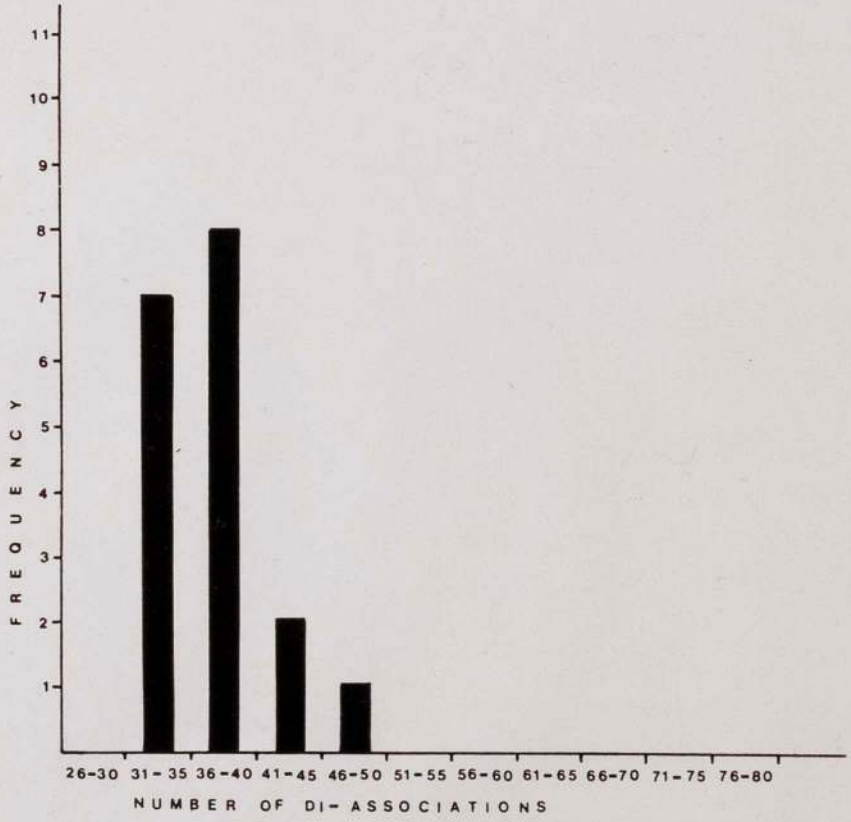


Figure 38 Histogram showing the frequency of the total number of diassociations in parents of normal control children.

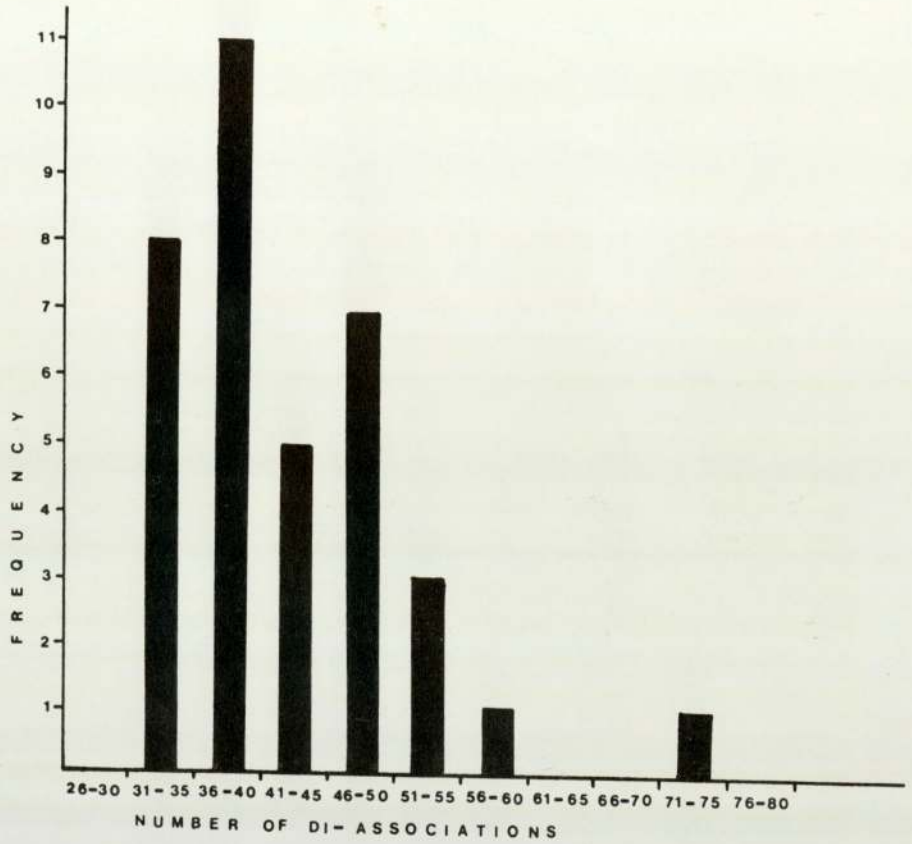


Figure 39 Histogram showing the frequency of the total number of diassociations in both parents and controls.

both parents and controls. When the figures were subjected to the t-test, it was demonstrated that the parents showed a significant increase in total di-associations when compared with the controls ($t=3.86$ $P < 0.001$). When the t-test was performed on type A di-associations, the results were even more significant ($t=5.47$ $P < 0.001$). With type B di-associations, the t-test did not reveal any difference between the two sets of results ($t=0.39$ $P > 0.05$).

Substantially lower figures are recorded in the summary of tri-associations in parents and controls (Table XVIII). Here, the total percentage of tri-associations in parents range from 2% to 11% and in the controls, between 0% and 9%. If the category of association is recorded, type A tri-associations in parents accounted for 1% to 8% of tri-associations compared with 0% to 5% in the controls. Type B tri-associations showed a range of 0% to 4% in parents and 0% to 4% in controls. Here again, the same pattern is seen as with the di-associations, the type B category being identical. However, with the type A category, the difference is not so marked as with the di-association figures.

In Tables XIX and XX, multi-association complexes are recorded. In general, there appears to be no difference in total percentage of associations or with type A and type B associations between parents and controls where four or more chromosomes are involved.

However, it is noted that in parents of mongol infants, five and six association complexes were observed, whilst in

PARENTS					CONTROLS				
S/A No.	Sex	Total %	Type A %	Type B %	S/A No.	Sex	Total %	Type A %	Type B %
5	F	6	4	2	13	F	5	4	1
6	M	3	3	0	14	M	6	3	3
15	M	4	2	2	33	F	0	0	0
16	F	5	3	2	34	M	1	1	0
19	F	3	1	2	41	F	5	4	1
20	M	2	2	0	42	M	6	4	2
21	M	11	7	4	43	F	4	4	0
22	F	5	3	2	44	M	5	1	4
23	M	7	5	2	45	M	6	3	3
24	F	9	6	3	46	F	3	2	1
25	F	10	8	2	47	M	9	5	4
26	M	10	7	3	48	F	0	0	0
27	F	6	5	1	51	M	3	1	2
28	M	5	3	2	52	F	2	2	0
31	F	8	7	1	59	M	4	1	3
32	M	10	7	3	60	F	0	0	0
39	F	8	4	4	61	M	2	0	2
40	M	4	2	2	62	F	1	1	0

	Total Tri-Associations	Type A Tri-Associations	Tri-Associations Involving 21:22 Configuration
Parents	118	30	47
Controls	62	10	16

Table XVIII Summary of the tri-associations observed in both parents and controls.

Four Associations					
No.	Sex	Type	Total	Type A	Type B
19	F	13-14-21-22	1	0	1
21	F	13-21-22-22	1	0	1
21	F	14-21-22-22	1	0	1
25	F	13-14-15-21	2	0	1
25	F	13-14-14-22	1	0	1
31	F	13-13-14-21	1	0	1

Five Associations					
No.	Sex	Type	Total	Type A	Type B
21	F	13-21-21-22-22	2	2	0
25	F	13-14-15-15-21	1	1	0

Six Associations					
No.	Sex	Type	Total	Type A	Type B
31	F	13-13-14-15-21-22	1	0	1

Table XIX Summary of associations involving four or more acrocentric chromosomes in parents of children with Down's Syndrome

Four Associations					
No.	Sex	Type	Total	Type A	Type B
14	M	13-14-21-21	1	1	0
33	F	13-14-14-21	1	1	0
41	F	14-14-15-21	1	1	0
42	M	14-14-15-21	1	0	1
59	M	13-15-22-22	1	1	0

Five Associations
No associations seen

Six Associations
No associations seen

Table XX Summary of associations involving four or more acrocentric associations in parents of normal children

the controls no multiple associations of this number were recorded.

In the results of the category of the types of association, the overall trend in total and type A associations was to show a definite increase in satellite associations in parents of mongol children. However, in the individual parents, a raised satellite association frequency and pattern can be seen in most of the recorded results. In the majority of parents, at least one parent shows a raised total percentage of satellite associations recorded which are in the type A associations.

In Figure 40 the number of specific associations observed in the 'D' group of chromosomes in parents and controls, are reproduced graphically.

Also demonstrated is the type of association, in relation to the total number observed.

It appears that there is a uniformity between parents and controls in the total number and types of associations seen involving the 'D' group of chromosomes. The indication in both samples is, for a greater percentage of associations involving the 13/14 and 14/15 chromosomes than with the other chromosomes within the group. It is also interesting to note that associations involving the homologous pairs remain constantly low.

Specific associations involving the 'D' and 'G' groups are seen in Figure 41. Here again, the total number and the type of association are relatively constant for both parents and controls. The trend in both samples is for a

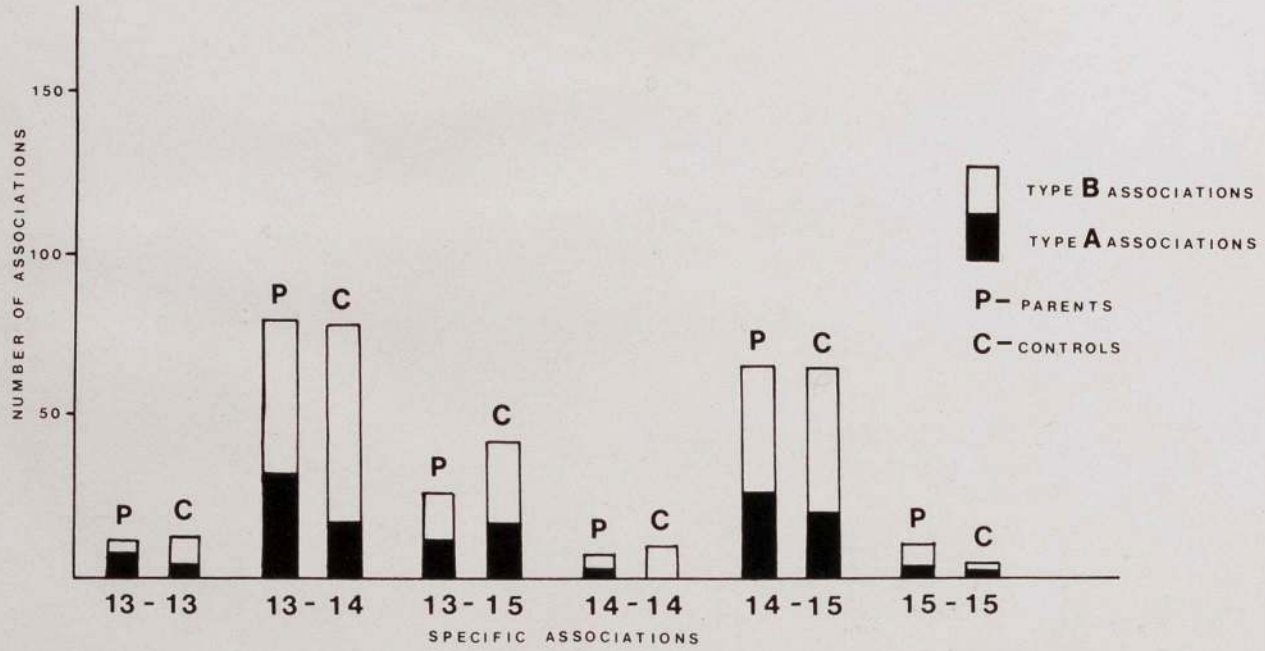


Figure 40 Graph showing the total number of specific satellite associations; type A and type B associations; which were observed in the D/D groups of chromosomes, in both parents and controls.

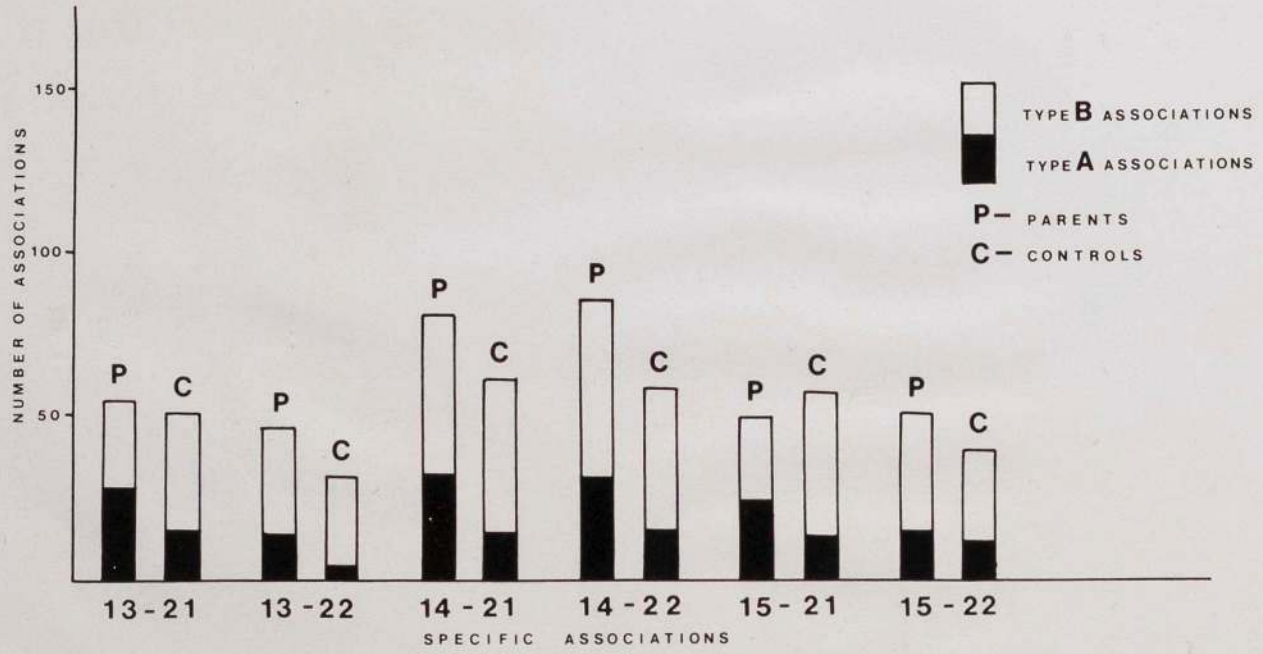


Figure 41 Graph showing the total number of specific satellite associations: type A and type B, which were observed in the D/G groups of chromosomes, in both parents and controls.

greater number of associations involving the 14/21 and 14/22 chromosomes, with a slightly higher total frequency in the parents, although the type B associations are virtually identical.

When the graph showing associations between the 'G' group of chromosomes (Figure 42) is produced, a striking difference in the results between parents and controls is instantly noticed. Associations involving the homologous pairs, i.e. 21/21 and 22/22 are considerably fewer than those between chromosomes 21 and 22. Further examination of type A and type B associations in parents and controls show a dramatic increase in type A associations in the parents involving chromosome 21 and 22. It can be seen that over three times as many type A associations are observed in parents than in the controls, whilst type B associations have, more or less, an identical number. When the t-test is applied to this configuration, the results for the type A associations are significant ($t=3.32$ $P < 0.001$) whereas the type B were not. Similarly, in the 21/21 and 22/22 specific associations, the number of type A associations are also raised in the parents.

When looking at the tri-associations involving numbers of the 'D' group of chromosomes, (Figure 43) a rather nondescript pattern emerges. No definitive indication is seen, other than to note that the number 14 chromosome appears to be involved in the association complex more often than any other chromosome.

In the graph showing tri-associations within the 'G'

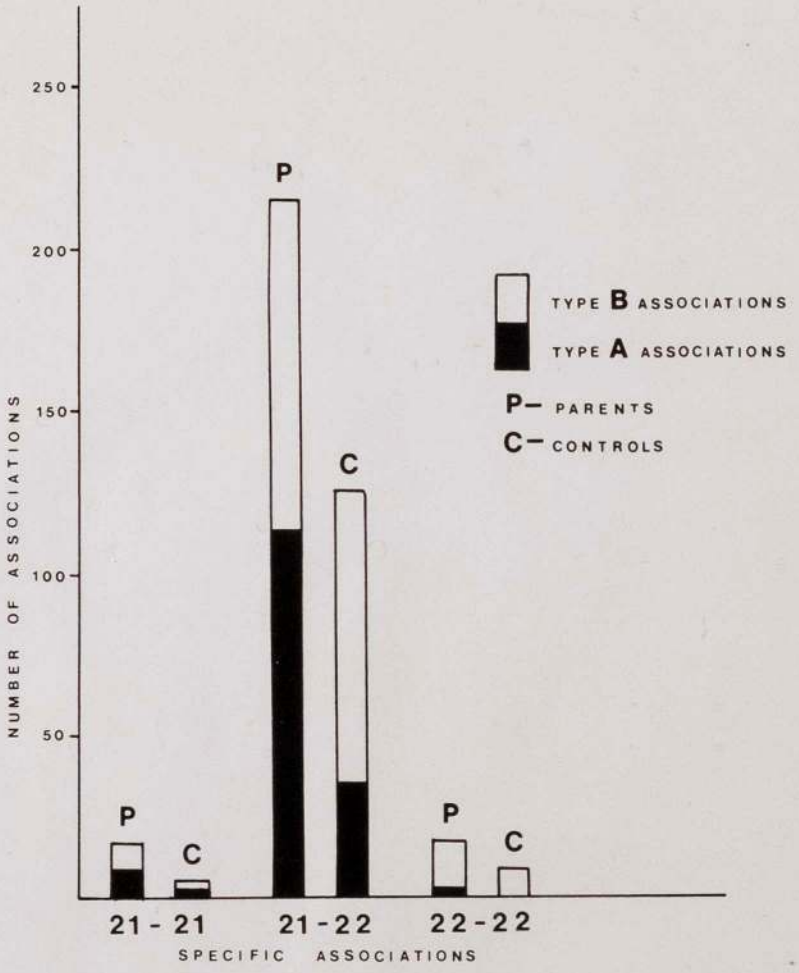


Figure 42 Graph showing the total number of specific satellite associations, type A and type B associations; which were observed in the G/G groups of chromosomes, in both parents and controls.

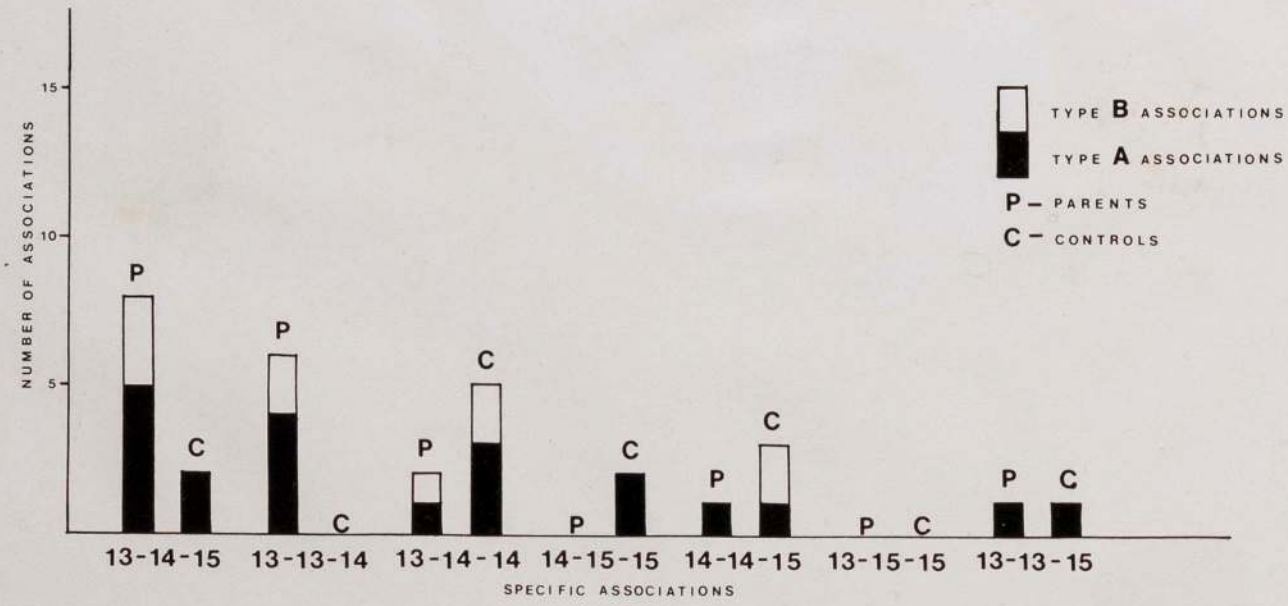


Figure 43 Graph showing the total number of specific satellite associations: type A and type B associations which were observed in the D/D/D group of chromosomes, in both parents and controls.

group (Figure 44), an increased frequency of type A associations is seen in the parents of mongol children, for the 21/22/22 configurations. Association complexes involving 21/21/22 show an identical number of type A associations. Here again, in the 21/22/22 tri-associations, the type A tri-associations are three times the number in the parents compared with the controls.

Figure 45 shows the specific tri-associations involving the 'D'/'G'/'G' groups. The trend in this graph shows that in the 13/21/22, 14/21/22 and 15/21/22, a greater total number of satellite associations are seen in the parents of mongol children. In all of the other combinations of this configuration, no obvious differences between the two samples are noted. The same pattern is seen with the 13/21/22, 14/21/22 and 15/21/22 as with the di-associations involving the 21/22 chromosomes (Figure 42). In each complex, the number of type A associations is virtually identical in the parents, and is at least twice the figure observed in the controls. It seems that the 21/22 configuration is the common denominator in these configurations, the other acrocentric chromosomes being involved merely by chance.

In the last graph (Figure 46), no definite pattern is seen, with the 'D'/'D'/'G' tri-association complexes. Perhaps one interesting feature is that the highest number of type A associations in both controls and parents is seen again when the 14 and 21 chromosomes are involved in the complex.

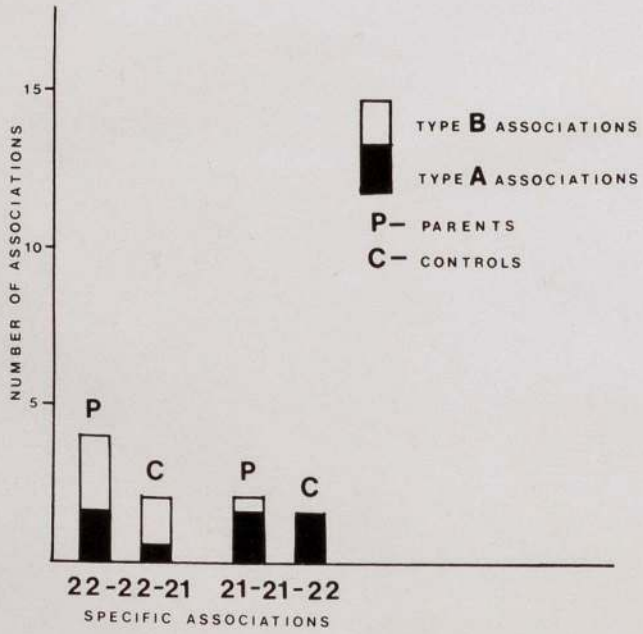


Figure 44 Graph showing the total number of specific satellite associations; type A and type B associations which were observed in the G/G/G group of chromosomes in both parents and controls.

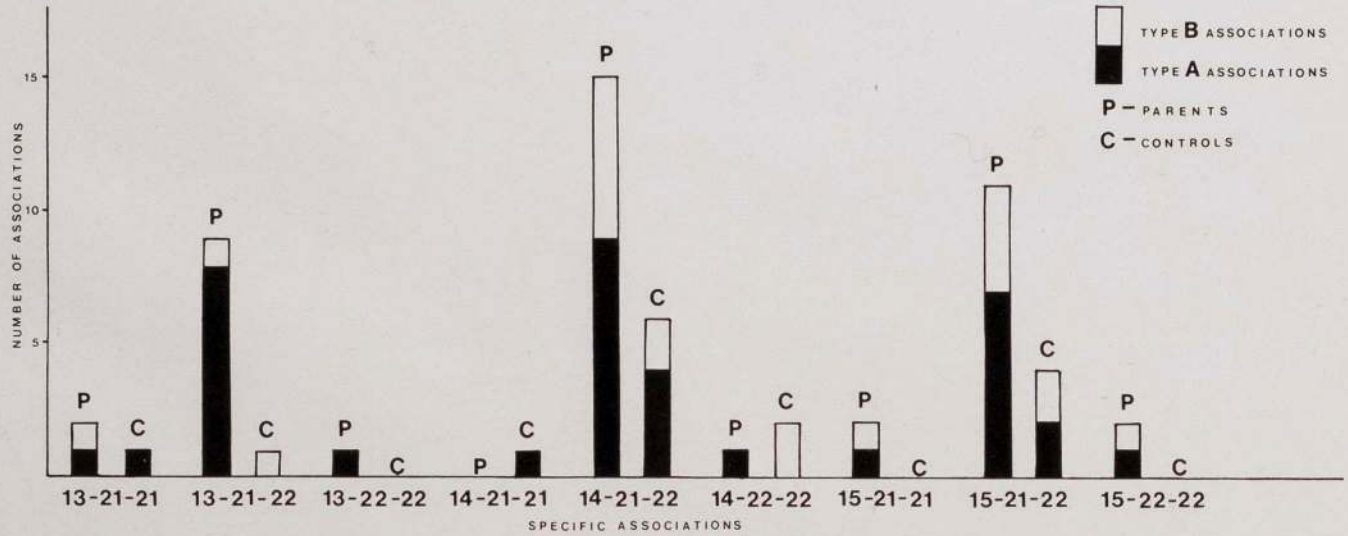


Figure 45 Graph showing the total number of specific satellite associations; type A and type B associations which were observed in the D/G/G groups of chromosomes, in both parents and controls.

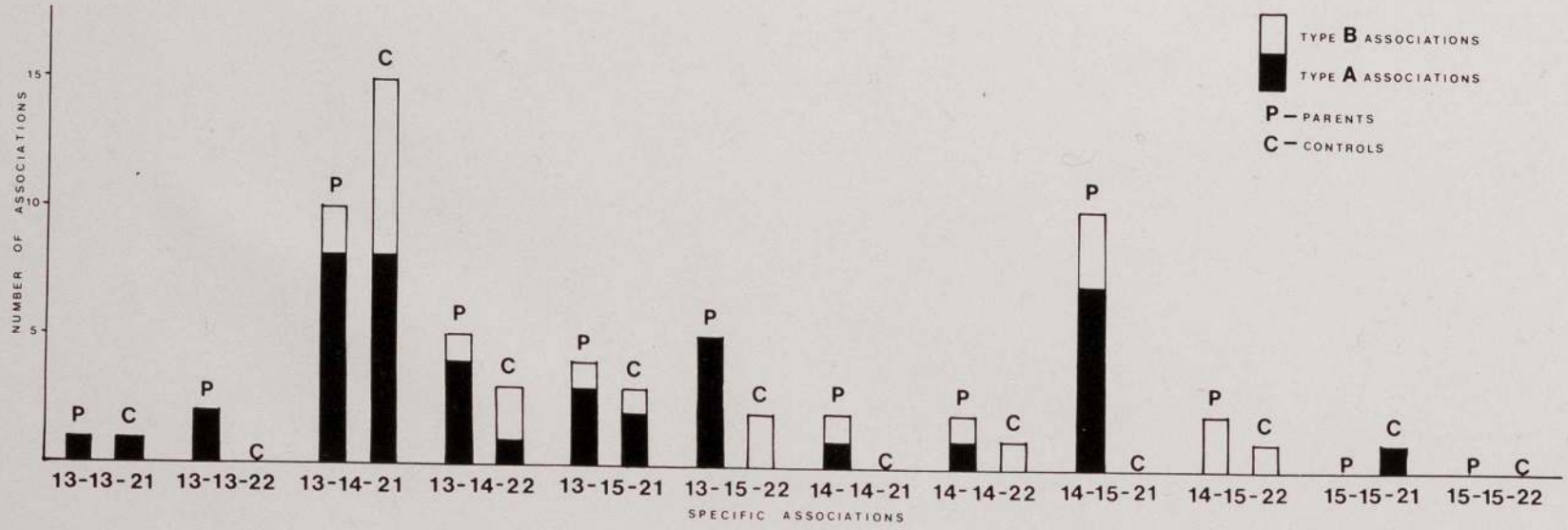


Figure 46 Graph showing the total number of specific satellite associations; type A and type B associations which were observed in the D/D/G groups of chromosomes, in both parents and controls.

The results in the second part of the study show some significant findings. The overall trend is to a higher frequency of satellite associations in the parents of mongol children. There does not appear to be any indication that the sex of the parent is involved in this finding. Perhaps the most definitive factor in these results, has been the identification of specific chromosomes and the categorisation of the various types of association. It is clearly shown that the frequency of associations involving the number 21 and 22 chromosomes is markedly increased in the parents of mongol children. This indication is underlined when the categories of associations are analysed. Here, a substantial increase in the type A associations is clearly demonstrated when chromosomes 21 and 22 are involved in the configurations.

IV. DISCUSSION

IV.i) General

Over the past fifteen years, there have been numerous studies (see page 25) concerning the effect of satellite association, either as a morphological variant, or as a factor in producing abnormal cell division. The inconsistency of the results can only serve to emphasise the extreme variability of this phenomenon. It is therefore important that a standardised technique and uniform criteria must be formulated before any real understanding can be gained.

The technical procedures used in the previous studies (Ferguson-Smith, 1961, Cohen and Shaw, 1967, Zang and Back, 1968, Nakagome, 1970 and Orye, 1974) have all varied, although the overall concepts have been identical. It would appear that the numerous different unknown combinations of variables, influence the association frequencies, and that different cells respond in diverse ways to various influences.

If the possible effect of variations according to race, sex, age and heredity factors are introduced, then the inconsistency in results can easily be understood. If, as well as the above factors, the differences in statistical analyses are considered, the problem of trying to evaluate a simple finding can assume mammoth proportions.

However, if from the previous studies, the correlated findings are used to formulate a baseline, any significant

results will be assumed to be correct for that series of experiments. In this way it has been shown that although the distribution of different types of associations vary from one individual to another, they are specific for a given subject, since different pairs of acrocentric chromosomes contribute in constant proportions (Schmid and Krone, 1974).

The phenomena of satellite association might also have a functional role. The satellited regions of the acrocentric chromosomes vary in size and shape, and as such, mean that each nucleolar organising region could be involved in diverse functional roles. Therefore, the extreme variants in the population will be maintained throughout a period of time, and will consistently show a strong tendency to satellite association, thus giving a strong indication that this tendency is genetically controlled. Present evidence suggests that each individual has a characteristic, or modal number of nucleolar organising regions. These can easily be demonstrated by using the silver nitrate techniques (Howell et al., 1975, Denton et al., 1976, Goodpasture and Bloom, 1976). These methods show a physical connection which is indicative of their participation together in nucleolar organisation.

It is without doubt that some form of physical communication exists between chromosomes in the interphase, the exact nature and function of which is still speculative. Hoskins (1968) brought forward evidence that human mitotic

chromosomes are bound together by chromosome to chromosome connectives. He showed that by using a micro-needle in living cells, each chromosome came out of the nucleus like a chain, each chromatid pair attached to the next, by a filament, directed towards the centromeric region.

Burkholder (1975) verified this finding in whole mount chromosome preparations, by observing that interchromosomal fibres were frequently seen extending from one chromosome to another in trypsin-treated preparations. However, he failed to see any such fibres in untrypsinised controls, and considered that perhaps these so-called fibres were artifacts produced by the overlapping of dispersed chromatin.

One possible explanation for the phenomena of satellite association could be that rupture of the interconnecting fibres occurs in the repetitive DNA sequences. Thus, certain interconnecting fibres would seem to break less easily, particularly in the case of the acrocentric chromosomes. This could mean that the cell has a greater chance of unequal cell division, thus producing aneuploidy. From this it can be postulated that chromosomes bearing abnormalities of the nucleolus organising regions may be more easily involved in the non-disjunction of chromosomes due to satellite association.

In the present study, the satellite association patterns seen in the hypotonic solution experiments, and those from parents of trisomy 'G' infants, share the same criteria, but with varying interpretations. In both experiments the con-

sistency in which the satellite association frequencies was observed, proves that this phenomenon is not an artifact but can be used as a reliable guide in morphological studies.

IV.ii) Satellite Association Patterns - Hypotonic Solutions

The results from the hypotonic experiments show that the choice of hypotonic solution is critical, when satellite association patterns are being analysed. The statement by Zang and Back (1968) that the standard technique of choice should be one that gives the highest frequency of associations, must be questioned. Surely, only in a technique that gives fewer true associations, with the lowest variance, must be the one of choice. Thus, only from this baseline can any conclusions be drawn as to their effective role in producing aneuploidy. In this respect it is worth comparing the results obtained using potassium chloride and those using water (1:3 Hanks/water solution).

The effect of water on the cell membrane is in itself far more traumatic and stringent than that of potassium chloride. It would appear that by using potassium chloride as a hypotonic solution, the chromosomes gradually disentangle themselves and pull apart as the cell swells in size. With the 1:3 Hanks/water hypotonic solution, the same process still occurs but much faster, so that the chromosomes are violently separated within a very short period of time. Hence, when looking at the satellite association frequencies, those cells which have been treated with the potassium chloride hypotonic solution will give a more accurate morphological picture than those treated with water. However, for the purpose of this study,

those associating 'D' and 'G' groups present after a 1:3 Hanks/water treatment, will serve to demonstrate those associations which exhibit particularly strong affinities between satellites.

Using 1:3 Hanks/water solution for the hypotonic treatment, enables any small increase in the satellite association frequency to be easily detected. Other authors have found a large variation in the cultural conditions which influence satellite association frequencies (Back and Zang, 1969, Nankin, 1970), as well as in the techniques of preparation (Back and Zang, 1969, Rozenkrantz and Fleck, 1969, Hoehn, Nagel and Krone, 1971).

It was found that up to the present time, no author has evaluated the effect of post cultural factors, especially the role of the hypotonic solution in relation to the frequency of associations. The variability in the results between the basic cultural methods indicate the possibility that it is the subsequent hypotonic solution that is the critical factor.

The concept that potassium chloride is actually involved in the alteration of the molecular arrangement of the chromosome might also be considered. This effect could be an integral step in the pre-treatment of the chromosomes for the G-banding methods. This pre-treatment of the chromosomes can involve proteolytic enzymes such as trypsin or varying salt solutions which, it is thought, rearrange the molecular architecture sufficiently for the Giemsa stain to selectively attach its molecules.

When the cells are treated with a hypotonic potassium chloride solution at 37°C, it is postulated that this, in some way, could be the start of this rearrangement. Comparison between banded preparations where the cells were treated with potassium chloride as the hypotonic solution and those treated with Hanks/water solution, show a better and more definitive banding structure.

Thus, if any serious analysis of satellite association patterns is to be studied, then the type of hypotonic treatment used in the harvesting of the cells must be taken into account. From this study it is seen that by using this standardised technique, the satellite association pattern for each individual person can be accurately determined. The reproducibility of the results serve to indicate this factor.

IV.iii) Satellite Association Patterns - Parents and Control

The effect of non-disjunction of chromosomes in producing a trisomic cell line is well known. Trisomy represents about half of all developmental chromosome anomalies found in human liveborns and spontaneous abortions. Its total incidence in human conceptions may be 3% or even higher (Polani and Jagiello, 1976). It is worth considering that of the three most common examples of autosomal trisomy in man, two involve the satellited acrocentric chromosomes (trisomy 'D' and trisomy 'G'). The third (trisomy 'E'), involves a chromosome whose short-arm often takes part in associations with satellited chromosomes.

When the DNA replication times of chromosomes are compared, the three groups of chromosomes which are the latest autosome replicators are the 'D', 'E' and 'G' groups. This means that any delay in separation will increase the chances of unequal division. Furthermore, chromosomes 13, 18, and 21 are the last of their groups to replicate. Banding studies have shown that the extra chromosome present in trisomy 'G' is a number 21, in trisomy 'D' it is 13 and in trisomy 'E' the extra chromosome present is 18. It must therefore be assumed that it is no coincidence that the rate of DNA replication plays an important part in the production of trisomic cell lines.

The aim of this study has been to look specifically at trisomy 'G' and to correlate between its incidence and

satellite association patterns. In man, most trisomies are related to advancing parental age, and because it has been shown that in trisomy 'G' the maternal age matters, this relationship is assumed for the other trisomies (except XYY). This maternal age factor may or may not directly involve satellite association patterns, although its role in this area may be far more closely connected than has been previously considered.

Evans (1967) suggested that the nucleolus becomes suspect with age, and that viral infection may reduce the capability of the nucleus for dissolution at division. Curtis and Cooke (1974) put forward the idea that only certain combinations of nucleoli may be conducive to non-disjunction, and that some chromosomes may be able to disrupt the fused nucleoli, whilst others cannot.

The involvement of the acrocentric chromosomes in the formation of nucleoli is well known. The morphological variants frequently observed on the short arms, stalk (secondary constriction) and satellites, provide a guide to its role, which could constitute an explanation for the variable frequency of associations in the population. Although the nucleolus organising regions have been assigned to the stalk (secondary constriction) of the chromosomes, it does not rule out the possibility of a number rDNA cistrons being present on the satellites themselves.

The hypothesis of this study postulates that there exists a certain predisposition among some individuals to

produce children with trisomy 'G' (mongolism). From the results of this study, it can be shown that there exists a significant difference in certain satellite association patterns in the parents of trisomy 'G' infants when compared with normal control parents. The relevance of this finding within the non-age related group of parents, suggests that there is a possible connection between these two factors.

If this predisposition exists among the general population, why should the recent statistics (Goad et al., 1976) show a seasonal variation in the number of trisomy 'G' children born to mothers under 34 years of age? One possible explanation for this would be that a combination of either a viral or allergenic component combined with a susceptibility to increased specific satellite associations might be the answer in such individuals. As this seasonal variation occurs between April and October, numerous other factors such as diet, radiation etc., could be implicated. Whatever factor provides the stimulus, the variation produced is sufficient to maintain the statistical incidence of non-age related mongolism.

The role of immunological response may be such a factor in this respect. The results of Frolov (1975) indicate that the frequency of satellite associations decreased proportionally to the increase in intensity of the immune response after smallpox vaccinations. Similarly, Hansson (1975a) found an increase in satellite association frequencies in patients with hypothyroidism. This would

appear to indicate that the mechanics of satellite association is relatively easily affected by exogenous agents, although it might mean only a reduction in the length of the time in interphase.

The role of the nucleolar organising regions (N.O.R.) on the stalks of the satellites might play an important role in the predisposition of certain acrocentric chromosomes to associate. If there was some variability in the quantitative numbers of N.O.R.'s present, then the inter-action of these chromosomes would be different. The possibility that these regions are more hypersensitive to certain external factors in some members of the population is suggested. This would account for the variability in satellite association frequencies found in random samples.

It is postulated that certain individuals have a greater number of N.O.R.'s present on the 'D' and 'G' groups of chromosomes, although on evidence found in this study, it is put forward that only those on the 'G' group are relevant. These might be represented in the form of "active areas", participating in, or near to, the nucleolar membrane during interphase (Figure 47). As a result of this, their involvement and subsequent potential damage, e.g. by viral infection, would be greater (Figure 48). It could be that these active areas are in fact participating ribosomal DNA cistrons, and that their functional role is in some way linked to the number present in each individual.

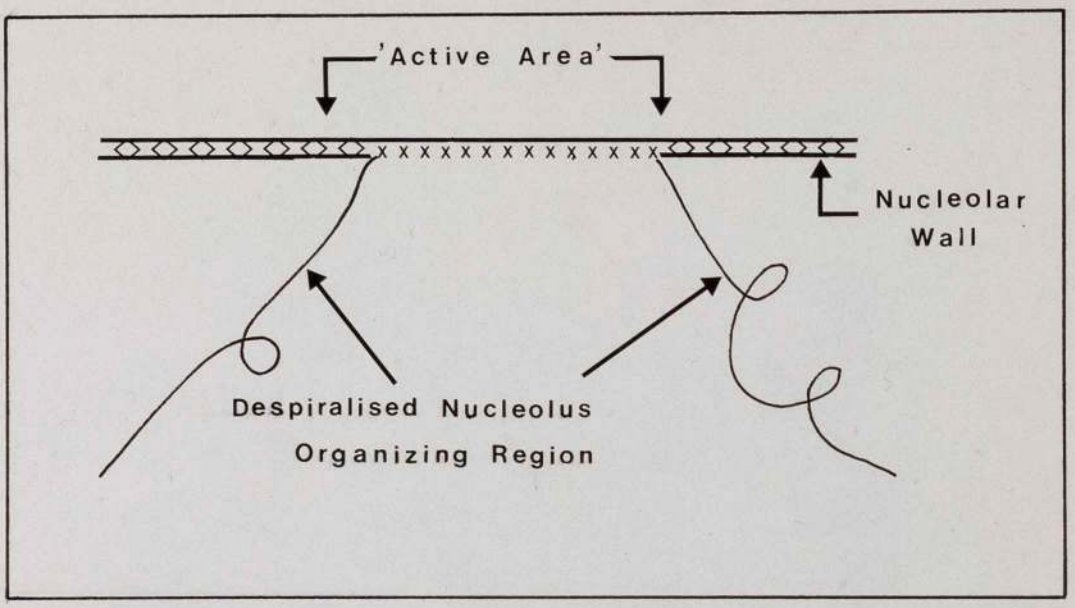


Figure 47 Proposed involvement of "active areas" on nucleolar membrane.

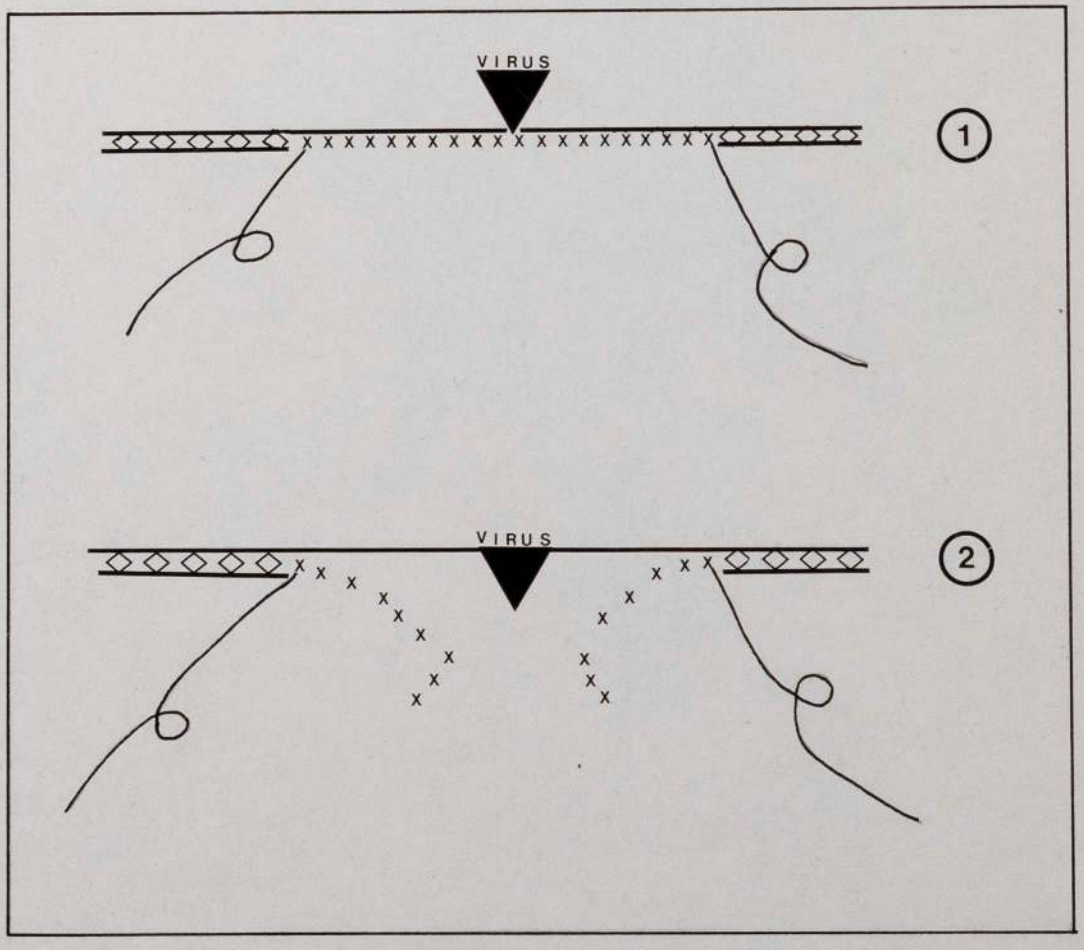


Figure 48 Possible cause of damage to DNA by a viral agent.

The suggestion that some form of viral infection is involved in the aetiology of Down's Syndrome requires further investigation. It is well known that viral agents can cause chromosome damage *in vivo*, chicken pox, measles, mumps (Aula, 1965), hepatitis (Aya and Makino, 1966), and rubella (Kuroki *et al.*, 1966) have all been implicated. The presence of these might well cause damage to the proposed "active areas", causing breakage or rearrangement of genetic material. If the number of areas vary, then the effect of the viral DNA would be proportional to that number present on the chromosome. Thus, the greater the number of N.O.R. "active areas", the greater the chance of malfunction in the subsequent cell divisions.

The effect caused by such a virus, whether it is of a specific type or molecular weight, would be disruptive, not only to the immediate DNA material, but to the function of the nucleolus. This could also be the same for all of the other possible factors, previously mentioned. Another consideration would be the exact location of the nucleolus in relation to the nuclear membrane. This, together with the number of participating chromosomes or fused nucleoli, might mean that the number of potential agents is selective for specific somatic conditions.

Recent research (Stahl *et al.*, 1976) has shown that by using *in situ* hybridisation techniques, ribosomal cistrons have been visualised on the periphery of the nucleoli. It was found that when the nucleoli were small

and numerous, one or two granules are seen in contact with the circumference of each nucleolus. In the absence of a fibrillar centre, localisation of the ribosomal cistrons is more diffuse, and may correspond to the entire nucleolar surface. From these observations, they deduced that the short arms of the acrocentric chromosomes are closely associated with the nucleolus and consequently make up part of the nucleolar-associated chromatin.

Another way in which this predisposition could be shown to manifest itself is when both parents exhibit a greater specific affinity of the 'G' group to associate. In these results, the stronger type 'A' associations between G21 and G22 chromosomes would appear to be the vector of the possible non-disjunction, and as such increase the chances of this event occurring. However, when one parent only has a higher incidence of this type 'A', G21-22 association, the chances are reduced as compared with the above, but still greater than those of normal parents. Thus, when another factor is introduced, and non-disjunction occurs, it is perhaps this group which is responsible for showing such a seasonal variation in the incidence of non-age related mongolism.

It is clearly seen from the results of this study that no sex selectivity exists in the cases analysed of parents of non-age related trisomy 'G'. As such, G21 trisomy may equally be the result of non-disjunction on either the

maternal or paternal side. This endorses the fact that such an error could occur at either the first or second meiosis, or rarely at the first mitotic division of the zygote, with a resultant disappearance of the monosomic cell line.

Over the past five years, differential staining methods and polymorphisms have been used for the detection of the parental origin of the extra G21 chromosome in cases of trisomy 'G'. Altogether, 62 cases have been analysed which were informative with respect to the non-disjunction of chromosomes 21 in oogenesis or spermatogenesis, as well as in the first or second meiotic division (Langenbeck et al., 1976). Results showed that the origin of the G21 was significantly more frequent as a result of an error occurring in oogenesis (43 cases) than in spermatogenesis (19 cases). It must be noted however, that all age groups were included in the sample.

As far as determining whether there was any preference of the malsegregation occurring at either the first or second meiotic divisions, the results were conflicting. From the cases analysed, no significant difference was observed between the non-disjunction occurring during first or second meiotic divisions. However, mathematical analysis and data from the literature showed that trisomy 'G' is caused between 5 to 10 times more frequently by a malsegregation in the first meiotic division.

The role of the G22 chromosome in satellite association patterns needs to be discussed. From the results in

this study it is seen that there are twice the number of type 'A' associations found in parents of mongol children when a 21:22 association is involved in the configuration. Analysis of the results in the series of Curtis and Cooke (1974), Mattei et al., (1974), Taysi (1975) and Hansson (1975) also show this feature. This particular observation is perhaps the most significant in any of the results, and its presence in any satellite association would possibly implicate its role in the etiology of chromosomal non-disjunction.

The pertinent question that needs to be answered however, regards the incidence of trisomy 22. As the hypothesis stands, an equal number of trisomy 21 and trisomy 22 cases should be born each year. This theory might well have been acceptable however, before the introduction of differential banding, if one assumes that no phenotypic differences exist. At present, there have been 17 cases of trisomy 22 reported (Penchaszadeh and Coco, 1975), all with inconsistent phenotypes not showing any features of the cliassical Down's Syndrome. This incidence is so low that some form of selectivity must be involved if the original hypothesis is to be correct.

There may be several reasons why the G22 chromosome is involved far less in trisomies than G21. Perhaps the most plausible and simplest explanation would be that the G22 pair replicate earlier than the G21 pair. This means that they would be complete, and separate, and that the chances of them being influenced by a partly replicated

G21 chromosome is unlikely. Alternatively, it may be purely mechanical as the G22 is larger, or perhaps a combination of both.

Although the sample used in this study is relatively small when compared to other larger series, the depth of analysis of individual patterns and categories is much greater. This would mean that any real significance of variation in the number of specific associations is accurately classified. It could be argued that a similar result might have been obtained by using a larger sample with fewer criteria. However, the use of a positional parameter to the existing definition of a satellite association is seen as an important addition, not only in detailed analysis, but in understanding its role in interphase.

It is recognised that all of the observations in this study were performed on mitotic cells, and that mitotic events do not necessarily correspond with those in meiosis. It would therefore be valuable to continue this study using meiotic material and to compare findings. It would be of particular interest to look at the chiasmata frequency in relation to parental age as well as the speed of dispersion of nucleolar material in both ovarian and testicular material.

Another future extension of this study would be in the use of the new silver methods to determine densiometrically, any variation in the nucleolar organising regions in

relation to the satellite association frequencies. By this, and the other banding methods, detailed observations utilising markers and polymorphisms on the acrocentric chromosomes should enable a better understanding of parental transmission to be formulated.

Mikkelsen (1976) in Denmark has found that there is an apparent increase in the number of Down's infants being born to younger parents. The explanation for this is not only in the improved diagnosis of the patients, but a possible environmental involvement. However, Fujita and Matsunaga (1976) in their survey over the past 30 years in Japan, found that there was no indication that the risk to younger mothers having Down's children was increased.

If these conflicting surveys are correct, perhaps further investigation into the differences in culture and life-style between these two countries needs to be examined. To comment on the local incidence of Down's children born to younger parents is perhaps unfair in the light of personal experience, but the trend is towards the findings of Mikkelsen (1976), and not those of the Japanese.

The obvious choice for investigation was the oral contraceptive pill. Since McQuarrie et al., (1970) first reported an increased number of chromosome aberrations in females on the pill, this subject has always generated discussion. Recent reports have now shown however, (Janerich et al., 1976 and Fuertes de la Haba et al., 1976),

that no significant differences exist between pill users, and that no evidence is established as to the effect of oral contraceptives and abnormal numerical and structural chromosome patterns. However, it would be of great interest to extend this survey to examine the time lapse between cessation of the oral contraceptive and the fertilisation of the ovum.

The reason why certain chromosomes show a selectivity towards non-disjunction remains speculative. However, what has been shown in this study is that in a selected group of the population, there exists a significant difference in satellite association behaviour. This finding is sufficiently important so as to suggest that it is a major factor in the aetiology of trisomy 'G' in non-age related parents. Whether the associations are as a result of an initial genetic predisposition, or as a reaction to indogenous or exogenous influences remains unknown. However, it is suggested that the interaction between satellited acrocentric chromosomes acts as a direct indicator for potential cell malsegregation, as a result of influences in the nucleolar organising regions.

V. SUMMARY OF CONCLUSIONS

1) The role of the post cultural hypotonic solution has been found to be of critical importance, especially if true associations are to be evaluated. The use of 25% Hanks/distilled water as the hypotonic solution provided fewer, true satellite associations. Thus, only those exhibiting the greatest attraction, i.e. the possible vectors of malsegregation, remain. The consistency of the results show that it is possible to evaluate accurately individual association patterns, and so detect any significant changes.

2) It is demonstrated that the frequency of satellite associations gradually rises with the increase in the molarity of the hypotonic solution. No effect on the frequency is seen in the cells subjected to the hypotonic solution for varying times.

3) Young parents of Down's infants showed the following characteristic satellite association patterns as compared to control parents:

   a) A statistically significant increase in frequency of 21:22 type A di-associations.

   b) A statistically significant increased frequency of tri-associations; a high proportion of which involved chromosomes 21 and 22, most often in type A associations.

4) There does not appear to be any indication that there is a sex predisposition towards any particular satellite association configuration. In both parents and controls, no difference was observed between the sexes within each group.

VI. APPENDIX I

VI.i) Culture Technique

a) Method

1) The culture is set up in 10cm^3 plastic culture tubes under sterile conditions as follows:

Parker TC199 medium	-	5cm^3
Foetal calf serum	-	0.75cm^3
Phytohaemagglutinin	-	0.05cm^3
Whole blood (heparinised)	-	0.4cm^3

2) The tubes are incubated at 37°C for 70 hours. All tubes are mixed twice daily.

3) 0.5cm^3 of 0.02% Colchicine is added to each tube, and incubation continued for a further 1½ hours at 37°C.

4) The cultures are transferred to centrifuge tubes and centrifuged at 1,000 r.p.m. for 5 minutes.

5) The supernate should be clear. This is carefully removed taking care not to disturb cell deposit.

6) 4cm^3 of pre-warmed hypotonic solution is then added to the tubes. This is placed on the shaker at 37°C for the required time.

7) The tubes are centrifuged for 7 minutes at 1,000 r.p.m.

8) The supernate is carefully removed.

9) The cells are fixed. Fresh fixative, Methanol/Glacial Acetic Acid (3:1) is cooled to $4^{o}C$. The fixative is then carefully added drop by drop on to the cell deposit and quickly mixed using a Pasteur pipette.

10) Leave for at least ½ hour before changing the fixative 3 times.

11) Centrifuge and remove supernatant fixative.

12) Add fresh fixative until correct dilution of cells obtained (slightly cloudy - approximately $0.5cm^{3}$).

13) Cells should not clump together.

14) Clean glass microscope slides are removed from ice-box in the refrigerator immediately prior to making slides.

15) The cell suspension is carefully mixed and dropped onto the slide from a height of approximately 12". The slide is held horizontally and the Pasteur pipette directly above it. Blowing the slide ensures a better distribution of the cells.

16) A total of six slides per case are made.

b) Solutions

1) Parker TC199 -
basic medium, single strength containing penicillin, streptomycin and sodium bicarbonate. Obtainable from: Burroughs Wellcome Ltd., Beckenham, Kent, BR3 3BS.

2) Foetal calf serum -
mycoplasma and virus screened. Obtainable from: Flow Laboratories, Irvine, Scotland, KA12 8NB.

3) Phytohaemagglutinin -
lyophilised - reconstituted with $5cm^3$ sterile distilled water. Obtainable from: Burroughs Wellcome Ltd., Beckenham, Kent, BR3 3BS.

4) Colcimid (Colchicine) -
aqueous solution (0.02%), Colcimid (Colchicine) 0.2g, sterile distilled water $1000cm^3$. Obtainable from: C.I.B.A. Chemicals Ltd.

5) Hypotonic solutions -
a) Potassium chloride - 0.075M aqueous, incubation time 8 minutes.
Potassium chloride - 0.5592g
Distilled water - $100cm^3$

b) Sodium citrate - 1% aqueous, incubation time 10 minutes.

Sodium citrate	-	1g
Distilled water	-	$100cm^3$

c) Hanks/water - 25% aqueous, incubation time 20 minutes.

Hanks balanced salt solution (Burroughs Wellcome Ltd.)	-	$25cm^3$
Distilled water	-	$75cm^3$

6) Fixative -

Glacial Acetic Acid	-	$10cm^3$
Methanol	-	$30cm^3$

VI.ii) Staining Techniques

1) Giemsa

a) Rinse slide in distilled water.

b) 1% aq. Giemsa solution - 5 minutes.

c) Rinse in distilled water, blot dry, mount in DPX.

Solutions - Giemsa - 1g

Distilled water - 100cm^3

Obtainable from: Searle-Gurr Products, High Wycombe, HP12 4HL.

2) Giemsa Banding

a) 2 x SSC solution is made using Sorrenson's buffer, equal volumes of A and B, as solvent. The pH is adjusted to 6.90.

b) Slides are immersed in 2 x SSC in glass troughs, and these are placed in a water bath at room temperature.

c) The water is allowed to reach 60^{o}C.

d) After 1½ hours in water bath, one slide from each case is removed and rinsed in ice cold 0.9% NaCl.

e) Trypsin solution (1 in 100 parts 0.9% NaCl) at 10^{o}C is placed onto the slides and left for 90 seconds.

f) This is quickly rinsed off using cold 0.9% NaCl.

g) The slides are stained using 1 in 10 Giemsa solution pH 6.8 for 5 minutes.

h) The slides are then dried, mounted in DPX, and examined to assess the affect of SSC and trypsin.

Further SSC treatment may be necessary. Exposure to trypsin may also need adjusting.

Until bands begin to appear, continue SSC treatment, this may take up to 3 hours.

When banding appears to be satisfactory, remove remaining slides and follow from 4 - 8.

Solutions -

i) 2 x SSC - sodium chloride (NaCl) - 17.53g

Tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$) - 8.82g

Buffered distilled water - $1000cm^3$

ii) Sorrenson's buffer A

Potassium dihydrogen orthophosphate - 0.454g

Distilled water - $1000cm^3$

iii) Sorrenson's buffer B

Di-potassium hydrogen orthophasphate - 0.473g

Distilled water - $1000cm^3$

iv) Trypsin - Bacto - Trypsin - reconstituted with $10cm^3$ sterile distilled water. Obtainable from: Difco Laboratories, West Molesey, KT8 OSE.

v) Giemsa - Gurr's improved R66 solution. Obtainable from: Searle-Gurr Products, High Wycombe, HP12 4HL.

3) Fluorescent Banding

a) Air dried slides are rehydrated using the following sequence:

70% alcohol	5 minutes
50% alcohol	5 minutes
Distilled water	5 minutes
Buffer (pH 7.4)	5 minutes

b) Slides are stained with Quinacrine Mustard solution for 40 - 45 minutes.

c) Rinse and leave the slides in buffer for:

Buffer (pH 7.4)	10 minutes
Buffer (pH 7.4)	5 minutes

d) Mount the slides leaving a very thin film of buffer between slide and coverslip; then seal.

Solutions -

i) McIlvain's buffer I -

0.1M citric acid	2.1g
Distilled water	$100cm^3$

ii) McIlvain's buffer II -

0.2M disodium hydrogen phosphate	7.16g
Distilled water	$100cm^3$

iii) McIlvain's buffer pH 7.4 -

Buffer I	$17.6cm^3$
Buffer II	$82.4cm^3$

iv) Quinacrine Mustard - 0.0050g
74.0P spirit $0.5cm^3$
Distilled water make up to $100cm^3$

4) Selective Silver Staining of Nucleolar Organising Regions

a) Slides are placed on a raised platform in a moisture-tight box.

b) The silver solution is flooded on to the slide, and then covered by a coverslip.

c) A small volume of distilled water is added to the bottom of the box to provide moisture, and the lid replaced.

d) The slides are incubated in this box for 18 hours (overnight) at $37^{\circ}C$.

e) Rinse the coverslip off with distilled water, wash slide well.

f) Stain the slides with 1% aq. Giemsa solution for 5 minutes.

g) The slides are then dried, mounted in DPX and then examined.

Solutions -

i) Silver solution -

Silver nitrate	1g
Distilled water pH 4.5-5.0	$2cm^3$

ii) Giemsa - 1g

Distilled water $100cm^3$

Obtainable from: Searle-Gurr Products, High Wycombe, HP12 4HL.

VII. APPENDIX II

VII.i) Results of Hypotonic Solution Experiments

			Percentage of Cells with one or more Satellite Associations		
Subject No.	Sex	Age	KCl	Sodium Citrate	Hanks with Distilled Water (1:3)
1	F	21	70	40	30
2	M	22	50	60	20
3	F	21	60	70	60
4	M	22	80	50	30
5	M	20	100	90	70
6	M	23	80	50	20
7	F	21	80	60	50
8	F	23	80	50	70
9	F	22	80	70	40
10	F	22	60	50	30
11	M	19	90	80	60
12	M	19	80	60	30
13	M	19	80	70	50
14	M	20	70	50	50
15	M	19	80	70	70
16	M	20	70	50	40
17	M	21	90	60	90
18	M	19	100	60	40
19	M	19	80	50	40
20	M	20	80	60	60
21	M	19	60	70	60
22	M	20	80	70	60
23	F	19	80	60	60
24	M	19	90	70	50
25	M	19	90	80	70
26	F	19	90	60	80
27	F	21	60	60	60
28	F	19	90	40	30
29	M	19	90	60	40
30	F	19	80	50	40
31	F	21	70	90	50
32	F	25	90	60	50
33	M	20	70	60	30
34	M	19	70	50	40
35	F	23	70	60	40

Table XXI Percentage of cells showing one or more satellite associations observed in 10 cells per subject

Hypotonic Treatment	Number of Controls Examined	Number of Metaphases Examined	Specific Group Associations Recorded								
			None	D/G	D/D	G/G	D/D/G	D/G/G	D/D/D	G/G/G	Others
Potassium Chloride	35	350	57	185	71	51	20	16	8	3	2
Tri-sodium Citrate	35	350	119	138	55	37	5	18	7	2	0
Hanks/ Water	35	350	171	87	48	40	9	6	1	1	0

Table XXII Comparison of the specific group satellite association patterns, as observed in the three hypotonic solutions

The Effect of Potassium Chloride (0.075M aq) as a Hypotonic Solution on Satellite Association Frequencies

No.	Sex	Age	G/G	G/D	D/D	D/D/D	D/D/G	G/G/G	D/G/G	Others
1	F	21	2	4	2	-	1	-	-	-
2	M	22	2	4	1	-	-	-	-	-
3	F	21	5	2	1	-	-	-	-	-
4	M	22	1	1	7	-	-	-	-	-
5	M	20	3	5	3	-	1	-	1	-
6	M	23	-	7	1	-	-	-	3	-
7	F	21	1	4	2	1	1	-	1	-
8	F	23	3	4	1	-	-	-	1	D/D/G/G
9	F	23	-	4	1	2	-	-	1	-
10	F	22	-	6	2	-	-	-	1	-
11	M	19	2	8	1	-	1	-	-	-
12	M	19	2	7	1	1	-	-	-	-
13	M	19	1	4	4	-	2	-	-	-
14	M	20	3	3	1	1	-	-	1	-
15	M	19	1	5	3	-	2	-	-	-
16	M	20	2	4	-	-	-	-	1	-
17	M	21	2	8	3	-	-	-	-	D/D/G/G
18	M	19	1	7	1	1	-	-	1	-
19	M	19	3	3	1	-	-	2	-	-
20	M	20	1	10	1	-	-	-	-	-
21	M	19	4	3	2	-	-	-	-	-
22	M	20	-	4	2	1	2	-	-	-
23	F	19	1	6	2	-	2	1	-	-
24	M	19	3	6	2	-	3	-	-	-
25	M	19	1	8	1	-	2	-	3	-
26	F	19	2	10	-	-	1	-	1	-
27	F	21	0	4	3	-	-	-	-	-
28	F	19	1	5	4	-	1	-	-	-
29	M	19	-	7	3	-	-	-	-	-
30	F	19	-	6	3	-	-	-	-	-
31	F	21	3	4	-	-	-	-	-	-
32	F	25	1	4	3	-	-	-	-	-
33	M	20	1	4	2	-	-	-	-	-
34	M	19	2	7	1	-	-	-	1	-
35	F	23	2	4	5	-	1	-	-	-

The Effect of Sodium Citrate (1% aq) as a Hypotonic Solution on Satellite Association Frequencies

No.	Sex	Age	G/G	G/D	D/D	D/D/D	D/D/G	G/G/G	G/G/D	Others
1	F	21	2	1	2	-	-	-	-	-
2	M	22	-	4	2	-	-	-	1	-
3	F	21	4	4	1	-	-	-	-	-
4	M	22	4	1	-	-	-	1	-	-
5	M	20	2	8	2	-	-	-	-	-
6	M	23	-	3	3	-	-	-	1	-
7	F	21	2	3	1	-	-	-	1	-
8	F	23	-	4	1	-	-	-	2	-
9	F	22	3	4	-	-	-	-	-	-
10	F	22	-	1	3	-	1	-	1	-
11	M	19	-	7	1	1	-	-	-	-
12	M	19	-	4	1	-	1	-	1	-
13	M	19	-	5	2	1	-	-	-	-
14	M	20	-	4	-	1	-	-	1	-
15	M	19	1	7	3	-	-	-	-	-
16	M	20	1	4	1	-	-	-	-	-
17	M	21	-	4	1	1	-	-	-	-
18	M	19	2	3	2	-	-	-	-	-
19	M	19	2	-	1	-	-	-	2	-
20	M	20	4	3	-	-	-	-	1	-
21	M	19	2	5	-	-	-	-	1	-
22	M	20	2	3	2	-	-	-	1	-
23	F	19	1	4	1	-	1	-	-	-
24	M	19	1	6	2	-	-	-	-	-
25	M	19	3	4	-	1	-	-	1	-
26	F	19	1	5	2	-	-	-	-	-
27	F	21	-	3	3	-	-	-	-	-
28	F	19	2	1	2	-	-	-	-	-
29	M	19	-	4	4	1	-	-	-	-
30	F	19	-	4	1	-	-	1	-	-
31	F	21	2	8	2	-	-	-	-	-
32	F	25	2	4	-	-	-	-	1	-
33	M	20	2	3	1	-	-	-	1	-
34	M	19	-	1	3	-	1	-	1	-
35	F	23	-	4	1	-	1	-	1	-

The Effect of Hanks Solution with Distilled Water (1:3) as a Hypotonic Solution on Satellite Association Frequencies

No.	Sex	Age	G/G	G/D	D/D	D/D/D	D/D/G	G/G/G	G/G/D	Others
1	F	21	-	1	2	-	-	-	-	-
2	M	22	-	-	1	-	1	-	-	-
3	F	21	2	1	3	-	-	-	-	-
4	M	22	-	1	1	-	1	-	-	-
5	M	20	-	6	1	-	-	-	-	-
6	M	23	1	1	-	-	-	-	-	-
7	F	21	-	4	2	-	-	-	-	-
8	F	23	2	4	-	-	-	-	1	-
9	F	22	-	4	-	-	-	-	-	-
10	F	22	1	1	1	-	-	-	-	-
11	M	19	-	3	4	-	-	-	1	-
12	M	19	1	2	-	-	-	-	-	-
13	M	19	2	-	4	-	-	-	-	-
14	M	20	1	4	-	-	-	-	-	-
15	M	19	3	3	3	-	-	-	-	-
16	M	20	3	4	-	-	-	-	-	-
17	M	21	-	6	3	-	2	-	-	-
18	M	19	1	2	1	-	-	-	-	-
19	M	19	1	2	-	-	-	-	1	-
20	M	20	3	5	-	-	-	-	-	-
21	M	19	4	4	-	-	-	-	-	-
22	M	20	1	2	3	-	1	-	-	-
23	F	19	1	5	1	1	1	-	-	-
24	M	19	1	2	1	-	2	-	-	-
25	M	19	2	2	-	-	-	1	2	-
26	F	19	1	4	4	-	-	-	-	-
27	F	21	-	2	4	-	-	-	-	-
28	F	19	-	1	2	-	-	-	-	-
29	M	19	2	1	1	-	-	-	-	-
30	F	19	1	1	3	-	-	-	-	-
31	F	21	1	2	1	-	-	-	1	-
32	F	25	2	2	-	-	-	-	-	-
33	M	20	1	1	1	-	-	-	-	-
34	M	19	2	1	-	-	1	-	-	-
35	F	23	-	3	1	-	-	-	-	-

VII.ii) Results of Satellite Association Patterns - Parents and Controls

	Number of Acrocentric Chromosomes Associating per Cell				
	None	2	3	4	Total
Fathers of children with Down's Syndrome	0.523	0.457	0.057	0.004	1.043
Control fathers of normal children	0.618	0.374	0.044	0.004	1.042
Total	1.142	0.832	0.102	0.008	2.085

No statistical significance between any of the comparisons (Mann and Whitney).

Table XXIII Mean frequency of satellite associations per cell (900 cells examined)

	Number of Acrocentric Chromosomes Associating per Cell				
	None	2	3	4	Total
Mothers of children with Down's Syndrome	0.507	0.471	0.071	0.008	1.057
Control mothers of normal children	0.601	0.373	0.023	0.001	0.998
Total	1.108	0.844	0.094	0.008	2.056

No statistical significance between any of the comparisons (Mann and Whitney).

Table XXIV Mean frequency of satellite associations per cell (900 cells examined)

Subject Number	Sex	Age	Number of Cells Examined	Number of Chromosomes in Association				
				None	2	3	4+	Total
5	F	22	100	44	54	6	0	60
6	M	23	100	46	51	3	0	54
15	M	34	100	38	74	4	0	78
16	F	32	100	58	41	5	0	46
19	F	20	100	54	47	3	1	51
20	M	22	100	46	48	2	0	50
21	F	21	100	39	53	11	4	68
22	M	23	100	62	38	5	0	43
23	F	22	100	60	37	7	0	44
24	M	24	100	48	46	9	0	45
25	M	29	100	49	44	10	4	58
26	F	26	100	47	48	10	0	58
27	F	29	100	44	57	6	0	63
28	M	29	100	59	41	5	0	46
31	F	28	100	54	41	8	2	51
32	M	30	100	60	36	10	0	46
39	F	34	100	35	46	8	0	54
40	M	34	100	65	34	4	0	38

Table XXV Distribution of the frequency of satellite associations in parents of children with Down's Syndrome (mongolism)

Subject Number	Sex	Age	Number of Cells Examined	Number of Chromosomes in Association				
				None	2	3	4+	Total
13	F	26	100	54	42	5	0	47
14	M	26	100	58	39	6	1	46
33	F	26	100	64	38	0	1	39
34	M	28	100	67	35	1	0	36
41	F	29	100	62	37	5	1	43
42	M	27	100	63	37	6	1	44
43	F	21	100	69	34	4	0	38
44	M	23	100	64	35	5	0	40
45	M	24	100	62	34	6	0	40
46	F	24	100	62	39	3	0	42
47	M	34	100	52	42	9	0	51
48	F	33	100	63	40	0	0	40
51	M	28	100	68	34	3	0	37
52	F	27	100	67	38	2	0	40
59	M	30	100	65	33	3	1	37
60	M	29	100	65	36	0	0	36
61	M	32	100	58	46	2	0	48
62	F	32	100	65	34	1	0	35

Table XXVI Distribution of the frequency of satellite associations in control parents of normal children

VII.iii Sample Details - Parents and Controls

S/A NUMBER	5	6
SEX	F	M
DATE OF BIRTH	1.12.50	23.11.49
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 20.8.73 F Downs Syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Nil Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant No	None relevant No
CHROMOSOME CONSTITUTION	46,XX	46,XY

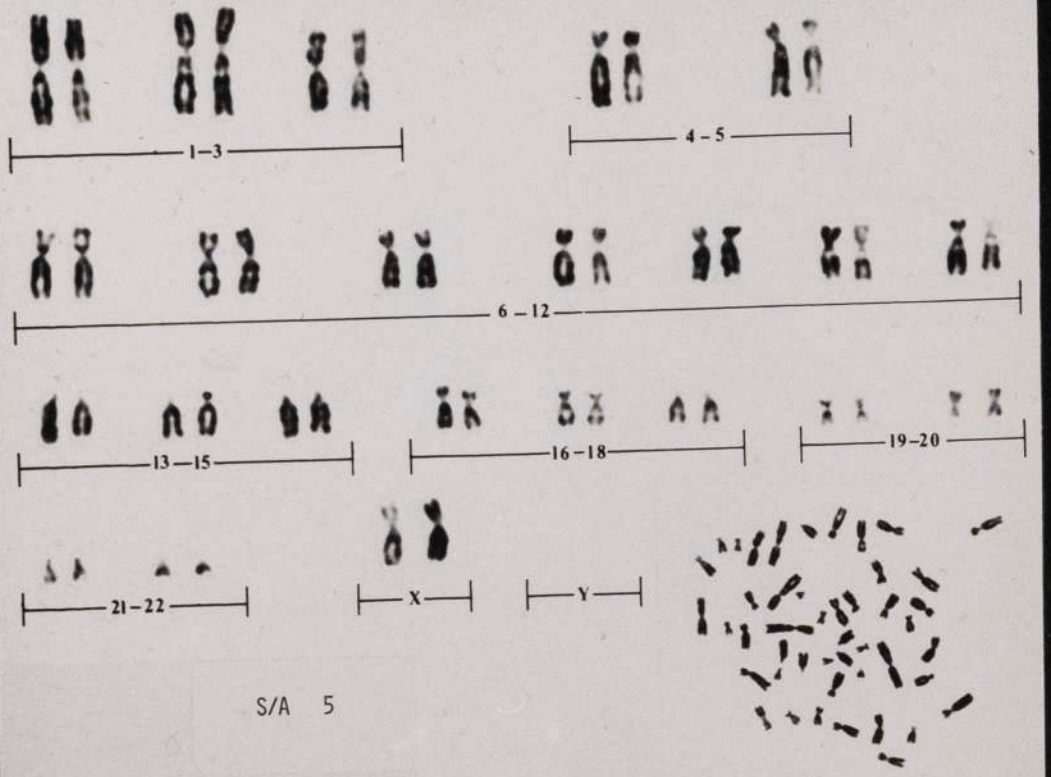
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14			
	13 - 15	1	3	4
	14 - 14			
	14 - 15		3	3
	15 - 15			
G/G	21 - 21	2	2	4
	21 - 22	8	10	18
	22 - 22		2	2
D/G	13 - 21	1		1
	13 - 22			
	14 - 21	1	2	3
	14 - 22	2	3	5
	15 - 21	4	4	8
	15 - 22		6	6
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21		1	1.
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

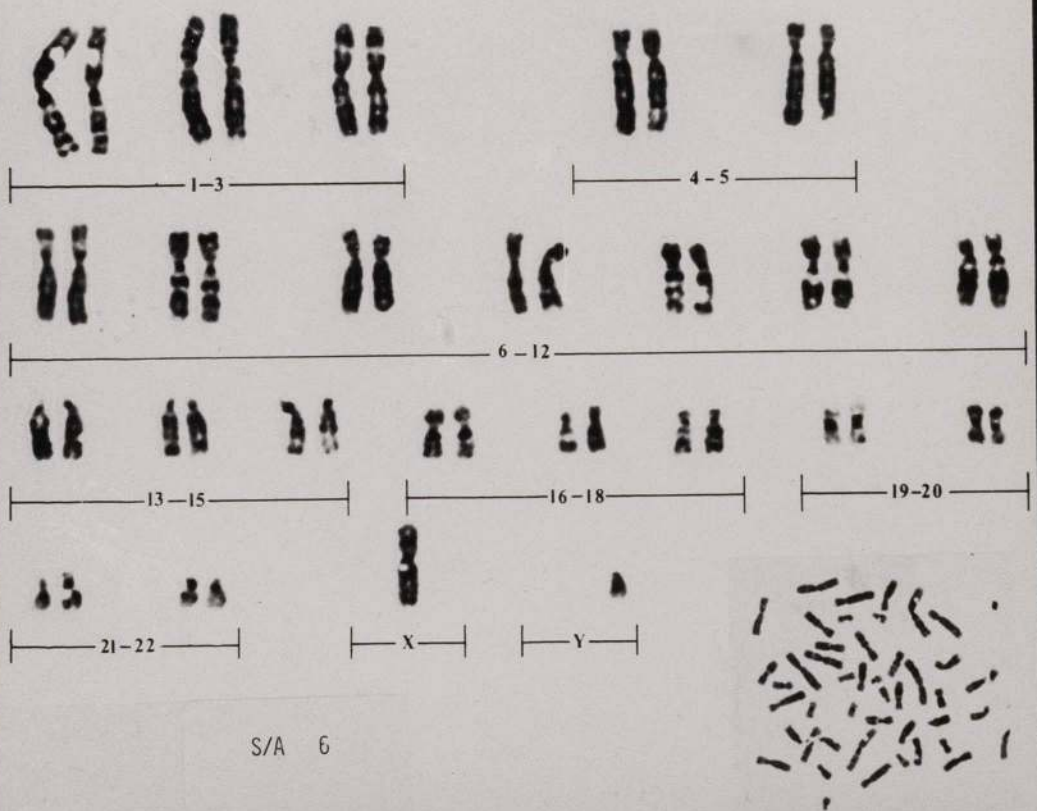
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	1		1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	2		2
	15 - 22 - 22			
D/D/D	13 - 14 - 15		1	1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21	1		1
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		2	2
	13 - 15			
	14 - 14			
	14 - 15		1	1
	15 - 15		1	1
G/G	21 - 21	1	1	2
	21 - 22	5	4	9
	22 - 22		4	4
D/G	13 - 21	6	3	9
	13 - 22	1	4	5
	14 - 21	2	1	3
	14 - 22	2	4	6
	15 - 21	2	3	5
	15 - 22	1	3	4
D/D/G	13 - 13 - 21			
	13 - 13 - 22	1		1
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1		1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 5


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 6

S/A NUMBER	15	16
SEX	M	F
DATE OF BIRTH	28.10.39	30.5.41
NATIONALITY	British	British
CHILDREN	1	2	3
Date of Birth	9.3.60	15.9.67	7.2.73
Sex	M	F	M
Details	Normal	Normal	Downs syndrome
AFFECTED PREGNANCY		
Drugs	Nil	
Accidents	Nil	
Radiation	Nil	
Other	Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

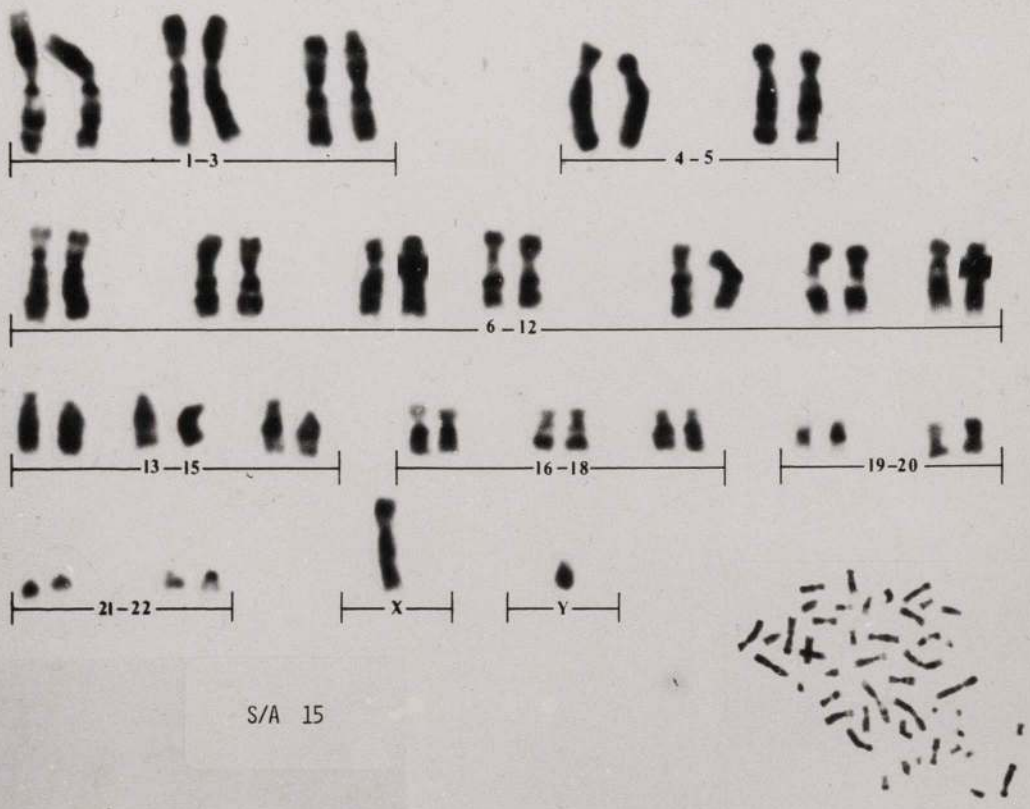
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	3	2	5
	13 - 15	2	2	4
	14 - 14	1		1
	14 - 15	8	2	10
	15 - 15			
G/G	21 - 21			
	21 - 22	8	7	15
	22 - 22			
D/G	13 - 21	1		1
	13 - 22	4	6	10
	14 - 21		2	2
	14 - 22	6	13	19
	15 - 21		1	1
	15 - 22	4	2	6
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22	1		1
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21		1	1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

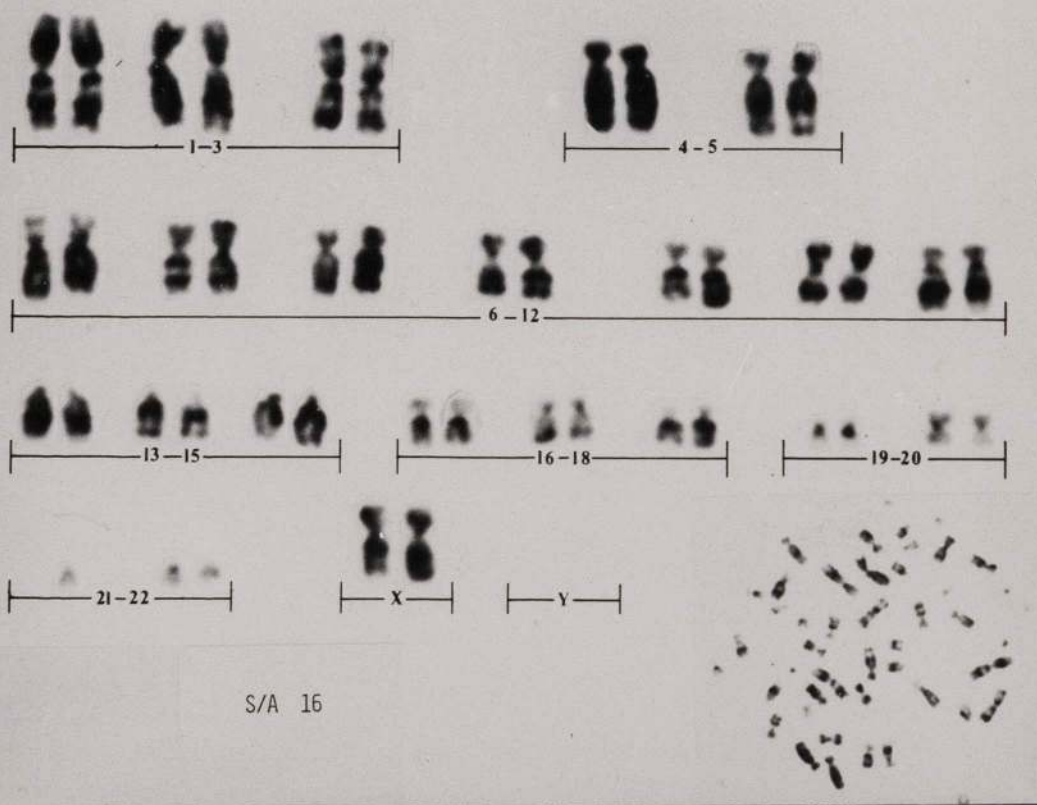
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	1		1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14		1	1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	1	2
	13 - 15		1	1
	14 - 14		1	1
	14 - 15		1	1
	15 - 15			
G/G	21 - 21			
	21 - 22	7	5	12
	22 - 22			
D/G	13 - 21		5	5
	13 - 22	2	3	5
	14 - 21	2	3	5
	14 - 22	2	1	3
	15 - 21	1	2	3
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22		1	1
	14 - 15 - 21	2		2
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	1		1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21		1	1
	21 - 21 - 22			
OTHER				


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 15


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 16

S/A NUMBER	19	20
SEX	F	M
DATE OF BIRTH	28.1.54	15.11.51
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 28.12.73 F Downs syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Iron only Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

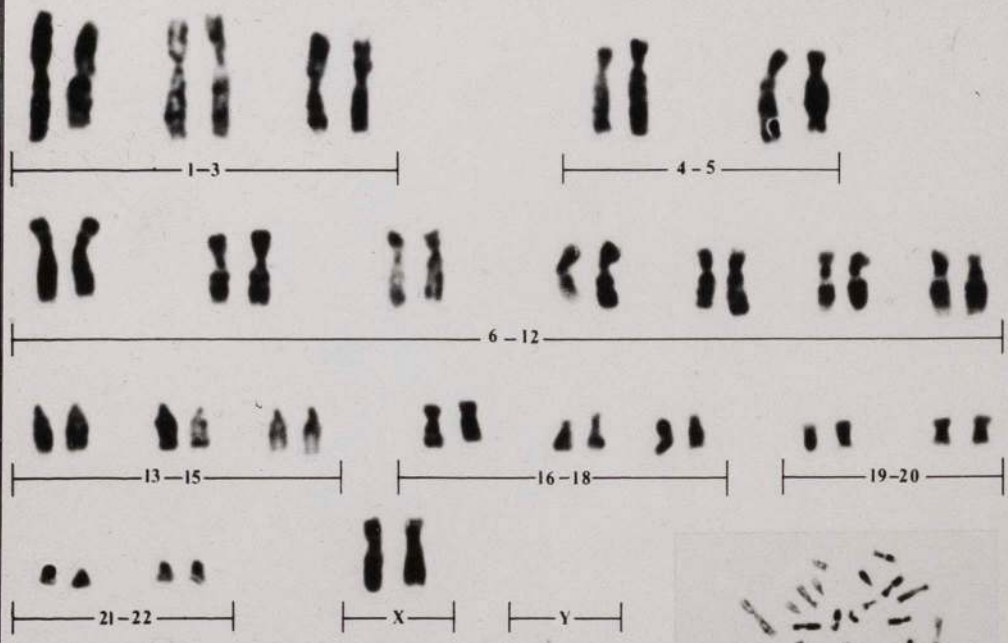
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1		1
	13 - 14	6	3	9
	13 - 15		1	1
	14 - 14			
	14 - 15	1	3	4
	15 - 15		1	1
G/G	21 - 21	1	1	2
	21 - 22	3	3	6
	22 - 22		4	4
D/G	13 - 21	1	1	2
	13 - 22		1	1
	14 - 21	1	5	6
	14 - 22	1	4	5
	15 - 21	1	1	2
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22		1	1
	15 - 15 - 21			
	15 - 15 - 22			

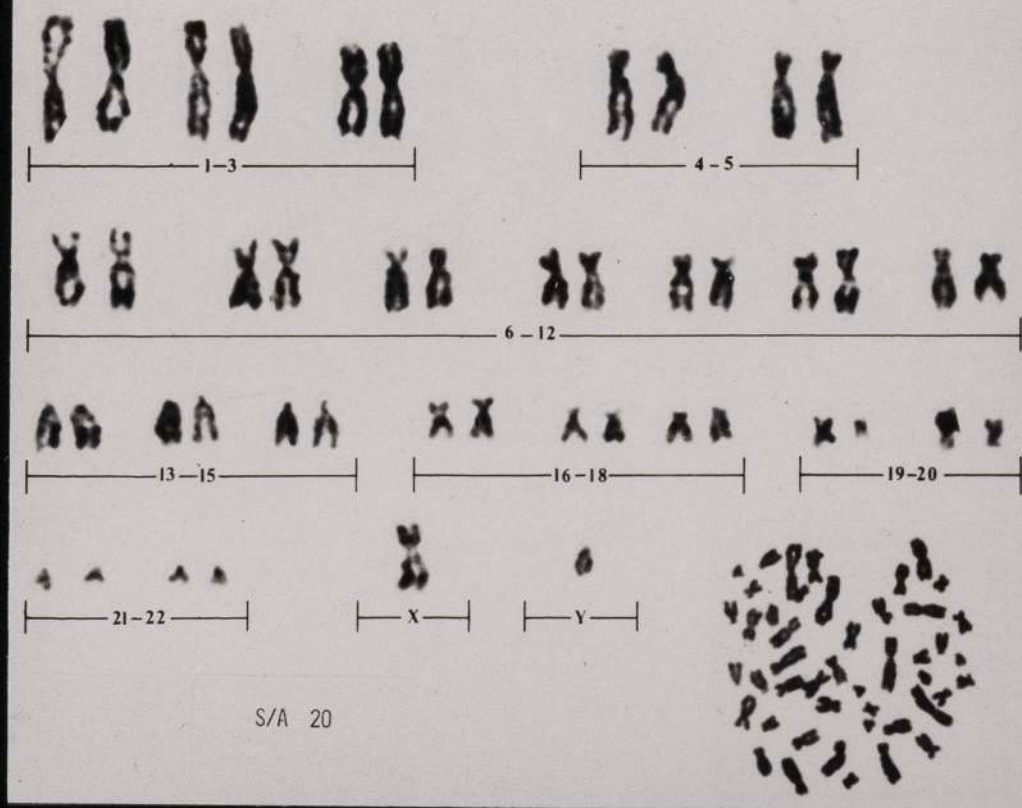
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22		1	1
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	13-14-22-21		1	1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	3	2	5
	13 - 15			
	14 - 14			
	14 - 15	1	2	3
	15 - 15			
G/G	21 - 21			
	21 - 22	8	10	18
	22 - 22	2		2
D/G	13 - 21	1	1	2
	13 - 22	1	1	2
	14 - 21	1	7	8
	14 - 22	3		3
	15 - 21	1		1
	15 - 22	1	3	4
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22	1		1
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19–20
21 – 22
X
Y

S/A 19


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19–20
21 – 22
X
Y

S/A 20

S/A NUMBER	21	22
SEX	F	M
DATE OF BIRTH	4.10.52	23.2.51
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 19.1.74 F Downs syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Nil Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

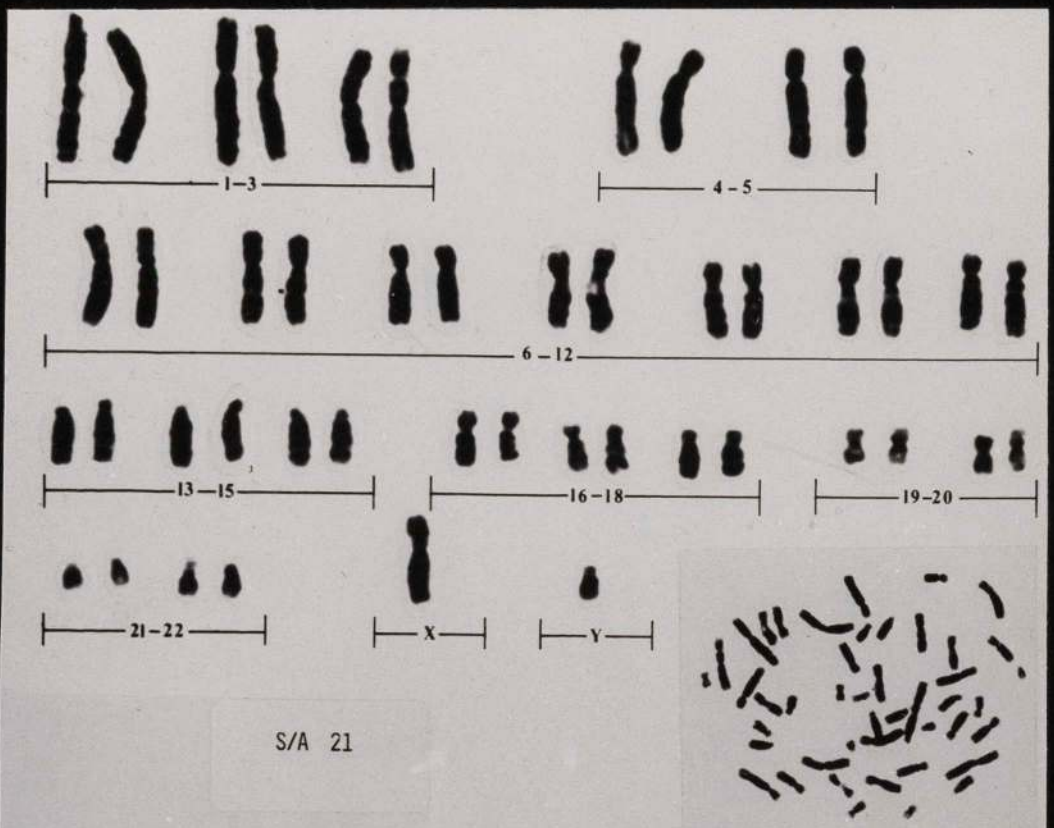
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		1	1
	13 - 15			
	14 - 14			
	14 - 15		2	2
	15 - 15		1	1
G/G	21 - 21		1	1
	21 - 22	23	11	34
	22 - 22		1	1
D/G	13 - 21	2		2
	13 - 22	1	1	2
	14 - 21		2	2
	14 - 22	2	2	4
	15 - 21	1	1	2
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22	1		1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

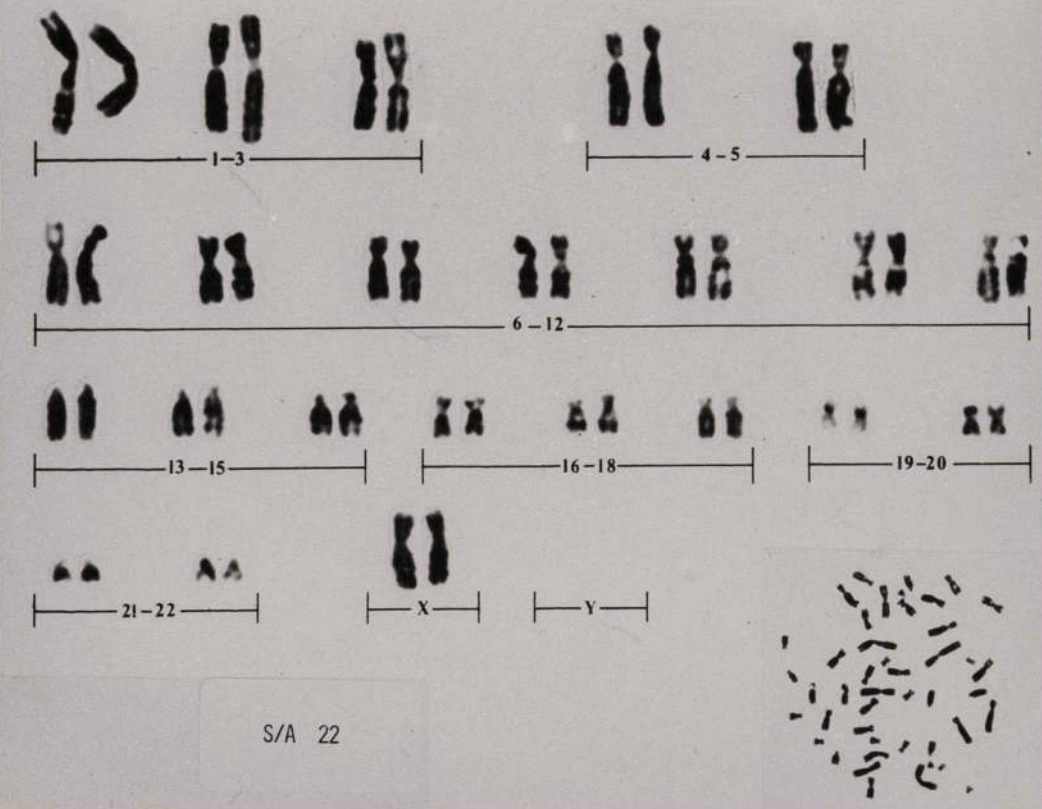
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	2		2
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	2	1	3
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1	2	3
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21	1	1	2
	21 - 21 - 22			
OTHER	21-22-22-13		1	1
	14-21-22-22		1	1
	13-21-21-22-22	) 1		1
	13-21-21-22-22	) 1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13		1	1
	13 - 14		4	4
	13 - 15		1	1
	14 - 14			
	14 - 15	3	1	4
	15 - 15		1	1
G/G	21 - 21			
	21 - 22	4	5	9
	22 - 22	1	1	2
D/G	13 - 21	1		1
	13 - 22		1	1
	14 - 21	1	2	3
	14 - 22	2	2	4
	15 - 21	2	1	3
	15 - 22	1	3	4
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21		1	1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22	1		1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	1		1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15		1	1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15	1		1
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 21


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 22

S/A NUMBER	23	24
SEX	F	M
DATE OF BIRTH	9.3.52	17.11.49
NATIONALITY	British	British
CHILDREN	1	2
Date of Birth	14.9.70	4.1.74
Sex	M	F
Details	Normal	Downs syndrome
AFFECTED PREGNANCY		
Drugs	Iron and vitamins	
Accidents	Nil	
Radiation	Nil	
Other	Nil	
FAMILY HISTORY		
Illness		
Affected Members	None relevant	None relevant
Other		
CHROMOSOME CONSTITUTION	46,XX	46,XY

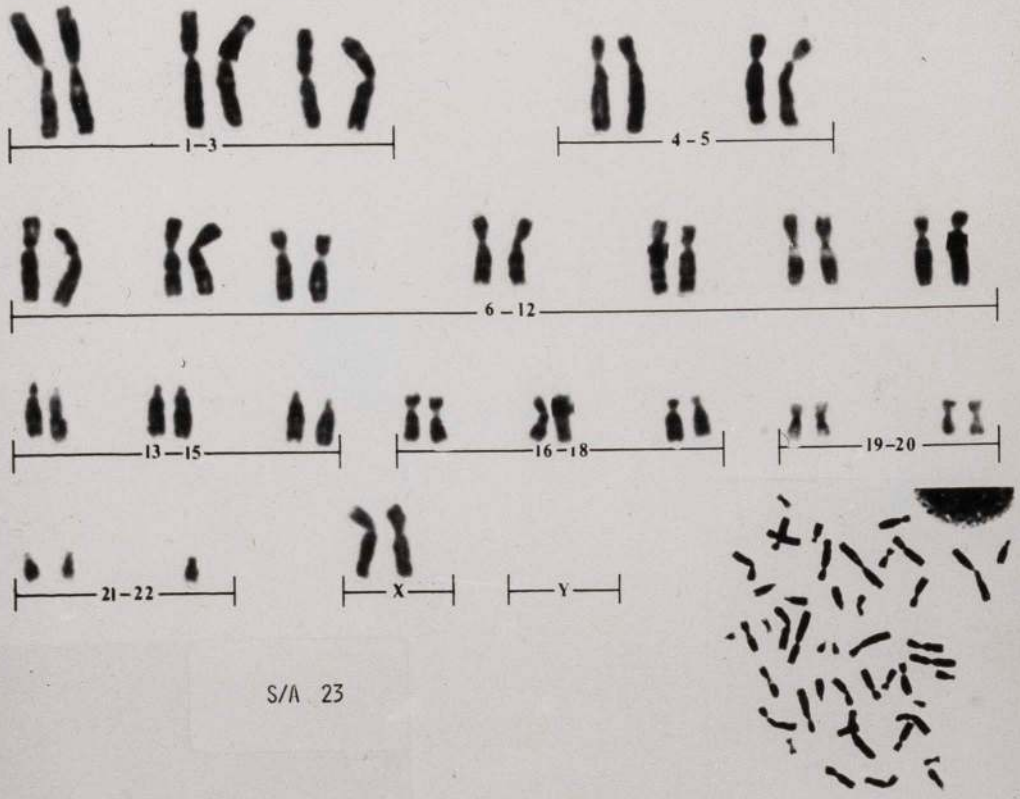
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13		1	1
	13 - 14	1	1	2
	13 - 15			
	14 - 14			
	14 - 15	1	1	2
	15 - 15			
G/G	21 - 21	2	2	4
	21 - 22	10	2	12
	22 - 22			
D/G	13 - 21	1	1	2
	13 - 22			
	14 - 21	1	3	4
	14 - 22	2	2	4
	15 - 21	3	2	5
	15 - 22	1		1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

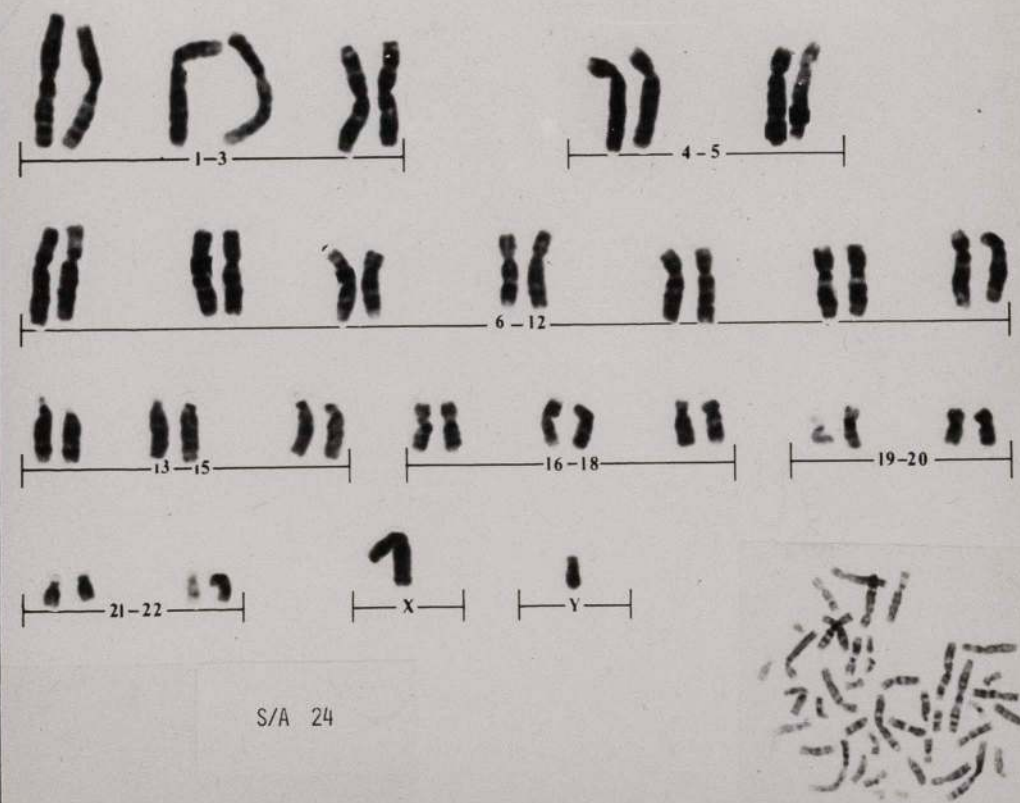
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	2		2
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1		1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21		2	2
	21 - 21 - 22	1		1
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	2	3
	13 - 14	3	3	6
	13 - 15	2		2
	14 - 14			
	14 - 15		7	7
	15 - 15			
G/G	21 - 21	1		1
	21 - 22	3	9	12
	22 - 22			
D/G	13 - 21	2	2	4
	13 - 22		3	3
	14 - 21	1	3	4
	14 - 22		1	1
	15 - 21			
	15 - 22	2	1	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	2		2
	13 - 14 - 22	1		1
	13 - 15 - 21	1		1
	13 - 15 - 22	1		1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21		1	1
	14 - 15 - 22		1	1
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22		1	1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13	1		1
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 23


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 24

S/A NUMBER	25	26
SEX	F	M
DATE OF BIRTH	21.7.47	2.2.45
NATIONALITY	Irish	British
CHILDREN Date of Birth Sex Details	1 26.1.74 M Downs syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Iron only Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant Not known	None relevant Not known
CHROMOSOME CONSTITUTION	46,XX	46,XY

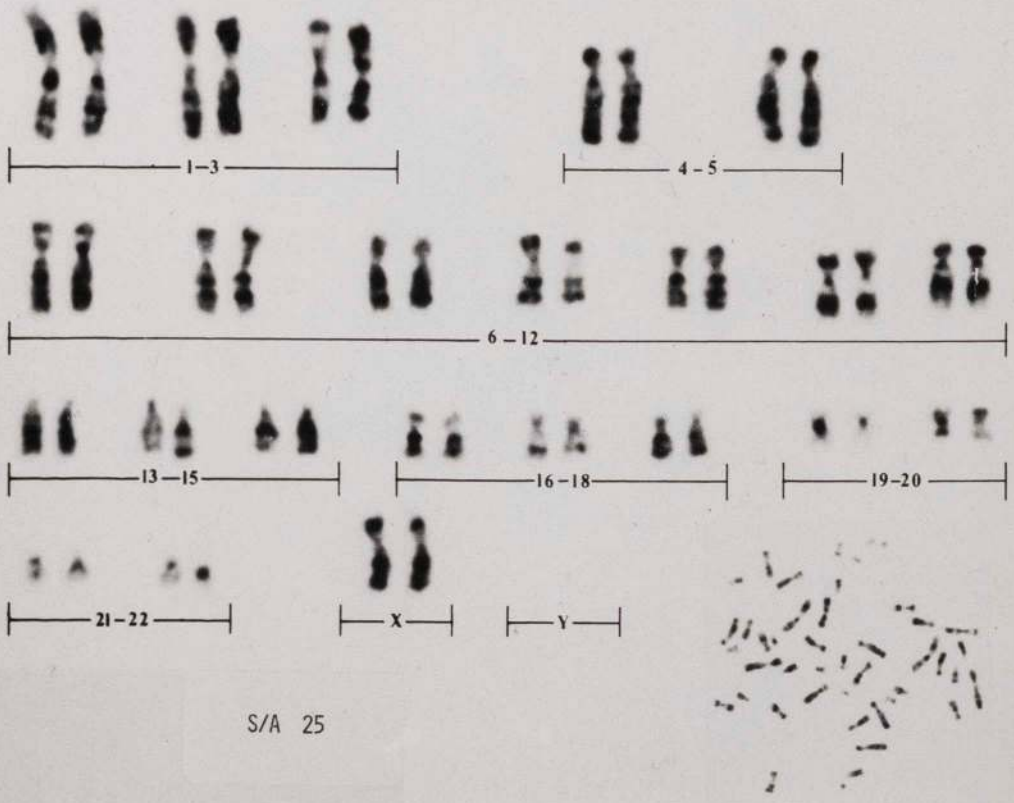
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1		1
	13 - 14	4	4	8
	13 - 15	1	1	2
	14 - 14	1	1	2
	14 - 15	5	5	10
	15 - 15	2		2
G/G	21 - 21			
	21 - 22	2		2
	22 - 22			
D/G	13 - 21	1	1	2
	13 - 22		1	1
	14 - 21	1	3	4
	14 - 22		3	3
	15 - 21	3	1	4
	15 - 22	2	1	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21		1	1
	13 - 14 - 22			
	13 - 15 - 21	1		1
	13 - 15 - 22	1		1
	14 - 14 - 21	1		1
	14 - 14 - 22			
	14 - 15 - 21	1	1	2
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

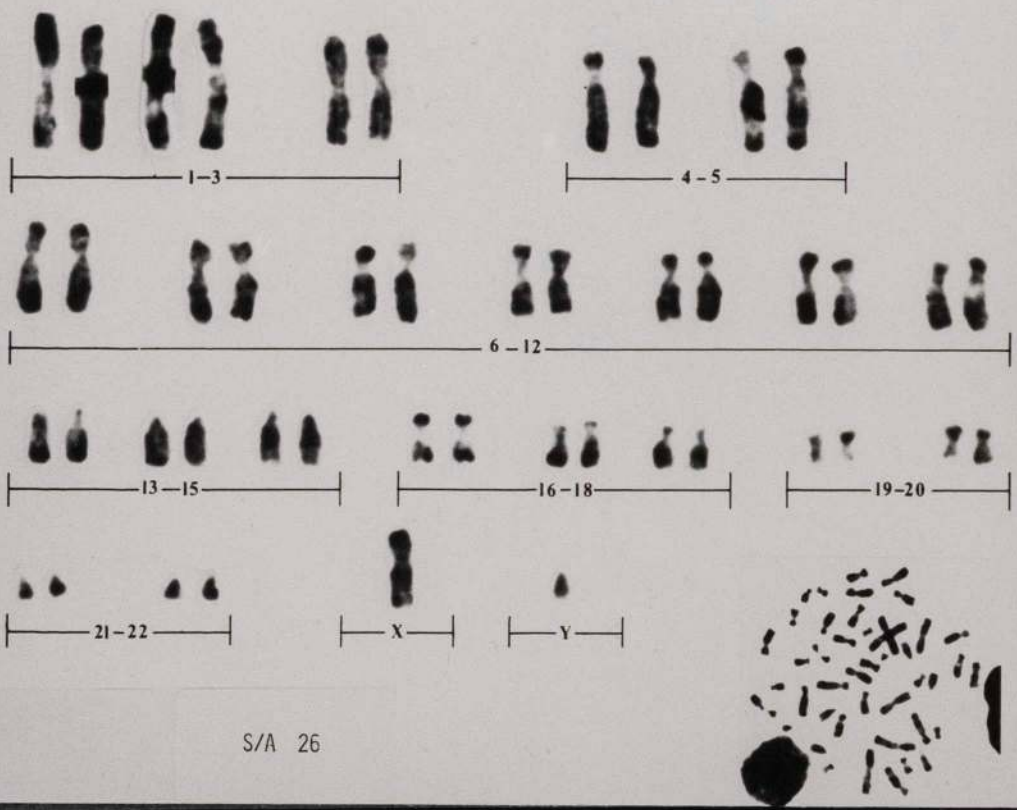
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15	2		2
	13 - 13 - 14	1		1
	13 - 14 - 14	1		1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	13-14-15-21	) 1		1
	13-14-15-21	) 1		1
	13-14-15-21-15	1		1
	13-14-14-22	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		1	1
	13 - 15		1	1
	14 - 14			
	14 - 15		2	2
	15 - 15	1		1
G/G	21 - 21			
	21 - 22	10	11	21
	22 - 22		2	2
D/G	13 - 21	1	1	2
	13 - 22	1	2	3
	14 - 21		8	8
	14 - 22	1	2	3
	15 - 21	1	2	3
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22	1		1
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21	1		1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22		1	1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	2		2
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22	1		1
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21	1	1	2
	21 - 21 - 22	1	1	2
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 25


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 26

S/A NUMBER	27	28
SEX	F	M
DATE OF BIRTH	12.1.45	3.5.45
NATIONALITY	British	British
CHILDREN	1	2
Date of Birth	6.10.67	20.6.69
Sex	F	M
Details	Normal	Downs syndrome
AFFECTED PREGNANCY		
Drugs	Nil	
Accidents	Nil	
Radiation	Nil	
Other	Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

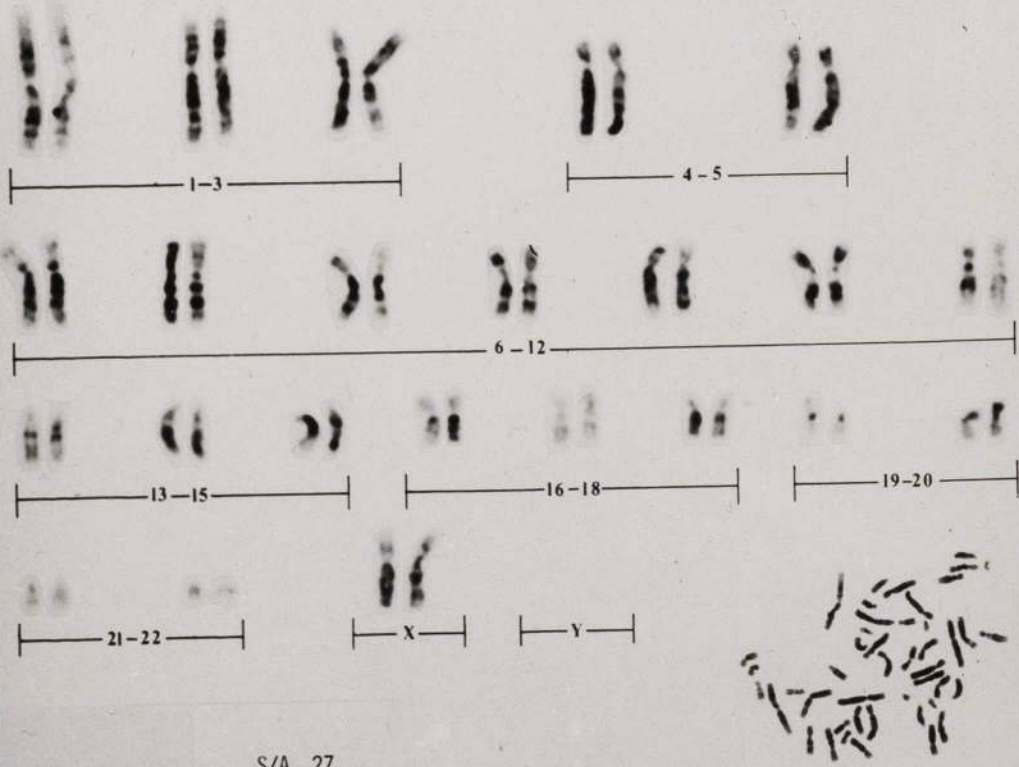
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1		1
	13 - 14	4	3	7
	13 - 15	1	2	3
	14 - 14		1	1
	14 - 15	5	3	8
	15 - 15	1		1
G/G	21 - 21	1		1
	21 - 22	5	3	8
	22 - 22		1	1
D/G	13 - 21	2	2	4
	13 - 22	1	2	3
	14 - 21	4	4	8
	14 - 22	2	3	5
	15 - 21	2	1	3
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21	1		1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

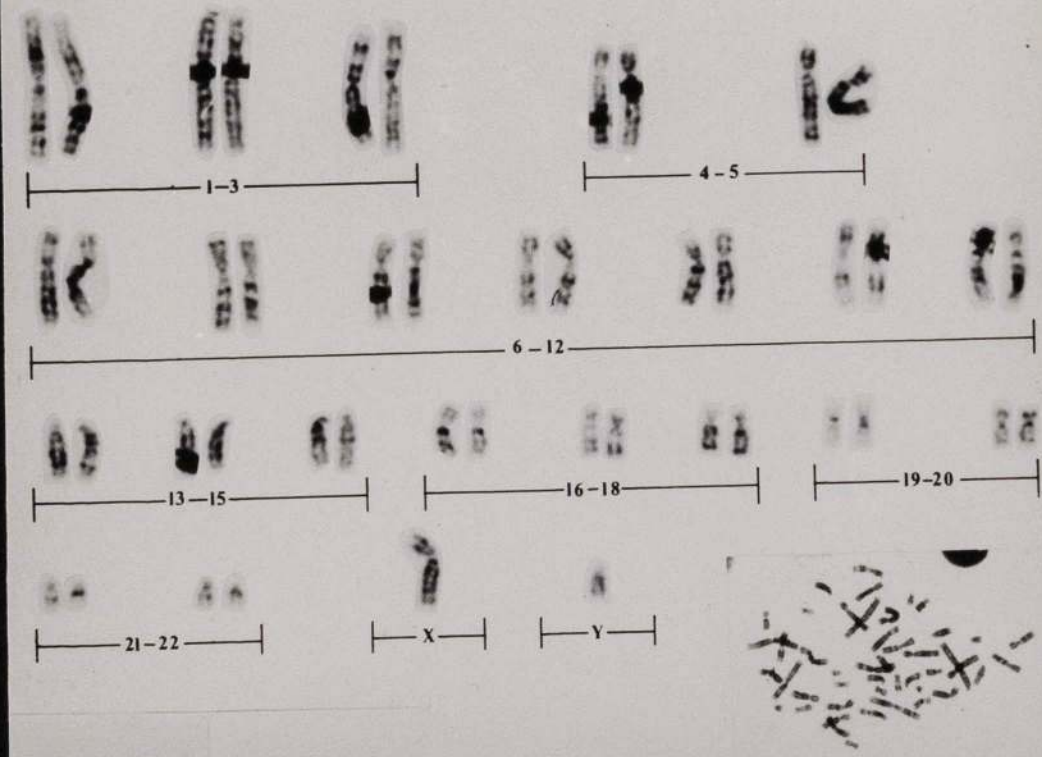
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21	1		1
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21	1		1
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14		1	1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	2		2
	13 - 14	2	2	4
	13 - 15			
	14 - 14			
	14 - 15	1	2	3
	15 - 15	1	1	2
G/G	21 - 21	1	1	2
	21 - 22	3	8	11
	22 - 22			
D/G	13 - 21		3	3
	13 - 22			
	14 - 21	2	3	5
	14 - 22	1	3	4
	15 - 21		2	2
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22	1		1
	14 - 21 - 21			
	14 - 21 - 22		1	1
	14 - 22 - 22	1		1
	15 - 21 - 21		1	1
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 27


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 28

S/A NUMBER	31	32
SEX	F	M
DATE OF BIRTH	21.6.46	20.12.43
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 6.11.70 M Downs syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Iron only Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	 Thrombosis None relevant	 None relevant None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

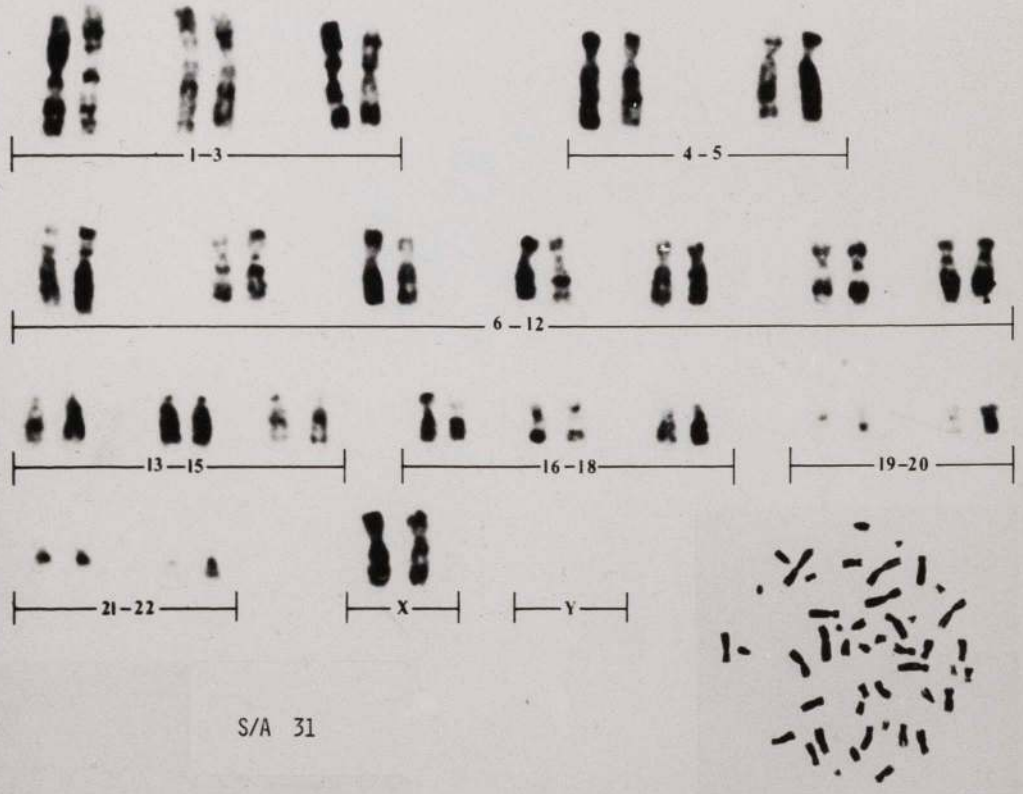
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	3	3	6
	13 - 15	4		4
	14 - 14			
	14 - 15	1	1	2
	15 - 15	1		1
G/G	21 - 21			
	21 - 22	2	3	5
	22 - 22			
D/G	13 - 21	3	2	5
	13 - 22	3	1	4
	14 - 21	1	4	5
	14 - 22	3	2	5
	15 - 21	2		2
	15 - 22		2	2
D/D/G	13 - 13 - 21	1		1
	13 - 13 - 22			
	13 - 14 - 21	2		2
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22	1		1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21	1		1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

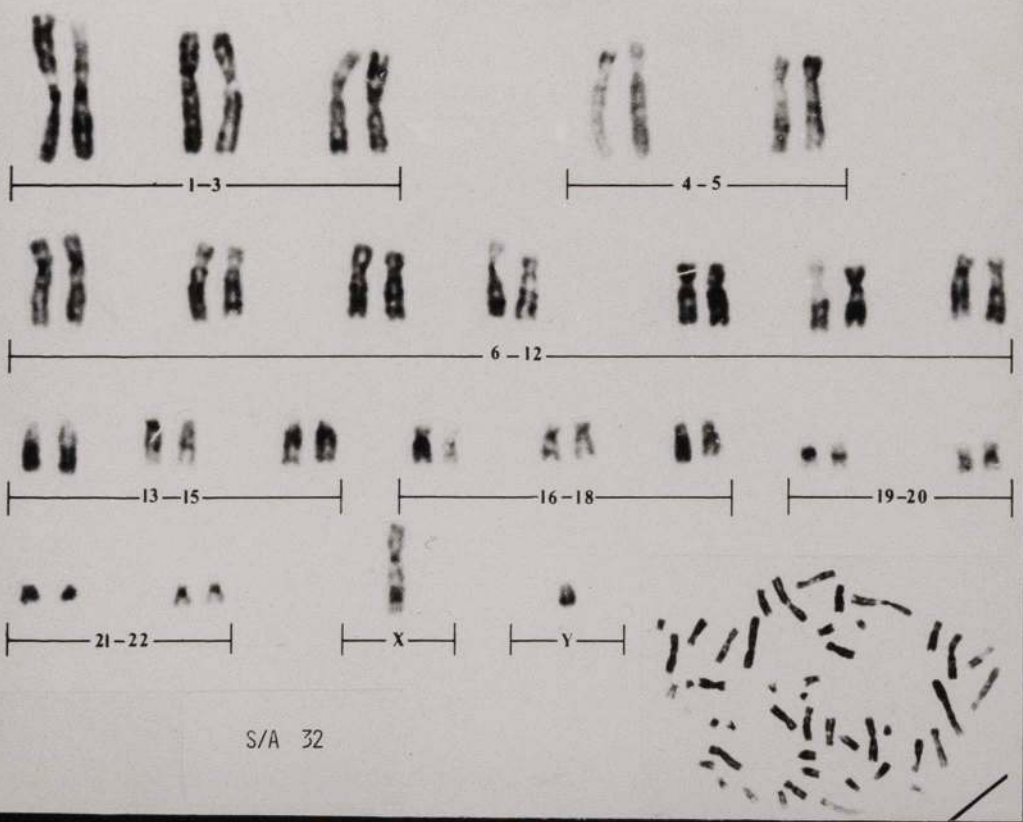
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22		1	1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14	1		1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	14-13-15-13-21-22	1		1
	13-14-21-13		1	1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	2		2
	13 - 14	2	2	4
	13 - 15	1	2	3
	14 - 14			
	14 - 15	1	2	3
	15 - 15			
G/G	21 - 21		1	1
	21 - 22	2	1	3
	22 - 22			
D/G	13 - 21	1	2	3
	13 - 22	1	1	2
	14 - 21	4	1	5
	14 - 22		3	3
	15 - 21	1	3	4
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22		1	1
	13 - 15 - 21	1		1
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22	1		1
	14 - 15 - 21	1		1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	2	2	4
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14	1		1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 31


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 32

S/A NUMBER	39	40
SEX	F	M
DATE OF BIRTH	15.8.40	9.2.40
NATIONALITY	British	British
CHILDREN	1	2
Date of Birth	14.8.70	25.6.74
Sex	F	M
Details	Normal	Downs syndrome
AFFECTED PREGNANCY		
Drugs	Iron only	
Accidents	Nil	
Radiation	Nil	
Other	Nil	
FAMILY HISTORY		
Illness	None relevant	None relevant
Affected Members		
Other		
CHROMOSOME CONSTITUTION	46,XX	46,XY

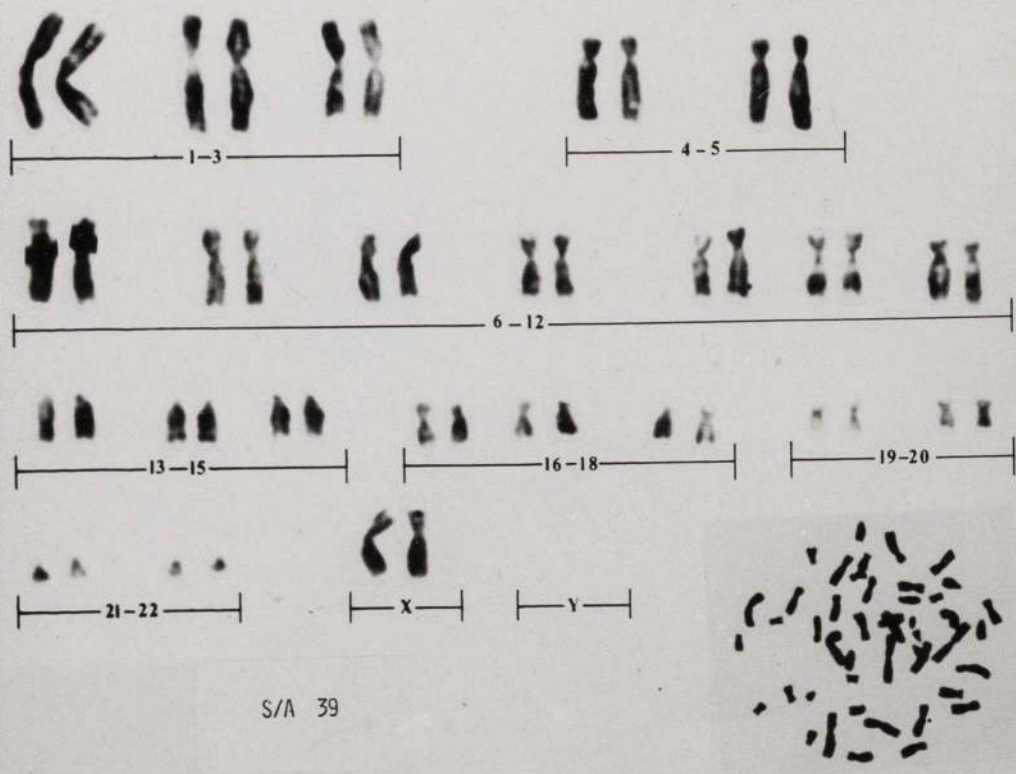
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	6	7
	13 - 15			
	14 - 14	2	2	4
	14 - 15	1		1
	15 - 15			
G/G	21 - 21			
	21 - 22	7	7	14
	22 - 22			
D/G	13 - 21	2	2	4
	13 - 22		4	4
	14 - 21	1	3	4
	14 - 22	1	4	5
	15 - 21		2	2
	15 - 22	1		1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21		1	1
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

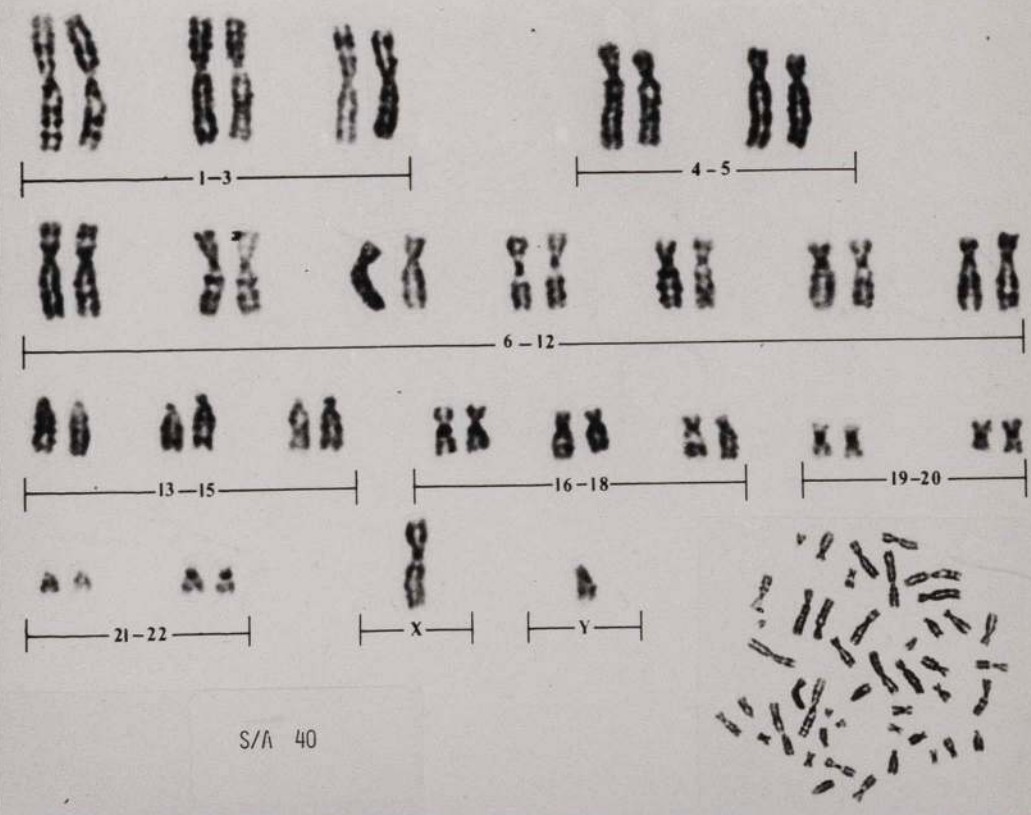
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21		1	1
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1	1	2
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14		1	1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22	1		1
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		8	8
	13 - 15	1		1
	14 - 14			
	14 - 15		1	1
	15 - 15			
G/G	21 - 21			
	21 - 22	3	2	5
	22 - 22			
D/G	13 - 21	2	2	4
	13 - 22	1	1	2
	14 - 21	3	1	4
	14 - 22	2	4	6
	15 - 21	1		1
	15 - 22		2	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22	1		1
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22		1	1
D/D/D	13 - 14 - 15			
	13 - 13 - 14	1		1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 39


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 40

S/A NUMBER	13	14
SEX	F	M
DATE OF BIRTH	2.5.47	16.3.47
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 M Normal	2 M Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

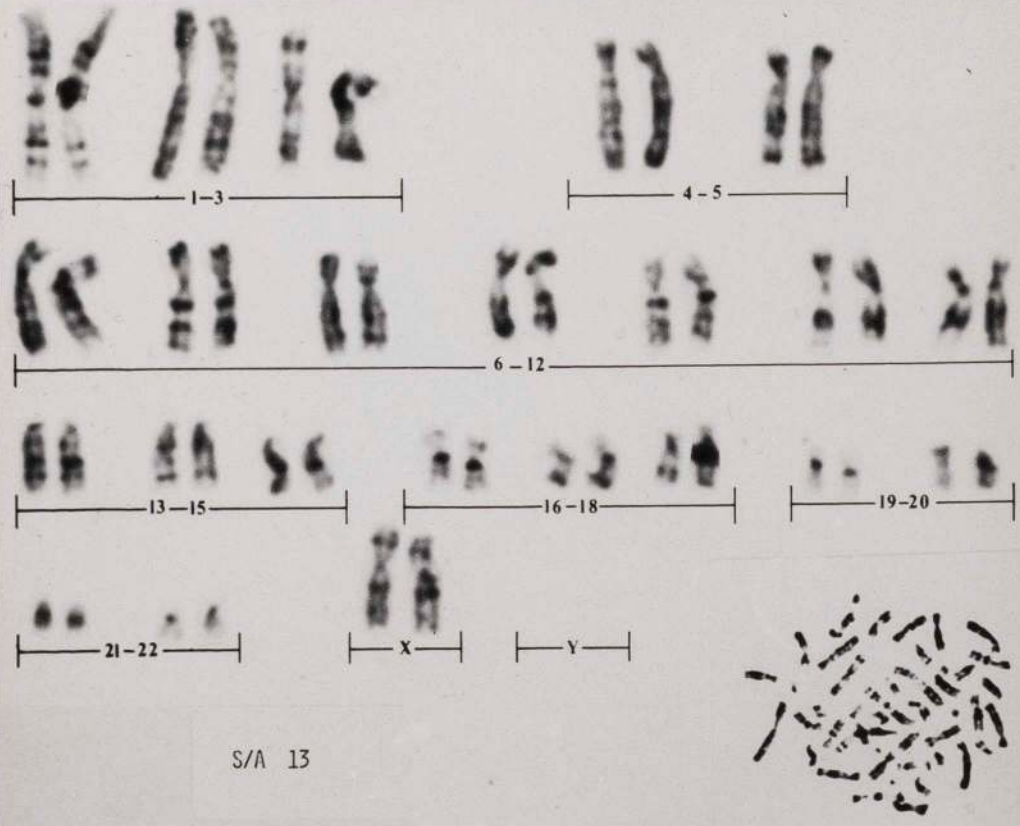
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	2	3
	13 - 14	2	5	7
	13 - 15		1	1
	14 - 14		1	1
	14 - 15		3	3
	15 - 15			
G/G	21 - 21	1	1	2
	21 - 22	2	4	6
	22 - 22		1	1
D/G	13 - 21		1	1
	13 - 22		2	2
	14 - 21		3	3
	14 - 22	1	4	5
	15 - 21	1	3	4
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22	1	1	2
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

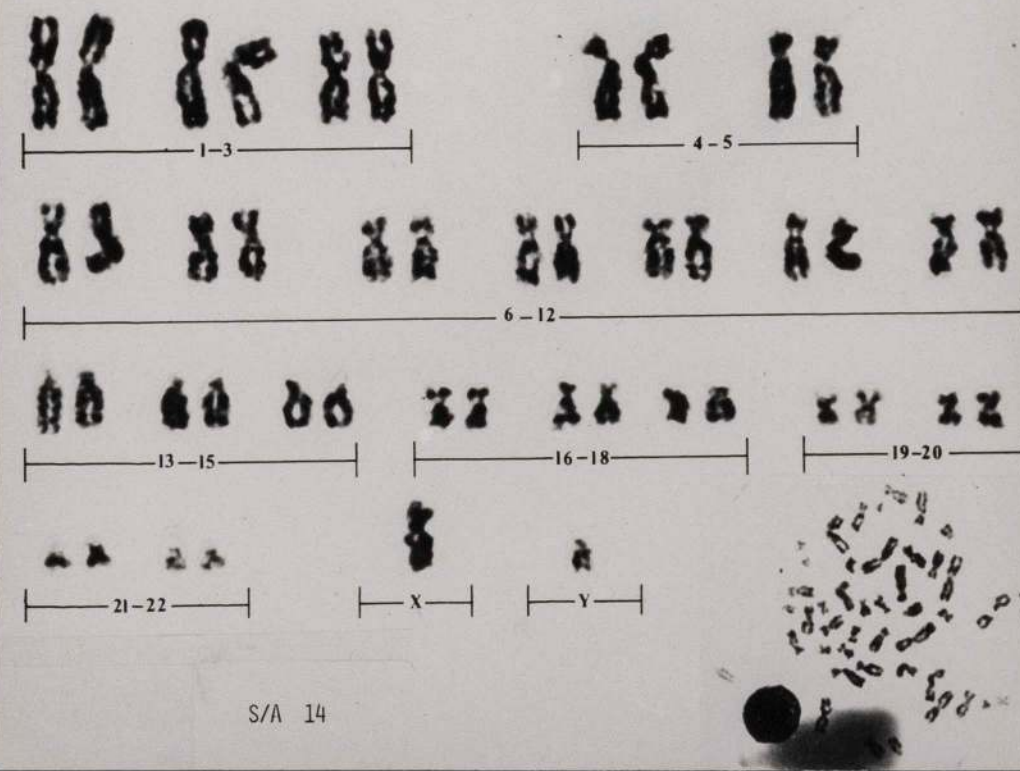
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14			
	13 - 14 - 14	1		1
	14 - 15 - 15			
	14 - 14 - 15	1		1
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	3	4
	13 - 15	1	1	2
	14 - 14			
	14 - 15	1	1	2
	15 - 15			
G/G	21 - 21			
	21 - 22		10	10
	22 - 22			
D/G	13 - 21	1	1	2
	13 - 22		1	1
	14 - 21	1	6	7
	14 - 22	3	1	4
	15 - 21	2	2	4
	15 - 22	2	1	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21		1	1
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22		1	1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15	1		1
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21		1	1
	21 - 21 - 22	1		1
OTHER	13-14-21-21	1		1


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 13


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 14

S/A NUMBER	33	34
SEX	F	M
DATE OF BIRTH	3.10.47	30.2.46
NATIONALITY	West Indian	West Indian
CHILDREN Date of Birth Sex Details	1 6.10.70 M Normal	2 9.5.74 M Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

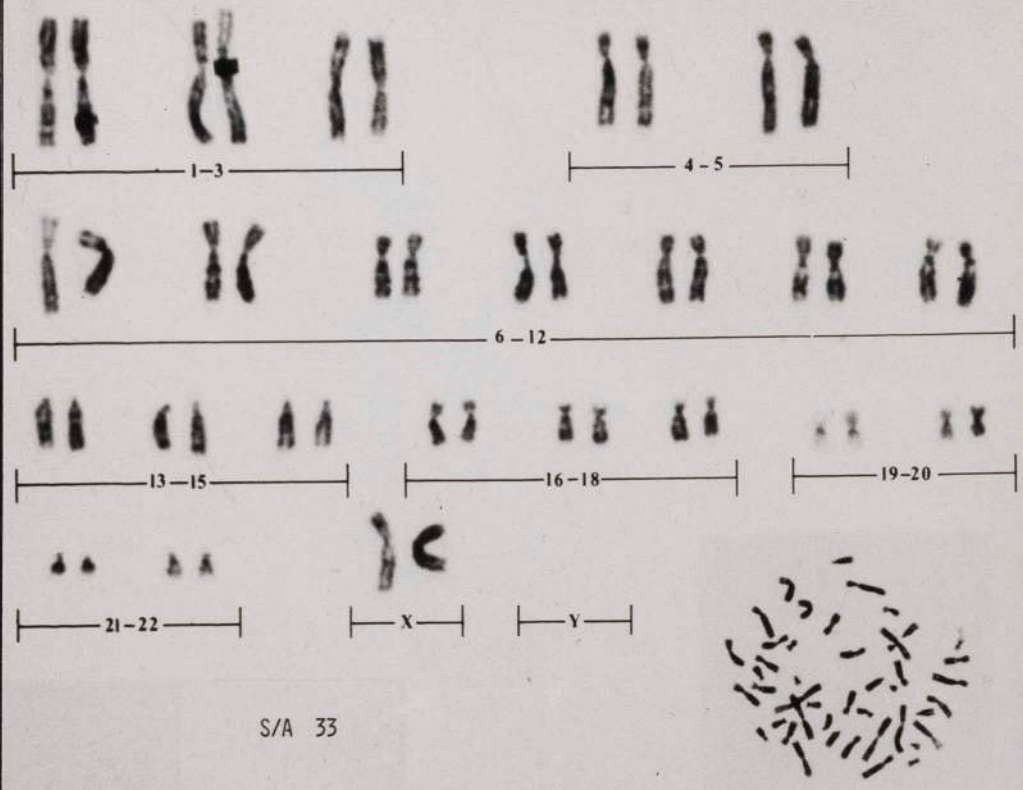
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	2	6	8
	13 - 15	2	1	3
	14 - 14			
	14 - 15	5	4	9
	15 - 15			
G/G	21 - 21			
	21 - 22		5	5
	22 - 22		1	1
D/G	13 - 21	1	2	3
	13 - 22			
	14 - 21	1	2	3
	14 - 22	1		1
	15 - 21	1	2	3
	15 - 22	1	1	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

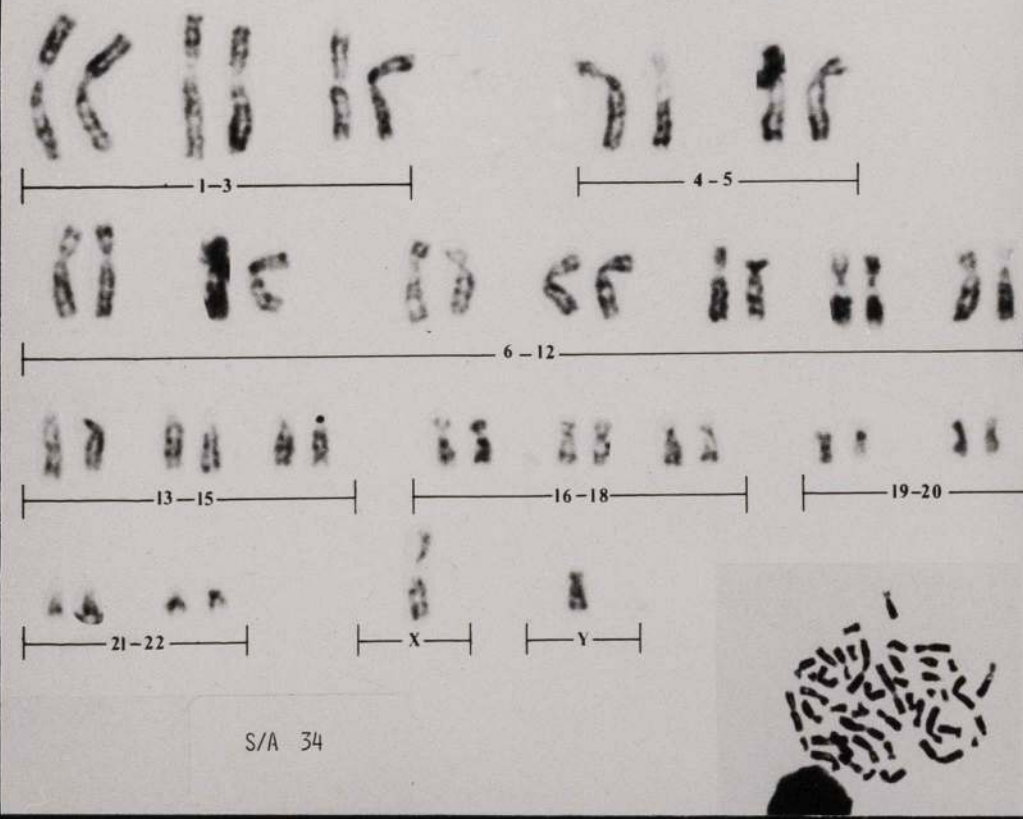
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	13-14-14-21	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	6	7
	13 - 15		2	2
	14 - 14			
	14 - 15	4	1	5
	15 - 15			
G/G	21 - 21			
	21 - 22	5	4	9
	22 - 22			
D/G	13 - 21	3	1	4
	13 - 22		1	1
	14 - 21	1	3	4
	14 - 22			
	15 - 21	1	1	2
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14	1		1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 33


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 34

S/A NUMBER	41	42
SEX	F	M
DATE OF BIRTH	7.8.45	10.6.47
NATIONALITY	British	British
CHILDREN	1	2	3
Date of Birth	23.11.66	31.10.68	19.3.72
Sex	M	F	M
Details	Normal	Normal	Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable		
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

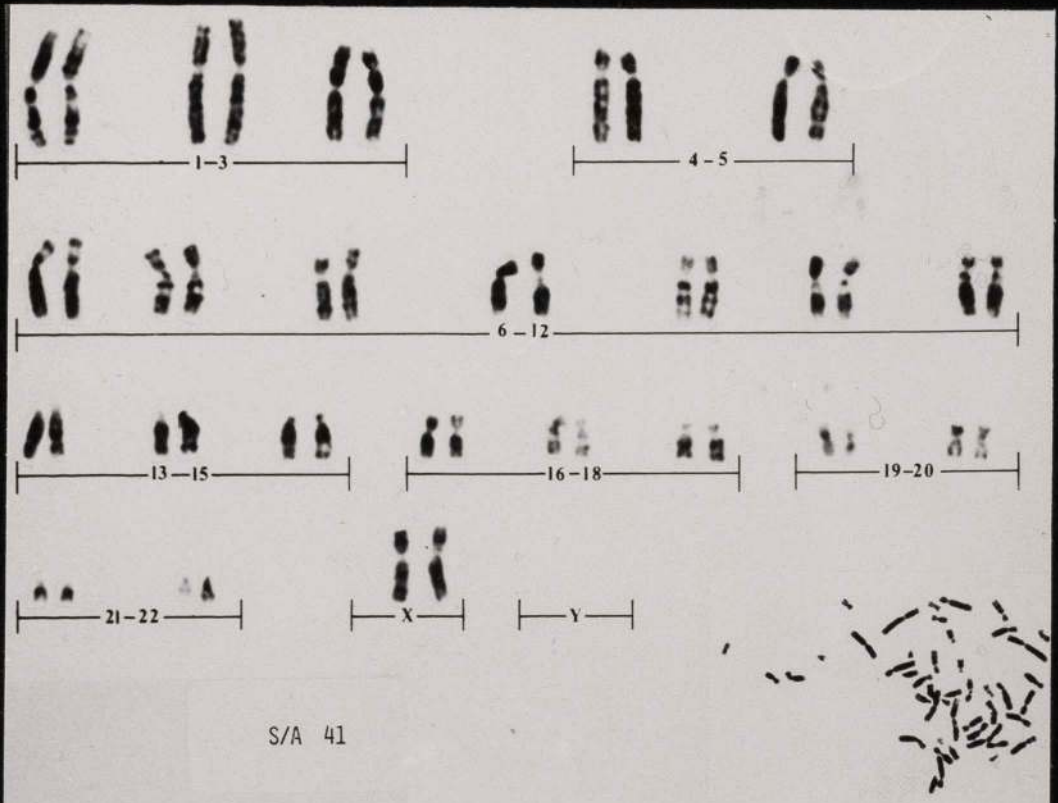
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	1	2
	13 - 14	1	4	5
	13 - 15	2	3	5
	14 - 14		1	1
	14 - 15	1	3	4
	15 - 15			
G/G	21 - 21			
	21 - 22		3	3
	22 - 22		1	1
D/G	13 - 21		3	3
	13 - 22		3	3
	14 - 21	1	2	3
	14 - 22	2		2
	15 - 21	1	4	5
	15 - 22			
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1	1	2
	13 - 14 - 22			
	13 - 15 - 21	1		1
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

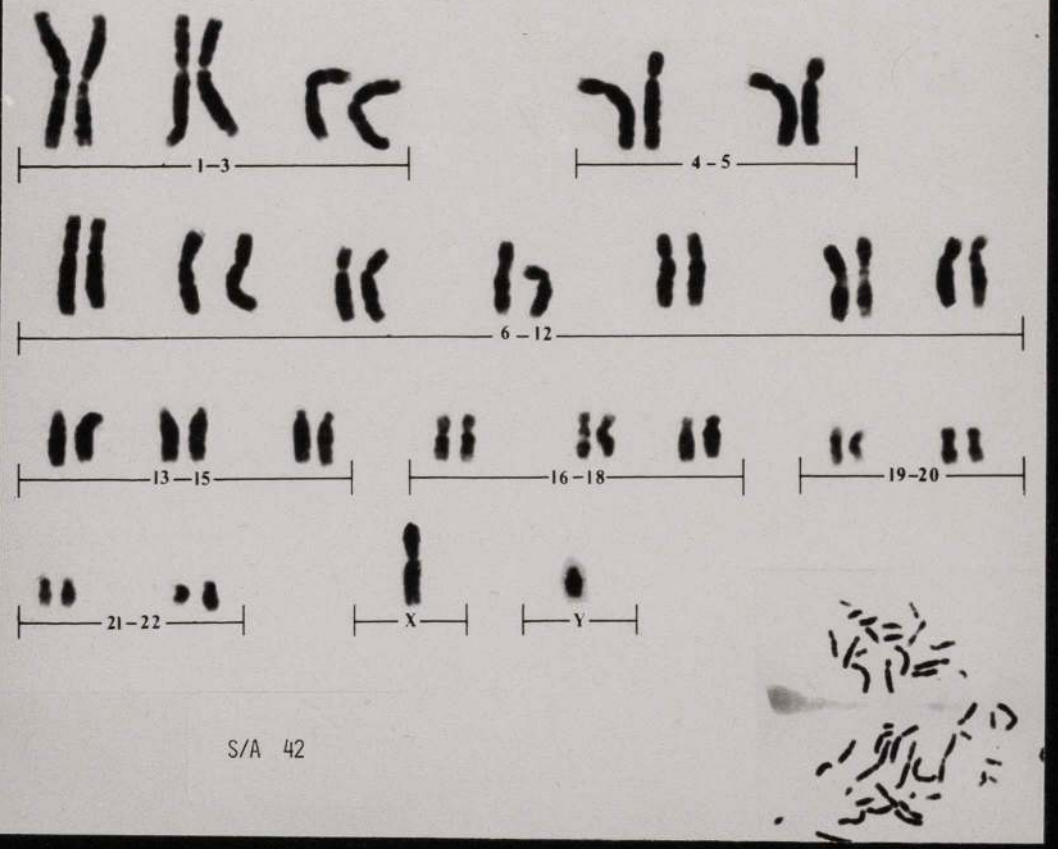
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1		1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	14-14-15-21	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	2	7	9
	13 - 15	3	2	5
	14 - 14		1	1
	14 - 15	3	3	6
	15 - 15		1	1
G/G	21 - 21			
	21 - 22	1	1	2
	22 - 22			
D/G	13 - 21		1	1
	13 - 22	1	1	2
	14 - 21	1	1	2
	14 - 22	2	2	4
	15 - 21	1	2	3
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1	1	2
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21	1		1
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1	1	2
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14	1		1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	14-14-15-21		1	1


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 41


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 42

S/A NUMBER	43	44
SEX	F	M
DATE OF BIRTH	28.10.52	22.7.50
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 22.6.70 F Normal	2 7.5.72 M Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

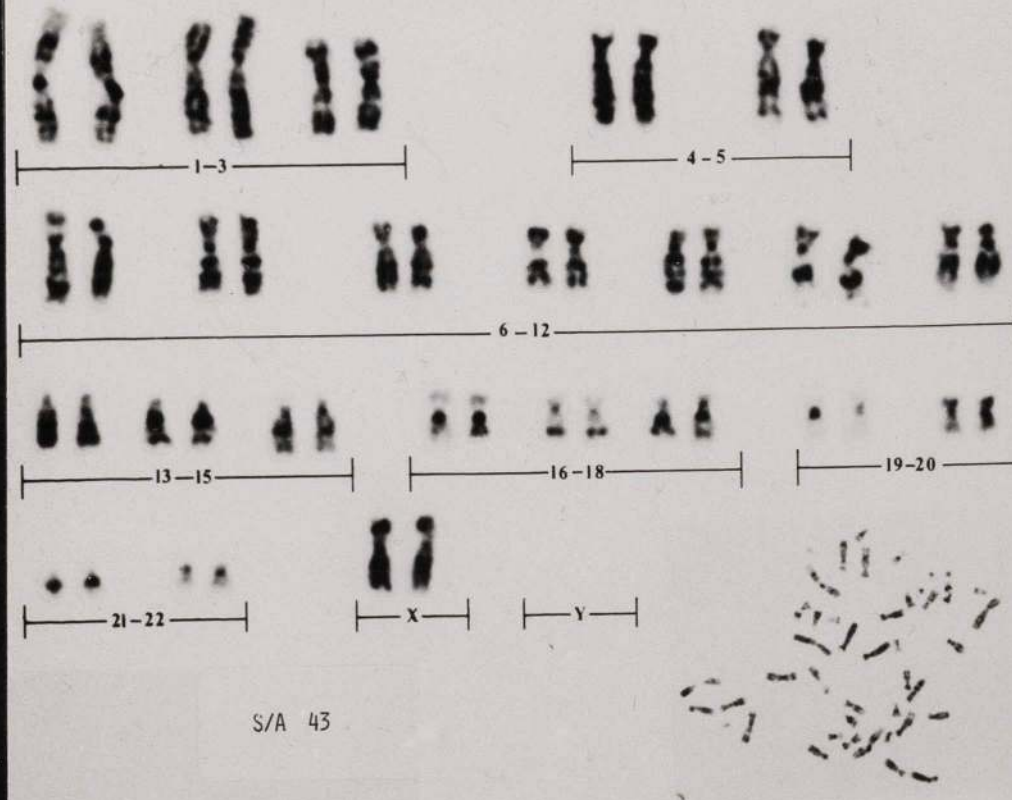
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13		1	1
	13 - 14		3	3
	13 - 15	1	3	4
	14 - 14		1	1
	14 - 15	1	2	3
	15 - 15		1	1
G/G	21 - 21			
	21 - 22		5	5
	22 - 22			
D/G	13 - 21	1	4	5
	13 - 22		1	1
	14 - 21		3	3
	14 - 22		2	2
	15 - 21		4	4
	15 - 22	1		1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21	1		1
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

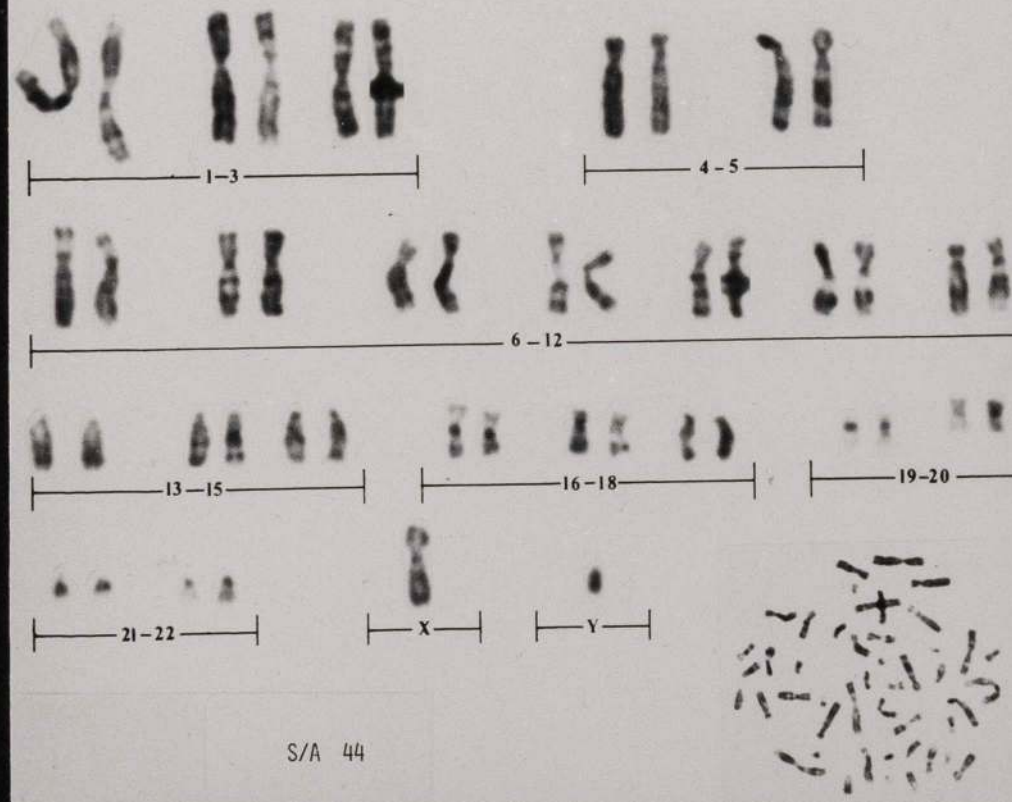
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1		1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13	1		1
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	3	4
	13 - 15		1	1
	14 - 14		2	2
	14 - 15		3	3
	15 - 15	1		1
G/G	21 - 21			
	21 - 22		5	5
	22 - 22			
D/G	13 - 21	2	1	3
	13 - 22			
	14 - 21			
	14 - 22	2	6	8
	15 - 21	1	4	5
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21		1	1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22		1	1
	14 - 14 - 21			
	14 - 14 - 22		1	1
	14 - 15 - 21			
	14 - 15 - 22		1	1
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22	1		1
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 43


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 44

S/A NUMBER	45	46
SEX	M	F
DATE OF BIRTH	17.1.50	7.12.49
NATIONALITY	Irish	Irish
CHILDREN Date of Birth Sex Details	1 7.2.69 M Normal	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

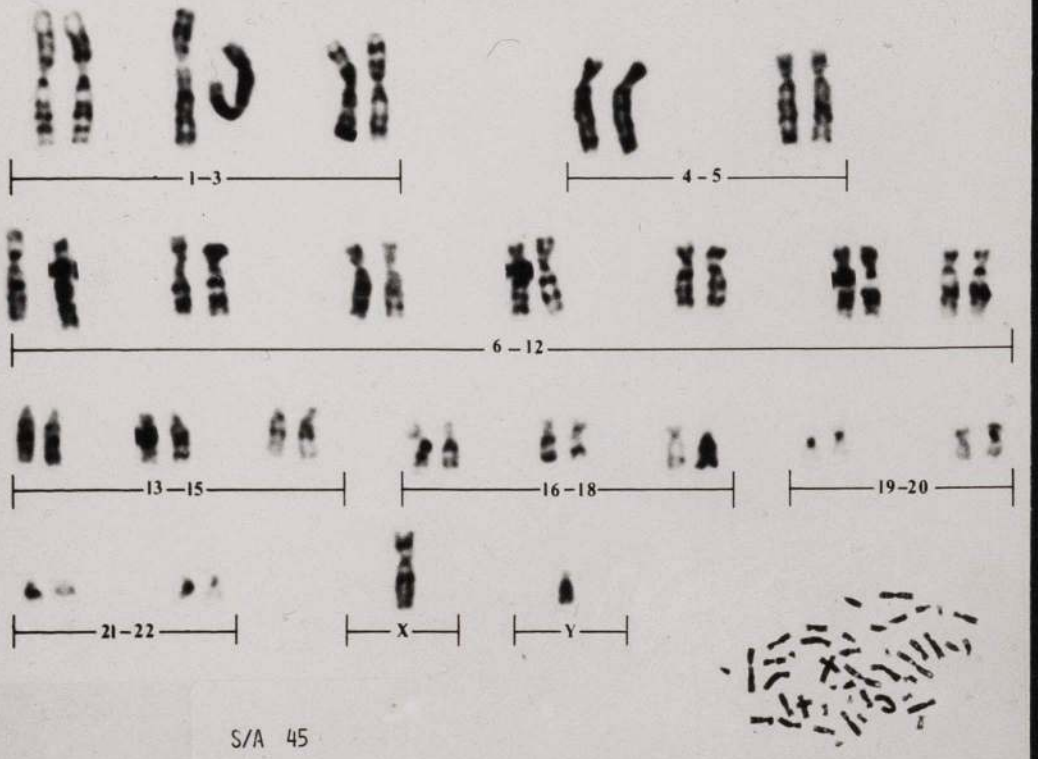
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	1	2
	13 - 14		7	7
	13 - 15		2	2
	14 - 14			
	14 - 15		3	3
	15 - 15			
G/G	21 - 21			
	21 - 22		2	2
	22 - 22			
D/G	13 - 21	1	2	3
	13 - 22		1	1
	14 - 21	1	3	4
	14 - 22	1	4	5
	15 - 21	1	2	3
	15 - 22		2	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	3	2	5
	13 - 14 - 22		1	1
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

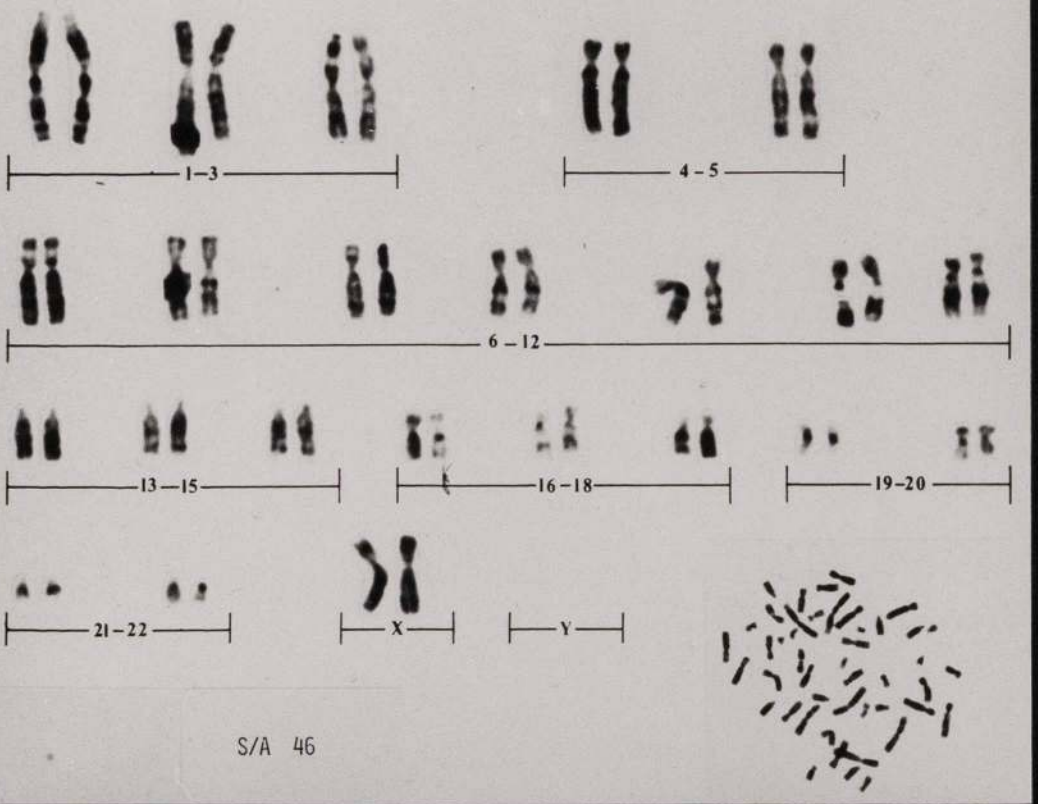
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	2	3
	13 - 15	2	2	4
	14 - 14			
	14 - 15	1	2	3
	15 - 15			
G/G	21 - 21			
	21 - 22	2	8	10
	22 - 22		1	1
D/G	13 - 21	3	1	4
	13 - 22		1	1
	14 - 21	3	1	4
	14 - 22		3	3
	15 - 21	1	3	4
	15 - 22	2		2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15	1		1
	14 - 14 - 15		1	1
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 45


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 46

S/A NUMBER	47	48
SEX	M	F
DATE OF BIRTH	1.8.39	15.6.40
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 14.2.65 F Normal	2 1.3.67 F Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

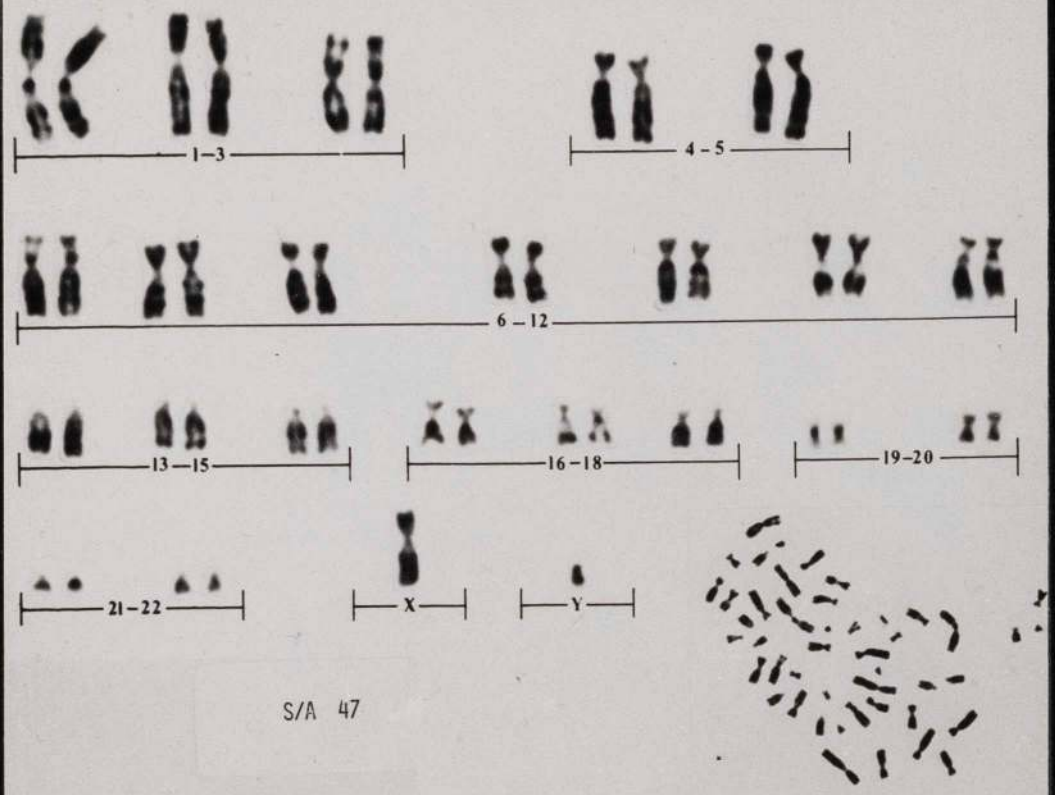
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13		2	2
	13 - 14		2	2
	13 - 15			
	14 - 14			
	14 - 15		1	1
	15 - 15			
G/G	21 - 21			
	21 - 22	7	7	14
	22 - 22		1	1
D/G	13 - 21	1	3	4
	13 - 22	2	3	5
	14 - 21	1	1	2
	14 - 22	1	5	6
	15 - 21	1	1	2
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22		1	1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

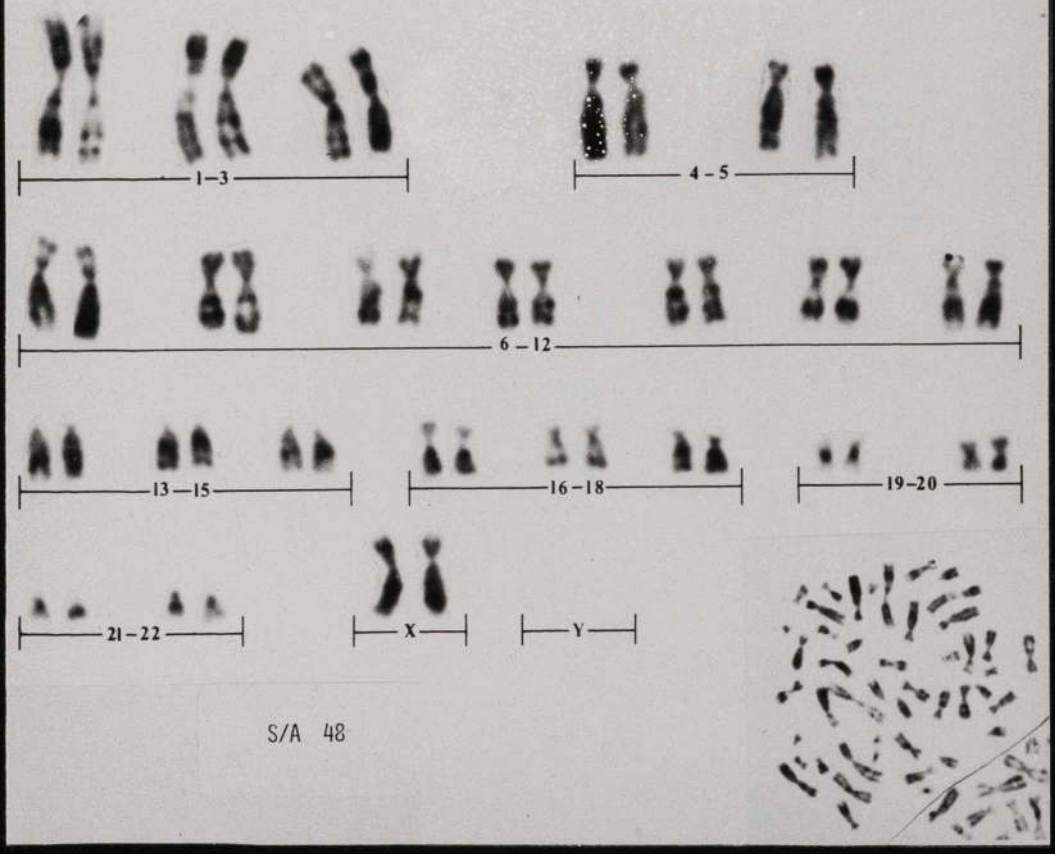
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21	1		1
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1	1	2
	14 - 22 - 22	1		1
	15 - 21 - 21			
	15 - 21 - 22		1	1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
	15 - 13 - 13			
G/G/G	22 - 22 - 21	1	1	2
	21 - 21 - 22	1		1
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	2	3	5
	13 - 15	1	4	5
	14 - 14			
	14 - 15	1	2	3
	15 - 15			
G/G	21 - 21	1		1
	21 - 22	3	1	4
	22 - 22			
D/G	13 - 21	3	2	5
	13 - 22			
	14 - 21	3	6	9
	14 - 22		2	2
	15 - 21		4	4
	15 - 22		2	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19 – 20
21 – 22
X
Y

S/A 47


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19 – 20
21 – 22
X
Y

S/A 48

S/A NUMBER	51	52
SEX	M	F
DATE OF BIRTH	20.3.46	5.8.47
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 4.1.72 M Normal	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

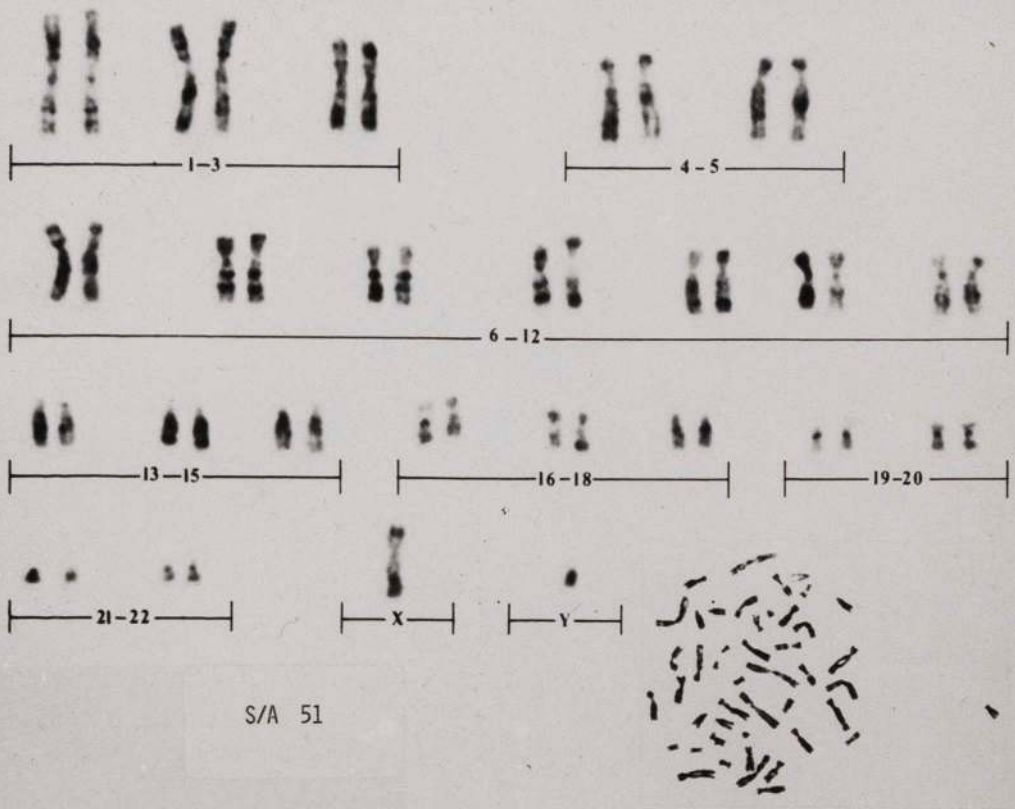
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1		1
	13 - 14	1	1	2
	13 - 15	1		1
	14 - 14		3	3
	14 - 15		3	3
	15 - 15			
G/G	21 - 21		1	1
	21 - 22		6	6
	22 - 22		2	2
D/G	13 - 21		3	3
	13 - 22		3	3
	14 - 21			
	14 - 22		5	5
	15 - 21	1		1
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

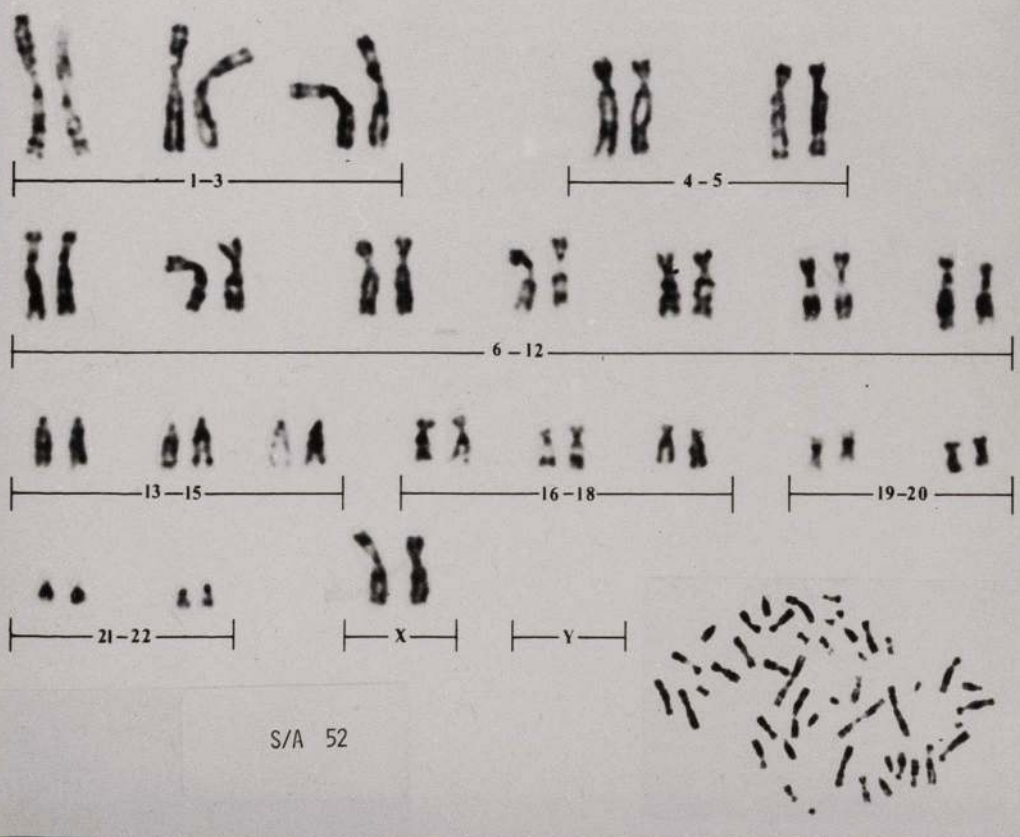
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14		1	1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21		1	1
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	1	2
	13 - 15			
	14 - 14		1	1
	14 - 15	3	4	7
	15 - 15	1		1
G/G	21 - 21			
	21 - 22	2	4	6
	22 - 22			
D/G	13 - 21		3	3
	13 - 22	1	2	3
	14 - 21		1	1
	14 - 22		3	3
	15 - 21	1	3	4
	15 - 22	5	2	7
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21	1		1
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22	1		1
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 51


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 52

S/A NUMBER	59	60
SEX	M	F
DATE OF BIRTH	25.12.43	19.3.45
NATIONALITY	British	British
CHILDREN	1	2	3
Date of Birth	11.7.66	14.12.67	13.2.74
Sex	F	F	M
Details	Normal	Normal	Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable		
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

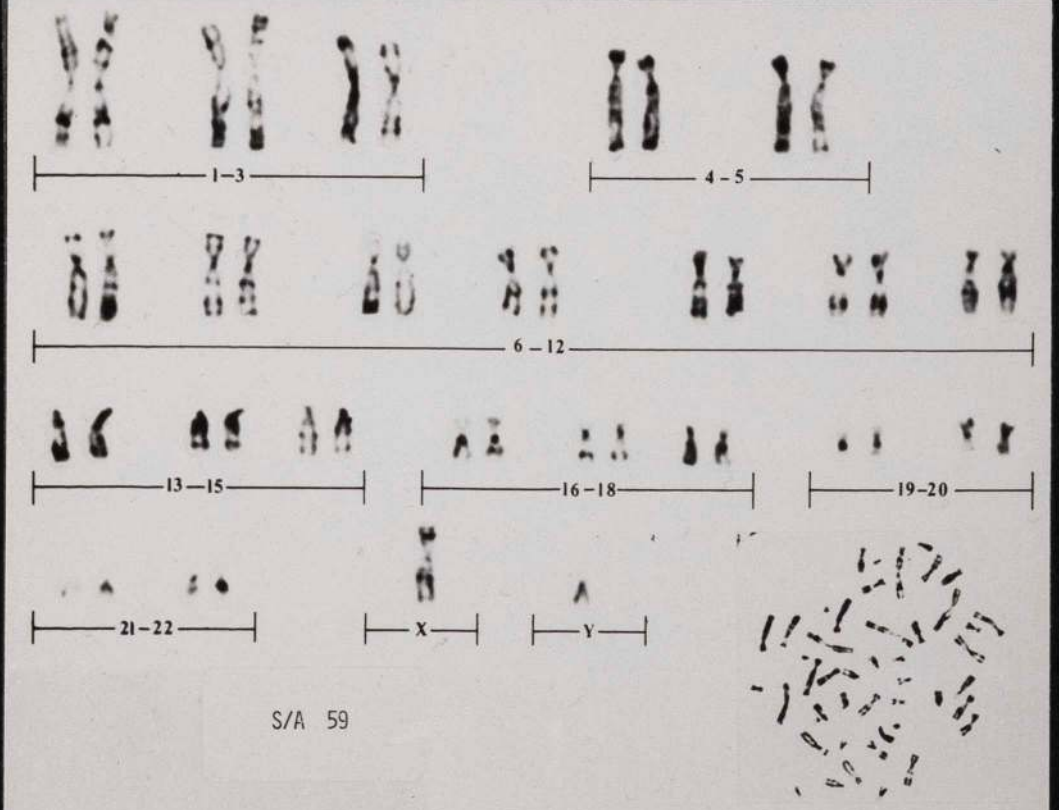
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	2	3	5
	13 - 15	1	1	2
	14 - 14			
	14 - 15	1	3	4
	15 - 15			
G/G	21 - 21			
	21 - 22	3	4	7
	22 - 22		1	1
D/G	13 - 21		2	2
	13 - 22		2	2
	14 - 21		1	1
	14 - 22	2	2	4
	15 - 21		2	2
	15 - 22		2	2
D/D/G	13 - 13 - 21	1		1
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

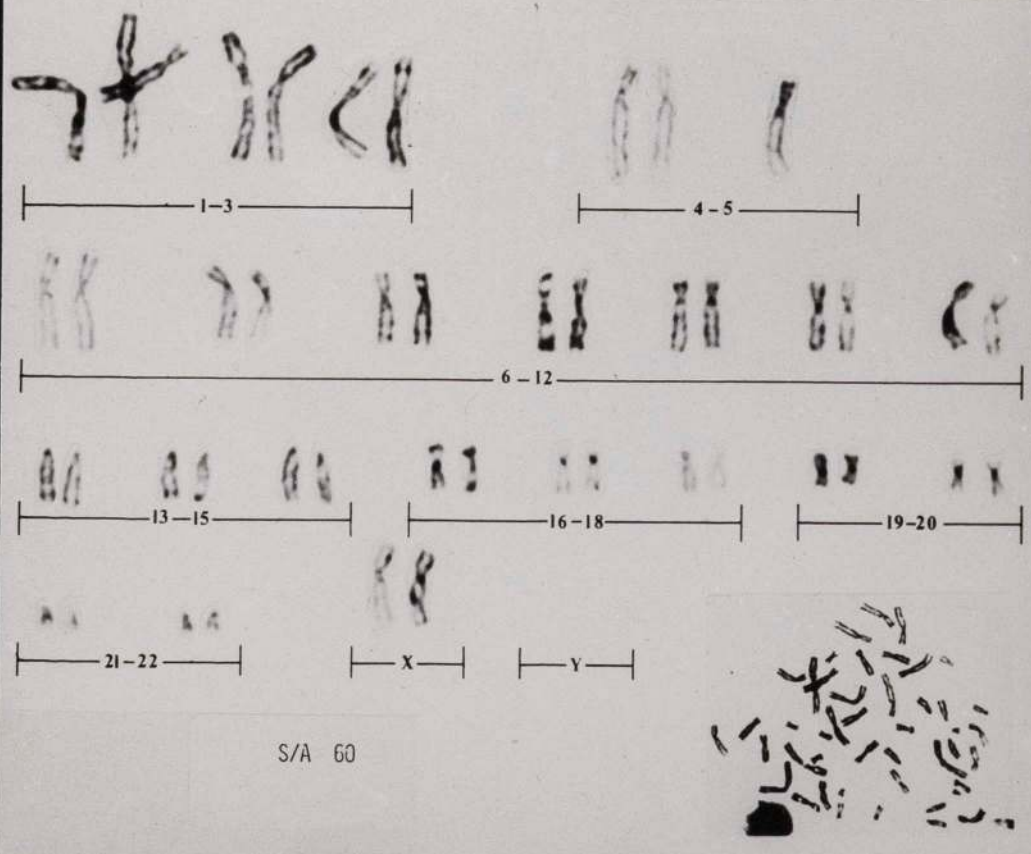
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22		1	1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14		1	1
	14 - 15 - 15			
	14 - 14 - 15		1	1
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	13-15-22-22	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	1	2
	13 - 14		4	4
	13 - 15	1	2	3
	14 - 14	2		2
	14 - 15		3	3
	15 - 15			
G/G	21 - 21		1	1
	21 - 22	2	3	5
	22 - 22		1	1
D/G	13 - 21	1	2	3
	13 - 22		2	2
	14 - 21		4	4
	14 - 22	1		1
	15 - 21		2	2
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 59


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 60

S/A NUMBER	61	62
SEX	M	F
DATE OF BIRTH	30.6.42	27.10.44
NATIONALITY	British	British
CHILDREN	1	2	3
Date of Birth	3.12.63	17.11.65	8.1.70
Sex	M	F	F
Details	Normal	Normal	Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable		
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

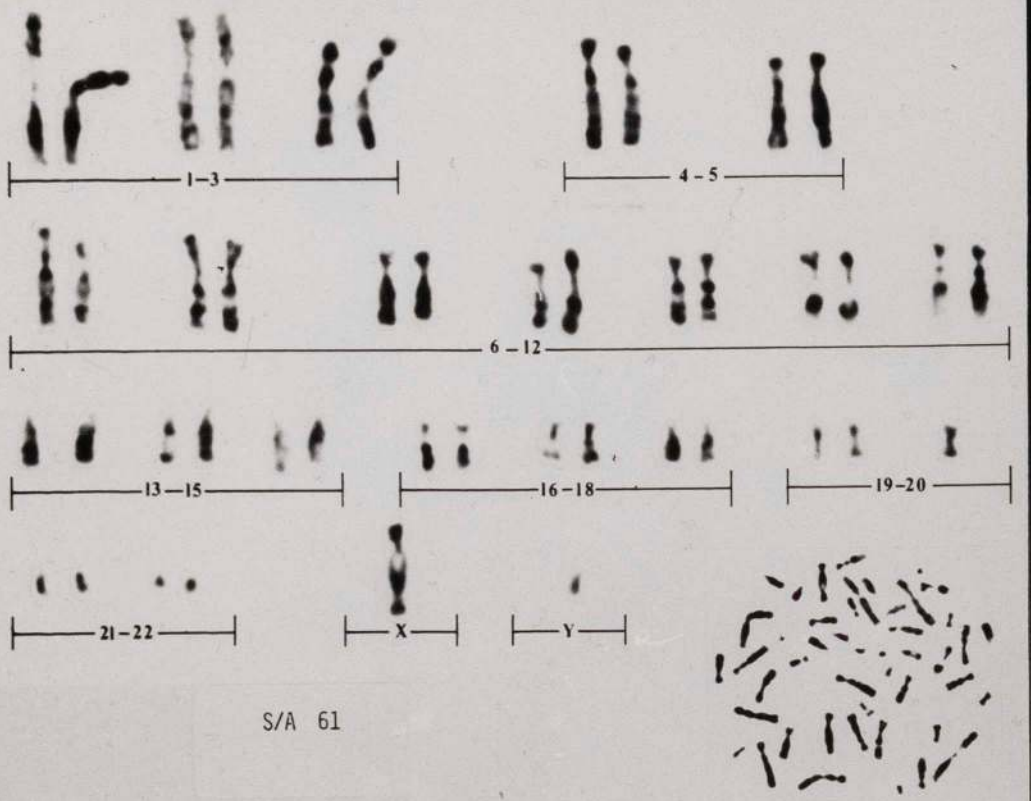
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14			
	13 - 15			
	14 - 14			
	14 - 15		2	2
	15 - 15			
G/G	21 - 21			
	21 - 22	6	12	18
	22 - 22		2	2
D/G	13 - 21		1	1
	13 - 22	1	3	4
	14 - 21	2	8	10
	14 - 22		2	2
	15 - 21	1	5	6
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21		2	2
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

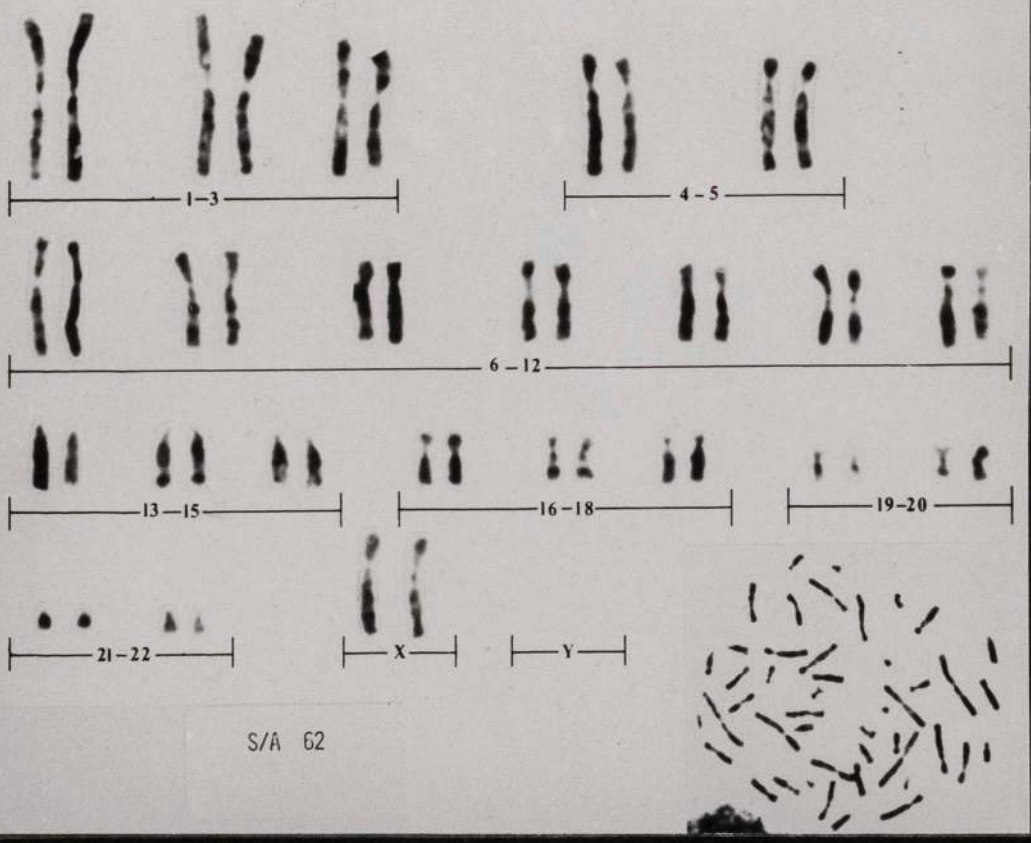
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		2	2
	13 - 15		3	3
	14 - 14			
	14 - 15		1	1
	15 - 15	1		1
G/G	21 - 21			
	21 - 22	3	8	11
	22 - 22			
D/G	13 - 21		2	2
	13 - 22		3	3
	14 - 21	1	3	4
	14 - 22		4	4
	15 - 21		1	1
	15 - 22		2	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 61


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 62

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THE RELATIONSHIP BETWEEN SATELLITE ASSOCIATION AND TRISOMY 'G'

A Thesis Submitted to

The Faculty of Science,

The University of Aston in Birmingham

by

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For the Degree of Master of Philosophy

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SUMMARY

A hypothesis was put forward, based on a personal observation, that there appeared to be an increased frequency of satellite associations between the acrocentric chromosomes in a young parent of a child with trisomy 'G'. It was therefore considered that there might be a predisposition towards trisomy in some families, and that satellite association was a possible factor in the production of such.

The literature covering the cytogenetics and technical significance of satellite association, together with the clinical, cytogenetical and parental age involvement in trisomy 'G', has been reviewed.

The design of this investigation was divided into two parts, namely methodological and clinical.

In the first part of the study a standardised technique was developed to provide the lowest frequency of satellite associations and accurate identification of participating chromosomes. Blood samples from thirty-five normal controls were used. A standard cultural method was employed to compare the satellite association frequencies observed in potassium chloride, sodium citrate and Hanks/water used as hypotonic solutions. Two further experiments were used to evaluate the effect of time and molarity of the hypotonic solutions on satellite association frequency. Hanks/water was used as the hypotonic solution of choice giving fewer satellite associations per cell.

The second part of this investigation dealt with the cytogenetic analysis of eighteen parents of regular Down's infants matched for age with control parents of normal children. The specific identification of the individual chromosomes involved, and the category of associations was recorded in both groups.

Application of the results to statistical analysis showed that in certain specific configurations, there was a significant increase in satellite association frequency in parents of Down's infants.

The possible role and causes of satellite association are discussed.

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I N D E X

THE RELATIONSHIP BETWEEN SATELLITE ASSOCIATION AND TRISOMY 'G'

I. INTRODUCTION

I.i Hypothesis

It is known that in families having one child with an autosomal trisomic anomaly, the chances of producing another child with a trisomy, even of a different type, are greater than average (Carter 1970). There seems therefore to be a predisposition towards trisomy in some families.

The formulation of the present hypothesis was stimulated by the personal observation that, in a young parent of a child with trisomy 'G' there was an unusually high number of D/G satellite associations in the metaphase spreads. The finding was sufficiently striking to suggest that it did not represent merely a chance exaggeration of the normal, but reflected an actual anomaly present in the chromosomes. It is suggested that this could be a mechanism which could account for the apparent familiar predisposition to aneuploidy.

The most frequent cause of anueploidy in man is non-disjunction where there is a failure in the separation of the chromosomes in the dividing cell. The most common of the autosomal trisomies is trisomy 'G' (Down's Syndrome or Mongolism) which has an incidence of one in seven hundred live births, and trisomy 'D' (Patau's Syndrome), which is seen in one in five thousand live births. Both of these anomalies stem from non-disjunction and both occur in the

chromosomes which show satellite association.

There are therefore, reasons for studying the phenomenon of satellite association as a possible factor in the production of trisomy in groups 'D' and 'G'. As satellite association is seen in normal metaphase spreads, the hypothesis requires that in the parents of children with these trisomies, a different degree of the normal affinity between associated chromosomes or a different quality of affinity possibly due to structural factors, is present in these chromosomes.

I.ii Review of Literature

a) Cytogenetics - General

In the human karyotype there are two groups of chromosomes which possess satellites. These so-called satellites appear on the short arms of the acrocentric chromosomes of the 'D' and 'G' groups (see Figure 1). It is known that these satellites are not artefacts of preparation but are structural features of these chromosomes, and as such are inherited. All of the ten acrocentric chromosomes probably bear satellites on their short arms. Howell *et al*., (1975) found that in apparently normal individuals, the number of acrocentric chromosomes which exhibited satellites, varied from person to person. However, each individual tended to have a consistent specific number of satellites, and it is thought that this constitutes a true variation.

It was in 1961 that the phenomenon of satellite association was first described in human mitotic metaphase spreads (Ferguson-Smith and Handmaker, 1961, Harnden, 1961, Ohno *et al*. 1961). It was observed that the satellited chromosomes assumed specific positions in the metaphase spreads showing an apparent affinity between the satellites (Figure 2). It was seen that any number of associations could occur involving all the satellited chromosomes. The possibility of this being a random non-specific artefact was eventually disproved: finally, Ferguson-Smith described it in meiotic chromosomes in 1964, where the association of bivalents of the acrocentric chromosomes was observed in

Figure 1

a) Normal female karyotype - giemsa stain.

b) Karyogram of the 'D' and 'G' groups of chromosomes.

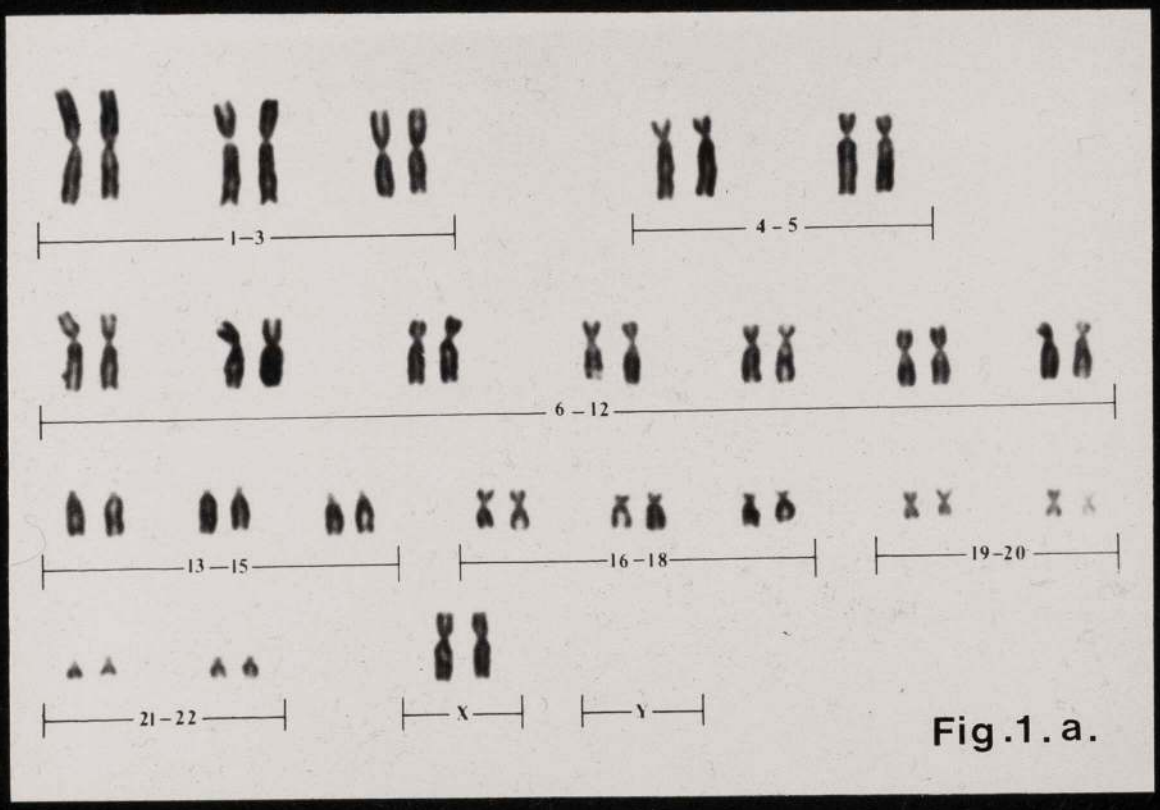


Fig.1.a.

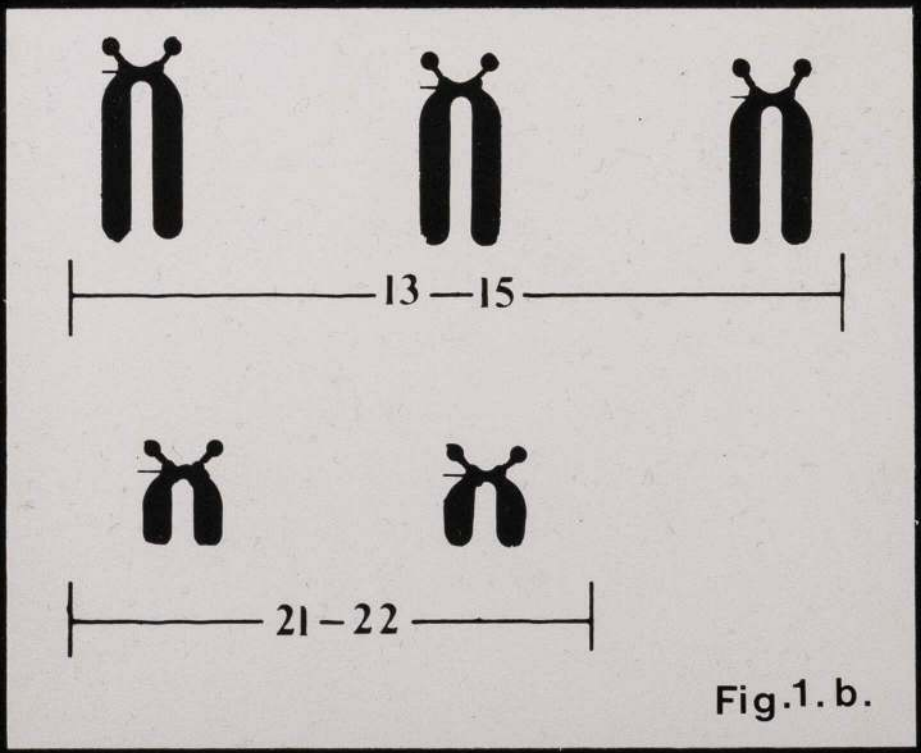


Fig.1.b.

Figure 2

Normal female metaphase spread - giemsa stain, showing apparent affinity between the acrocentric chromosomes.

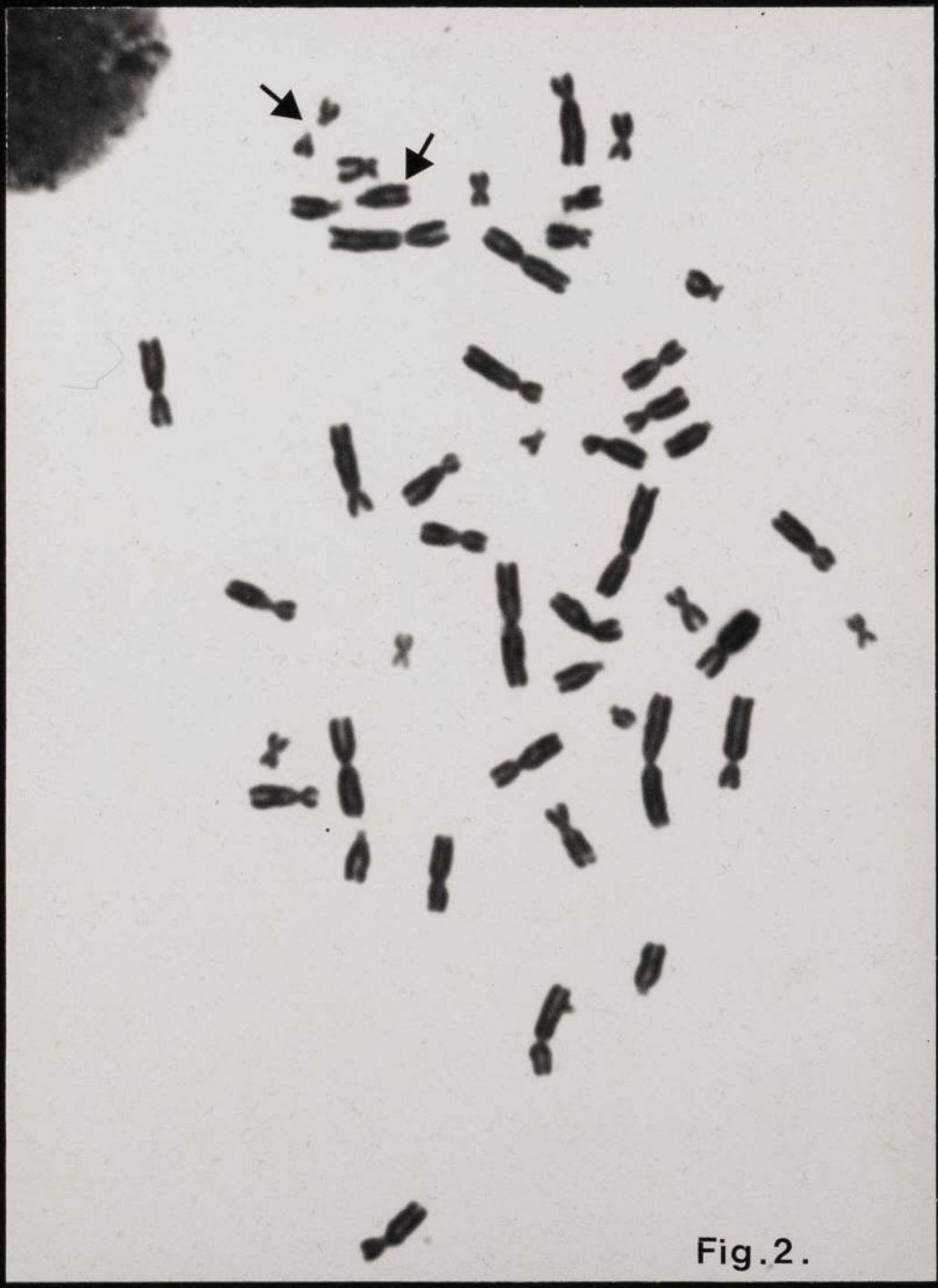


Fig.2.

human pachytene chromosomes, obtained from direct squash preparations.

The formation of satellite associations has often been attributed to the involvement of the satellited chromosomes in the nucleolar formation of the cell. It was postulated that the satellites were an integral part of the nucleolus and this involvement produced the adhesion needed to hold the satellites together during mitosis. If two or more nucleoli fuse to form one larger nucleolus this then would increase the chances of damage to the satellite segment with obvious risk of breakage (Ohno et al. 1961). Thus, if the breaks occur in more than one of the segments involved, the proximity of the broken ends might predispose to the translocation of material. This possibility might be the answer to the increased tendency of non-disjunction in the satellited chromosomes, e.g. trisomy 'G'. This is particularly relevant as autoradiographic studies have shown (Mikkelsen 1969) that chromosome D13, E18 and G21 are late in DNA replication as compared with the other autosomes.

The most common form of satellite association seen is the di-association where two satellited chromosomes are involved (see Figure 3). Less common is the tri-association, which, as the name implies, shows three satellited chromosomes involved (see Figure 4). In fact all combinations can occur until all of the ten acrocentrics are in association (see Figure 5). Occasionally, a non-satellited chromosome may be involved in an association. Perhaps the most common is A-1, where the centrimeric area appears to be the part to

Figure 3

Satellite association - Di-association - giemsa stain.

Figure 4

Satellite association - Tri-association - giemsa stain.

Figure 5

Satellite association - Multi-association - giemsa stain.

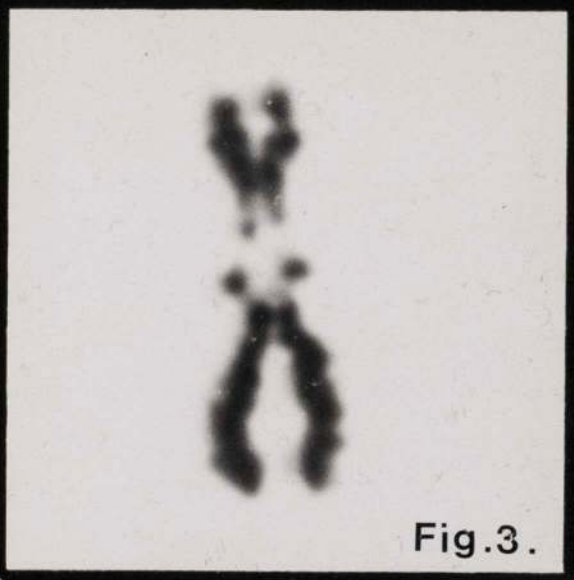


Fig.3.

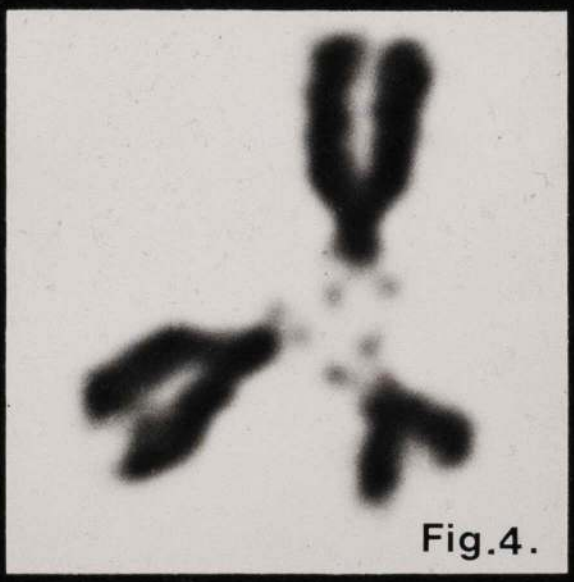


Fig.4.

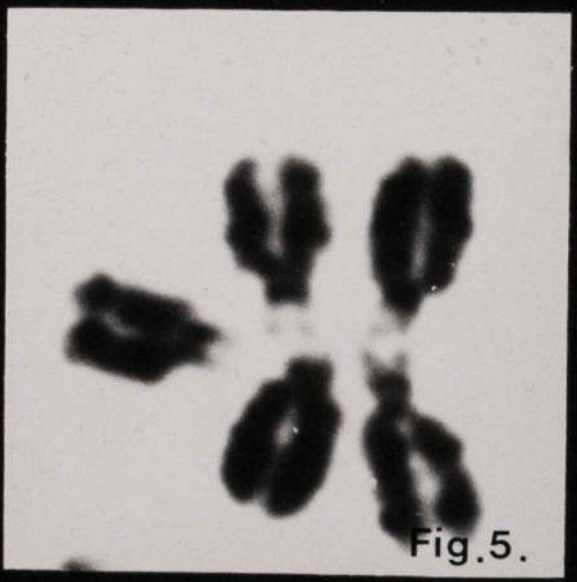


Fig.5.

which the satellites are most attracted.

b) Cytogenetics - Techniques

According to Ferguson-Smith and Handmaker (1961), satellite associations can be seen in up to 60% of normal mitotic metaphase spreads. Hansson (1970) found that the frequency varied between 62% to 80%, the mean value being 70.5% of normal cells had at least one satellite association present. However, Sasaki and Makino (1963) observed an increase in the number of secondary constrictions after culturing in a calcium free media, and this suggests that the cultural methods are likely to affect the frequency of the observed associations. Zang and Back (1967) confirmed that the frequency of association is influenced by the conditions of culture and the technique of preparation. Recently, Zhdanova and Deryagin (1975) have shown that all of the acrocentric chromosomes take part in the formation of satellite associations at the 52nd, 72nd and 90th hours of culture. They found that this occurs in the first mitosis with equal frequency, but in further mitoses, different association capacities appear. Zang and Back (1968) reported that the incidence of satellite association differed significantly between two of their experimental series. They compared the micro-method of Arakaki and Sparkes (1963) with the macro-method of Moorhead et al. (1960). In the micro-method, heparinized whole blood is inoculated into the basic culture medium, whereas in the macro-method, sedimented leucocyte rich plasma is used. Using TC. 199 as the basic culture media, 1,300 metaphases were examined in

the macro-method. In the micro-method, 1550 metaphases were analysed, these were grown in McCoys 5A media. It was found that in the latter series (the micro-method), all absolute counts of satellite associations were significantly lower; 37.8 $\pm$ 6.44 percent of available acrocentric chromosomes were involved in satellite association complexes in the macro-method, compared with 23.9 $\pm$ 4.09 percent in the micro-method. In their later paper (Back and Zang 1969) they compared several variants of the macro and micro-methods and their effect on the frequency and pattern of the satellite associations. These experiments included the same types of culture media as above, (macro-method using TC 199 and McCoys 5A and the micro-method again using McCoys 5A as the basic media). This time, their results gave the same satellite association frequency for both types of culture. Interesting differences were found, however, in the various qualitative properties and it was concluded that the ratio of the associating 'D' and 'G' groups were significantly influenced by the methodology of their experiments.

In a similar series of experiments, Hansson (1970 a,b) used TC 199 as the basic culture media for both micro and macro-methods. His results, however, were completely opposite to Zang and Back (1968,1969), showing an absolute count of satellite associations of 38.0 $\pm$ 3.21 percent in the macro-methods compared with 61.8 $\pm$ 3.12 percent in the micro-methods: 700 metaphases were analysed in each of the methods. He considered that the cultural factors influenced the outcome of the satellite association patterns significantly and that the discrepancy in the results of

Zang and Back (1969) with his own, might well be explained by the differences in the basic media used for the cultures. Hoehn, Nagel and Krone (1971) found that there was a decrease in the satellite association frequencies when the glucose concentration of the media was doubled.

Hansson (1970a) also questioned the addition of the nutrient serum or plasma to the basic medium. He used both human serum and calf serum in his experiments. However, no difference in satellite association patterns was observed. Back and Zang (1969), also searched for any small methodological differences in both of their series. The only difference noted was in the origin of the mitogenic agent, Phytohaemagglutinin (PHA). In their first series, the PHA was obtained from Difco whilst in their second series, Burroughs Wellcome were the suppliers. However, they considered that neither the PHA or the exposure of 5% CO_2 in air to the cultures to be as influential on the satellite association pattern as the cleanliness of the glass slides for the final preparation!

Both Zang and Back (1968), Back and Zang (1969) and Hansson (1970a,b) did not consider the subsequent hypotonic treatment in their experiments as having any influence on the satellite association pattern. However, the choice of hypotonic solutions was considered to be very important by Pyatkin et al. (1969, 1972) who found greater frequencies of aberrations in cells treated with potassium chloride than with Hanks solution in distilled water. In their work on the cytogenetics of bone marrow after gamma-radiation, they

found that at the lower level of radiation and in unexposed samples, the number of aberrant metaphases was 36% with 0.5% potassium chloride, compared with 22% with 25% Hanks solution in distilled water. The hypotonic solution used by Zang and Back (1968) and Back and Zang (1969) in all of their series was sodium citrate, the concentrations varying between 0.7% and 1%. Hansson (1970a,b, 1976b) used distilled water, added to three times the volume of the culture medium.

The effect of fixation upon the cells has not been thought to contribute to any significant changes in the association patterns. Zang and Back (1968), Back and Zang (1969) and Hansson (1970a,b, 1975a,b) in all of their experiments have used glacial actic acid as the main constituent of the fixative. Zang and Back (1968) and Back and Zang (1969) used the conventional glacial acetic acid/methyl alcohol (3:1) solution whilst Hansson (1970a,b, 1975a, b) used a glacial acetic acid/1N hydrochloric acid (9:1) mixture.

However, Back and Zang (1969) did find an increase in the satellite association patterns when the fixed cells were resuspended in a 70% glacial acetic acid, rather than the glacial acetic acid/methyl alcohol mixture. No explanation was given for this finding other than to endorse their original statement that the actual mechanical procedure in producing the slides was critical. Flame drying of the smear, differences in the temperature and thickness of the water film on the slide and blowing on the slides to help spread the cells were some examples given as

possible influences affecting the number of associations seen (Back and Zang, 1967, Hoehn, Nagel and Krone, 1971). It was considered that preparation of the smears by one individual would help minimise some of the inevitable sources of error in the preparative technique.

It can be seen from these results that the cause of satellite association patterns is a complex phenomenon the nature of which is still largely unknown, but that the technical factors on culturing the cells can influence the outcome significantly.

c) Cytogenetics - Nuclear Fine Structure

Many authors have thought there might be some correlation between satellite association and chromosomal non-disjunction. (Ferguson-Smith and Handmaker 1961a,b, 1963, Harnden 1961, Ohno et al. 1961, Ferguson-Smith 1964, Kiossoglou et al. 1964, Tips et al. 1964, Lyons et al. 1965, Zellweger and Abbo 1965, Abbo et al. 1966, Mertz and Prempree 1966, Zellweger et al. 1966, Evans 1967). The most frequent cause of aneuploidy in man is non-disjunction and this phenomenon might provide one possible explanation for the translocation and non-disjunction in mitosis and meiosis. Hansson (1975a) showed a significantly increased number of satellite associations between the number 14 and 21 chromosomes in cases of hypothyroidism. This is particularly relevant as there appears to be an increased frequency of hypothyroidism in mothers of mongol children, which would greatly increase the risk of non-disjunction occurring.

It is known that the satellited chromosomes are involved in the formation of the nucleolus (see Figure 6) (Hsu et al. 1965) and that the regions below the satellites are the loci of ribosomal DNA, synthesising nucleolar material (Henderson et al. 1972). Indeed, Ferguson-Smith and Handmaker in 1961 used the term "nucleolar chromosomes" when describing the acrocentric 'D' and 'G' groups of chromosomes. It was noted that the satellites on the short arms were in frequent association with the nucleoli during mitotic prophase (Ohno et al. 1961). Furthermore, the association of their bivalents with nucleoli in the pachytene of spermatogenesis (Ferguson-Smith 1963, Hungerford 1971) and oogenesis (Stahl and Luciani 1972) has been reported. This would clearly assign the function of nucleolus formation to the satellites. However, correlation between biochemical and cytogenetical findings of r-DNA in nucleolus organising regions, (Dittes et al. 1975), shows that the amount of r-DNA in the human genome is not primarily a function of the number of acrocentric chromosomes. More recently Zankl and Zang (1972) proved that the loss of two or more acrocentric chromosomes significantly reduced the number of nucleoli present.

At the fine structural level, Kasten and Strasser (1966) observed that perinucleolar chromatin often intrudes directly into the nucleolar mass and by in-situ DNA-RNA hybridization techniques, the ribosomal cistrons have been assigned to the satellites (Henderson et al. 1972, Bross and Krone 1972). This has been demonstrated by differential staining of the

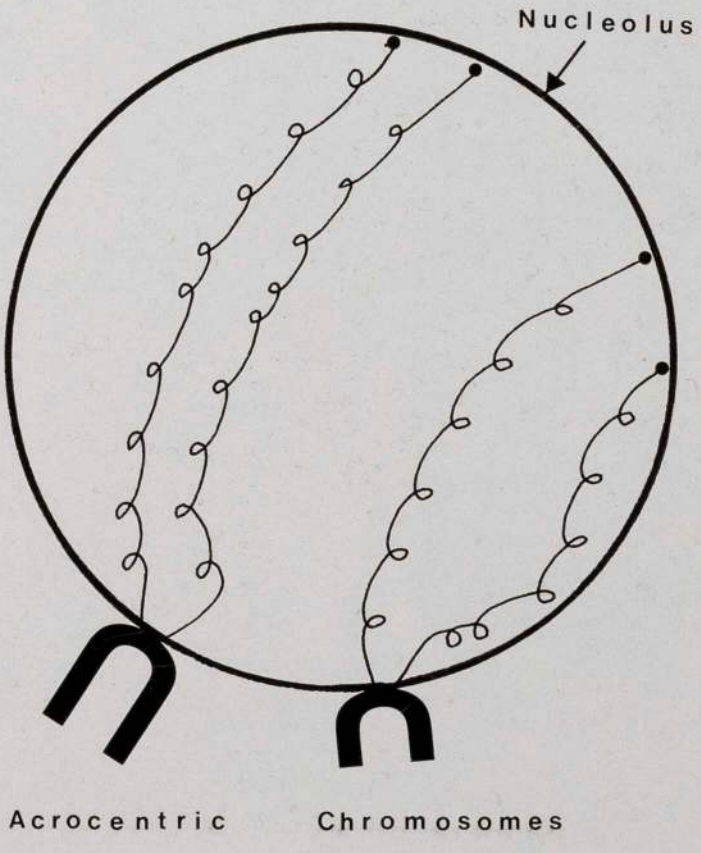


Figure 6 Diagram showing the acrocentric chromosomes attached to a nucleoli as they appear in early prophase. The nucleolus-organising regions are widely stretched. (After Ohno et al, 1961).

satellite regions by Matsui and Sasaki (1973), Howell et al. (1975), Denton et al. (1976) and Goodpasture and Bloom (1976) (Figure 7).

Threads connecting satellites are occasionally visible, for example, Zang and Back (1968) reported them in 10% of associations. Lampert et al. (1969) and Du Praw (1969) showed the presence of inter-satellite fibres by electron microscopy. These consisted of a type 'B' nucleoprotein. In order for these fibres to become visible to the light microscope, a large bundle of such fibres would be needed, hence, any connecting threads between associating chromosomes are probably present more often than actually seen. Henderson et al. (1973) using in-situ hybridization techniques, found that in 18 out of 105 metaphases examined, there appeared to be connections between the satellite regions of different acrocentric chromosomes.

Altered association frequencies of individual acrocentric marker chromosomes having enlarged, tandem (duplicate) satellites or elongated short arms (stalk), have also been reported. Bauchinger and Schmid, 1969, Rocchi et al. 1971, Gigliani et al. 1972, De Capoa et al. 1973, Schmid and Krone 1974) compared the relationship between these polymorphic variants and the satellite association patterns. Their results confirmed that acrocentric chromosomes possessing two satellites (tandem) and those having elongated short arms (stalk or nucleolar constriction) associated more frequently. However, those with short arm deficiencies associated less (or not at all)

Figure 7

Differential staining of satellite regions by the silver method of Goodpasture and Bloom (1976).

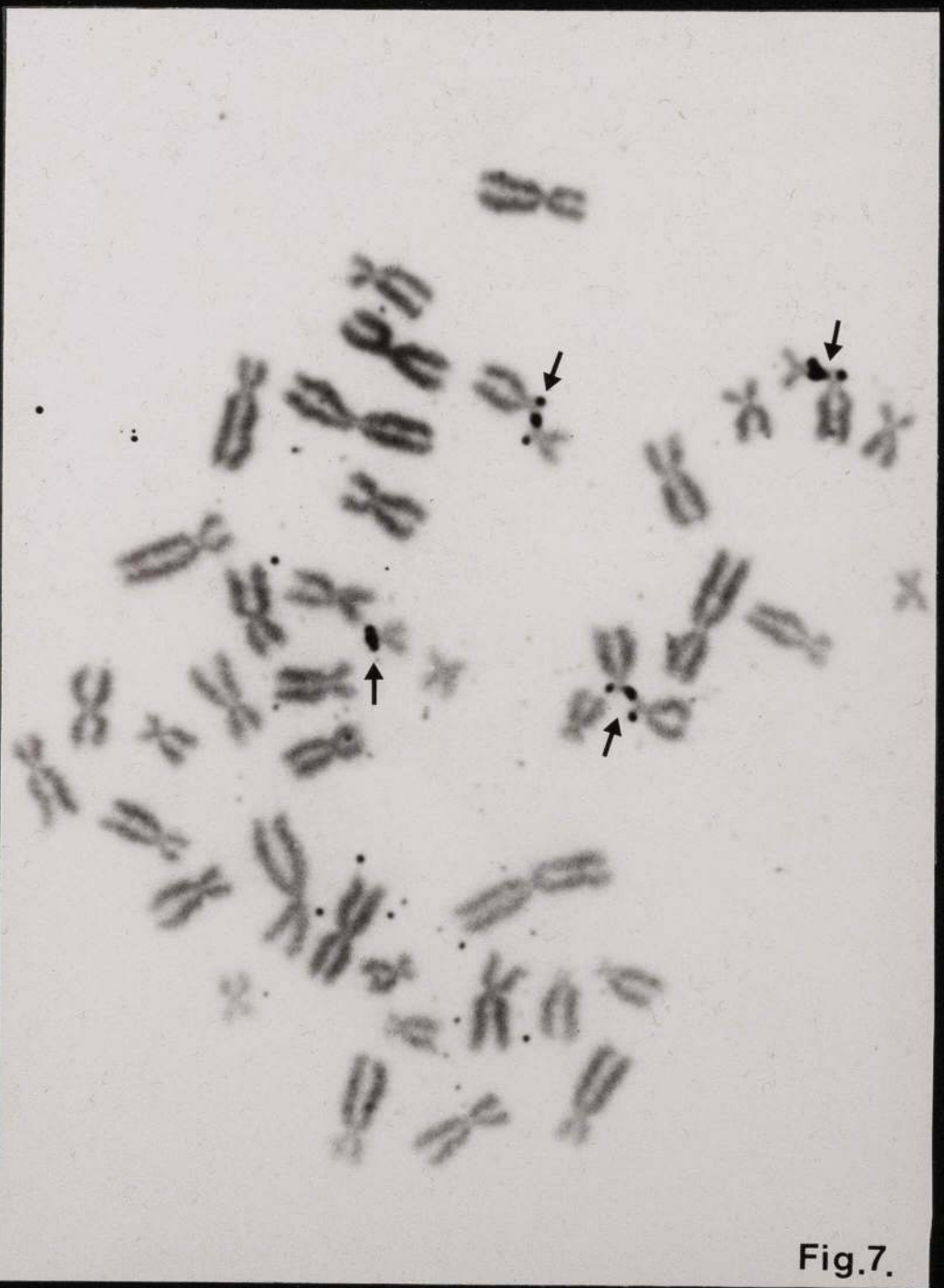


Fig.7.

than the "normal" acrocentrics. They concluded by assuming that an acrocentric chromosome participates in the formation of a nucleolus more often if it has a longer nucleolar constriction with satellites, than those without.

Contrary to this, Zankl and Zang (1974) observed a decreased frequency of association in their series of 130 acrocentric marker chromosomes (Dp+, Gp+). However, with the enlarged satellited markers (DS+, GS+), they too found increased association patterns but only with the DS+ type. They considered that the differences in the satellite association patterns of the marker chromosomes may indicate an involvement of the nucleolus-organising regions into some structural re-arrangement of the short arm or satellite regions. In fact recent work by Henderson and Attwood (1976) show a definite correlation between the amount of r-DNA and the frequency of participation in satellite associations seen in double satellited acrocentric chromosomes.

When nucleoli fuse, the mechanical stretching might lead to the breakage of the nucleolus forming segment of the chromosome (Ohno et al. 1961). It has been demonstrated that an increase of nucleolar area can be produced by the addition of thioacetamide to human embryonic fibroblast cultures (Zhdanova 1974). Observations on the subsequent metaphase spreads revealed a significant increase in the satellite association patterns. Hence, when the nucleoli amalgamate at the beginning of G1 and are isolated during cell division, non-disjunction could occur, especially if

these fused nucleoli persist throughout. Thus, if the nucleolar organising region is damaged, and breakage occurs, the proximity of the broken ends would predispose to the increased tendency of non-disjunction involving the satellited 'D' and 'G' groups of chromosomes. In fact, an increased percentage of acrocentric chromosomes with satellite variants have been observed in mongols and mothers of mongols (Zankl and Zang 1974). This is particularly relevant, as trisomy 'G' or Down's Syndrome (Mongolism) is the commonest autosomal trisomy in man with an overall incidence of one in seven hundred live births.

I.iii Down's Syndrome (Mongolism)

a) General

Down's Syndrome or Mongolism, was first described by John Langford Down in 1866 who called the condition "mongolian idiocy". However, Seguin in 1846 appears to have referred to the syndrome under the description of "furfuraceous cretins", and as such, was not recognised as distinct from other forms of mental subnormality. Almost certainly, however, it had been known to exist long before then, possibly as far back as the seventh century (Brothwell 1960), while some sixteenth and seventeenth century paintings have depicted infants with mongoloid features. There has been much speculation as to its aetiology, and studies in different parts of the world show that the condition occurs in all national and racial groups.

In 1932 Waardenburg and then Penrose in 1939, suggested that mongolism might be caused by a chromosome abnormality. This was not validated until 1959 when Lejeune, Gautier and Turpin demonstrated in nine affected children, the presence of 47 chromosomes in every cell. Down's Syndrome was thus shown to be associated with an additional chromosome. This extra chromosome was found to be always identical with the 'G' group of chromosomes. Modern banding techniques have proved that this extra chromosome is in fact a number 21, (Figures 8 and 9). Down's Syndrome is now described as Trisomy 21 and in this form of the condition is found in approximately 90% of all cases, these are described as "regular mongols". In the other 10% of cases, the extra

Figure 8

Giemsa banded karyotype of a regular trisomy 21 female.

Figure 9

Quinacrine fluorescent banded karyotype of a regular trisomy 21 female.

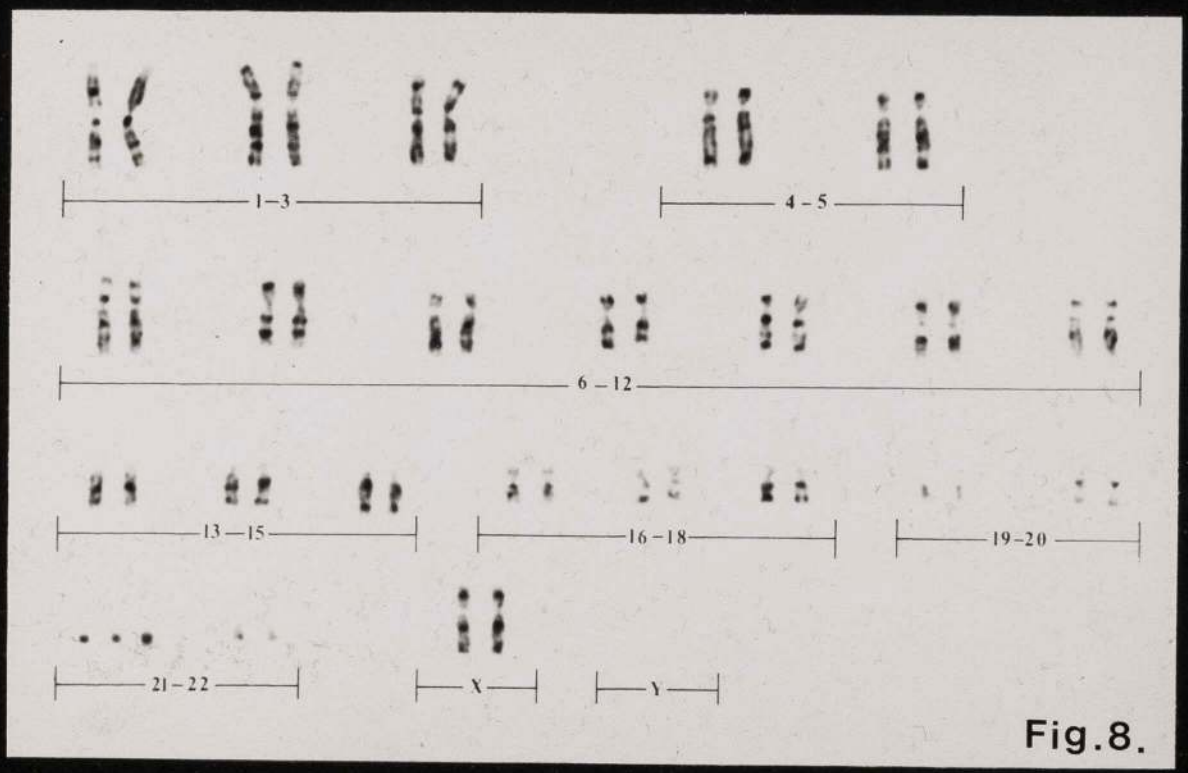


Fig.8.

Fig.9.

chromosome is fused to another chromosome belonging to either the 'D' or 'G' groups (Figures 10 and 11). The term used for this group is called "translocation mongols", which can, though not always, be familial in origin.

Children suffering from Down's Syndrome are unique in many ways, and as such, few remain undiagnosed. The affected children look alike and are markedly different from both normal and other retarded children (Figure 12). Penrose and Smith (1966) have compared the frequency of physical signs quoted by different authors for Down's Syndrome in the newborn. The ten most characteristic signs are given in Table I. The diagnosis in the newborn is more difficult than in later life, and as expected with an abnormality present in every cell nucleus in the body, the signs and symptoms are widely spread. It has been shown that the complications of Down's Syndrome can sometimes lead to failure to thrive in the neonatal period, the commonest causes being congenital heart lesions or duodenal obstructions. The association of Down's Syndrome with leukaemia has also been described. Holland et al. (1962) found that the death rate from leukaemia in Down's Syndrome to be eighteen times that of the general population. The type of leukaemia found in such cases are predominantly lymphatic or myelogenous. This finding is particularly interesting in the case of chronic myeloid leukaemia, where an abnormal G22 chromosome is seen as a diagnostic feature.

Tan et al. (1973) and Lippitt and Fridowich (1973) established that the locus for the enzyme superoxide dismutase

Figure 10

Giemsa banded karyotype of a G/G (21:21) translocation female.

Figure 11

Giemsa banded karyotype of a D/G (14:21) translocation female.

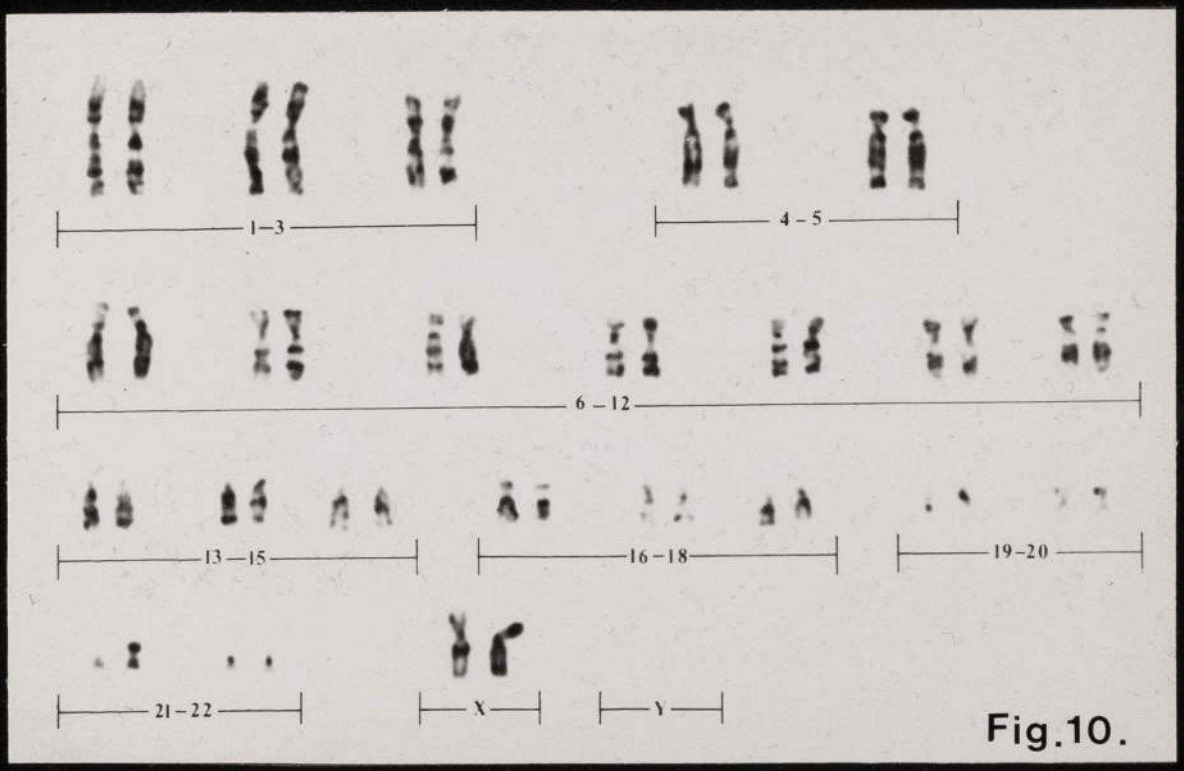

1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

Fig.10.

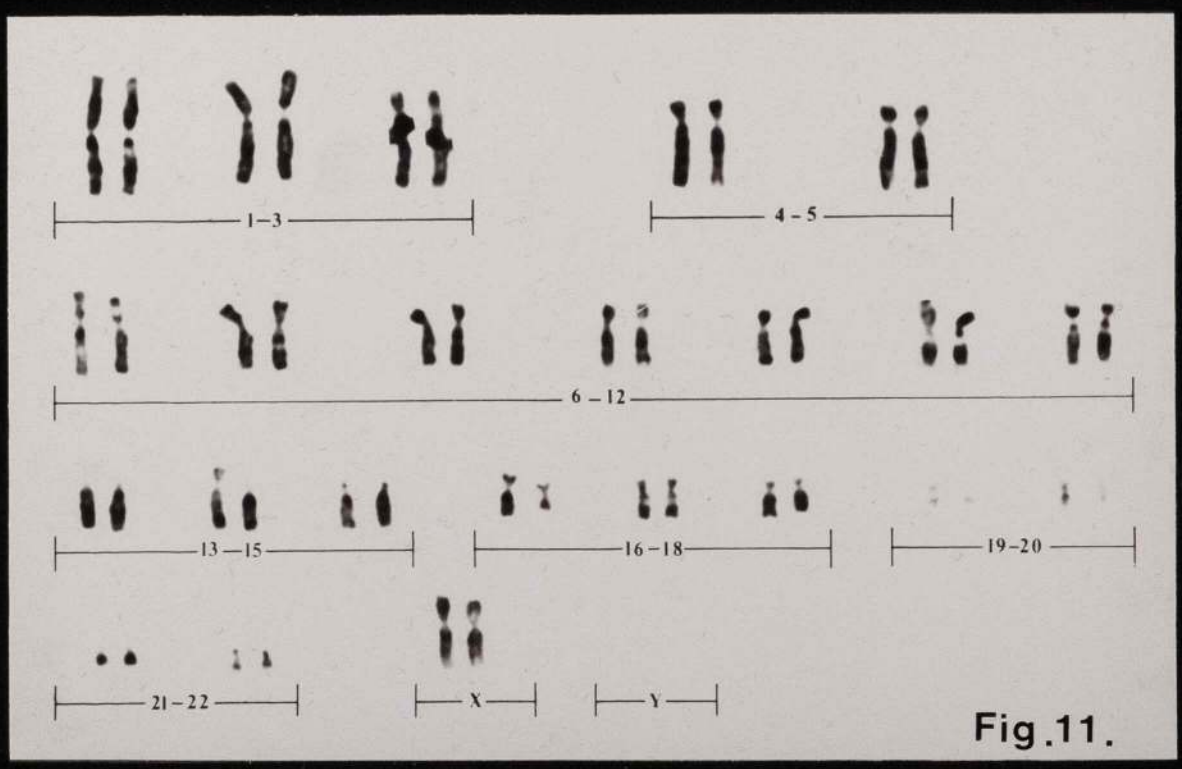

1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

Fig.11.

Figure 12

Typical physical appearance of a Down's Syndrome child.

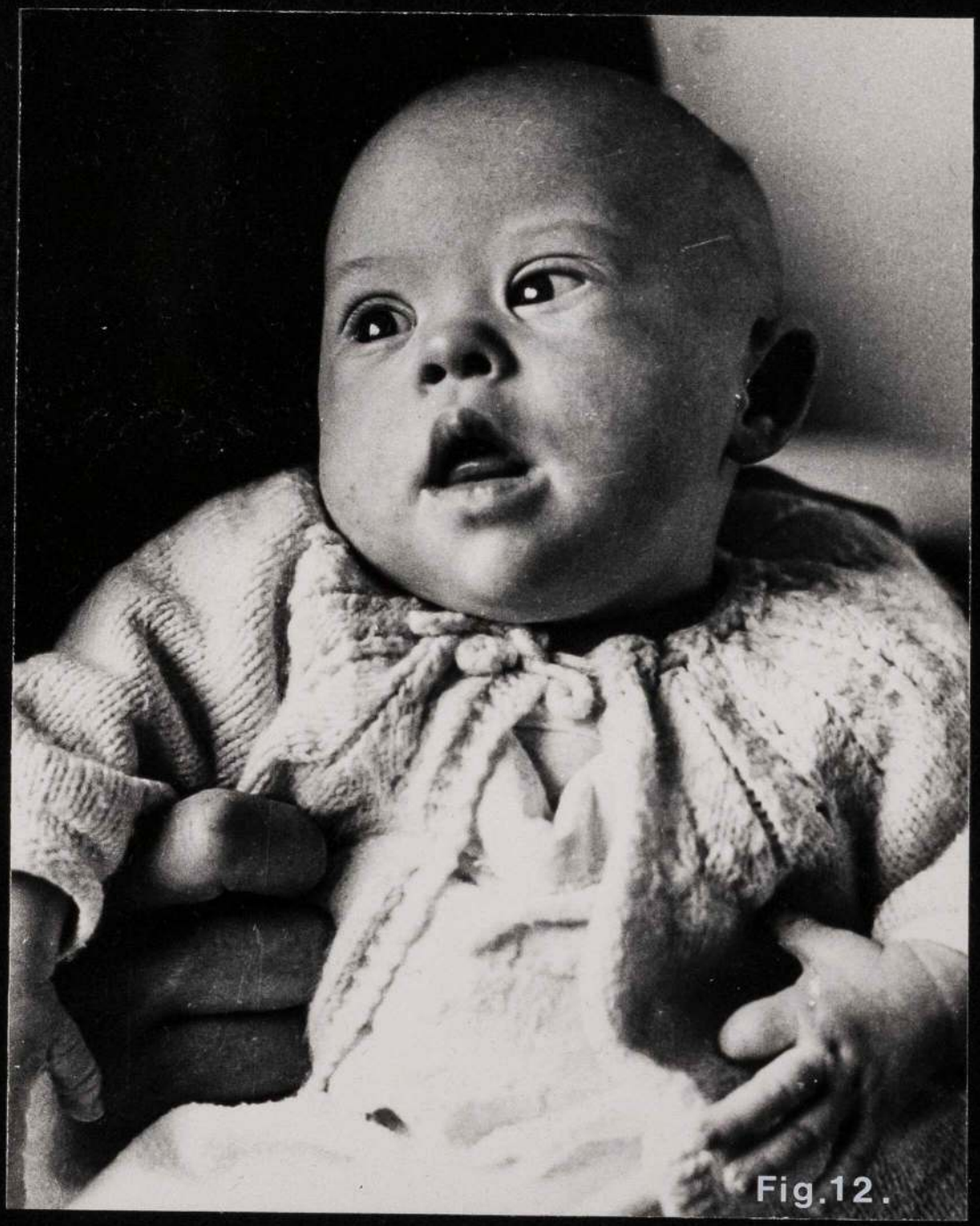


Fig.12.

Physical Signs	Frequency (%)
Flat facial profile	89
Lack of Moro reflex	82
Abundant skin (neck)	81
Oblique palpebral fissures	80
Hyperextensibility	77
Hypotonia	77
Flat occiput	74
Small teeth	71
Short broad hands	69
High arched palate	67
Dysplastic pelvis	67
Dysplastic ears	62
Furrowed tongue	59
Dysplastic fifth middle phalanx	58
Four-finger crease	54
Curved fifth finger	48
Epicanthic folds	28

Table I The most common physical characteristics of Down's Syndrome

(SOD-A) is carried on chromosome 21. Further work on this enzyme (Sinet et al. 1974, Sichitiu 1974 and Crosti et al. 1976) has demonstrated increased activity in trisomic 21 cells, to show the presence of a simple gene dosage effect. The exact location of this gene on chromosome 21 will be of value in cases of partial trisomy 21. Lejeune (1976) considers that the loci for the clinical appearance of Down's Syndrome is situated on the long arm of chromsome 21. Recently, Poissonnier et al. (1976) reported a case of an abnormal chromosome 21 (in which a duplication of segment 21q21→ 21q22.2 occurred) which showed all of the signs of Down's Syndrome. When the phenotype was compared with that of other partial and total trisomy 21 cases, it was postulated that the characteristic features of mongolism and in particular mental retardation, is due to trisomy 21q22.1 and perhaps 21q22.2 (Figure 13).

b) Satellite Association Involvement

Several workers have noticed cases of increased satellite association in families in which trisomy 'G' occurs. Zellweger et al. (1966) found in four families of translocation mongolism that both of the affected children and their parents had a raised satellite association pattern. A statistically significant increase in the satellite association patterns was noted between normal and mongol children by Rosenkranz et al. (1969). However, neither Froland and Mikkelsen, (1964) nor Zang and Back (1966) could show any significant increase in the frequency of

Figure 13

a) Diagrammatic representation of the banding patterns on the X, Y, D, E, F and G groups of chromosomes.

b) Enlargement of the G21 and G22 banding patterns to show regions and bands.

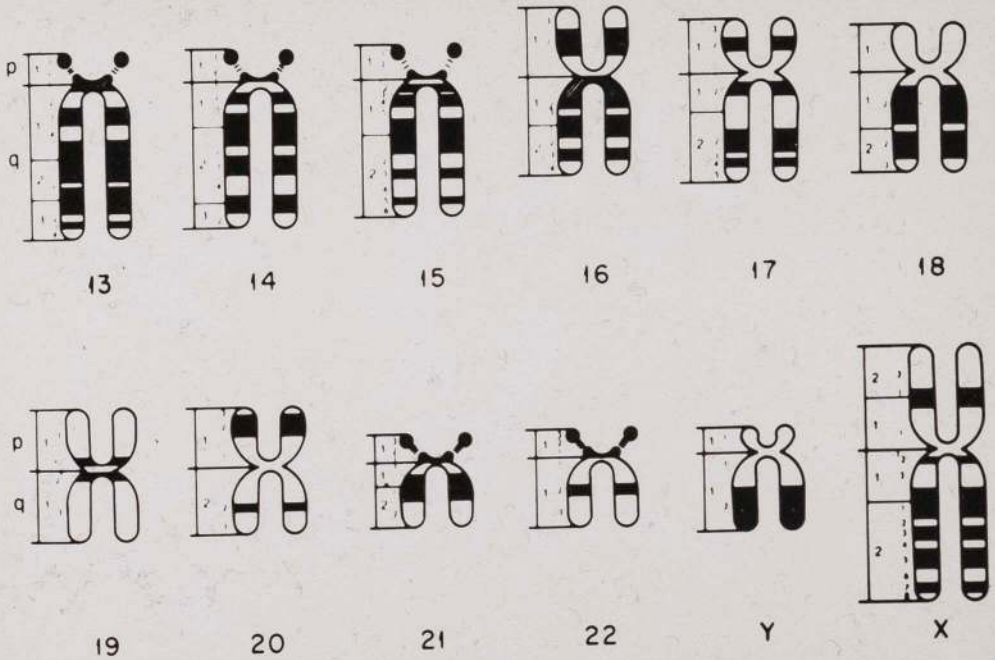


Fig.13 a.

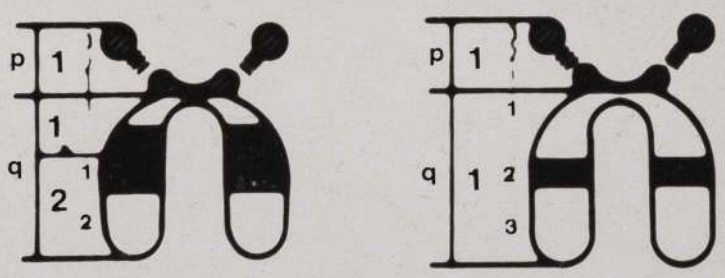


Fig.13 b.

satellite association in mothers of mongols or children, compared with the normal population. More recently, Cooke and Curtis (1974) were not able to establish any definite pattern although a number of significant differences emerged between parents and controls which needed further study. Taysi (1975) studied the association patterns using Giemsa banding techniques from chromosomally normal parents and parents of regular mongol children. His results showed that chromosomes 21 and 22 were involved in satellite associations more frequently than any of the other acrocentric chromosomes. However, he did not find any difference between the parental and normal control groups in relation to these frequencies.

However, Hansson and Mikkelsen (1974) found a significantly increased satellite association pattern involving the 21 chromosome in mothers of regular mongol children as compared with mothers of chromosomally normal children. This tendency was also observed in mothers of children with Robertsonian translocation children by Hansson, (1975) and Mikkelsen et al. (1975). Broustet et al. noted a higher than average incidence of satellite association in the parents of a sibship with trisomy 21 and monosomy X. Mattei et al. (1974) found in parents of trisomies, lowered associations involving chromosome number 15 and increased associations with number 22.

c) Parental Age

The association between Down's Syndrome and parental age

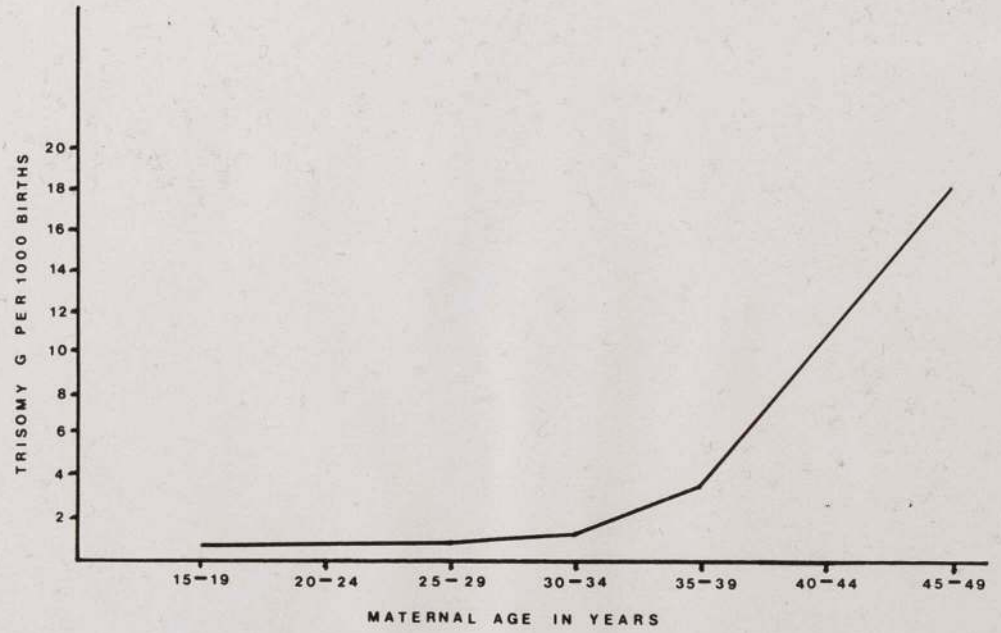


Figure 14 Graph showing the relationship between the incidence of Down's Syndrome (mongolism) and the maternal age (after Levine, 1971).

has been known for some time, with Frazer and Mitchell (1876) first producing evidence of a later maternal age effect. Subsequently, Penrose (1932) and Jenkins (1932) confirmed this finding showing that the paternal age was irrelevant (but see page). Penrose (1934) was then able to show that the late maternal age effect was independent of parity (Figure 14). Other than this, no significant etiological factor has so far been confirmed and the significance of this relationship is unknown, but it might represent a decrease in meiotic efficiency with the increasing age of the oocyte. German (1968), postulated that this relationship might be explained by a delayed fertilization in older women due to spasmodic or decreased frequency of coitus. However, Penrose and Berg (1968), Cannings and Cannings (1968) and Matsunaga and Maruyama (1969) have shown by their results that German's hypothesis cannot account for this relationship unless some other factors are related to age, other than coital intervals.

Viral infection (Coleman and Stoller 1962), fluoride concentration (Rappaport 1963) and atmospheric pollution (Greenberg 1964) have all been reported as a possible contributary factor in the aetiology of Down's Syndrome. In recent years, radiation (Schuman and Gullen 1970, Wald and Turner, 1970) and hepatitis (McDonald 1972) have been suggested. Carr (1967, 1970) reported an increased frequency of triploidy among abortions in mothers who had ceased to take oral contraceptives less than 6 months before conception. The number of trisomies was not increased, but McQuarrie (1970)

found an increased incidence of chromosome breakage and satellite associations in 23 female patients using oral contraceptives.

The paternal role in the aetiology of Down's Syndrome has also been questioned. Sasaki and Hara (1973) and Uchida (1973) have both reported cases in which it was demonstrated that the extra G21 chromosome was of paternal origin. In both cases, a brilliantly fluorescent satellite was used as a marker to determine the origin of the extra chromosome. Paternal mosaicism is discounted as no metaphase spreads with the extra G21 chromosome were observed in 1,000 cells examined. In two recent surveys using fluorescent G21 marker chromosomes, Wagenblicher et al. (1976) and Mikkelsen et al. (1976) found that in 34 parents of mongol children, the extra G21 chromosome was of maternal origin in 21 cases and of paternal origin in 13.

It is known that certain families are more prone to the occurrence of chromosome aneuploidy and that the recurrence risk figures for Down's Syndrome (mongolism) appear to be greater than chance alone (Table II), especially when the maternal age is under 35 years at the birth of the first trisomy. From the graph showing age distribution of mothers of children with Down's Syndrome (mongolism) in Sweden, Australia and England, this consistency can be seen (Figure 15). The term "non-age related mongolism" can be used to describe such a group of cases. Indeed, recent epidemiological evidence has shown (Lindsjo 1974) that a statistically constant number of cases

MATERNAL AGE GROUPS	INCIDENCE OF MONGOLISM	
	IN POPULATION	IN FAMILIES AFTER ONE TRISOMY-21 CHILD
15 - 19	1/2,400	1/800
20 - 24	1/1,500	1/500
25 - 29	1/1,200	1/400
30 - 34	1/900	1/300
35 - 39	1/300	1/100
40 - 44	1/100	1/30
45 - 49	1/40	1/10

Table II Calculated risk values for Down's Syndrome (mongolism) in the general population and in families after one affected child. (From Allen et al. 1974)

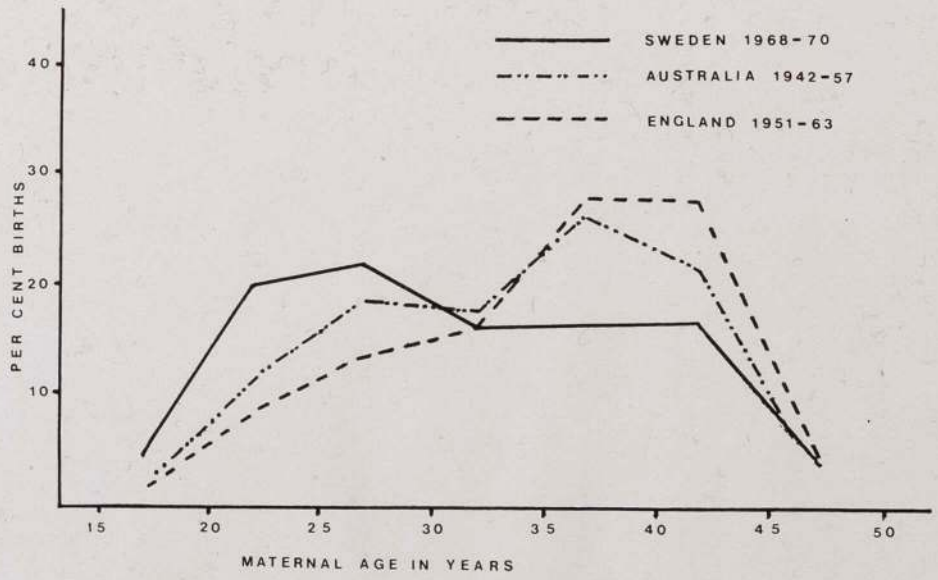


Figure 15 Graph showing the age distribution of mothers of children with Down's Syndrome (mongolism). (After Linsjo 1974).

were born to parents up to 35 years of age.

The figures relating to the overall incidence of Down's Syndrome, have fallen from a 1:650 (Carter 1951, Penrose 1954, Hall 1964) to a 1:755 in the latest Swedish figures (Lindsjo 1974). A meaningful comparison between these figures and the ages of all mothers must be taken into account (Figure 16). The shift towards a lower maternal age in the Swedish material from a median of 27 years to a median of 25 years over the past 10 years is interesting. This is clearly reflected in the distribution of mongol children in relation to maternal age graph (Figure 14). A minor peak occurs between 25 and 29 years of age for "non-age related mongolism", in the British and Australian figures. However, with the Swedish data, the trend is reversed, showing the relevant peak between 25 and 29 years, which cannot be described as age-related. In their series, Penrose and Smith (1966) divided mothers of mongol infants into two classes; those showing no shift to maternal age and those who do not. Moran (1974) questions these classes statistically, but does confirm a small group of mothers not showing a maternal age shift.

In a recent survey, Goad et al. (1976) examined 40,371 newborn infants and found a seasonal variation in the incidence of mongol children born to mothers under 35 years of age. In any one year, it was found that there were three times as many mongol children born between the months of May and October than in the rest of the year. However,

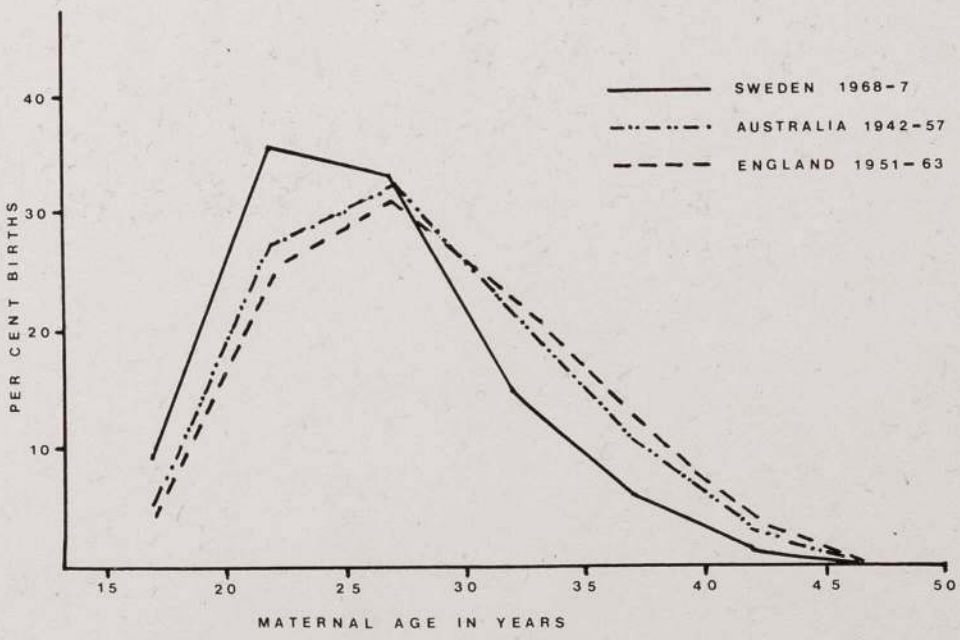


Figure 16 Graph showing the age distribution of mothers of all liveborn children. (After Linsjo, 1974).

no seasonal difference was observed in mongol children born to mothers aged 35 and over. They concluded that three distinct components can be statistically identified regarding the incidence of trisomy 21: 1) a steady endemic component in mothers under 35 years, 2) an epidemic component in mothers under 35 years and 3) a stable, larger component for mothers aged 35 and over.

I.iv The Design and Objectives of this Investigation

This investigation can be divided broadly into two sections: methodological and clinical.

The distribution pattern of acrocentric chromosomes *in vivo* remains unknown. In the first part, therefore, the object was to look closely at the effect that a hypotonic solution had on cultured cells, with regard to their satellite association patterns. Three hypotonic solutions were compared so as to obtain the lowest number of satellite associations observed in thirty-five normal males and females. From the results, a standardised technique was evolved to produce a base line from which any significant increase in association patterns could be detected.

Before any conclusions could be drawn about the effects of the various hypotonic solutions, specific criteria as to the exact definition of a satellite association had to be formulated. These criteria had to incorporate the distance, number and arrangements of the association chromosomes, so that they could then be used as a standard for all of the observations throughout the investigation.

In order to identify accurately the chromosomes involved in satellite associations, a specific form of staining is required. There are basically two techniques which can precisely identify individual chromosomes, these are fluorescence microscopy or light microscopy with critical staining. These methods are far from being infallable, and some modification was found to be necessary in order to give reproducible results for accurate

standardisation. Both of these techniques were assessed for consistency and the most suitable were selected.

When the first part of this investigation had been completed, a standard technique was developed to provide the lowest frequency of satellite associations in normal metaphase spreads, together with the accurate identification of the chromosomes involved. Only in this way was it possible to detect any significant differences in the individual patterns in the second part of the study.

The second part of the investigation deals with the clinical aspects involving parents of regular mongol children and normal controls. Blood samples from both parents of mongol children and parents of normal children were taken, with their consent. The parents of normal children were, as near as possible, matched for age with the parents of the mongol children. Confirmation of the diagnosis of mongolism of the children of the parents in this survey was carried out by the same cultural method as standard.

The specific identification of the individual chromosomes involved and the category of associations was recorded in both groups. Thus, if any significant differences in satellite association patterns were likely to be present between parents of mongol children and parents of normal children in this sample, they should be easily detected by these procedures.

II. EXPERIMENTAL PROCEDURES

II.i Experimental Design and Methods

a) Satellite Association Patterns - The Effect of Hypotonic Solutions

The first part of this study is to determine the effect of hypotonic solutions on satellite association patterns. Normally in metaphase spreads, there are always random associations, or chance proximities of the 'D' and 'G' chromosomes. The cultural technique was controlled so as to give the minimum number of chance associations. This was necessary before true, non-random associations could be evaluated. As previously reported, the basic type of culture method did not appear to influence the satellite association patterns (Zang and Back 1968), Back and Zang 1969, Hansson 1970, Curtis and Cooke, 1974) so the subsequent hypotonic solutions were examined.

The three hypotonic solutions used were potassium chloride, trisodium citrate and 25% Hanks solution, diluted with distilled water. The results from each of these procedures would indicate whether the hypotonic treatment on the cells has a major influence on the number of satellite associations per cell. It would also demonstrate which solution gave the minimum number of associations per cell. This hypotonic solution would then be used for all cultures in the second part of the study.

In order to define what constitutes a true association, of standard criteria are needed. It was therefore, decided

that two criteria would be used:

a) the satellited ends of the associating acrocentric chromosomes had to be directed towards each other, and

b) the distance between one or both of the satellites of the associating acrocentric chromosomes should not exceed the width of 1 chromatid. If the distance was greater (even if they were directed towards each other) they were excluded (see Figure 17).

Chromosome preparations were obtained from short term cultures according to the method of Arakaki and Sparkes (1963) with some modifications. TC.199 (Burroughs Wellcome) was used as the culture medium, with 15% calf serum together with Penicillin B.P. and Streptomycin B.P. (Flow Laboratories). Phytohaemagglutinin (Burroughs Wellcome) was used as the mitogenic agent. Whole blood was inoculated with aseptic technique into sterile plastic cultures tubes, each sample having three tubes (A.B. and C.). The cultures were then incubated for 70 hours at a temperature of 37°C. They were agitated at least twice per day. Colcimid (CIBA) (Colchicine 0.02%) was then added and further incubation at 37°C for 1½ hours was continued. The cultures were then transferred to a glass centrifuge tube and centrifuged at 1,000 r.p.m. for 5 minutes. After centrifugation, the supernate was discarded and the cell deposit subjected to the varying hypotonic treatments.

Cells from the first culture (A) were treated with a

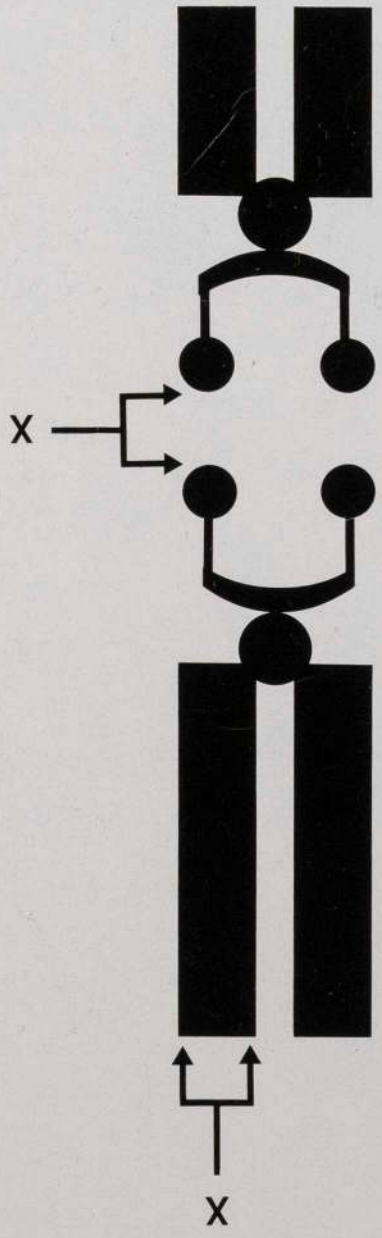


Figure 17 Satellite association criteria - the distance between one or both of the satellites should not exceed the width of one chromatid (X).

pre-warmed solution of potassium chloride (0.075M.aq.) for 8 minutes at 37°C with continuous agitation.

Cells from the second culture (B) were treated with a pre-warmed solution of sodium citrate (1% aq.) for 10 minutes at 37°C with continuous agitation.

Cells from the third culture (C) were treated with a pre-warmed Hanks distilled water solution (1:3) for 20 minutes at 37°C with continuous agitation.

All the cultures were then centrifuged at 1,000 r.p.m. for 5 minutes, the supernate discarded, and the cells fixed by adding a freshly prepared ice-cold acetic acid/methanol (1:3) fixative, drop by drop from a Pasteur pipette on to the cell deposit whilst shaking carefully. When approximately 5ml. of fixative had been added, the cell suspension was left at room temperature for ½ hour. They were then centrifuged for 5 minutes at 1,000 r.p.m., the supernate discarded and the cell deposit resuspended in fresh fixative. This washing procedure was continued twice more until the supernate was clear and the deposit of cells clean, finally resuspending in approximately 0.5ml. fixative to produce a slightly opaque cell suspension. Slides were then prepared by adding one drop of this cell suspension from a Pasteur pipette on to an inclined pre-cooled clean, glass slide, and blown gently to obtain even cell distribution until dry. Six slides per culture were made. (A more detailed account of the whole technique can be found in Appendix I).

One slide per culture (total 105 slides) was stained with Giemsa (1% aq.) for 5 minutes, rinsed in distilled water, blotted dry and mounted in DPX.

Full microscopic analysis on 15 metaphase spreads was performed on each slide, one representative cell was photographed and a chromosome karyotype constructed. This was to confirm that all of the controls had a normal chromosome complement. A further 10 metaphase spreads from each slide were then analysed for their satellite association frequencies. The criteria previously described was used and the types of satellite associations found were categorized in this order: D-G; D-D; G-G; D-D-G; D-D-D; D-G-G; G-G-G; Others; and Total. The results of the frequencies found in each of the three hypotonic solutions were recorded.

From the results of the above experiment (see page 60) it was decided to look at the effect of potassium chloride further. Two further experiments were devised to see:

a) if the molarity of the solution had any effect upon the satellite association frequency, and

b) if the time of exposure also affected the satellite association frequencies.

In the first experiment, five cultures were set up from a single blood sample from a known chromosomally normal male control, and cultured as in the standard technique used in the first experiment. For the hypotonic stage, however, each culture was treated with a different solution of potassium chloride of the molarities outlined in Table III.

	Culture A	Culture B	Culture C	Culture D	Culture E
Molarity of KCl	0.02M	0.04M	0.06M	0.08M	0.10M
pH of KCl	7.10	6.75	6.60	6.50	6.45
Chloride Ions	20	40	75	90	101

Table III Composition of solutions of varying molarities of potassium chloride

	Culture X	Culture Y	Culture Z
Time in 0.075M KCl	4 mins.	8 mins.	16 mins.

Table IV Varying incubation times of the cultures in 0.075M potassium chloride

The pH and chloride ions of the solutions were determined in the laboratory using a Corning-Eel pH meter (model 109) and by a Corning-Eel chloride meter (model 920).

All of the cultures were incubated at 37°C for 8 minutes with continuous agitation. The fixation, staining and microscopic analysis of the satellite association frequencies were carried out exactly as in the previous experiment, and the results recorded.

In the second experiment, using the same male control, three cultures were set up and cultured as in the standard technique as in the previous experiments. For the hypotonic stage, each culture was treated with pre-warmed 0.075M KCl and incubated at 37°C for different times, as outlined in Table IV.

The fixation, staining and microscopic analysis of the satellite association frequencies was carried out exactly as in the previous experiments and the results obtained recorded.

b) Satellite Association Patterns - Parents and Controls

For the second part of the study, further criteria has to be laid down so that a more specific assessment of the participating acrocentric chromosomes involved in satellite associations can be established. The concept of what actually constitutes a satellite association is the same as that specified in the first section (see page 39), but with a more critical observation on the positions of the

associating chromosomes. It was decided to group the types of associations into two categories:

Type A (Figure 18) - where both satellites and chromatids are positioned directly towards each other. i.e. the angle between chromatids is 180^{o}.

Type B (Figure 19) - where both satellites and chromatids are positioned obliquely to each other. i.e. the angle between chromatids is less than 180^{o}.

Thus, the positional category will be recorded as either type A or type B for each satellite association counted.

The second important criteria required is to identify each individual chromosome involved in a satellite association. This is achieved by staining the chromosomes so that each homologous pair is sufficiently different from each other so as to be accurately identified. It was at first thought that the identification would have to be by autoradiography, but this has been superseded by the use of specialised banding methods.

Caspersson(1970) was the first to use a fluorescent dye (Quinacrine dihydrochloride) for accurately identifying each chromosome by a definite banding pattern which was characteristic for each homologous pair. The banding

Figure 18

Satellite association criteria: Type A a) as represented in a di-association, b) as represented in a tri-association.

Figure 19

Satellite association criteria: Type B a) as represented in a di-association, b) as represented in a tri-association.

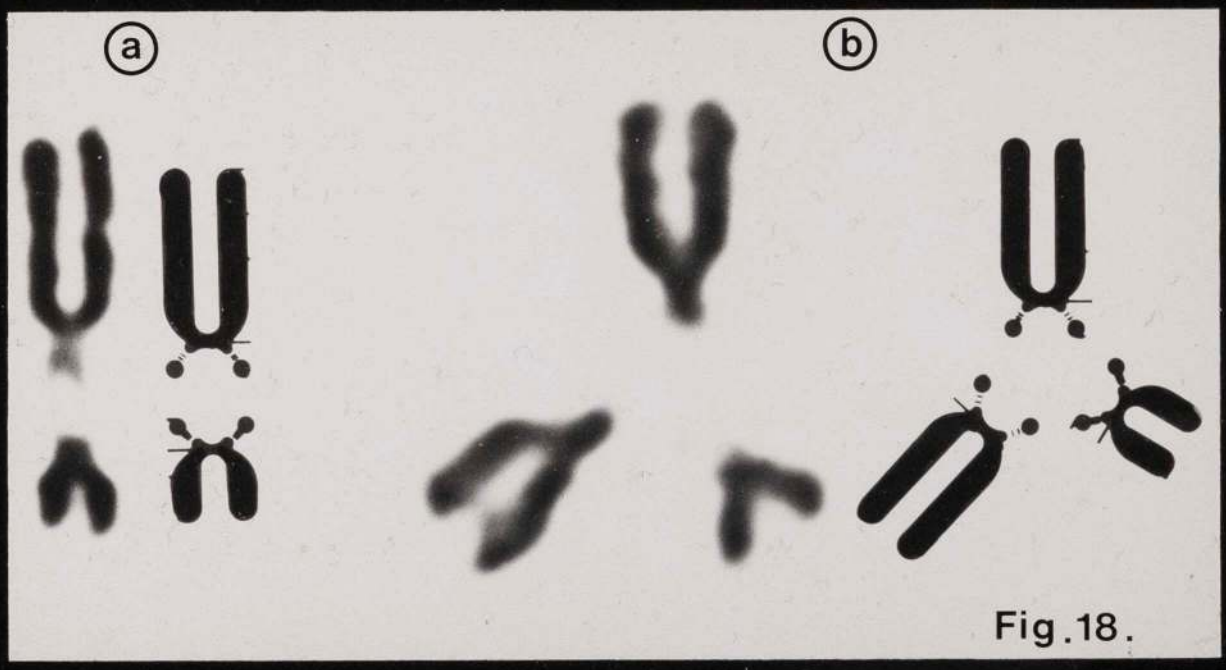


Fig.18.

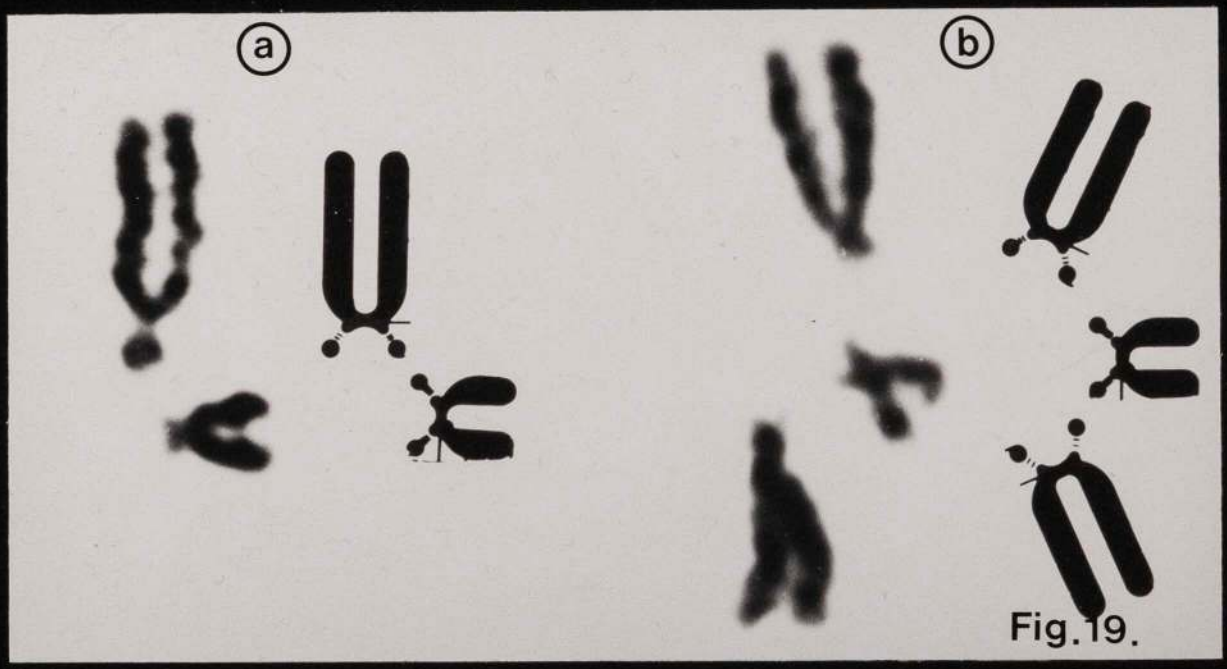


Fig.19.

pattern for the 'D' and 'G' groups are shown in Figure 20. It was thought that this method would be used and was carefully considered. However, it has the disadvantage that the dye is susceptible to fading and that a high quality fluorescence microscope is required to produce acceptable results.

It was, therefore, decided to try another banding method using Giemsa as the staining agent. This technique was first described by Sumner et al. (1971) and consisted of a pre-treatment of the slide in a salt solution (Acetic-Saline), followed by staining in a Giemsa solution (G.T.Gurr). This method failed to produce consistent results and so a modified technique (Seabright, 1971) was tried. In this method the slides were pre-treated with a trypsin solution before the Giemsa stain. However, after further experiments with this method it was found that although it gave consistently precise identification of individual chromosomes, the morphology was grossly changed. This made it difficult to see the satellite associations, and impossible to categorize them (Figure 21).

An improved quinacrine derivative (quinacrine mustard) became available in this country in 1973, and also access to a good fluorescence microscope became possible. More experiments continued in parallel with a method which combined the acetic-saline-giemsa with the trypsin-giemsa methods (Richardson and Gallimore 1973). Comparison between the two methods showed that both gave consistently good identification of the individual chromosomes without any

Figure 20

The 'D' and 'G' groups of chromosomes showing the individual giemsa banding patterns as observed microscopically and drawn diagrammatically.

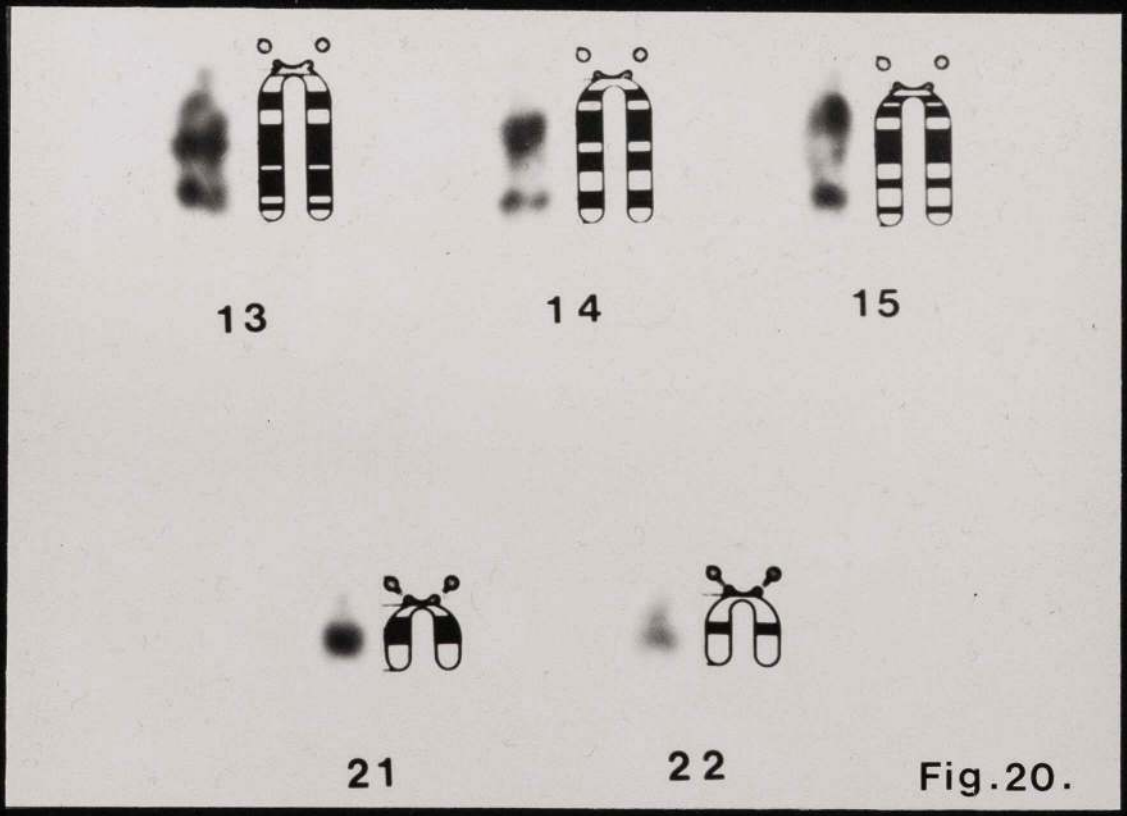


Fig.20.

Figure 21

Normal male metaphase spread after treatment with the trypsin-giemsa method.

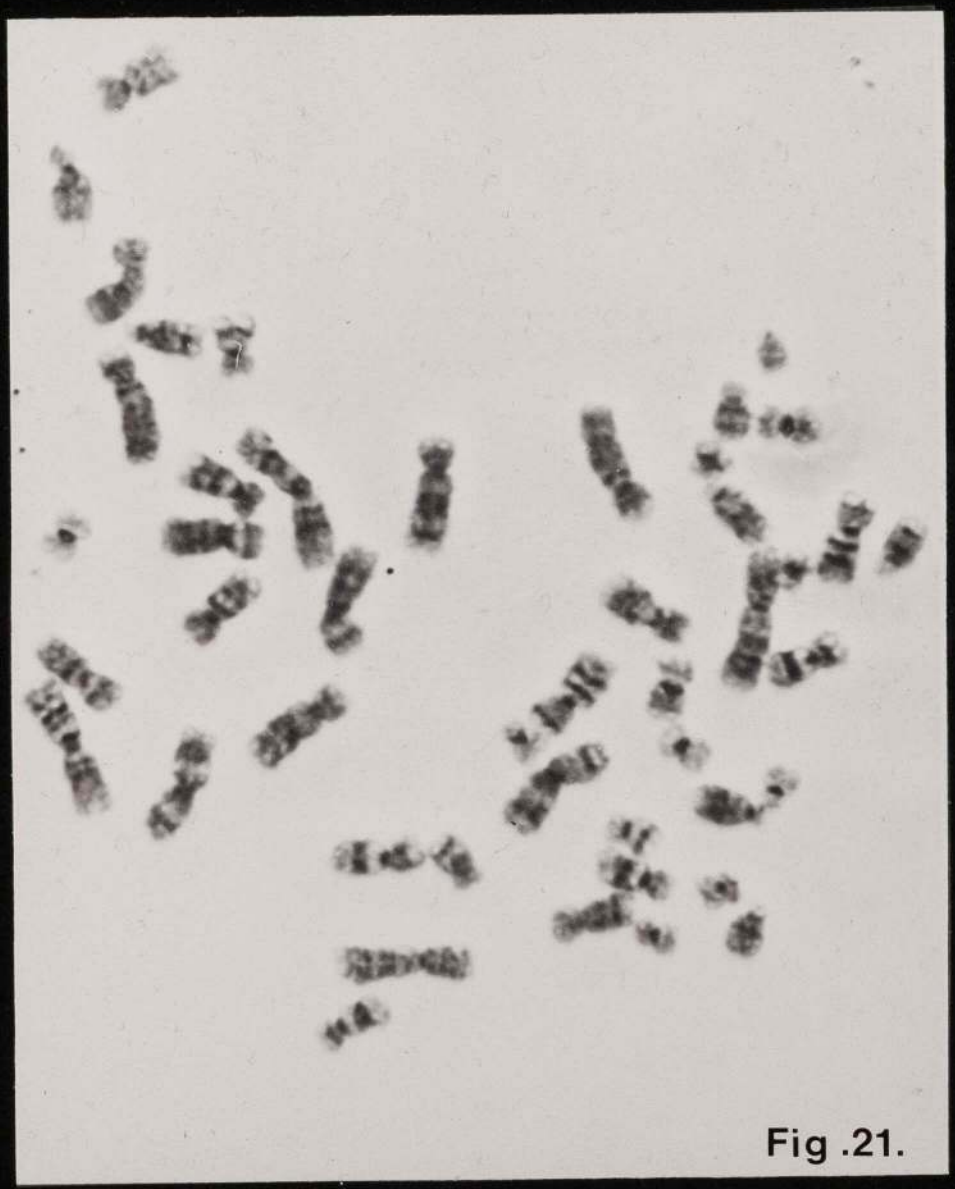


Fig .21.

alteration to their morphology. However, the modified Giemsa technique gave better photographic reproduction, together with a more permanent preparation than that of the fluorescence method (Figure 22).

It was, therefore, decided to use the modified Giemsa method for staining all of the material in this investigation. Further slight modifications were made, and the reproduction improved. Basically, the method consisted of first pre-treating the slides at 60°C with sodium chloride/sodium citrate solution (pH 6.8). This was then followed by a trypsin-saline solution at 10°C, and finally stained in Giemsa (10% aq.) stain, they were then blotted dry and mounted. (A more detailed account of the method is presented in Appendix I).

As in the first part of the study, the chromosomes were studied in short-term cultures to a modified method of Arakaki and Sparkes (1963). TC.199 was used as the basic culture medium, with 15% calf serum together with Penicillin and Streptomycin. Phytohaemagglutinin was used as the mitogenic stimulant. Whole blood was innoculated with aseptic technique into 10ml. sterile plastic culture tubes. Each sample was innoculated into two tubes. The cultures were then incubated for 70 hours at a temperature of 37°C. They were agitated at least twice per day. Colcimid (Colchicine) was then added and further incubation at 37°C for 1½ hours was continued. The cultures were then transferred to a glass centrifuge tube and centrifuged at 1,000 r.p.m. for 5 minutes. After centrifugation, the supernate

Figure 22

The 'D' and 'G' groups of chromosomes showing the individual fluorescent banding patterns as observed microscopically and drawn diagrammatically.

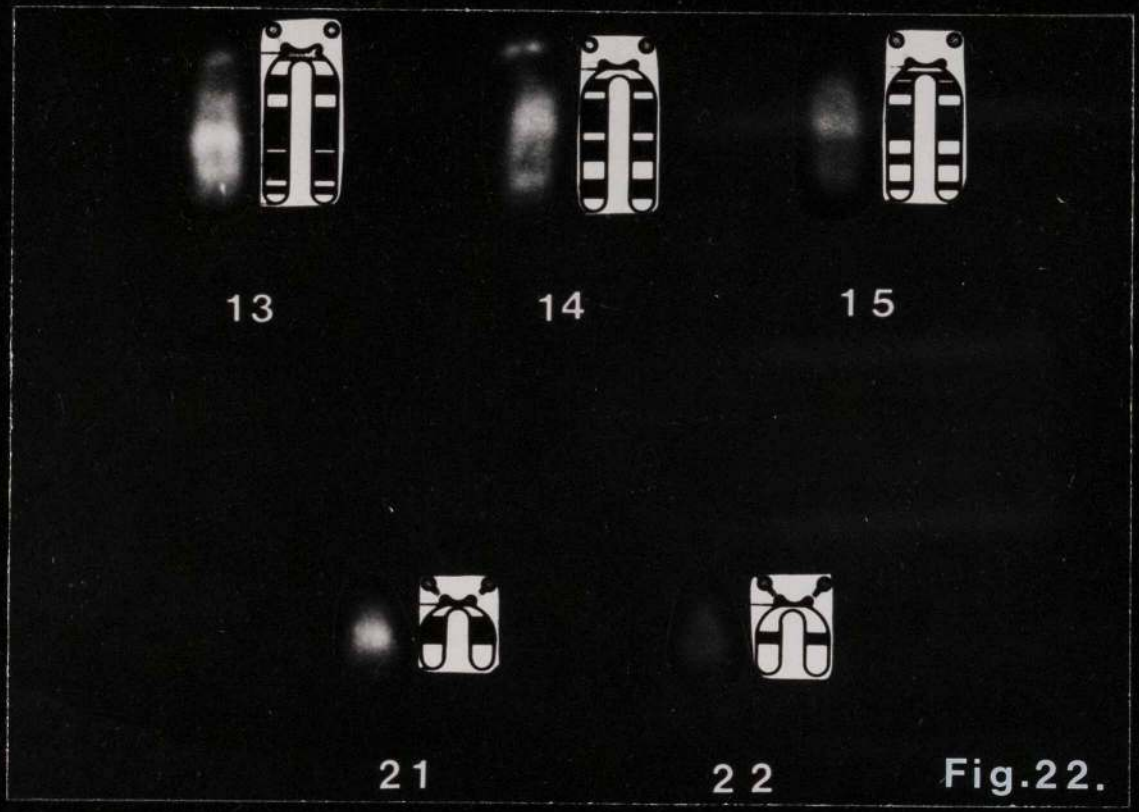


Fig.22.

was discarded and to the cell deposit, the hypotonic solution added.

The hypotonic solution used and corresponding exposure times will be those that in the first part of this study gave the lowest overall number of satellite associations per metaphase spread. After this treatment, fixation and slide preparation was completed in exactly the same manner as previously described in the earlier experiments (see Appendix I). Six slides per culture were made.

The unstained slides were screened using phase-contrast microscopy to determine whether the cultures had grown satisfactorily enough so as to provide sufficient metaphase spreads for examination. If of poor quality and insufficient, a repeat culture from the original blood sample was again set up. If this failed, no further examination on this sample was done.

Four slides from each case were then stained with the Giemsa banding technique (Richardson and Gallimore 1973) as described earlier (see page 50). Full microscopic analysis on 15 metaphase spreads was routinely performed on each case. One representative cell was photographed and a chromosome karyotype constructed. All of the microscopy and photomicroscopy was carried out on a Zeiss Photomicroscope III using the X6.3 Apochromat for screening, and the X100 Planapochromat for the final analysis. The film used for the photomicroscopy was Kodak 'Recordak Microfile'; this was developed in Kodak D-11 developer and fixed in Kodafix. The negatives were enlarged to whole plate size (16cm. x 22cm.)

printed on Kodak WSG paper, developed in Kodak D163 and fixed in Kodafix.

When all of the cells from the parents of mongol children and the control parents were confirmed chromosomally normal, the actual analysis of the satellite association patterns could begin. From each case, 100 normal metaphase spreads of good quality were examined, and the following observations recorded:

a) the number of associations seen in each cell.

b) specific identification of each individual acrocentric chromosome participating in an association.

c) the category of the association.

Strict adherence to the criteria previously described was followed in all observations.

II.ii Experimental Sample

a) The Effect of Satellite Association Patterns - Hypotonic Solutions

A total number of 35 normal controls were used for this part of the experiment. These consisted of 21 male and 14 female volunteers, ages ranging from 19 to 25 years with a mean age of 20.4 $\pm$ 0.27 years; all were chromosomally normal (see Table V). A sample of 5ml. venous blood was taken into a lithium heparin tube from each individual. This was given a consecutive number to be used throughout the experiment as the only means of identification.

For the molarity and pH experiments, a 5ml. venous blood sample was taken in a lithium heparin tube from a chromosomally normal male aged 34 years with no family history of chromosome abnormalities.

b) Satellite Association Patterns - Parents and Controls

Blood samples were obtained from parents of mongol children and normal control parents. A 5ml. venous blood sample was taken into a lithium heparin tube from each individual, and was treated identically, and issued with a consecutive number. This number was the only means of identification throughout the procedure, thus giving no opportunity for bias during the analysis of the chromosome spreads. This type of blind trial is extremely important in that no preconceived ideas on the satellite association patterns can be seen until all of the experimental data is

complete.

A form was designed and issued, so as to obtain as much information as possible, regarding the history of both parents of mongol children, and for the normal controls. The following details were provided in confidence:

surname, forename, address, date of birth, nationality, general practitioner, children (d-o-b, sex, details), pregnancy (drugs, accidents, radiation etc.) and family history (illnesses, affected siblings etc.)

This information was recorded on one form for both husband and wife (full sample details are recorded in Appendix II).

The co-operation of consultant paediatricians, clinical geneticists, obstetricians and gynaecologists in the Birmingham and Solihull Hospitals was secured. The Down's Babies Association was also approached, and kindly assisted in providing volunteer parents willing to donate a blood sample for this investigation. For the purpose of this study, parents up to the age of 35 years were used, having a mean age of 26.7 $\pm$ 1.10 years (see Table VI). This enables the "non-age related" groups of parents only (see page 30) to be evaluated.

The controls, namely parents of chromosomally normal children, were matched for age and parity as near as possible. Blood samples were obtained from married colleagues, friends and parents visiting children on the surgical wards at East Birmingham Hospital. All of the normal controls were volunteers and at no time was any

coercion practiced in order to obtain the specimens. The average age for the control group was 27.6 $\pm$ 0.79 years (see Table VII).

Difficulty in obtaining specimens was at first encountered. One problem was that samples received sometimes only came from one parent, usually the mother. This situation improved, and throughout the time of the study, 56 parents of mongol children (i.e. 28 couples) were received. Where possible, samples from the mongol children of the families concerned were also forwarded.

Control samples from parents of normal children were also difficult to obtain. It was first thought that volunteers from the family planning clinic might be a source of such material. However, this proved to be a very unsatisfactory source and was abandoned. Finally, a total number of twenty-four parents (i.e. twelve couples) were obtained.

Number	Sex	Age	Karyotype
1	F	21	46,XX
2	M	22	46,XY
3	F	21	46,XX
4	M	22	46,XY
5	M	20	46,XY
6	M	23	46,XY
7	F	21	46,XX
8	F	23	46,XX
9	F	23	46,XX
10	F	22	46,XX
11	M	19	46,XY
12	M	19	46,XY
14	M	20	46,XY
15	M	19	46,XY
16	M	20	46,XY
17	M	21	46,XY
18	M	19	46,XY
19	M	19	46,XY
20	M	20	46,XY
21	M	19	46,XY
22	M	20	46,XY
23	F	19	46,XX
24	M	19	46,XY
25	M	19	46,XY
26	F	19	46,XX
27	F	21	46,XX
28	F	19	46,XX
29	M	19	46,XY
30	F	19	46,XX
31	F	21	46,XX
32	F	25	46,XX
33	M	20	46,XY
34	M	19	46,XY
35	F	23	46,XX
Mean age: 20.4 ± 0.27 years			

Table V Composition of sample - Control group for hypotonic solutions experiment

Number	Sex	Age	Karyotype	Infant
5	F	22	46,XX	47,XX,G21+
6	M	23	46,XY	
15	M	34	46,XY	47,XY,G21+
16	F	32	46,XX	
19	F	20	46,XX	47,XX,G21+
20	M	22	46,XY	
21	F	21	46,XX	47,XX,G21+
22	M	23	46,XY	
23	F	22	46,XX	47,XX,G21+
24	M	24	46,XY	
25	M	29	46,XY	47,XY,G21+
26	F	26	46,XX	
27	F	29	46,XX	47,XY,G21+
28	M	29	46,XY	
31	F	28	46,XX	47,XY,G21+
32	M	30	46,XY	
39	F	34	46,XX	47,XY,G21+
40	M	34	46,XY	
Mean age: 26.7 ± 1.10 years				

Samples used in the experiment from parents of children with Down's Syndrome

Reason	No.	Comment
Failed cultures	16	(either one or both failed)
Maternal/paternal age 35 years or above	20	(either one or both older)
Abnormal maternal karyotype	1	(46,XXt (1:17) (q21:q21)

Samples not included in the experiment from parents of children with Down's Syndrome

Table VI Composition of sample - parents of children with Down's Syndrome (mongolism)

Number	Sex	Age	Karyotype
13	F	26	46,XX
14	M	26	46,XY
33	F	26	46,XX
34	M	28	46,XY
41	F	29	46,XX
42	M	27	46,XY
43	F	21	46,XX
44	M	23	46,XY
45	M	24	46,XY
46	F	24	46,XX
47	M	34	46,XY
48	F	33	46,XX
51	M	28	46,XY
52	F	27	46,XX
59	M	30	46,XY
60	F	29	46,XX
61	M	32	46,XY
62	F	30	46,XX
Mean age: 27.6 $\pm$ 0.79 years			

Samples used in the experiment from control parents of normal children

Reason	No.	Comment
Failed cultures	4	(either one or both failed)
Maternal/paternal age 35 years or above	2	(either one or both failed)

Samples not included in the experiment from control parents of normal children

Table VII Composition of sample - control parents of normal children

III. RESULTS

III.i) Satellite Association Patterns - The Effect of Hypotonic Solutions

The results obtained from this series of experiments should indicate whether the hypotonic treatment has any effect on the satellite association patterns.

It can be seen that the number of satellite associations observed in metaphase spreads after treatment with the three hypotonic solutions, vary considerably (Appendix II, Table XXI) When potassium chloride was used as the hypotonic solution, the frequency histogram (Figure 23) of satellite associations showing one or more associations, ranged from 50% to 100% in each cell examined, with a modal value of 80-90% of cells. With sodium citrate (Figure 24) the values were lower, ranging from 40% to 100%, this time with a mode of 60-69% of cells.

The shape of the frequency histogram when water/Hanks solution was used shows a nominal modal value of 40-49% (Figure 25). If all the observations using the three hypotonic solutions are combined, the frequency histogram (Figure 26) clearly demonstrates a mode of 60-69% of cells that are involved in satellite associations.

In all of the metaphase spreads examined, those using potassium chloride as the hypotonic solution always showed a higher frequency of satellite associations than with the other two solutions. When a high frequency of associations was recorded with potassium chloride, the corresponding

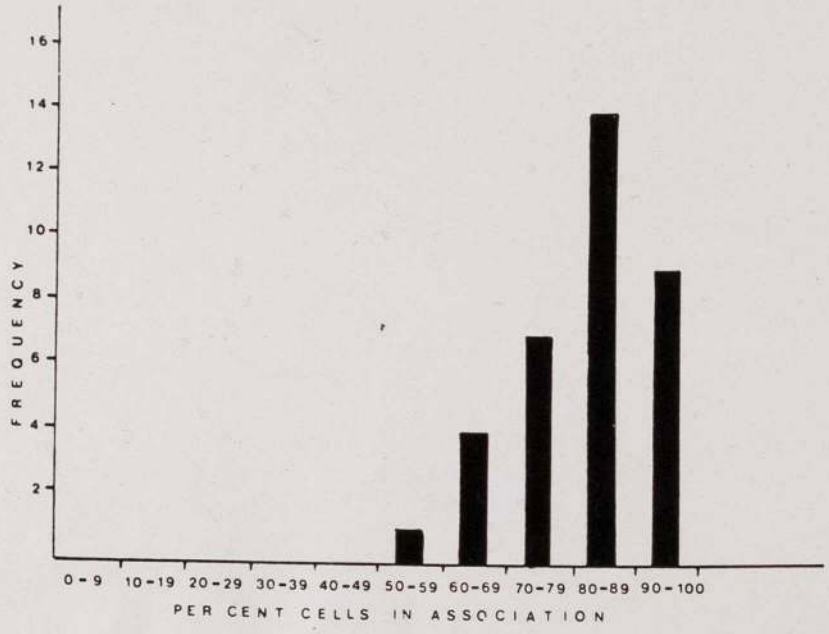


Figure 23

Histogram showing the frequency of one or more satellite associations per cell observed using potassium chloride as the hypotonic solution.

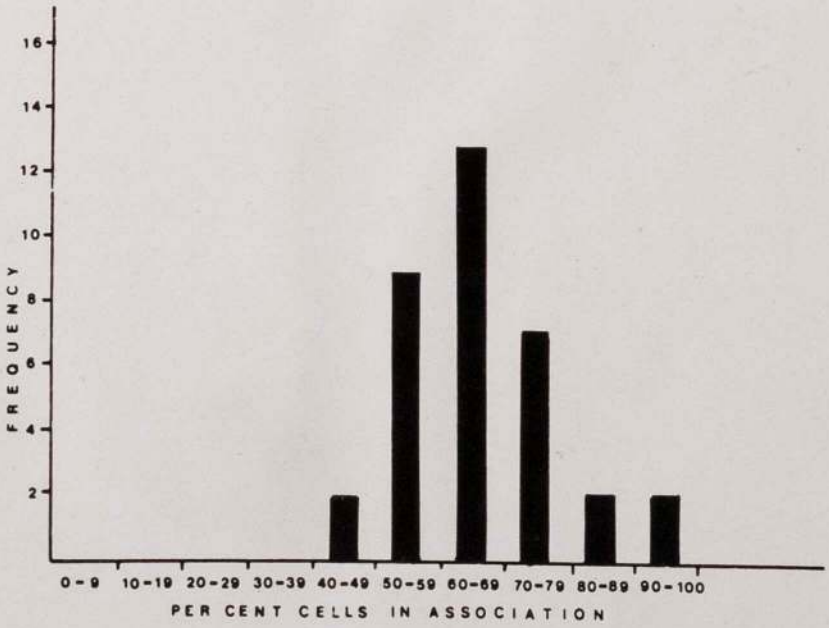


Figure 24

Histogram showing the frequency of one or more satellite associations per cell observed using sodium citrate as the hypotonic solution.

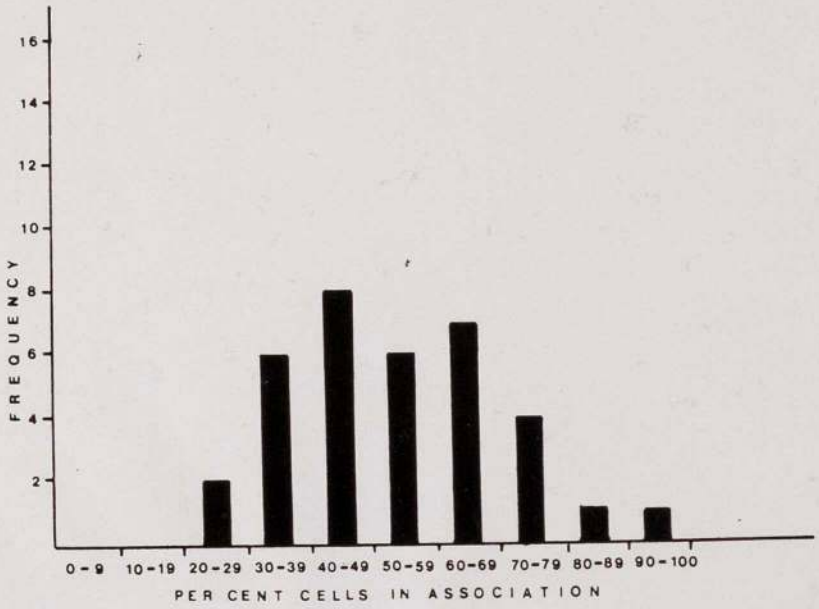


Figure 25

Histogram showing the frequency of one or more satellite associations per cell observed using water/Hanks as the hypotonic solution.

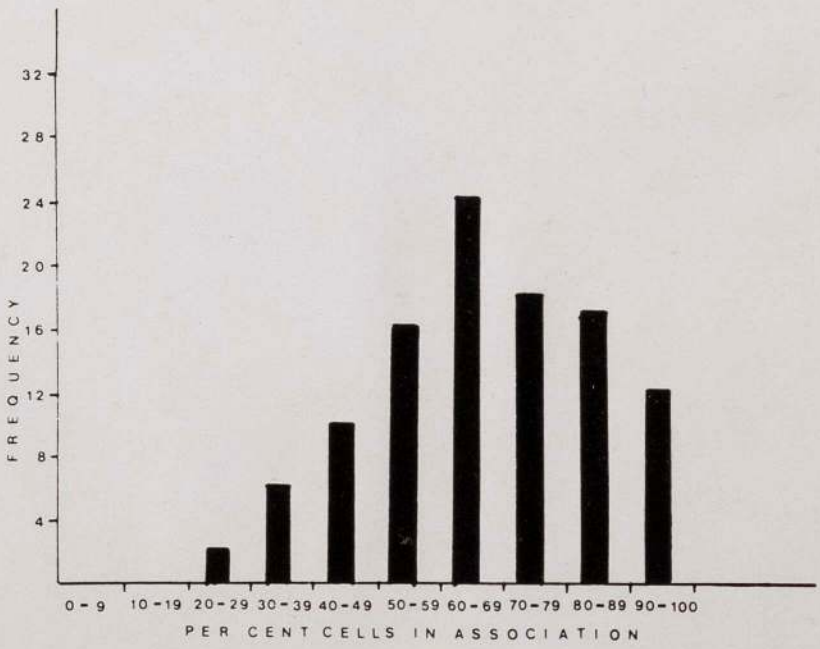


Figure 26

Histogram showing the frequency of one or more satellite associations per cell observed in all of the hypotonic solutions used.

figures with sodium citrate and distilled water, although lower, were also raised.

Comparison of the frequency between the hypotonic solutions (Table VIII) confirms the above finding. This shows that when potassium chloride is used as the hypotonic solution, the frequency of associations observed is consistently higher than with the other two solutions. The standard errors calculated for each of the solutions show a particularly consistent figure considering the sample size and variability in interpretive results.

When the specific group satellite associations are recorded (Appendix II, Table XXII) the same pattern emerges. If frequency histograms are constructed (Figures 27, 28, 29, 30) it can be clearly seen that in all cases, potassium chloride treated cultures have a higher frequency of specific associations than those treated with either sodium citrate or distilled water/Hanks solutions. Di-associations between the 'D' and 'G' groups of chromosomes are far more frequent than those involving the 'D' and 'D' and 'G' and 'G' groups. A greater number of tri-associations between the members of the 'D' and 'G' groups are also seen when compared with those involving the same groups. One interesting result is seen in the groups of di-associations involving the 'G'-'G' group. Here, there is less variability between the solutions, compared with the other groups.

The results from the experiment to determine whether the molarity of the potassium chloride solution affected

Hypotonic Treatment	Number of Controls Examined	Number of Metaphases Examined	Percentage of Metaphases Showing Satellite Associations
Potassium Chloride	35	350	78.28 ± 1.98
Tri-Sodium Citrate	35	350	61.14 ± 2.04
Hanks/ Water	35	350	48.85 ± 2.86

Table VIII Comparison of the frequency of satellite associations observed in the three hypotonic solutions

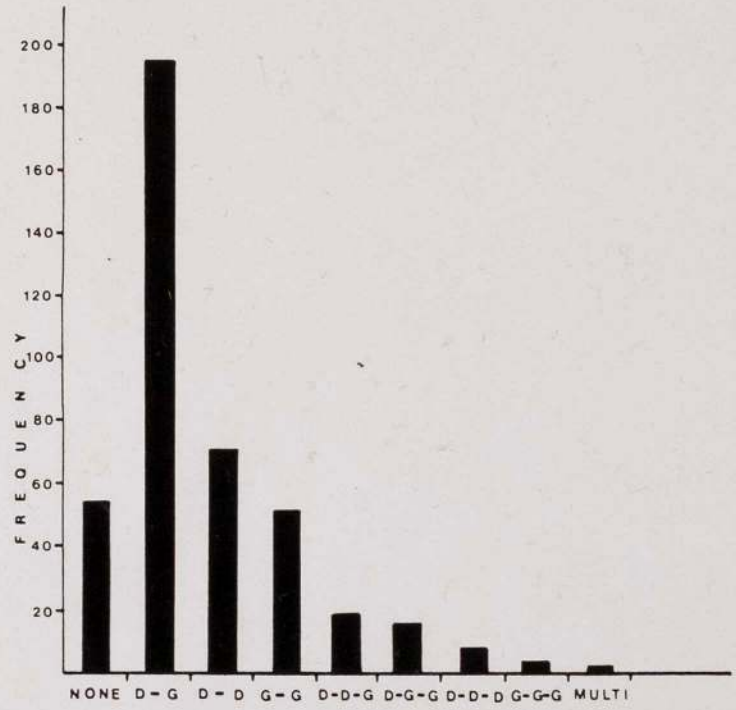


Figure 27

Histogram showing the frequency of the 'D' and 'G' groups in association using potassium chloride as the hypotonic solution.

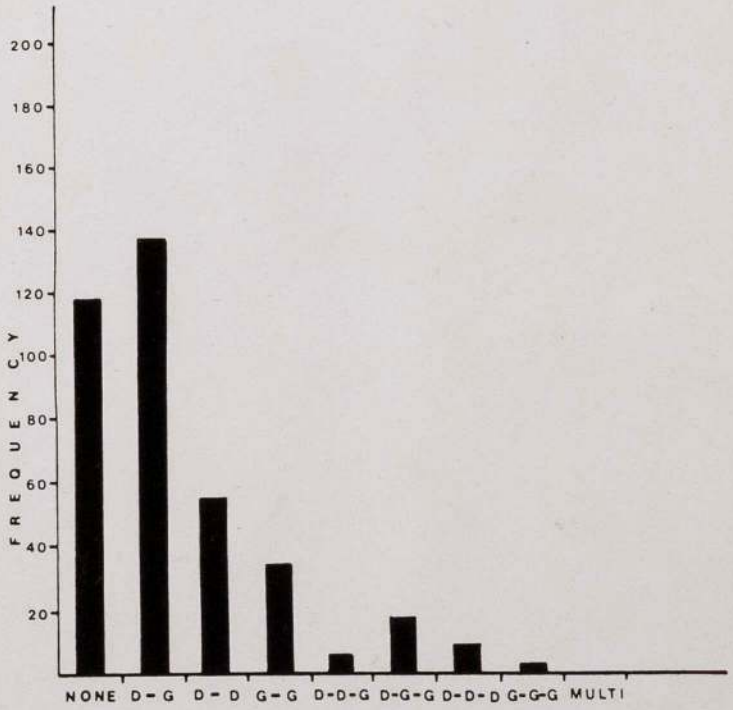


Figure 28

Histogram showing the frequency of the 'D' and 'G' groups in association using sodium citrate as the hypotonic solution.

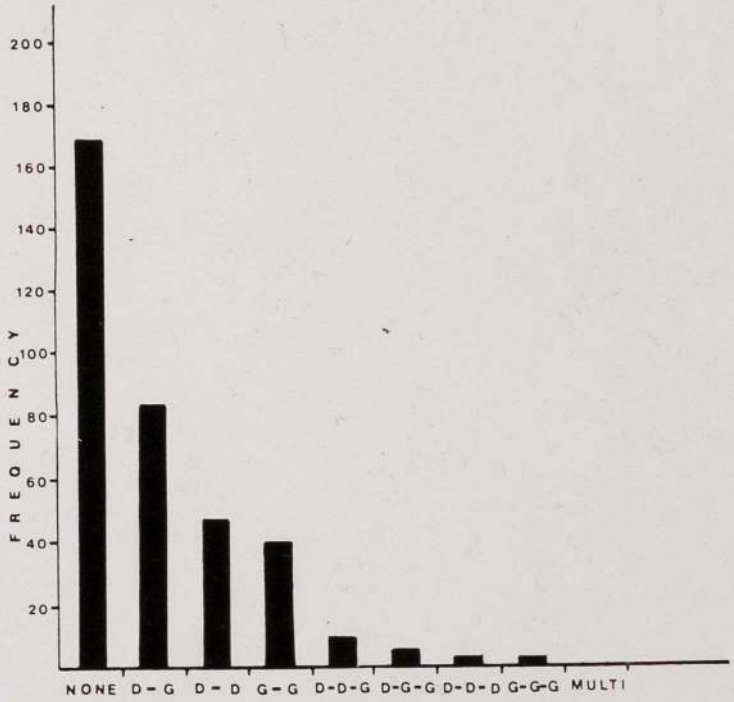


Figure 29

Histogram showing the frequency of the 'D' and 'G' groups in association using water/Hanks as the hypotonic solution.

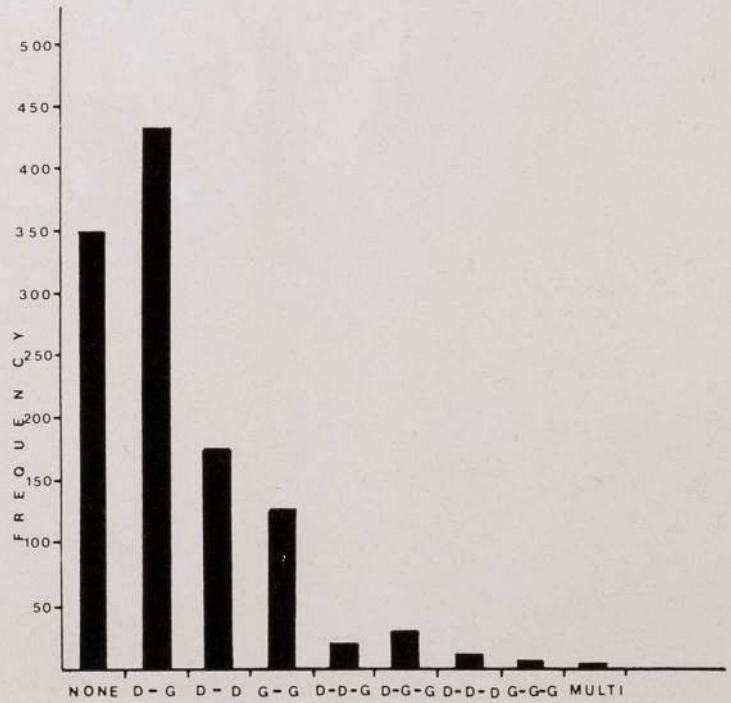


Figure 30

Histogram showing the frequency of the 'D' and 'G' groups in association in all of the solutions used.

the frequency of satellite associations, showed some interesting points (Table IX). The consistent number of associations recorded in 100 cells from each of the 0.04M, 0.06M and 0.08M solutions is remarkable. The trend would appear to show that the frequency of association increases with the molarity of the solution. This is a surprising result, as the opposite effect would usually be expected. Here again, the highest number of specific group associations involved di-associations between the 'D' and 'G' groups.

In the experiment to determine whether the time exposed to potassium chloride (0.075M) had any effect upon the satellite association frequency, it was found that there was no difference in the number of associations seen (Table X). The number of metaphase spreads showing satellite associations after 4, 8 and 16 minutes hypotonic treatment is remarkably constant. The number of specific group associations also show the same consistent numbers. The pattern of associations demonstrate the same trend as in all of the previous experiments, namely that the 'D'/'G' groups associate more frequently than any of the other combinations.

In summarising the results in the first part of this study, it can be seen that the hypotonic solution used to treat the cells, does significantly affect the satellite association frequency. Cells treated with potassium chloride show a higher incidence of associations than with

Molarity of Potassium Chloride	No. of Metaphases Examined	No. of Metaphases Showing Satellite Association	No. of Specific Group Satellite Associations seen in all Metaphase Spreads							
			D/G	D/D	G/G	D/D/G	D/G/G	D/D/D	G/G/G	Others
0.02M	100	Chromosomes too condensed for accurate analysis								
0.04M	100	78	34	13	20	4	3	4	0	0
0.06M	100	80	34	18	9	4	7	3	1	4
0.08M	100	83	29	18	26	1	6	3	0	0
0.10M	100	90	41	20	11	8	9	2	0	1

Table IX Satellite association frequency in relation to the molarity of potassium chloride used as a hypotonic solution

Time	No. of Metaphases Examined	No. of Metaphases Showing Satellite Association	No. of Specific Group Satellite Associations seen in all Metaphase Spreads							
			D/G	D/D	G/G	D/D/G	D/G/G	D/D/D	G/G/G	Others
4 min.	100	80	32	17	16	4	6	0	2	3
8 min.	100	78	31	17	17	4	6	1	1	1
16 min.	100	79	34	13	21	4	4	1	0	2

Table X Satellite association frequency in relation to the time exposed to 0.075M potassium chloride hypotonic solution

those treated with sodium citrate or Hanks/distilled water (1:3). It was, therefore, decided to use Hanks/distilled water (1:3) as the hypotonic solution of choice, for the standard technique. The consistency of the results show that it is possible to evaluate accurately, individual association patterns and so detect any significant changes.

III.ii Satellite Association Patterns - Parents and Controls

The main experimental procedures are those which set out to test whether specific satellite association patterns recorded in cells from parents of mongol children were significantly different to normal control parents. The results were tabulated for distribution, frequency and categorisation of satellite associations and histograms constructed.

In Figure 31 a frequency histogram was constructed to show the range of associations per cell in parents of affected children. When compared with a similar histogram (Figure 32) showing the range of associations per cell in parents of normal control children, it is seen that there are fewer cells without satellite associations in the parents than in the controls. However, this difference is made up by an increase in cells exhibiting two associating chromosomes in the parents than in control samples.

In Table XI the mean frequency of satellite associations per cell can be compared between the parents of infants with Down's Syndrome and control parents of normal children. It can be seen from this that the highest number of associations involve two acrocentric chromosomes. These show a mean frequency of 0.478 in the parents, compared with 0.378 in the controls. There are fewer associations involving three acrocentric chromosomes. The parents show a mean frequency of 0.054 associations per cell compared with 0.037 in the controls. Cells showing four or more

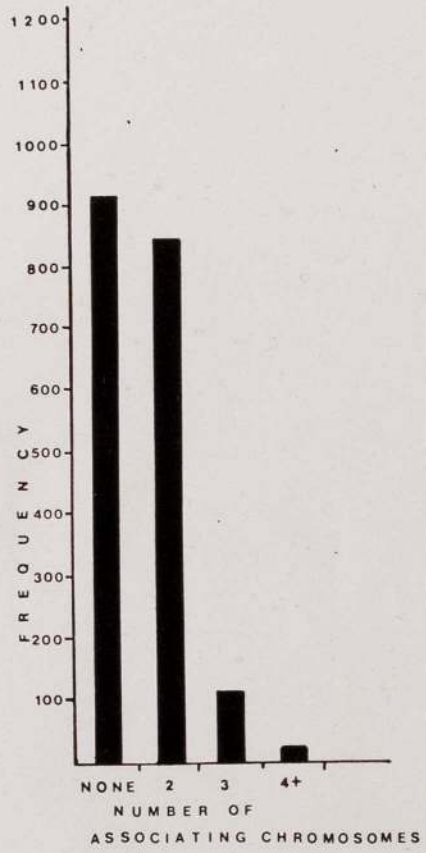


Figure 31 Histogram showing the frequency of association of 'D' and 'G' group chromosomes in parents of Down's children.

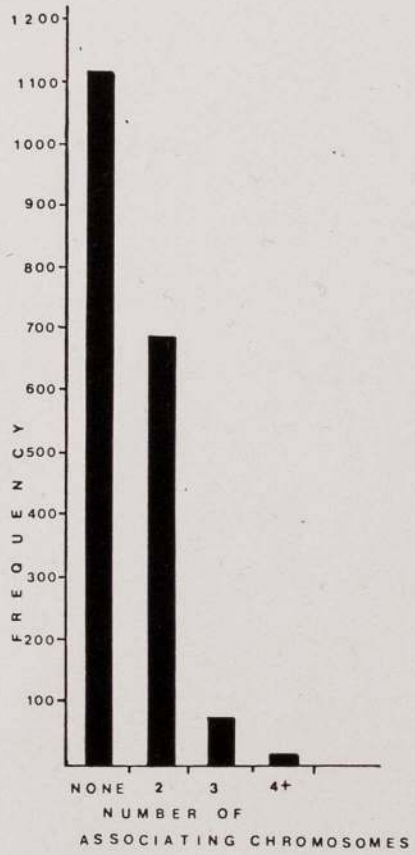


Figure 32 Histogram showing the frequency of association of 'D' and 'G' group chromosomes in parents of normal control children.

	Parents	Controls
Number of cells with 1 satellite association per cell	0.478	0.378
Number of cells with 2 satellite associations per cell	0.054	0.037
Number of cells with 3 or more satellite associations per cell	0.0017	0.0016
Total:	0.534	0.416

Table XI Mean frequency of associations per cell (900 cells per group).

These frequencies are not significant at the P 0.05 level.

(Mann-Whitney test)

association complexes give 0.0017 associations per cell in the parents and 0.0016 per cell in the controls. In all of these results, the overall trend is for the parents of mongol children to show a higher relative figure than those in the control sample. However, when the Mann-Whitney test was applied to test the significance of these results, it was clearly shown that these frequencies were not significant at the $P > 0.05$ level.

The same indication is seen in Tables XII and XIII, where the numbers of satellite associations seen per cell are compared. In 1,800 cells analysed from the parents, there were 861 cells showing one association, 98 showing two associations and 3 with three or more associations per cell. This can be compared with 680 cells with one association, 67 with two and 1 with three or more associations per cell in the control group.

If the above results are divided into maternal and paternal groups (Tables XIV and XV) any potential sex involvement will be demonstrated. In Table XIV mothers of mongol children are compared with mothers of normal controls in relation to the number of chromosomes associating per cell. Here again, the trend is for the mothers of affected children to show a higher number of associations per cell than mothers of normal children. This is clearly seen in all sections of the Table.

Table XV shows the same type of comparison between the fathers of mongol children and fathers of normal children. A similar pattern is observed as above, namely that the

Numbers of Satellite Associations Found in a Total of 1,800 Cells	
Number of cells with 1 satellite association per cell	861
Number of cells with 2 satellite associations per cell	98
Number of cells with 3 + satellite associations per cell	3

Table XII Summary of the frequency of satellite associations seen in 1,800 cells from the parents of children with Down's Syndrome (mongolism)

Numbers of Satellite Associations Found in a Total of 1,800 Cells	
Number of cells with 1 satellite association per cell	680
Number of cells with 2 satellite associations per cell	67
Number of cells with 3 + satellite associations per cell	1

Table XIII Summary of the frequency of satellite associations seen in 1,800 cells from the control parents of normal children

	No.of Acrocentric Chromosomes Associating per Cell				
	None	2	3	4+	Total
Fathers of children with Down's Syndrome	471	412	52	4	939
Control fathers of normal children	557	337	40	4	938
Total:	1028	749	92	8	1877

Table XIV Comparison between fathers of Down's infants and normal controls

	No.of Acrocentric Chromosomes Associating per Cell				
	None	2	3	4+	Total
Mothers of children with Down's Syndrome	457	424	64	7	952
Control mothers of normal children	541	336	21	1	899
Total:	998	760	85	8	1851

Table XV Comparison between mothers of Down's infants and normal controls

fathers of affected infants show a marked increase in association patterns compared with the fathers of normal infants.

If the two sets of results (Tables XIV and XV) are compared with each other, it can be seen that in all associations involving two chromosomes, the figures obtained show a striking similarity between mothers of affected children and controls and fathers of affected children and controls.

Frequency histograms were constructed from the above figures for both mothers of affected children and mothers of normal control children (Figures 33 and 34). Other histograms were drawn for the fathers of these children and for the fathers of normal control children (Figures 35 and 36). From these histograms it can be seen that both sets of parents and controls show an almost identical shape, and that in each case the differences in shape are wholly as a result of the increased number of di-associations in both parents.

Table XVI gives a summary of the di-associations seen in parents and controls, with special regard to the category of association. It can be seen that the total number of di-associations counted in 100 cells vary in individual parents from 34 to 74. The control parents show a range from 33 to 46 in 100 cells counted. If these di-associations are categorised into two types - A and B, it can be seen that in the parents, type A associations varied between 13 and 37 of the di-associations, whilst in

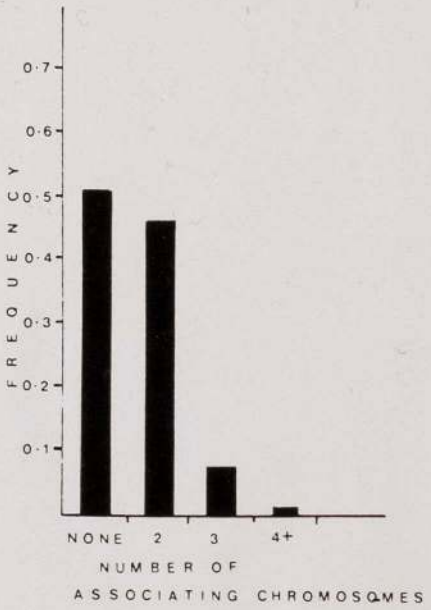


Figure 33

Histogram showing the mean frequency per cell of acrocentric chromosomes in association in mothers of children with Down's Syndrome.

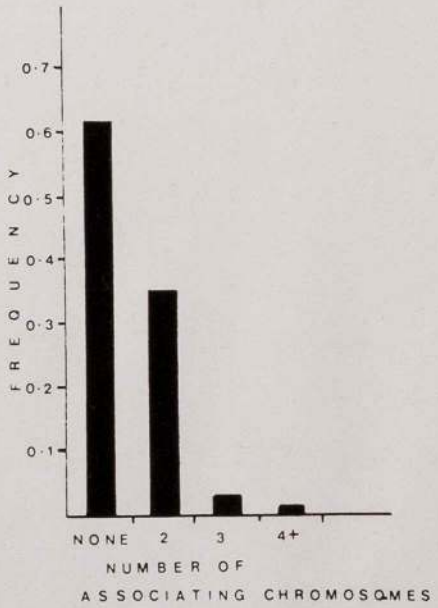


Figure 34

Histogram showing the mean frequency per cell of acrocentric chromosomes in association in mothers of normal control children.

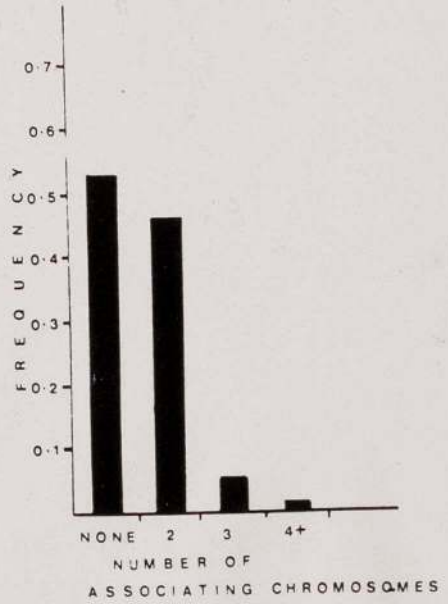


Figure 35

Histogram showing the mean frequency per cell of acrocentric chromosomes in association in fathers of children with Down's Syndrome.

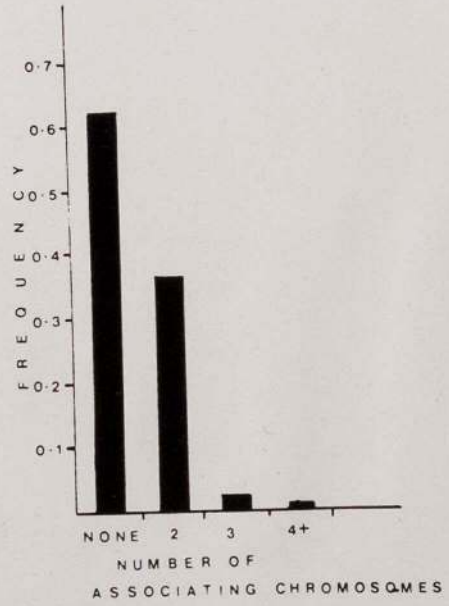


Figure 36

Histogram showing the mean frequency per cell of acrocentric chromosomes in association in fathers of normal control children.

PARENTS					CONTROLS				
Subject No.	Sex	Total	Type A	Type B	Subject No.	Sex	Total	Type A	Type B
5	F	54	19	35	13	F	42	9	33
6	M	51	20	31	14	M	39	12	27
15	M	74	37	37	33	F	38	14	24
16	F	41	16	25	34	M	35	15	20
19	F	47	16	31	41	F	37	9	28
20	M	48	22	26	42	M	37	14	23
21	M	53	29	24	43	F	34	4	30
22	F	38	15	23	44	M	35	8	27
23	F	37	22	15	45	M	34	5	29
24	M	46	15	31	46	F	39	15	24
25	F	46	23	23	47	M	42	13	29
26	M	48	15	33	48	F	40	14	26
27	F	57	30	27	51	M	34	4	30
28	M	41	14	27	52	F	38	14	24
31	F	41	23	18	59	M	33	10	23
32	M	36	15	21	60	F	36	8	28
39	F	46	16	30	61	M	46	10	36
40	M	34	13	21	62	F	34	5	29

Table XVI Summary of the total number of di-associations observed in both parents and controls (100 cells per subject)

the controls, type A associations ranged from 4 to 15 of the di-associations examined. Type B associations varied between 15 and 37 in the parents, and between 23 and 36 in the controls.

In Table XVII, the above di-association figures are broken down into percentages of the total number of satellite associations observed in each case. In the parents, the number of di-associations ranged from 77.94% to 96.00% of the total number counted. In the controls, the number of di-associations ranged from 82.35% to 100.00% of the total number counted. Here again, when these di-associations are categorised into types A and B, the percentage of type A seen in the parents varied from 31.25% to 59.45% whilst in the controls between 11.76% and 42.85% of the di-associations were of the category.

If frequency histograms of total di-associations seen in parents and controls are constructed (Figures 37 and 38), it is seen that the shape of these histograms show normal distribution in both individual groups. This finding is verified when both results are combined (Figure 39).

From the above figures it can be seen that the same trend continues as in the other tabulations, in that parents of mongol children show a higher incidence in the total number of di-associations than those observed in the control sample. However, if these associations are broken down into the two types of association, it is seen that only in the type A category of di-associations is there any significant increase. The type B category is identical for

PARENTS					CONTROLS				
Subject No.	Sex	Total %	Type A %	Type B %	Subject No.	Sex	Total %	Type A %	Type B %
5	F	90.00	35.18	64.81	13	F	89.36	21.42	78.57
6	M	94.44	39.21	60.78	14	M	84.78	30.76	69.23
15	M	94.48	50.00	50.00	33	F	97.43	36.84	63.15
16	F	89.13	39.02	60.97	34	M	97.22	42.85	57.14
19	F	92.15	34.04	65.95	41	F	86.24	24.32	75.67
20	M	96.00	45.83	54.16	42	M	84.09	37.83	62.16
21	M	77.94	54.71	45.28	43	F	89.47	11.76	88.23
22	F	88.37	39.47	47.91	44	M	87.50	22.85	77.14
23	F	84.09	59.45	40.54	45	M	85.00	14.70	85.29
24	M	83.63	32.60	67.39	46	F	92.85	38.46	61.53
25	F	79.31	50.00	50.00	47	M	82.35	30.95	69.04
26	M	82.75	31.25	68.75	48	F	100.00	35.00	65.00
27	F	90.47	52.63	47.36	51	M	91.89	11.76	88.23
28	M	89.13	34.14	65.85	52	F	95.00	36.84	63.15
31	F	80.39	56.09	43.90	59	M	89.18	30.30	69.69
32	M	78.26	41.66	58.33	60	F	100.00	22.22	77.77
39	F	85.18	34.78	65.21	61	M	95.83	21.73	78.26
40	M	89.47	38.23	61.76	62	F	97.14	14.70	85.29

Table XVII Summary of the di-associations observed in both parents and controls (100 cells per subject) expressed as a percentage of the total number of associations counted

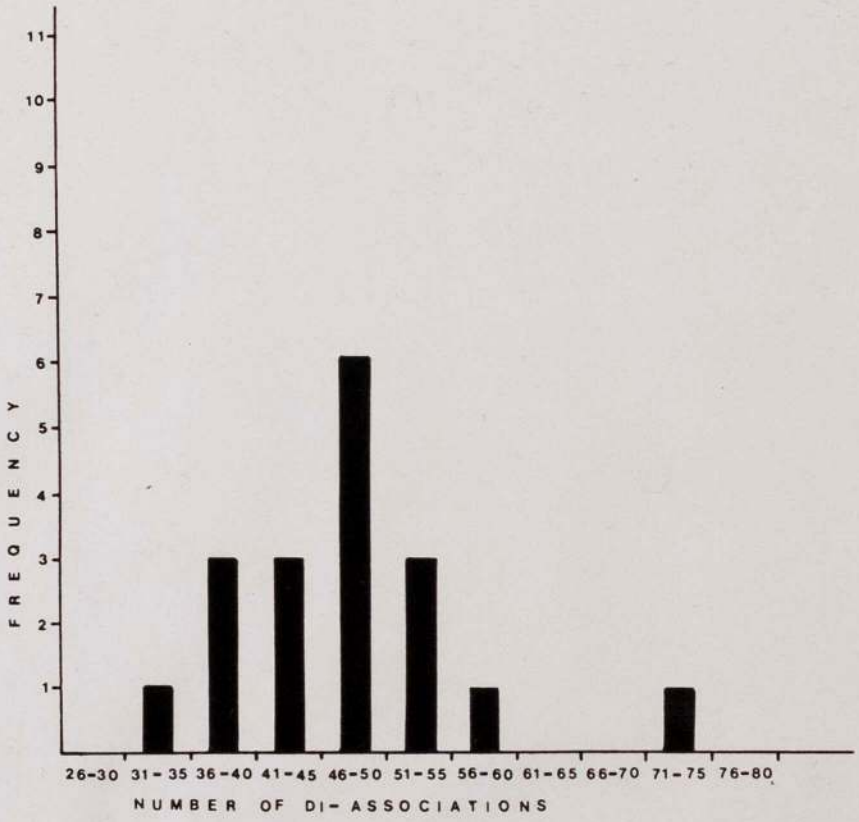


Figure 37 Histogram showing the frequency of the total number of diassociations in parents of children with Down's Syndrome.

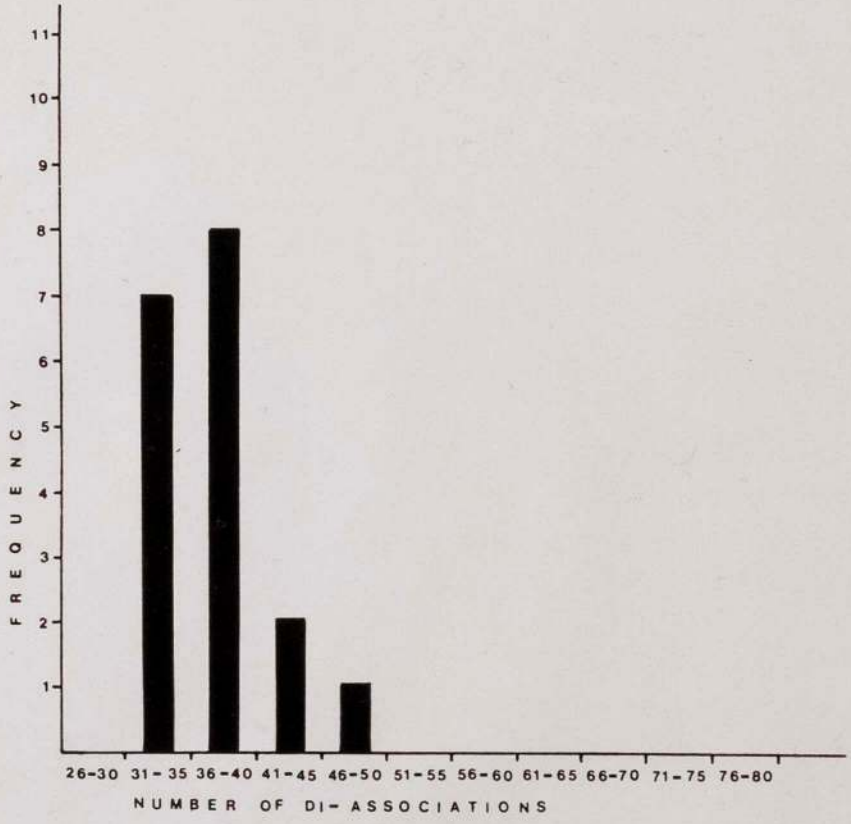


Figure 38 Histogram showing the frequency of the total number of diassociations in parents of normal control children.

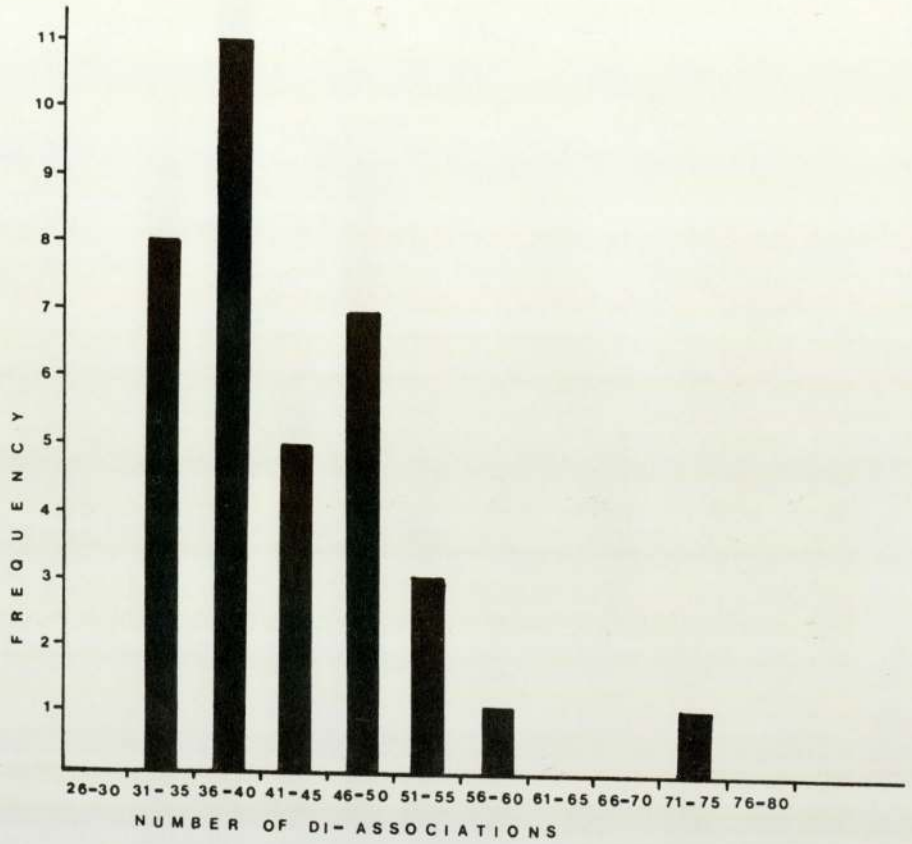


Figure 39 Histogram showing the frequency of the total number of diassociations in both parents and controls.

both parents and controls. When the figures were subjected to the t-test, it was demonstrated that the parents showed a significant increase in total di-associations when compared with the controls ($t=3.86$ $P < 0.001$). When the t-test was performed on type A di-associations, the results were even more significant ($t=5.47$ $P < 0.001$). With type B di-associations, the t-test did not reveal any difference between the two sets of results ($t=0.39$ $P > 0.05$).

Substantially lower figures are recorded in the summary of tri-associations in parents and controls (Table XVIII). Here, the total percentage of tri-associations in parents range from 2% to 11% and in the controls, between 0% and 9%. If the category of association is recorded, type A tri-associations in parents accounted for 1% to 8% of tri-associations compared with 0% to 5% in the controls. Type B tri-associations showed a range of 0% to 4% in parents and 0% to 4% in controls. Here again, the same pattern is seen as with the di-associations, the type B category being identical. However, with the type A category, the difference is not so marked as with the di-association figures.

In Tables XIX and XX, multi-association complexes are recorded. In general, there appears to be no difference in total percentage of associations or with type A and type B associations between parents and controls where four or more chromosomes are involved.

However, it is noted that in parents of mongol infants, five and six association complexes were observed, whilst in

PARENTS					CONTROLS				
S/A No.	Sex	Total %	Type A %	Type B %	S/A No.	Sex	Total %	Type A %	Type B %
5	F	6	4	2	13	F	5	4	1
6	M	3	3	0	14	M	6	3	3
15	M	4	2	2	33	F	0	0	0
16	F	5	3	2	34	M	1	1	0
19	F	3	1	2	41	F	5	4	1
20	M	2	2	0	42	M	6	4	2
21	M	11	7	4	43	F	4	4	0
22	F	5	3	2	44	M	5	1	4
23	M	7	5	2	45	M	6	3	3
24	F	9	6	3	46	F	3	2	1
25	F	10	8	2	47	M	9	5	4
26	M	10	7	3	48	F	0	0	0
27	F	6	5	1	51	M	3	1	2
28	M	5	3	2	52	F	2	2	0
31	F	8	7	1	59	M	4	1	3
32	M	10	7	3	60	F	0	0	0
39	F	8	4	4	61	M	2	0	2
40	M	4	2	2	62	F	1	1	0

	Total Tri-Associations	Type A Tri-Associations	Tri-Associations Involving 21:22 Configuration
Parents	118	30	47
Controls	62	10	16

Table XVIII Summary of the tri-associations observed in both parents and controls.

Four Associations					
No.	Sex	Type	Total	Type A	Type B
19	F	13-14-21-22	1	0	1
21	F	13-21-22-22	1	0	1
21	F	14-21-22-22	1	0	1
25	F	13-14-15-21	2	0	1
25	F	13-14-14-22	1	0	1
31	F	13-13-14-21	1	0	1

Five Associations					
No.	Sex	Type	Total	Type A	Type B
21	F	13-21-21-22-22	2	2	0
25	F	13-14-15-15-21	1	1	0

Six Associations					
No.	Sex	Type	Total	Type A	Type B
31	F	13-13-14-15-21-22	1	0	1

Table XIX Summary of associations involving four or more acrocentric chromosomes in parents of children with Down's Syndrome

Four Associations					
No.	Sex	Type	Total	Type A	Type B
14	M	13-14-21-21	1	1	0
33	F	13-14-14-21	1	1	0
41	F	14-14-15-21	1	1	0
42	M	14-14-15-21	1	0	1
59	M	13-15-22-22	1	1	0

Five Associations
No associations seen

Six Associations
No associations seen

Table XX Summary of associations involving four or more acrocentric associations in parents of normal children

the controls no multiple associations of this number were recorded.

In the results of the category of the types of association, the overall trend in total and type A associations was to show a definite increase in satellite associations in parents of mongol children. However, in the individual parents, a raised satellite association frequency and pattern can be seen in most of the recorded results. In the majority of parents, at least one parent shows a raised total percentage of satellite associations recorded which are in the type A associations.

In Figure 40 the number of specific associations observed in the 'D' group of chromosomes in parents and controls, are reproduced graphically.

Also demonstrated is the type of association, in relation to the total number observed.

It appears that there is a uniformity between parents and controls in the total number and types of associations seen involving the 'D' group of chromosomes. The indication in both samples is, for a greater percentage of associations involving the 13/14 and 14/15 chromosomes than with the other chromosomes within the group. It is also interesting to note that associations involving the homologous pairs remain constantly low.

Specific associations involving the 'D' and 'G' groups are seen in Figure 41. Here again, the total number and the type of association are relatively constant for both parents and controls. The trend in both samples is for a

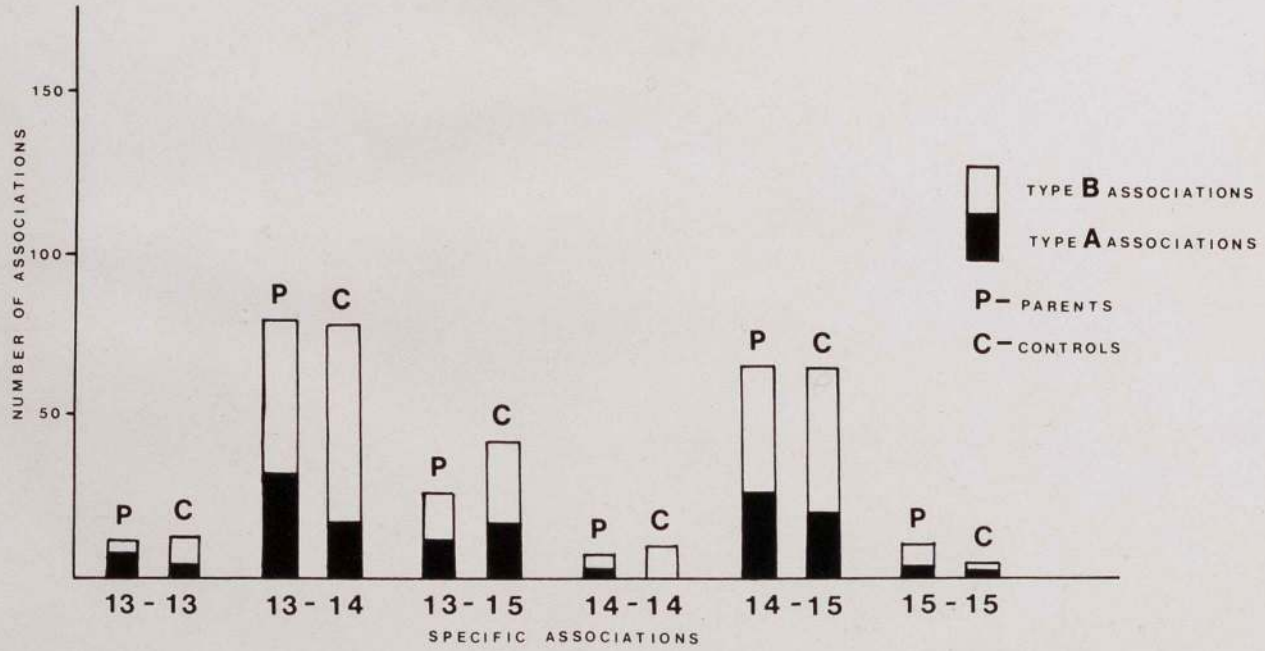


Figure 40 Graph showing the total number of specific satellite associations; type A and type B associations; which were observed in the D/D groups of chromosomes, in both parents and controls.

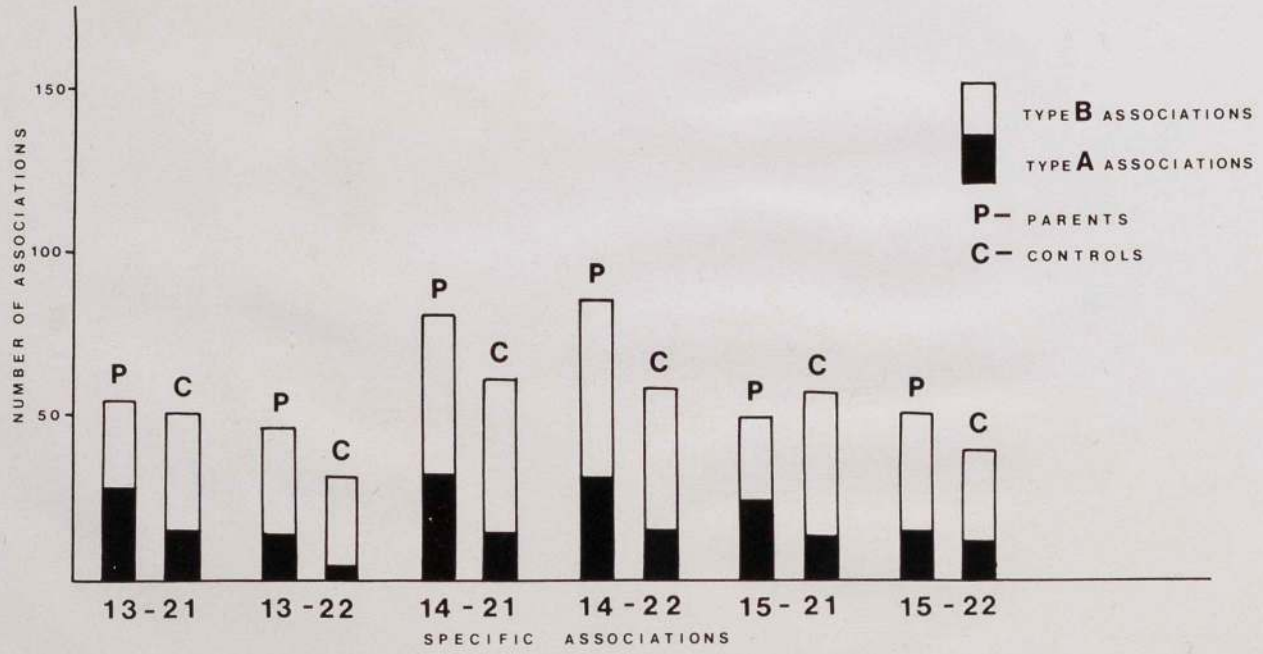


Figure 41 Graph showing the total number of specific satellite associations: type A and type B, which were observed in the D/G groups of chromosomes, in both parents and controls.

greater number of associations involving the 14/21 and 14/22 chromosomes, with a slightly higher total frequency in the parents, although the type B associations are virtually identical.

When the graph showing associations between the 'G' group of chromosomes (Figure 42) is produced, a striking difference in the results between parents and controls is instantly noticed. Associations involving the homologous pairs, i.e. 21/21 and 22/22 are considerably fewer than those between chromosomes 21 and 22. Further examination of type A and type B associations in parents and controls show a dramatic increase in type A associations in the parents involving chromosome 21 and 22. It can be seen that over three times as many type A associations are observed in parents than in the controls, whilst type B associations have, more or less, an identical number. When the t-test is applied to this configuration, the results for the type A associations are significant ($t=3.32$ $P < 0.001$) whereas the type B were not. Similarly, in the 21/21 and 22/22 specific associations, the number of type A associations are also raised in the parents.

When looking at the tri-associations involving numbers of the 'D' group of chromosomes, (Figure 43) a rather nondescript pattern emerges. No definitive indication is seen, other than to note that the number 14 chromosome appears to be involved in the association complex more often than any other chromosome.

In the graph showing tri-associations within the 'G'

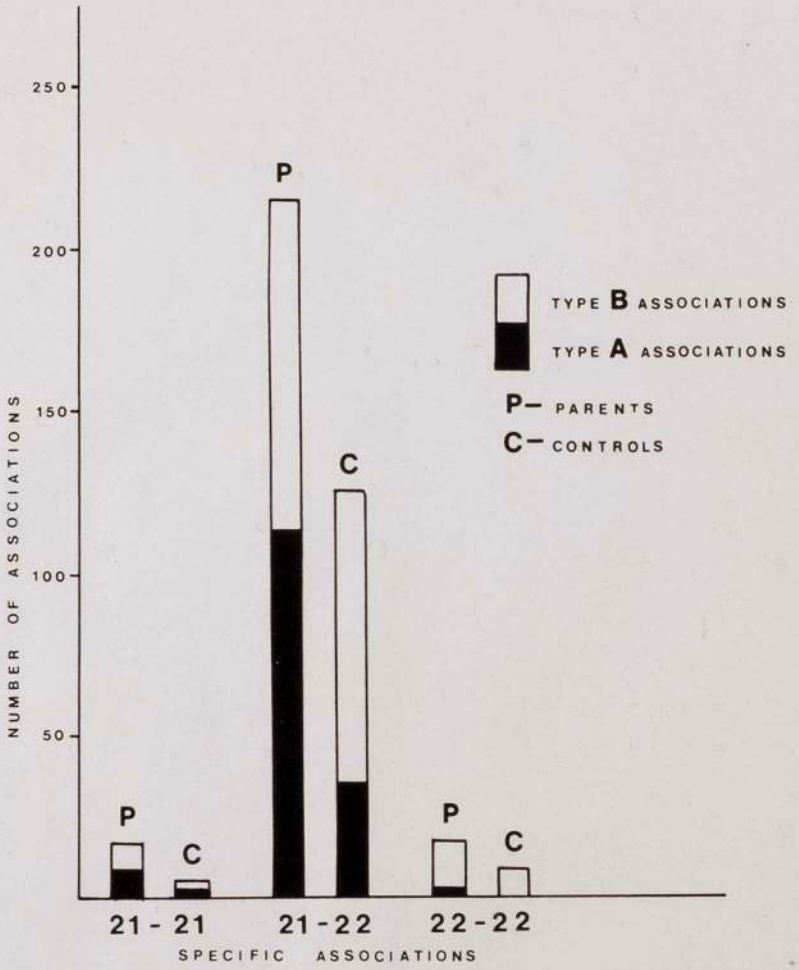


Figure 42 Graph showing the total number of specific satellite associations, type A and type B associations; which were observed in the G/G groups of chromosomes, in both parents and controls.

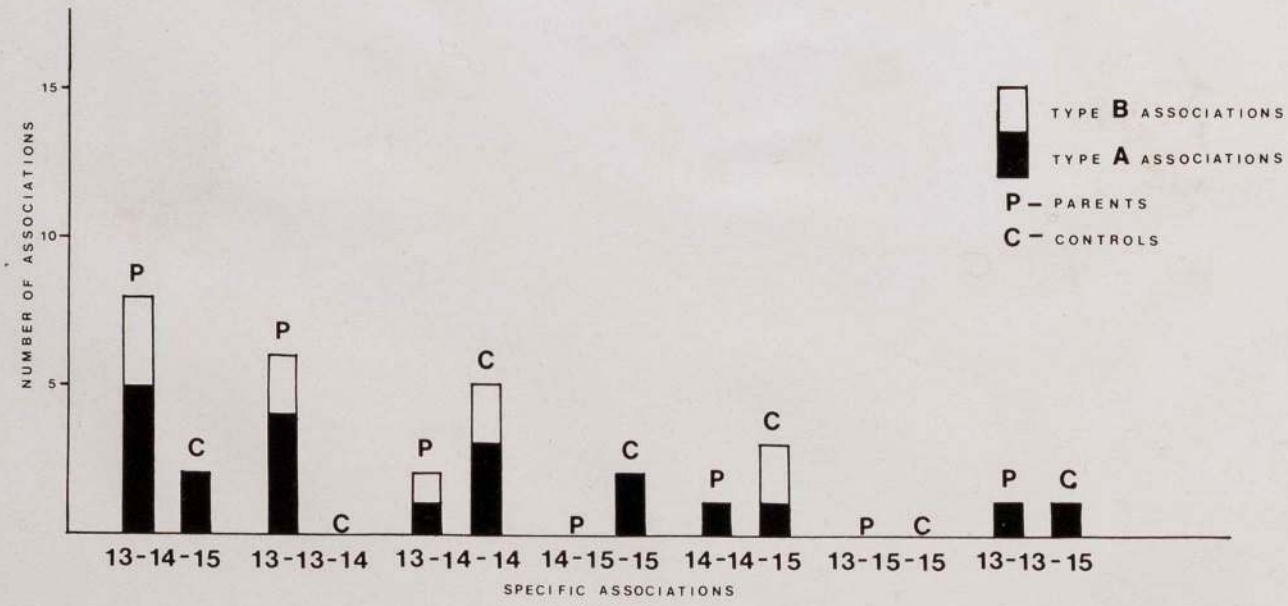


Figure 43 Graph showing the total number of specific satellite associations; type A and type B associations which were observed in the D/D/D group of chromosomes, in both parents and controls.

group (Figure 44), an increased frequency of type A associations is seen in the parents of mongol children, for the 21/22/22 configurations. Association complexes involving 21/21/22 show an identical number of type A associations. Here again, in the 21/22/22 tri-associations, the type A tri-associations are three times the number in the parents compared with the controls.

Figure 45 shows the specific tri-associations involving the 'D'/'G'/'G' groups. The trend in this graph shows that in the 13/21/22, 14/21/22 and 15/21/22, a greater total number of satellite associations are seen in the parents of mongol children. In all of the other combinations of this configuration, no obvious differences between the two samples are noted. The same pattern is seen with the 13/21/22, 14/21/22 and 15/21/22 as with the di-associations involving the 21/22 chromosomes (Figure 42). In each complex, the number of type A associations is virtually identical in the parents, and is at least twice the figure observed in the controls. It seems that the 21/22 configuration is the common denominator in these configurations, the other acrocentric chromosomes being involved merely by chance.

In the last graph (Figure 46), no definite pattern is seen, with the 'D'/'D'/'G' tri-association complexes. Perhaps one interesting feature is that the highest number of type A associations in both controls and parents is seen again when the 14 and 21 chromosomes are involved in the complex.

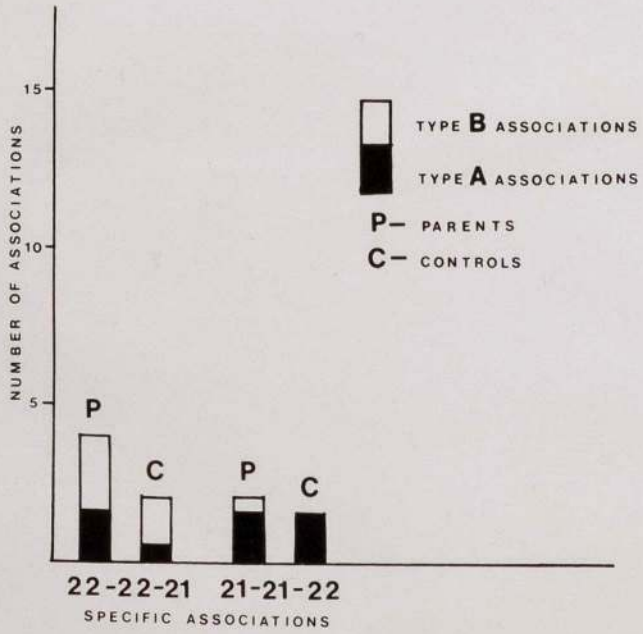


Figure 44 Graph showing the total number of specific satellite associations; type A and type B associations which were observed in the G/G/G group of chromosomes in both parents and controls.

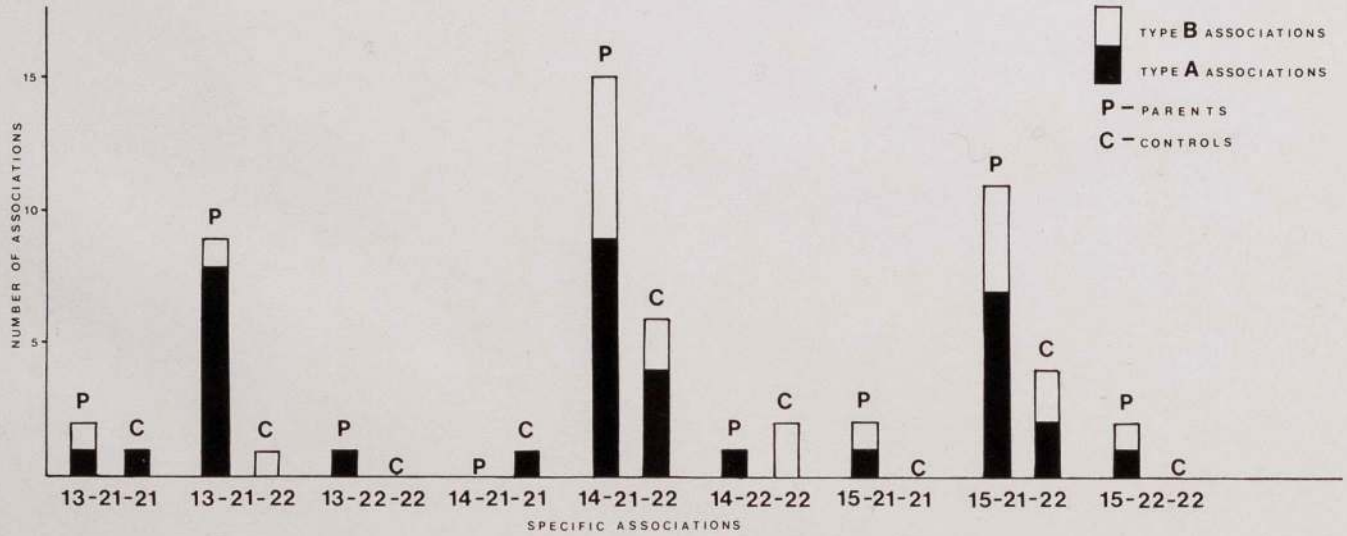


Figure 45 Graph showing the total number of specific satellite associations; type A and type B associations which were observed in the D/G/G groups of chromosomes, in both parents and controls.

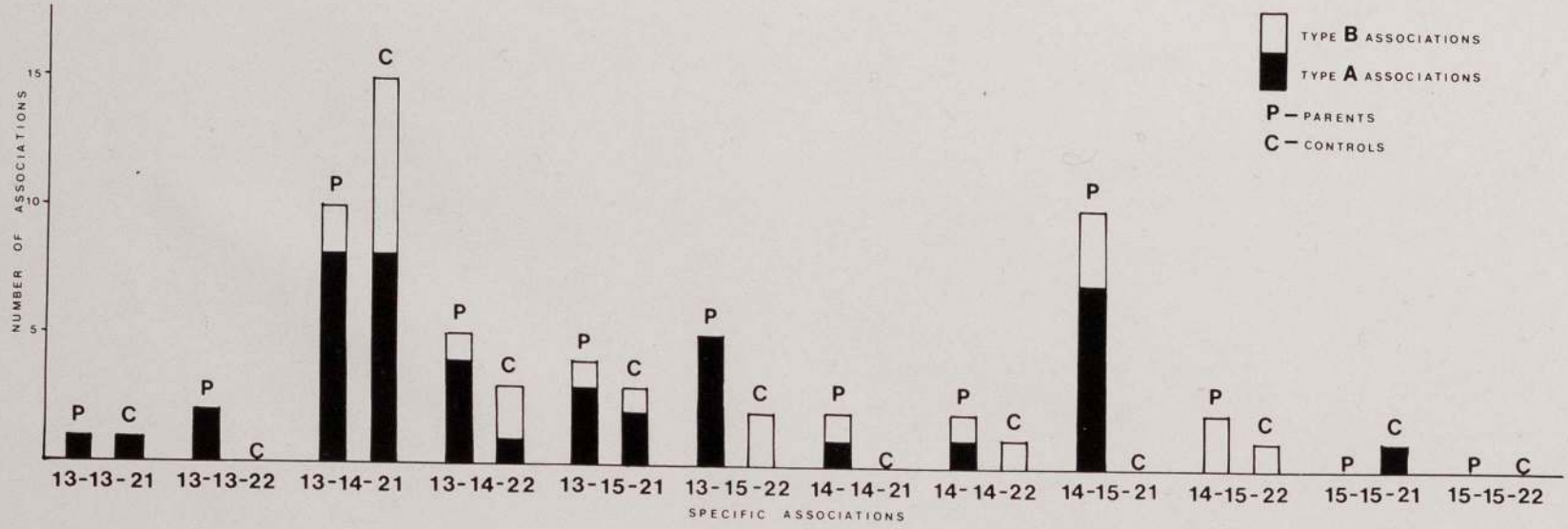


Figure 46 Graph showing the total number of specific satellite associations; type A and type B associations which were observed in the D/D/G groups of chromosomes, in both parents and controls.

The results in the second part of the study show some significant findings. The overall trend is to a higher frequency of satellite associations in the parents of mongol children. There does not appear to be any indication that the sex of the parent is involved in this finding. Perhaps the most definitive factor in these results, has been the identification of specific chromosomes and the categorisation of the various types of association. It is clearly shown that the frequency of associations involving the number 21 and 22 chromosomes is markedly increased in the parents of mongol children. This indication is underlined when the categories of associations are analysed. Here, a substantial increase in the type A associations is clearly demonstrated when chromosomes 21 and 22 are involved in the configurations.

IV. DISCUSSION

IV.i) General

Over the past fifteen years, there have been numerous studies (see page 25) concerning the effect of satellite association, either as a morphological variant, or as a factor in producing abnormal cell division. The inconsistency of the results can only serve to emphasise the extreme variability of this phenomenon. It is therefore important that a standardised technique and uniform criteria must be formulated before any real understanding can be gained.

The technical procedures used in the previous studies (Ferguson-Smith, 1961, Cohen and Shaw, 1967, Zang and Back, 1968, Nakagome, 1970 and Orye, 1974) have all varied, although the overall concepts have been identical. It would appear that the numerous different unknown combinations of variables, influence the association frequencies, and that different cells respond in diverse ways to various influences.

If the possible effect of variations according to race, sex, age and heredity factors are introduced, then the inconsistency in results can easily be understood. If, as well as the above factors, the differences in statistical analyses are considered, the problem of trying to evaluate a simple finding can assume mammoth proportions.

However, if from the previous studies, the correlated findings are used to formulate a baseline, any significant

results will be assumed to be correct for that series of experiments. In this way it has been shown that although the distribution of different types of associations vary from one individual to another, they are specific for a given subject, since different pairs of acrocentric chromosomes contribute in constant proportions (Schmid and Krone, 1974).

The phenomena of satellite association might also have a functional role. The satellited regions of the acrocentric chromosomes vary in size and shape, and as such, mean that each nucleolar organising region could be involved in diverse functional roles. Therefore, the extreme variants in the population will be maintained throughout a period of time, and will consistently show a strong tendency to satellite association, thus giving a strong indication that this tendency is genetically controlled. Present evidence suggests that each individual has a characteristic, or modal number of nucleolar organising regions. These can easily be demonstrated by using the silver nitrate techniques (Howell et al., 1975, Denton et al., 1976, Goodpasture and Bloom, 1976). These methods show a physical connection which is indicative of their participation together in nucleolar organisation.

It is without doubt that some form of physical communication exists between chromosomes in the interphase, the exact nature and function of which is still speculative. Hoskins (1968) brought forward evidence that human mitotic

chromosomes are bound together by chromosome to chromosome connectives. He showed that by using a micro-needle in living cells, each chromosome came out of the nucleus like a chain, each chromatid pair attached to the next, by a filament, directed towards the centromeric region.

Burkholder (1975) verified this finding in whole mount chromosome preparations, by observing that interchromosomal fibres were frequently seen extending from one chromosome to another in trypsin-treated preparations. However, he failed to see any such fibres in untrypsinised controls, and considered that perhaps these so-called fibres were artifacts produced by the overlapping of dispersed chromatin.

One possible explanation for the phenomena of satellite association could be that rupture of the interconnecting fibres occurs in the repetitive DNA sequences. Thus, certain interconnecting fibres would seem to break less easily, particularly in the case of the acrocentric chromosomes. This could mean that the cell has a greater chance of unequal cell division, thus producing aneuploidy. From this it can be postulated that chromosomes bearing abnormalities of the nucleolus organising regions may be more easily involved in the non-disjunction of chromosomes due to satellite association.

In the present study, the satellite association patterns seen in the hypotonic solution experiments, and those from parents of trisomy 'G' infants, share the same criteria, but with varying interpretations. In both experiments the con-

sistency in which the satellite association frequencies was observed, proves that this phenomenon is not an artifact but can be used as a reliable guide in morphological studies.

IV.ii) Satellite Association Patterns - Hypotonic Solutions

The results from the hypotonic experiments show that the choice of hypotonic solution is critical, when satellite association patterns are being analysed. The statement by Zang and Back (1968) that the standard technique of choice should be one that gives the highest frequency of associations, must be questioned. Surely, only in a technique that gives fewer true associations, with the lowest variance, must be the one of choice. Thus, only from this baseline can any conclusions be drawn as to their effective role in producing aneuploidy. In this respect it is worth comparing the results obtained using potassium chloride and those using water (1:3 Hanks/water solution).

The effect of water on the cell membrane is in itself far more traumatic and stringent than that of potassium chloride. It would appear that by using potassium chloride as a hypotonic solution, the chromosomes gradually disentangle themselves and pull apart as the cell swells in size. With the 1:3 Hanks/water hypotonic solution, the same process still occurs but much faster, so that the chromosomes are violently separated within a very short period of time. Hence, when looking at the satellite association frequencies, those cells which have been treated with the potassium chloride hypotonic solution will give a more accurate morphological picture than those treated with water. However, for the purpose of this study,

those associating 'D' and 'G' groups present after a 1:3 Hanks/water treatment, will serve to demonstrate those associations which exhibit particularly strong affinities between satellites.

Using 1:3 Hanks/water solution for the hypotonic treatment, enables any small increase in the satellite association frequency to be easily detected. Other authors have found a large variation in the cultural conditions which influence satellite association frequencies (Back and Zang, 1969, Nankin, 1970), as well as in the techniques of preparation (Back and Zang, 1969, Rozenkrantz and Fleck, 1969, Hoehn, Nagel and Krone, 1971).

It was found that up to the present time, no author has evaluated the effect of post cultural factors, especially the role of the hypotonic solution in relation to the frequency of associations. The variability in the results between the basic cultural methods indicate the possibility that it is the subsequent hypotonic solution that is the critical factor.

The concept that potassium chloride is actually involved in the alteration of the molecular arrangement of the chromosome might also be considered. This effect could be an integral step in the pre-treatment of the chromosomes for the G-banding methods. This pre-treatment of the chromosomes can involve proteolytic enzymes such as trypsin or varying salt solutions which, it is thought, rearrange the molecular architecture sufficiently for the Giemsa stain to selectively attach its molecules.

When the cells are treated with a hypotonic potassium chloride solution at 37°C, it is postulated that this, in some way, could be the start of this rearrangement. Comparison between banded preparations where the cells were treated with potassium chloride as the hypotonic solution and those treated with Hanks/water solution, show a better and more definitive banding structure.

Thus, if any serious analysis of satellite association patterns is to be studied, then the type of hypotonic treatment used in the harvesting of the cells must be taken into account. From this study it is seen that by using this standardised technique, the satellite association pattern for each individual person can be accurately determined. The reproducibility of the results serve to indicate this factor.

IV.iii) Satellite Association Patterns - Parents and Control

The effect of non-disjunction of chromosomes in producing a trisomic cell line is well known. Trisomy represents about half of all developmental chromosome anomalies found in human liveborns and spontaneous abortions. Its total incidence in human conceptions may be 3% or even higher (Polani and Jagiello, 1976). It is worth considering that of the three most common examples of autosomal trisomy in man, two involve the satellited acrocentric chromosomes (trisomy 'D' and trisomy 'G'). The third (trisomy 'E'), involves a chromosome whose short-arm often takes part in associations with satellited chromosomes.

When the DNA replication times of chromosomes are compared, the three groups of chromosomes which are the latest autosome replicators are the 'D', 'E' and 'G' groups. This means that any delay in separation will increase the chances of unequal division. Furthermore, chromosomes 13, 18, and 21 are the last of their groups to replicate. Banding studies have shown that the extra chromosome present in trisomy 'G' is a number 21, in trisomy 'D' it is 13 and in trisomy 'E' the extra chromosome present is 18. It must therefore be assumed that it is no coincidence that the rate of DNA replication plays an important part in the production of trisomic cell lines.

The aim of this study has been to look specifically at trisomy 'G' and to correlate between its incidence and

satellite association patterns. In man, most trisomies are related to advancing parental age, and because it has been shown that in trisomy 'G' the maternal age matters, this relationship is assumed for the other trisomies (except XYY). This maternal age factor may or may not directly involve satellite association patterns, although its role in this area may be far more closely connected than has been previously considered.

Evans (1967) suggested that the nucleolus becomes suspect with age, and that viral infection may reduce the capability of the nucleus for dissolution at division. Curtis and Cooke (1974) put forward the idea that only certain combinations of nucleoli may be conducive to non-disjunction, and that some chromosomes may be able to disrupt the fused nucleoli, whilst others cannot.

The involvement of the acrocentric chromosomes in the formation of nucleoli is well known. The morphological variants frequently observed on the short arms, stalk (secondary constriction) and satellites, provide a guide to its role, which could constitute an explanation for the variable frequency of associations in the population. Although the nucleolus organising regions have been assigned to the stalk (secondary constriction) of the chromosomes, it does not rule out the possibility of a number rDNA cistrons being present on the satellites themselves.

The hypothesis of this study postulates that there exists a certain predisposition among some individuals to

produce children with trisomy 'G' (mongolism). From the results of this study, it can be shown that there exists a significant difference in certain satellite association patterns in the parents of trisomy 'G' infants when compared with normal control parents. The relevance of this finding within the non-age related group of parents, suggests that there is a possible connection between these two factors.

If this predisposition exists among the general population, why should the recent statistics (Goad et al., 1976) show a seasonal variation in the number of trisomy 'G' children born to mothers under 34 years of age? One possible explanation for this would be that a combination of either a viral or allergenic component combined with a susceptibility to increased specific satellite associations might be the answer in such individuals. As this seasonal variation occurs between April and October, numerous other factors such as diet, radiation etc., could be implicated. Whatever factor provides the stimulus, the variation produced is sufficient to maintain the statistical incidence of non-age related mongolism.

The role of immunological response may be such a factor in this respect. The results of Frolov (1975) indicate that the frequency of satellite associations decreased proportionally to the increase in intensity of the immune response after smallpox vaccinations. Similarly, Hansson (1975a) found an increase in satellite association frequencies in patients with hypothyroidism. This would

appear to indicate that the mechanics of satellite association is relatively easily affected by exogenous agents, although it might mean only a reduction in the length of the time in interphase.

The role of the nucleolar organising regions (N.O.R.) on the stalks of the satellites might play an important role in the predisposition of certain acrocentric chromosomes to associate. If there was some variability in the quantitative numbers of N.O.R.'s present, then the inter-action of these chromosomes would be different. The possibility that these regions are more hypersensitive to certain external factors in some members of the population is suggested. This would account for the variability in satellite association frequencies found in random samples.

It is postulated that certain individuals have a greater number of N.O.R.'s present on the 'D' and 'G' groups of chromosomes, although on evidence found in this study, it is put forward that only those on the 'G' group are relevant. These might be represented in the form of "active areas", participating in, or near to, the nucleolar membrane during interphase (Figure 47). As a result of this, their involvement and subsequent potential damage, e.g. by viral infection, would be greater (Figure 48). It could be that these active areas are in fact participating ribosomal DNA cistrons, and that their functional role is in some way linked to the number present in each individual.

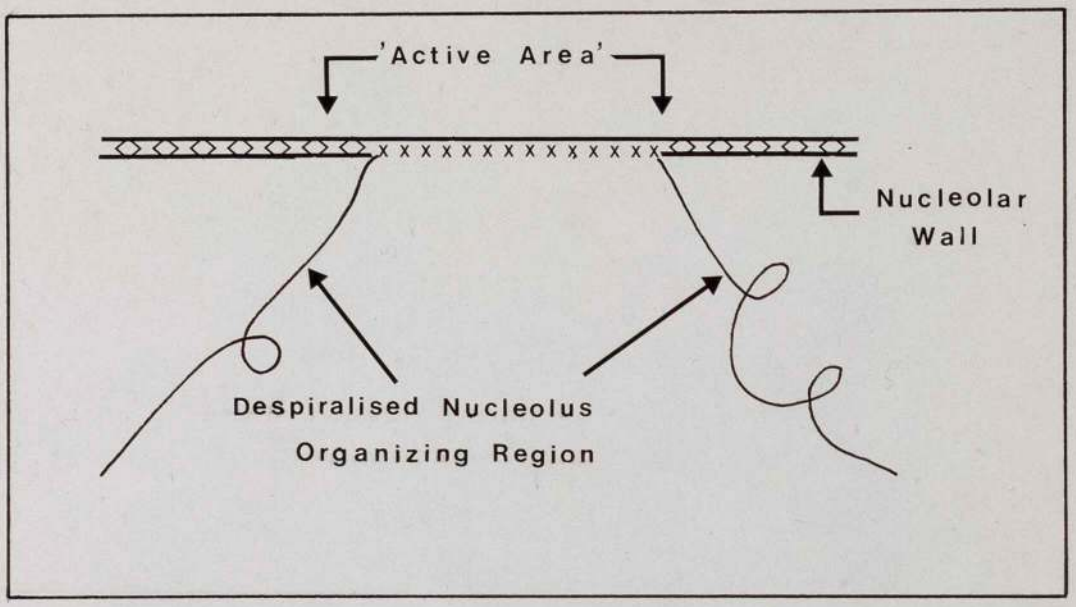


Figure 47 Proposed involvement of "active areas" on nucleolar membrane.

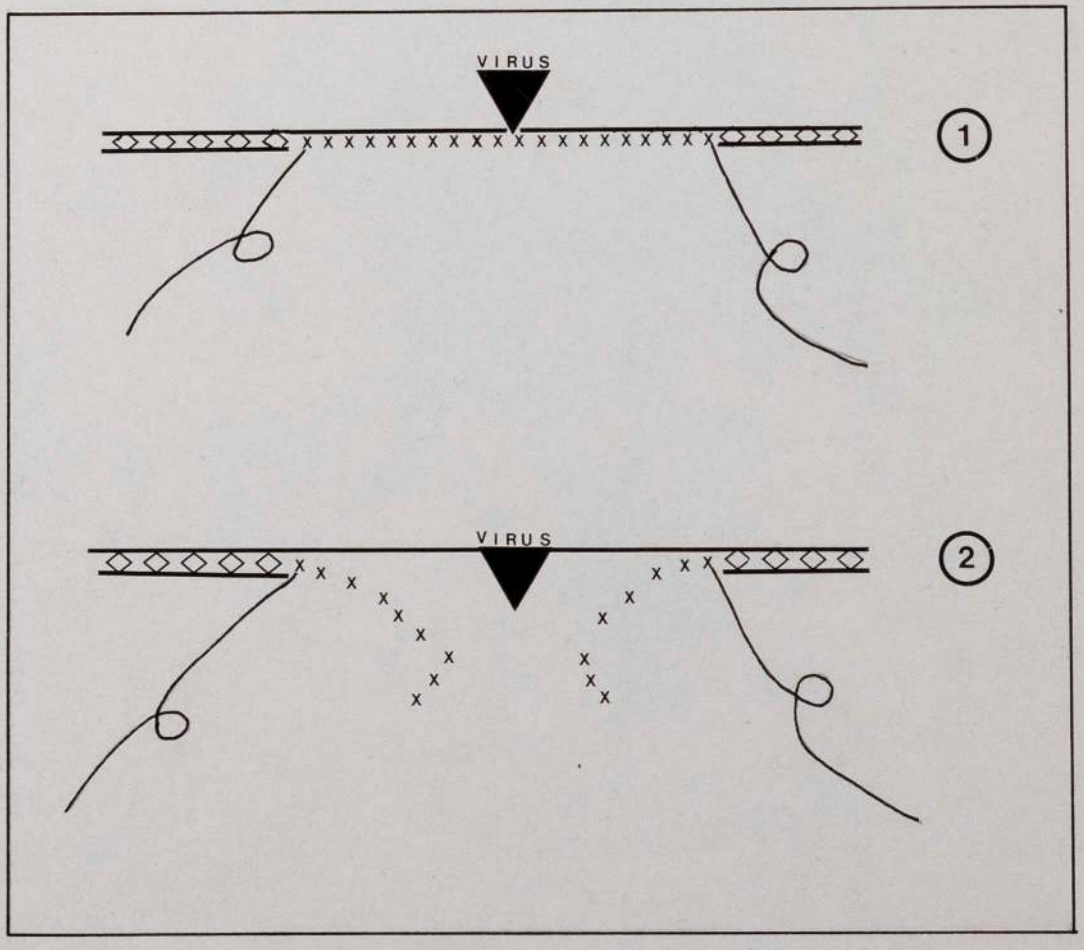


Figure 48 Possible cause of damage to DNA by a viral agent.

The suggestion that some form of viral infection is involved in the aetiology of Down's Syndrome requires further investigation. It is well known that viral agents can cause chromosome damage *in vivo*, chicken pox, measles, mumps (Aula, 1965), hepatitis (Aya and Makino, 1966), and rubella (Kuroki *et al.*, 1966) have all been implicated. The presence of these might well cause damage to the proposed "active areas", causing breakage or rearrangement of genetic material. If the number of areas vary, then the effect of the viral DNA would be proportional to that number present on the chromosome. Thus, the greater the number of N.O.R. "active areas", the greater the chance of malfunction in the subsequent cell divisions.

The effect caused by such a virus, whether it is of a specific type or molecular weight, would be disruptive, not only to the immediate DNA material, but to the function of the nucleolus. This could also be the same for all of the other possible factors, previously mentioned. Another consideration would be the exact location of the nucleolus in relation to the nuclear membrane. This, together with the number of participating chromosomes or fused nucleoli, might mean that the number of potential agents is selective for specific somatic conditions.

Recent research (Stahl *et al.*, 1976) has shown that by using *in situ* hybridisation techniques, ribosomal cistrons have been visualised on the periphery of the nucleoli. It was found that when the nucleoli were small

and numerous, one or two granules are seen in contact with the circumference of each nucleolus. In the absence of a fibrillar centre, localisation of the ribosomal cistrons is more diffuse, and may correspond to the entire nucleolar surface. From these observations, they deduced that the short arms of the acrocentric chromosomes are closely associated with the nucleolus and consequently make up part of the nucleolar-associated chromatin.

Another way in which this predisposition could be shown to manifest itself is when both parents exhibit a greater specific affinity of the 'G' group to associate. In these results, the stronger type 'A' associations between G21 and G22 chromosomes would appear to be the vector of the possible non-disjunction, and as such increase the chances of this event occurring. However, when one parent only has a higher incidence of this type 'A', G21-22 association, the chances are reduced as compared with the above, but still greater than those of normal parents. Thus, when another factor is introduced, and non-disjunction occurs, it is perhaps this group which is responsible for showing such a seasonal variation in the incidence of non-age related mongolism.

It is clearly seen from the results of this study that no sex selectivity exists in the cases analysed of parents of non-age related trisomy 'G'. As such, G21 trisomy may equally be the result of non-disjunction on either the

maternal or paternal side. This endorses the fact that such an error could occur at either the first or second meiosis, or rarely at the first mitotic division of the zygote, with a resultant disappearance of the monosomic cell line.

Over the past five years, differential staining methods and polymorphisms have been used for the detection of the parental origin of the extra G21 chromosome in cases of trisomy 'G'. Altogether, 62 cases have been analysed which were informative with respect to the non-disjunction of chromosomes 21 in oogenesis or spermatogenesis, as well as in the first or second meiotic division (Langenbeck et al., 1976). Results showed that the origin of the G21 was significantly more frequent as a result of an error occurring in oogenesis (43 cases) than in spermatogenesis (19 cases). It must be noted however, that all age groups were included in the sample.

As far as determining whether there was any preference of the malsegregation occurring at either the first or second meiotic divisions, the results were conflicting. From the cases analysed, no significant difference was observed between the non-disjunction occurring during first or second meiotic divisions. However, mathematical analysis and data from the literature showed that trisomy 'G' is caused between 5 to 10 times more frequently by a malsegregation in the first meiotic division.

The role of the G22 chromosome in satellite association patterns needs to be discussed. From the results in

this study it is seen that there are twice the number of type 'A' associations found in parents of mongol children when a 21:22 association is involved in the configuration. Analysis of the results in the series of Curtis and Cooke (1974), Mattei et al., (1974), Taysi (1975) and Hansson (1975) also show this feature. This particular observation is perhaps the most significant in any of the results, and its presence in any satellite association would possibly implicate its role in the etiology of chromosomal non-disjunction.

The pertinent question that needs to be answered however, regards the incidence of trisomy 22. As the hypothesis stands, an equal number of trisomy 21 and trisomy 22 cases should be born each year. This theory might well have been acceptable however, before the introduction of differential banding, if one assumes that no phenotypic differences exist. At present, there have been 17 cases of trisomy 22 reported (Penchaszadeh and Coco, 1975), all with inconsistent phenotypes not showing any features of the cliassical Down's Syndrome. This incidence is so low that some form of selectivity must be involved if the original hypothesis is to be correct.

There may be several reasons why the G22 chromosome is involved far less in trisomies than G21. Perhaps the most plausible and simplest explanation would be that the G22 pair replicate earlier than the G21 pair. This means that they would be complete, and separate, and that the chances of them being influenced by a partly replicated

G21 chromosome is unlikely. Alternatively, it may be purely mechanical as the G22 is larger, or perhaps a combination of both.

Although the sample used in this study is relatively small when compared to other larger series, the depth of analysis of individual patterns and categories is much greater. This would mean that any real significance of variation in the number of specific associations is accurately classified. It could be argued that a similar result might have been obtained by using a larger sample with fewer criteria. However, the use of a positional parameter to the existing definition of a satellite association is seen as an important addition, not only in detailed analysis, but in understanding its role in interphase.

It is recognised that all of the observations in this study were performed on mitotic cells, and that mitotic events do not necessarily correspond with those in meiosis. It would therefore be valuable to continue this study using meiotic material and to compare findings. It would be of particular interest to look at the chiasmata frequency in relation to parental age as well as the speed of dispersion of nucleolar material in both ovarian and testicular material.

Another future extension of this study would be in the use of the new silver methods to determine densiometrically, any variation in the nucleolar organising regions in

relation to the satellite association frequencies. By this, and the other banding methods, detailed observations utilising markers and polymorphisms on the acrocentric chromosomes should enable a better understanding of parental transmission to be formulated.

Mikkelsen (1976) in Denmark has found that there is an apparent increase in the number of Down's infants being born to younger parents. The explanation for this is not only in the improved diagnosis of the patients, but a possible environmental involvement. However, Fujita and Matsunaga (1976) in their survey over the past 30 years in Japan, found that there was no indication that the risk to younger mothers having Down's children was increased.

If these conflicting surveys are correct, perhaps further investigation into the differences in culture and life-style between these two countries needs to be examined. To comment on the local incidence of Down's children born to younger parents is perhaps unfair in the light of personal experience, but the trend is towards the findings of Mikkelsen (1976), and not those of the Japanese.

The obvious choice for investigation was the oral contraceptive pill. Since McQuarrie et al., (1970) first reported an increased number of chromosome aberrations in females on the pill, this subject has always generated discussion. Recent reports have now shown however, (Janerich et al., 1976 and Fuertes de la Haba et al., 1976),

that no significant differences exist between pill users, and that no evidence is established as to the effect of oral contraceptives and abnormal numerical and structural chromosome patterns. However, it would be of great interest to extend this survey to examine the time lapse between cessation of the oral contraceptive and the fertilisation of the ovum.

The reason why certain chromosomes show a selectivity towards non-disjunction remains speculative. However, what has been shown in this study is that in a selected group of the population, there exists a significant difference in satellite association behaviour. This finding is sufficiently important so as to suggest that it is a major factor in the aetiology of trisomy 'G' in non-age related parents. Whether the associations are as a result of an initial genetic predisposition, or as a reaction to indogenous or exogenous influences remains unknown. However, it is suggested that the interaction between satellited acrocentric chromosomes acts as a direct indicator for potential cell malsegregation, as a result of influences in the nucleolar organising regions.

V. SUMMARY OF CONCLUSIONS

1) The role of the post cultural hypotonic solution has been found to be of critical importance, especially if true associations are to be evaluated. The use of 25% Hanks/distilled water as the hypotonic solution provided fewer, true satellite associations. Thus, only those exhibiting the greatest attraction, i.e. the possible vectors of malsegregation, remain. The consistency of the results show that it is possible to evaluate accurately individual association patterns, and so detect any significant changes.

2) It is demonstrated that the frequency of satellite associations gradually rises with the increase in the molarity of the hypotonic solution. No effect on the frequency is seen in the cells subjected to the hypotonic solution for varying times.

3) Young parents of Down's infants showed the following characteristic satellite association patterns as compared to control parents:

   a) A statistically significant increase in frequency of 21:22 type A di-associations.

   b) A statistically significant increased frequency of tri-associations; a high proportion of which involved chromosomes 21 and 22, most often in type A associations.

4) There does not appear to be any indication that there is a sex predisposition towards any particular satellite association configuration. In both parents and controls, no difference was observed between the sexes within each group.

VI. APPENDIX I

VI.i) Culture Technique

a) Method

1) The culture is set up in 10cm^3 plastic culture tubes under sterile conditions as follows:

Parker TC199 medium	-	5cm^3
Foetal calf serum	-	0.75cm^3
Phytohaemagglutinin	-	0.05cm^3
Whole blood (heparinised)	-	0.4cm^3

2) The tubes are incubated at 37°C for 70 hours. All tubes are mixed twice daily.

3) 0.5cm^3 of 0.02% Colchicine is added to each tube, and incubation continued for a further 1½ hours at 37°C.

4) The cultures are transferred to centrifuge tubes and centrifuged at 1,000 r.p.m. for 5 minutes.

5) The supernate should be clear. This is carefully removed taking care not to disturb cell deposit.

6) 4cm^3 of pre-warmed hypotonic solution is then added to the tubes. This is placed on the shaker at 37°C for the required time.

7) The tubes are centrifuged for 7 minutes at 1,000 r.p.m.

8) The supernate is carefully removed.

9) The cells are fixed. Fresh fixative, Methanol/Glacial Acetic Acid (3:1) is cooled to $4^{o}C$. The fixative is then carefully added drop by drop on to the cell deposit and quickly mixed using a Pasteur pipette.

10) Leave for at least $\frac{1}{2}$ hour before changing the fixative 3 times.

11) Centrifuge and remove supernatant fixative.

12) Add fresh fixative until correct dilution of cells obtained (slightly cloudy - approximately $0.5cm^{3}$).

13) Cells should not clump together.

14) Clean glass microscope slides are removed from ice-box in the refrigerator immediately prior to making slides.

15) The cell suspension is carefully mixed and dropped onto the slide from a height of approximately 12". The slide is held horizontally and the Pasteur pipette directly above it. Blowing the slide ensures a better distribution of the cells.

16) A total of six slides per case are made.

b) Solutions

1) Parker TC199 -
basic medium, single strength containing penicillin, streptomycin and sodium bicarbonate. Obtainable from: Burroughs Wellcome Ltd., Beckenham, Kent, BR3 3BS.

2) Foetal calf serum -
mycoplasma and virus screened. Obtainable from: Flow Laboratories, Irvine, Scotland, KA12 8NB.

3) Phytohaemagglutinin -
lyophilised - reconstituted with $5cm^3$ sterile distilled water. Obtainable from: Burroughs Wellcome Ltd., Beckenham, Kent, BR3 3BS.

4) Colcimid (Colchicine) -
aqueous solution (0.02%), Colcimid (Colchicine) 0.2g, sterile distilled water $1000cm^3$. Obtainable from: C.I.B.A. Chemicals Ltd.

5) Hypotonic solutions -
a) Potassium chloride - 0.075M aqueous, incubation time 8 minutes.
Potassium chloride - 0.5592g
Distilled water - $100cm^3$

b) Sodium citrate - 1% aqueous, incubation time 10 minutes.

Sodium citrate	-	1g
Distilled water	-	100cm^3

c) Hanks/water - 25% aqueous, incubation time 20 minutes.

Hanks balanced salt solution (Burroughs Wellcome Ltd.)	-	25cm^3
Distilled water	-	75cm^3

6) Fixative -

Glacial Acetic Acid	-	10cm^3
Methanol	-	30cm^3

VI.ii) Staining Techniques

1) Giemsa

a) Rinse slide in distilled water.

b) 1% aq. Giemsa solution - 5 minutes.

c) Rinse in distilled water, blot dry, mount in DPX.

Solutions - Giemsa - 1g

Distilled water - 100cm^3

Obtainable from: Searle-Gurr Products, High Wycombe, HP12 4HL.

2) Giemsa Banding

a) 2 x SSC solution is made using Sorrenson's buffer, equal volumes of A and B, as solvent. The pH is adjusted to 6.90.

b) Slides are immersed in 2 x SSC in glass troughs, and these are placed in a water bath at room temperature.

c) The water is allowed to reach 60^{o}C.

d) After 1½ hours in water bath, one slide from each case is removed and rinsed in ice cold 0.9% NaCl.

e) Trypsin solution (1 in 100 parts 0.9% NaCl) at 10^{o}C is placed onto the slides and left for 90 seconds.

f) This is quickly rinsed off using cold 0.9% NaCl.

g) The slides are stained using 1 in 10 Giemsa solution pH 6.8 for 5 minutes.

h) The slides are then dried, mounted in DPX, and examined to assess the affect of SSC and trypsin.

Further SSC treatment may be necessary. Exposure to trypsin may also need adjusting.

Until bands begin to appear, continue SSC treatment, this may take up to 3 hours.

When banding appears to be satisfactory, remove remaining slides and follow from 4 - 8.

Solutions -

i) 2 x SSC - sodium chloride (NaCl) - 17.53g

Tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$) - 8.82g

Buffered distilled water - $1000cm^3$

ii) Sorrenson's buffer A

Potassium dihydrogen orthophosphate - 0.454g

Distilled water - $1000cm^3$

iii) Sorrenson's buffer B

Di-potassium hydrogen orthophasphate - 0.473g

Distilled water - $1000cm^3$

iv) Trypsin - Bacto - Trypsin - reconstituted with $10cm^3$ sterile distilled water. Obtainable from: Difco Laboratories, West Molesey, KT8 OSE.

v) Giemsa - Gurr's improved R66 solution. Obtainable from: Searle-Gurr Products, High Wycombe, HP12 4HL.

3) Fluorescent Banding

a) Air dried slides are rehydrated using the following sequence:

70% alcohol	5 minutes
50% alcohol	5 minutes
Distilled water	5 minutes
Buffer (pH 7.4)	5 minutes

b) Slides are stained with Quinacrine Mustard solution for 40 - 45 minutes.

c) Rinse and leave the slides in buffer for:

Buffer (pH 7.4)	10 minutes
Buffer (pH 7.4)	5 minutes

d) Mount the slides leaving a very thin film of buffer between slide and coverslip; then seal.

Solutions -

i) McIlvain's buffer I -

0.1M citric acid	2.1g
Distilled water	$100cm^3$

ii) McIlvain's buffer II -

0.2M disodium hydrogen phosphate	7.16g
Distilled water	$100cm^3$

iii) McIlvain's buffer pH 7.4 -

Buffer I	$17.6cm^3$
Buffer II	$82.4cm^3$

iv) Quinacrine Mustard - 0.0050g
74.0P spirit $0.5cm^3$
Distilled water make up to $100cm^3$

4) Selective Silver Staining of Nucleolar Organising Regions

a) Slides are placed on a raised platform in a moisture-tight box.

b) The silver solution is flooded on to the slide, and then covered by a coverslip.

c) A small volume of distilled water is added to the bottom of the box to provide moisture, and the lid replaced.

d) The slides are incubated in this box for 18 hours (overnight) at $37^{\circ}C$.

e) Rinse the coverslip off with distilled water, wash slide well.

f) Stain the slides with 1% aq. Giemsa solution for 5 minutes.

g) The slides are then dried, mounted in DPX and then examined.

Solutions -

i) Silver solution -

Silver nitrate	1g
Distilled water pH 4.5-5.0	$2cm^3$

ii) Giemsa - 1g

Distilled water $100cm^3$

Obtainable from: Searle-Gurr Products, High Wycombe, HP12 4HL.

VII. APPENDIX II

VII.i) Results of Hypotonic Solution Experiments

			Percentage of Cells with one or more Satellite Associations		
Subject No.	Sex	Age	KCl	Sodium Citrate	Hanks with Distilled Water (1:3)
1	F	21	70	40	30
2	M	22	50	60	20
3	F	21	60	70	60
4	M	22	80	50	30
5	M	20	100	90	70
6	M	23	80	50	20
7	F	21	80	60	50
8	F	23	80	50	70
9	F	22	80	70	40
10	F	22	60	50	30
11	M	19	90	80	60
12	M	19	80	60	30
13	M	19	80	70	50
14	M	20	70	50	50
15	M	19	80	70	70
16	M	20	70	50	40
17	M	21	90	60	90
18	M	19	100	60	40
19	M	19	80	50	40
20	M	20	80	60	60
21	M	19	60	70	60
22	M	20	80	70	60
23	F	19	80	60	60
24	M	19	90	70	50
25	M	19	90	80	70
26	F	19	90	60	80
27	F	21	60	60	60
28	F	19	90	40	30
29	M	19	90	60	40
30	F	19	80	50	40
31	F	21	70	90	50
32	F	25	90	60	50
33	M	20	70	60	30
34	M	19	70	50	40
35	F	23	70	60	40

Table XXI Percentage of cells showing one or more satellite associations observed in 10 cells per subject

Hypotonic Treatment	Number of Controls Examined	Number of Metaphases Examined	Specific Group Associations Recorded								
			None	D/G	D/D	G/G	D/D/G	D/G/G	D/D/D	G/G/G	Others
Potassium Chloride	35	350	57	185	71	51	20	16	8	3	2
Tri-sodium Citrate	35	350	119	138	55	37	5	18	7	2	0
Hanks/ Water	35	350	171	87	48	40	9	6	1	1	0

Table XXII Comparison of the specific group satellite association patterns, as observed in the three hypotonic solutions

The Effect of Potassium Chloride (0.075M aq) as a Hypotonic Solution on Satellite Association Frequencies

No.	Sex	Age	G/G	G/D	D/D	D/D/D	D/D/G	G/G/G	D/G/G	Others
1	F	21	2	4	2	-	1	-	-	-
2	M	22	2	4	1	-	-	-	-	-
3	F	21	5	2	1	-	-	-	-	-
4	M	22	1	1	7	-	-	-	-	-
5	M	20	3	5	3	-	1	-	1	-
6	M	23	-	7	1	-	-	-	3	-
7	F	21	1	4	2	1	1	-	1	-
8	F	23	3	4	1	-	-	-	1	D/D/G/G
9	F	23	-	4	1	2	-	-	1	-
10	F	22	-	6	2	-	-	-	1	-
11	M	19	2	8	1	-	1	-	-	-
12	M	19	2	7	1	1	-	-	-	-
13	M	19	1	4	4	-	2	-	-	-
14	M	20	3	3	1	1	-	-	1	-
15	M	19	1	5	3	-	2	-	-	-
16	M	20	2	4	-	-	-	-	1	-
17	M	21	2	8	3	-	-	-	-	D/D/G/G
18	M	19	1	7	1	1	-	-	1	-
19	M	19	3	3	1	-	-	2	-	-
20	M	20	1	10	1	-	-	-	-	-
21	M	19	4	3	2	-	-	-	-	-
22	M	20	-	4	2	1	2	-	-	-
23	F	19	1	6	2	-	2	1	-	-
24	M	19	3	6	2	-	3	-	-	-
25	M	19	1	8	1	-	2	-	3	-
26	F	19	2	10	-	-	1	-	1	-
27	F	21	0	4	3	-	-	-	-	-
28	F	19	1	5	4	-	1	-	-	-
29	M	19	-	7	3	-	-	-	-	-
30	F	19	-	6	3	-	-	-	-	-
31	F	21	3	4	-	-	-	-	-	-
32	F	25	1	4	3	-	-	-	-	-
33	M	20	1	4	2	-	-	-	-	-
34	M	19	2	7	1	-	-	-	1	-
35	F	23	2	4	5	-	1	-	-	-

The Effect of Sodium Citrate (1% aq) as a Hypotonic Solution on Satellite Association Frequencies

No.	Sex	Age	G/G	G/D	D/D	D/D/D	D/D/G	G/G/G	G/G/D	Others
1	F	21	2	1	2	-	-	-	-	-
2	M	22	-	4	2	-	-	-	1	-
3	F	21	4	4	1	-	-	-	-	-
4	M	22	4	1	-	-	-	1	-	-
5	M	20	2	8	2	-	-	-	-	-
6	M	23	-	3	3	-	-	-	1	-
7	F	21	2	3	1	-	-	-	1	-
8	F	23	-	4	1	-	-	-	2	-
9	F	22	3	4	-	-	-	-	-	-
10	F	22	-	1	3	-	1	-	1	-
11	M	19	-	7	1	1	-	-	-	-
12	M	19	-	4	1	-	1	-	1	-
13	M	19	-	5	2	1	-	-	-	-
14	M	20	-	4	-	1	-	-	1	-
15	M	19	1	7	3	-	-	-	-	-
16	M	20	1	4	1	-	-	-	-	-
17	M	21	-	4	1	1	-	-	-	-
18	M	19	2	3	2	-	-	-	-	-
19	M	19	2	-	1	-	-	-	2	-
20	M	20	4	3	-	-	-	-	1	-
21	M	19	2	5	-	-	-	-	1	-
22	M	20	2	3	2	-	-	-	1	-
23	F	19	1	4	1	-	1	-	-	-
24	M	19	1	6	2	-	-	-	-	-
25	M	19	3	4	-	1	-	-	1	-
26	F	19	1	5	2	-	-	-	-	-
27	F	21	-	3	3	-	-	-	-	-
28	F	19	2	1	2	-	-	-	-	-
29	M	19	-	4	4	1	-	-	-	-
30	F	19	-	4	1	-	-	1	-	-
31	F	21	2	8	2	-	-	-	-	-
32	F	25	2	4	-	-	-	-	1	-
33	M	20	2	3	1	-	-	-	1	-
34	M	19	-	1	3	-	1	-	1	-
35	F	23	-	4	1	-	1	-	1	-

The Effect of Hanks Solution with Distilled Water (1:3) as a Hypotonic Solution on Satellite Association Frequencies

No.	Sex	Age	G/G	G/D	D/D	D/D/D	D/D/G	G/G/G	G/G/D	Others
1	F	21	-	1	2	-	-	-	-	-
2	M	22	-	-	1	-	1	-	-	-
3	F	21	2	1	3	-	-	-	-	-
4	M	22	-	1	1	-	1	-	-	-
5	M	20	-	6	1	-	-	-	-	-
6	M	23	1	1	-	-	-	-	-	-
7	F	21	-	4	2	-	-	-	-	-
8	F	23	2	4	-	-	-	-	1	-
9	F	22	-	4	-	-	-	-	-	-
10	F	22	1	1	1	-	-	-	-	-
11	M	19	-	3	4	-	-	-	1	-
12	M	19	1	2	-	-	-	-	-	-
13	M	19	2	-	4	-	-	-	-	-
14	M	20	1	4	-	-	-	-	-	-
15	M	19	3	3	3	-	-	-	-	-
16	M	20	3	4	-	-	-	-	-	-
17	M	21	-	6	3	-	2	-	-	-
18	M	19	1	2	1	-	-	-	-	-
19	M	19	1	2	-	-	-	-	1	-
20	M	20	3	5	-	-	-	-	-	-
21	M	19	4	4	-	-	-	-	-	-
22	M	20	1	2	3	-	1	-	-	-
23	F	19	1	5	1	1	1	-	-	-
24	M	19	1	2	1	-	2	-	-	-
25	M	19	2	2	-	-	-	1	2	-
26	F	19	1	4	4	-	-	-	-	-
27	F	21	-	2	4	-	-	-	-	-
28	F	19	-	1	2	-	-	-	-	-
29	M	19	2	1	1	-	-	-	-	-
30	F	19	1	1	3	-	-	-	-	-
31	F	21	1	2	1	-	-	-	1	-
32	F	25	2	2	-	-	-	-	-	-
33	M	20	1	1	1	-	-	-	-	-
34	M	19	2	1	-	-	1	-	-	-
35	F	23	-	3	1	-	-	-	-	-

VII.ii) Results of Satellite Association Patterns - Parents and Controls

	Number of Acrocentric Chromosomes Associating per Cell				
	None	2	3	4	Total
Fathers of children with Down's Syndrome	0.523	0.457	0.057	0.004	1.043
Control fathers of normal children	0.618	0.374	0.044	0.004	1.042
Total	1.142	0.832	0.102	0.008	2.085

No statistical significance between any of the comparisons (Mann and Whitney).

Table XXIII Mean frequency of satellite associations per cell (900 cells examined)

	Number of Acrocentric Chromosomes Associating per Cell				
	None	2	3	4	Total
Mothers of children with Down's Syndrome	0.507	0.471	0.071	0.008	1.057
Control mothers of normal children	0.601	0.373	0.023	0.001	0.998
Total	1.108	0.844	0.094	0.008	2.056

No statistical significance between any of the comparisons (Mann and Whitney).

Table XXIV Mean frequency of satellite associations per cell (900 cells examined)

Subject Number	Sex	Age	Number of Cells Examined	Number of Chromosomes in Association				
				None	2	3	4+	Total
5	F	22	100	44	54	6	0	60
6	M	23	100	46	51	3	0	54
15	M	34	100	38	74	4	0	78
16	F	32	100	58	41	5	0	46
19	F	20	100	54	47	3	1	51
20	M	22	100	46	48	2	0	50
21	F	21	100	39	53	11	4	68
22	M	23	100	62	38	5	0	43
23	F	22	100	60	37	7	0	44
24	M	24	100	48	46	9	0	45
25	M	29	100	49	44	10	4	58
26	F	26	100	47	48	10	0	58
27	F	29	100	44	57	6	0	63
28	M	29	100	59	41	5	0	46
31	F	28	100	54	41	8	2	51
32	M	30	100	60	36	10	0	46
39	F	34	100	35	46	8	0	54
40	M	34	100	65	34	4	0	38

Table XXV Distribution of the frequency of satellite associations in parents of children with Down's Syndrome (mongolism)

Subject Number	Sex	Age	Number of Cells Examined	Number of Chromosomes in Association				
				None	2	3	4+	Total
13	F	26	100	54	42	5	0	47
14	M	26	100	58	39	6	1	46
33	F	26	100	64	38	0	1	39
34	M	28	100	67	35	1	0	36
41	F	29	100	62	37	5	1	43
42	M	27	100	63	37	6	1	44
43	F	21	100	69	34	4	0	38
44	M	23	100	64	35	5	0	40
45	M	24	100	62	34	6	0	40
46	F	24	100	62	39	3	0	42
47	M	34	100	52	42	9	0	51
48	F	33	100	63	40	0	0	40
51	M	28	100	68	34	3	0	37
52	F	27	100	67	38	2	0	40
59	M	30	100	65	33	3	1	37
60	M	29	100	65	36	0	0	36
61	M	32	100	58	46	2	0	48
62	F	32	100	65	34	1	0	35

Table XXVI Distribution of the frequency of satellite associations in control parents of normal children

VII.iii Sample Details - Parents and Controls

S/A NUMBER	5	6
SEX	F	M
DATE OF BIRTH	1.12.50	23.11.49
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 20.8.73 F Downs Syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Nil Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant No	None relevant No
CHROMOSOME CONSTITUTION	46,XX	46,XY

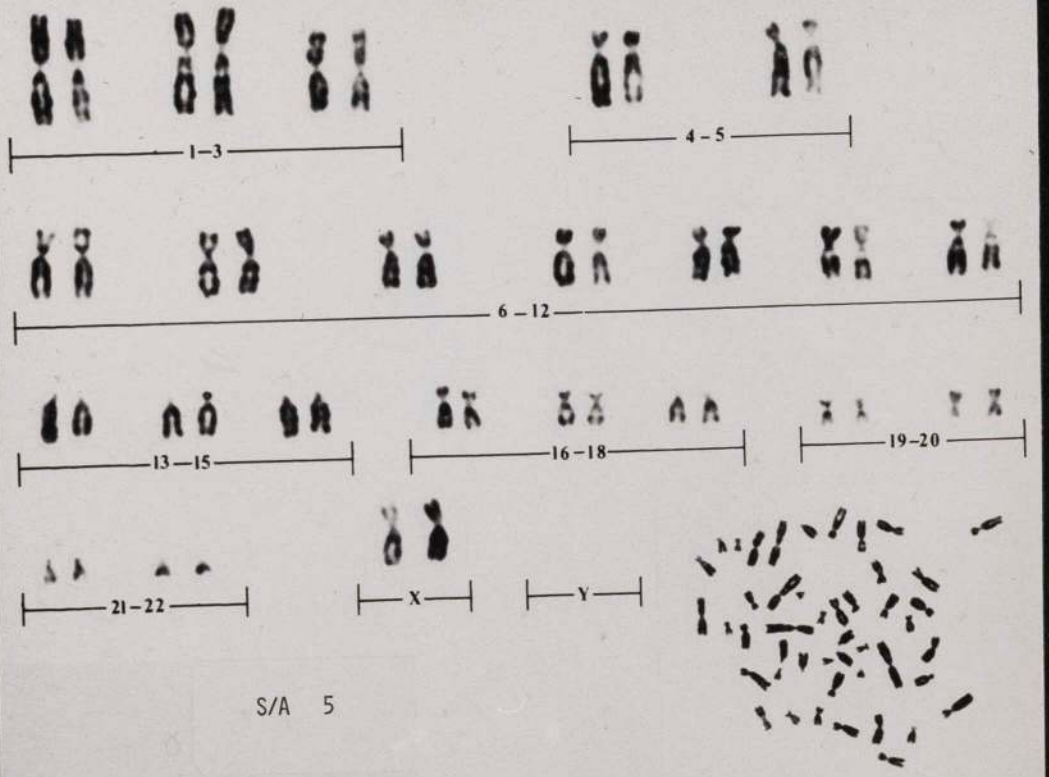
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14			
	13 - 15	1	3	4
	14 - 14			
	14 - 15		3	3
	15 - 15			
G/G	21 - 21	2	2	4
	21 - 22	8	10	18
	22 - 22		2	2
D/G	13 - 21	1		1
	13 - 22			
	14 - 21	1	2	3
	14 - 22	2	3	5
	15 - 21	4	4	8
	15 - 22		6	6
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21		1	1.
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	1		1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	2		2
	15 - 22 - 22			
D/D/D	13 - 14 - 15		1	1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21	1		1
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		2	2
	13 - 15			
	14 - 14			
	14 - 15		1	1
	15 - 15		1	1
G/G	21 - 21	1	1	2
	21 - 22	5	4	9
	22 - 22		4	4
D/G	13 - 21	6	3	9
	13 - 22	1	4	5
	14 - 21	2	1	3
	14 - 22	2	4	6
	15 - 21	2	3	5
	15 - 22	1	3	4
D/D/G	13 - 13 - 21			
	13 - 13 - 22	1		1
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1		1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				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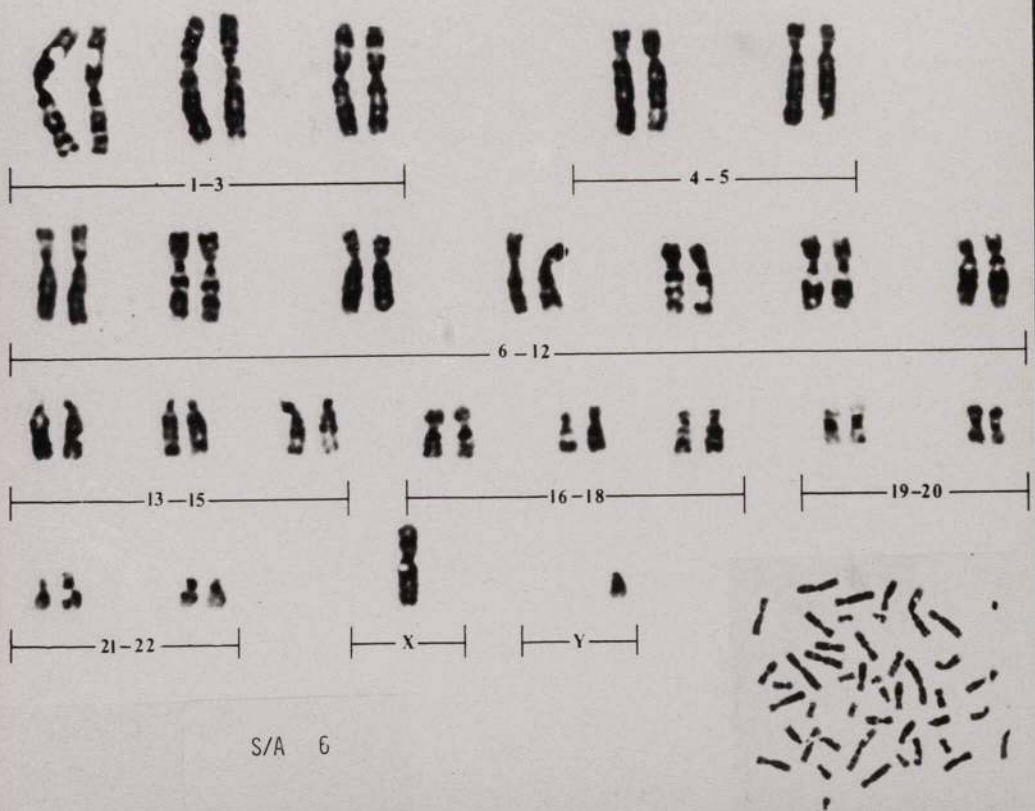
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 5


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 6

S/A NUMBER	15		16
SEX	M		F
DATE OF BIRTH	28.10.39		30.5.41
NATIONALITY	British		British
CHILDREN	1	2	3
Date of Birth	9.3.60	15.9.67	7.2.73
Sex	M	F	M
Details	Normal	Normal	Downs syndrome
AFFECTED PREGNANCY			
Drugs	Nil		
Accidents	Nil		
Radiation	Nil		
Other	Nil		
FAMILY HISTORY			
Illness			
Affected Members	None relevant		None relevant
Other			
CHROMOSOME CONSTITUTION	46,XY		46,XX

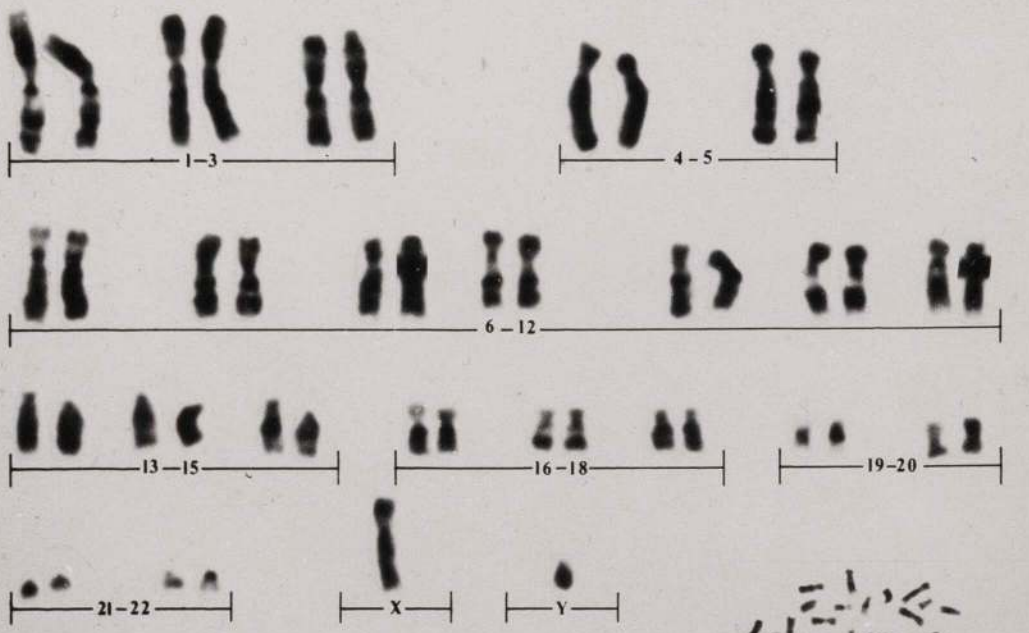
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	3	2	5
	13 - 15	2	2	4
	14 - 14	1		1
	14 - 15	8	2	10
	15 - 15			
G/G	21 - 21			
	21 - 22	8	7	15
	22 - 22			
D/G	13 - 21	1		1
	13 - 22	4	6	10
	14 - 21		2	2
	14 - 22	6	13	19
	15 - 21		1	1
	15 - 22	4	2	6
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22	1		1
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21		1	1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	1		1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14		1	1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	1	2
	13 - 15		1	1
	14 - 14		1	1
	14 - 15		1	1
	15 - 15			
G/G	21 - 21			
	21 - 22	7	5	12
	22 - 22			
D/G	13 - 21		5	5
	13 - 22	2	3	5
	14 - 21	2	3	5
	14 - 22	2	1	3
	15 - 21	1	2	3
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22		1	1
	14 - 15 - 21	2		2
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	1		1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21		1	1
	21 - 21 - 22			
OTHER				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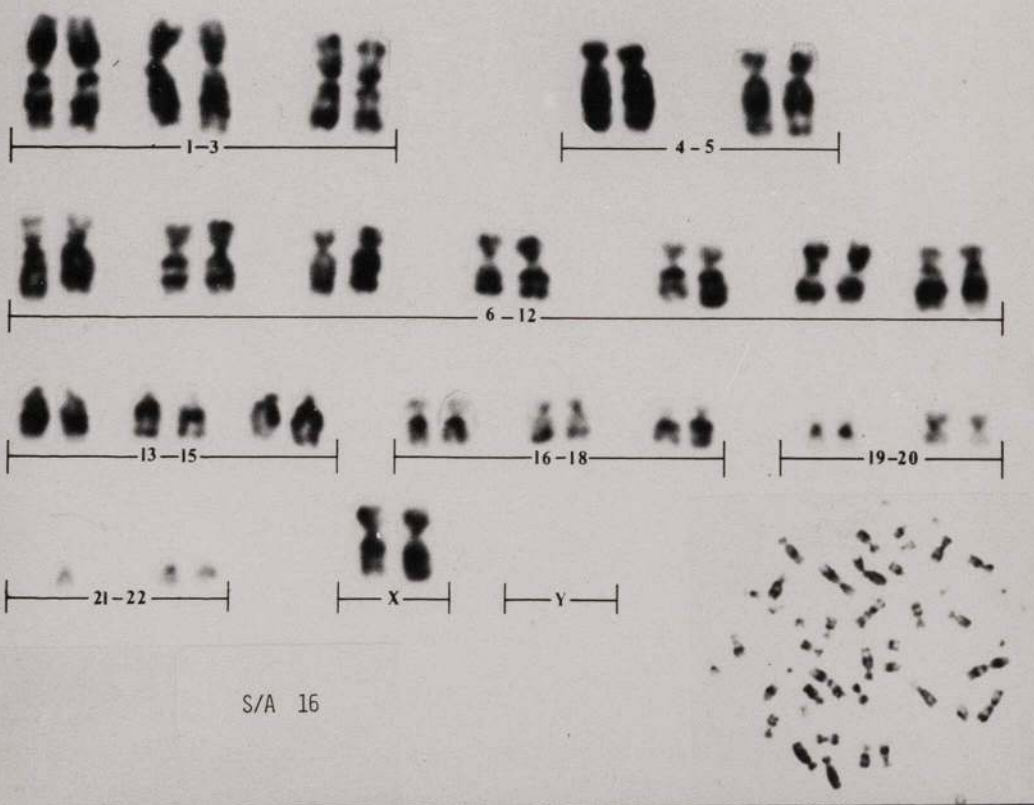
6 – 12
13 – 15
16 – 18
19–20
21 – 22
X
Y

S/A 15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4 – 5
6 – 12
13 – 15
16 – 18
19–20
21 – 22
X
Y

S/A 16

S/A NUMBER	19	20
SEX	F	M
DATE OF BIRTH	28.1.54	15.11.51
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 28.12.73 F Downs syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Iron only Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

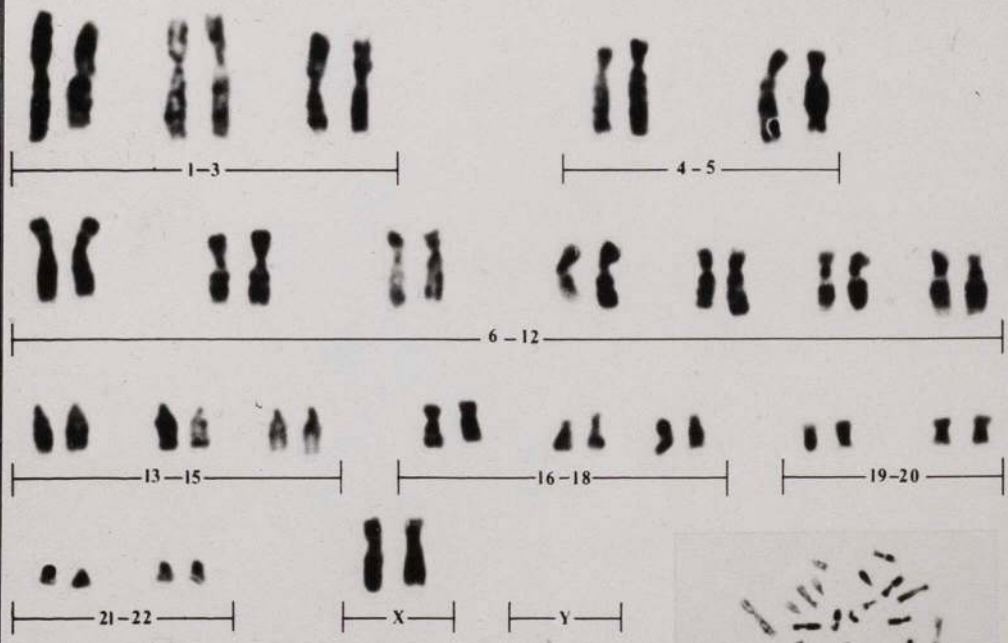
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1		1
	13 - 14	6	3	9
	13 - 15		1	1
	14 - 14			
	14 - 15	1	3	4
	15 - 15		1	1
G/G	21 - 21	1	1	2
	21 - 22	3	3	6
	22 - 22		4	4
D/G	13 - 21	1	1	2
	13 - 22		1	1
	14 - 21	1	5	6
	14 - 22	1	4	5
	15 - 21	1	1	2
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22		1	1
	15 - 15 - 21			
	15 - 15 - 22			

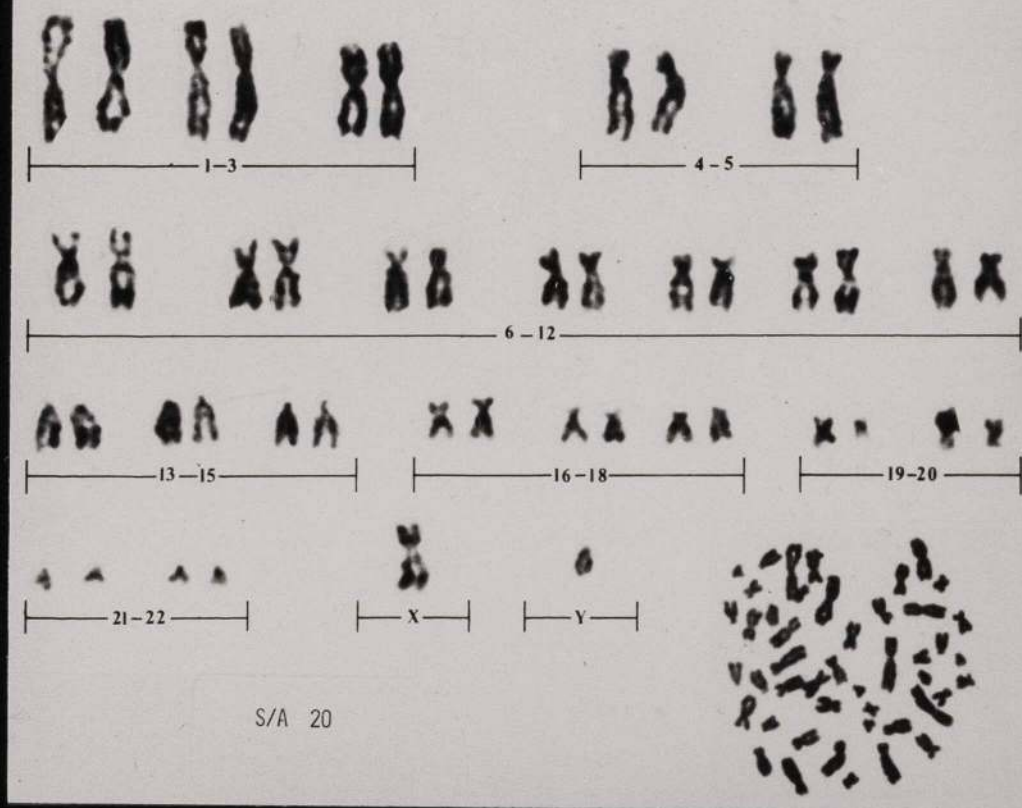
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22		1	1
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	13-14-22-21		1	1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	3	2	5
	13 - 15			
	14 - 14			
	14 - 15	1	2	3
	15 - 15			
G/G	21 - 21			
	21 - 22	8	10	18
	22 - 22	2		2
D/G	13 - 21	1	1	2
	13 - 22	1	1	2
	14 - 21	1	7	8
	14 - 22	3		3
	15 - 21	1		1
	15 - 22	1	3	4
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22	1		1
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19–20
21 – 22
X
Y

S/A 19


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19–20
21 – 22
X
Y

S/A 20

S/A NUMBER	21	22
SEX	F	M
DATE OF BIRTH	4.10.52	23.2.51
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 19.1.74 F Downs syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Nil Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

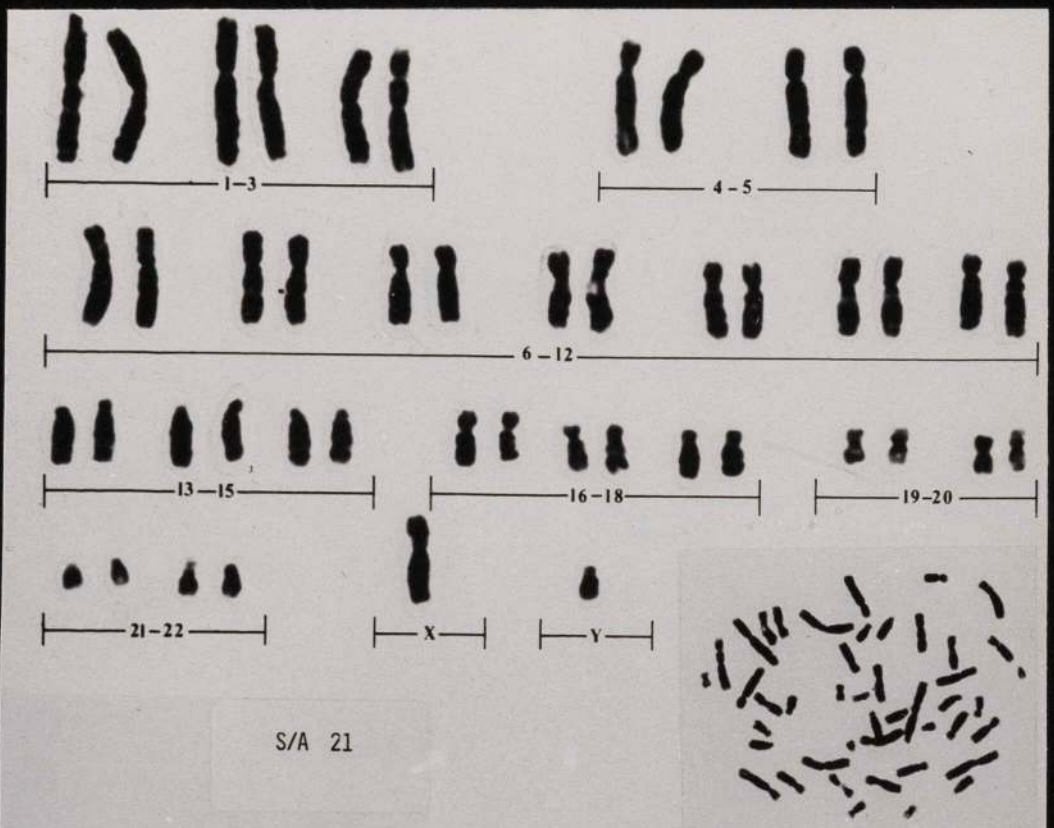
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		1	1
	13 - 15			
	14 - 14			
	14 - 15		2	2
	15 - 15		1	1
G/G	21 - 21		1	1
	21 - 22	23	11	34
	22 - 22		1	1
D/G	13 - 21	2		2
	13 - 22	1	1	2
	14 - 21		2	2
	14 - 22	2	2	4
	15 - 21	1	1	2
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22	1		1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

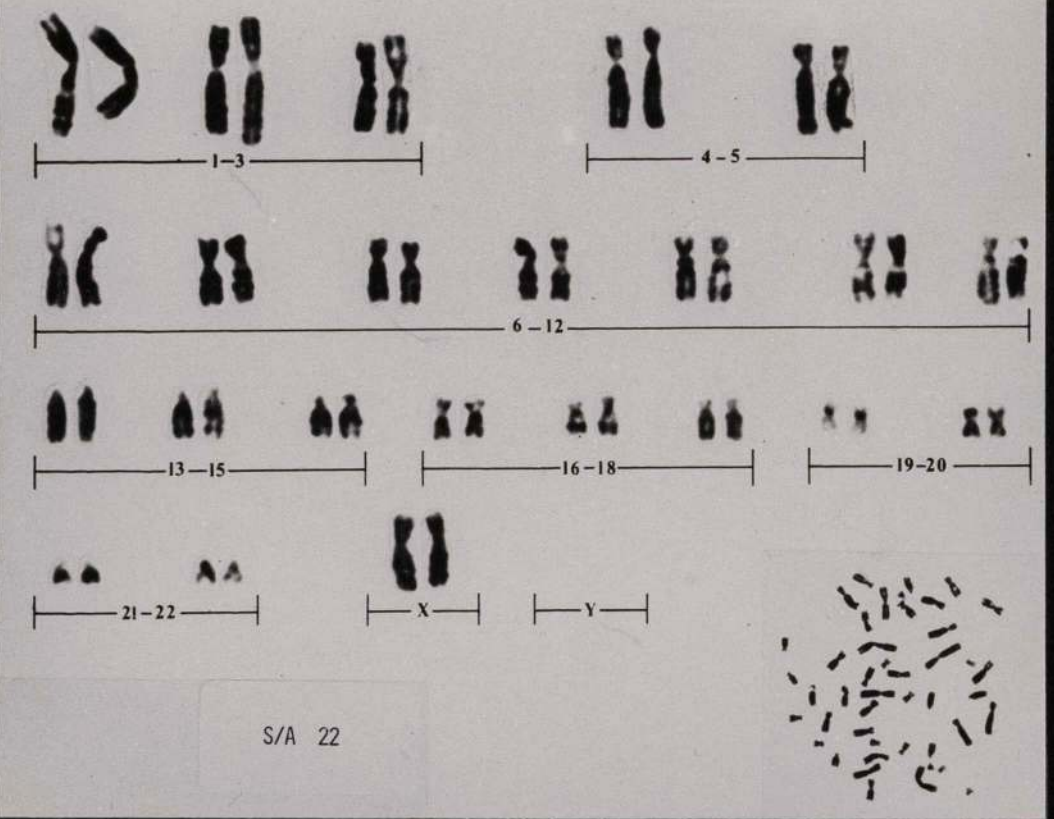
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	2		2
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	2	1	3
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1	2	3
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21	1	1	2
	21 - 21 - 22			
OTHER	21-22-22-13		1	1
	14-21-22-22		1	1
	13-21-21-22-22)	1		1
	13-21-21-22-22)	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13		1	1
	13 - 14		4	4
	13 - 15		1	1
	14 - 14			
	14 - 15	3	1	4
	15 - 15		1	1
G/G	21 - 21			
	21 - 22	4	5	9
	22 - 22	1	1	2
D/G	13 - 21	1		1
	13 - 22		1	1
	14 - 21	1	2	3
	14 - 22	2	2	4
	15 - 21	2	1	3
	15 - 22	1	3	4
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21		1	1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22	1		1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	1		1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15		1	1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15	1		1
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 21


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 22

S/A NUMBER	23	24
SEX	F	M
DATE OF BIRTH	9.3.52	17.11.49
NATIONALITY	British	British
CHILDREN	1	2
Date of Birth	14.9.70	4.1.74
Sex	M	F
Details	Normal	Downs syndrome
AFFECTED PREGNANCY		
Drugs	Iron and vitamins	
Accidents	Nil	
Radiation	Nil	
Other	Nil	
FAMILY HISTORY		
Illness		
Affected Members	None relevant	None relevant
Other		
CHROMOSOME CONSTITUTION	46,XX	46,XY

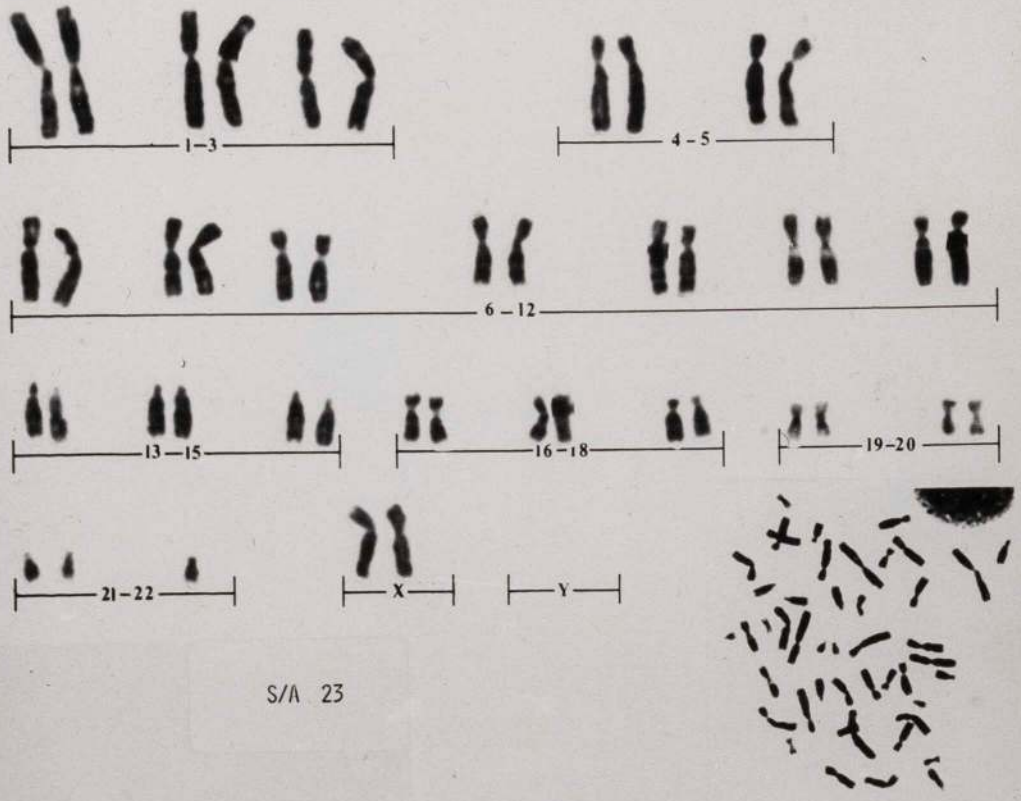
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13		1	1
	13 - 14	1	1	2
	13 - 15			
	14 - 14			
	14 - 15	1	1	2
	15 - 15			
G/G	21 - 21	2	2	4
	21 - 22	10	2	12
	22 - 22			
D/G	13 - 21	1	1	2
	13 - 22			
	14 - 21	1	3	4
	14 - 22	2	2	4
	15 - 21	3	2	5
	15 - 22	1		1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

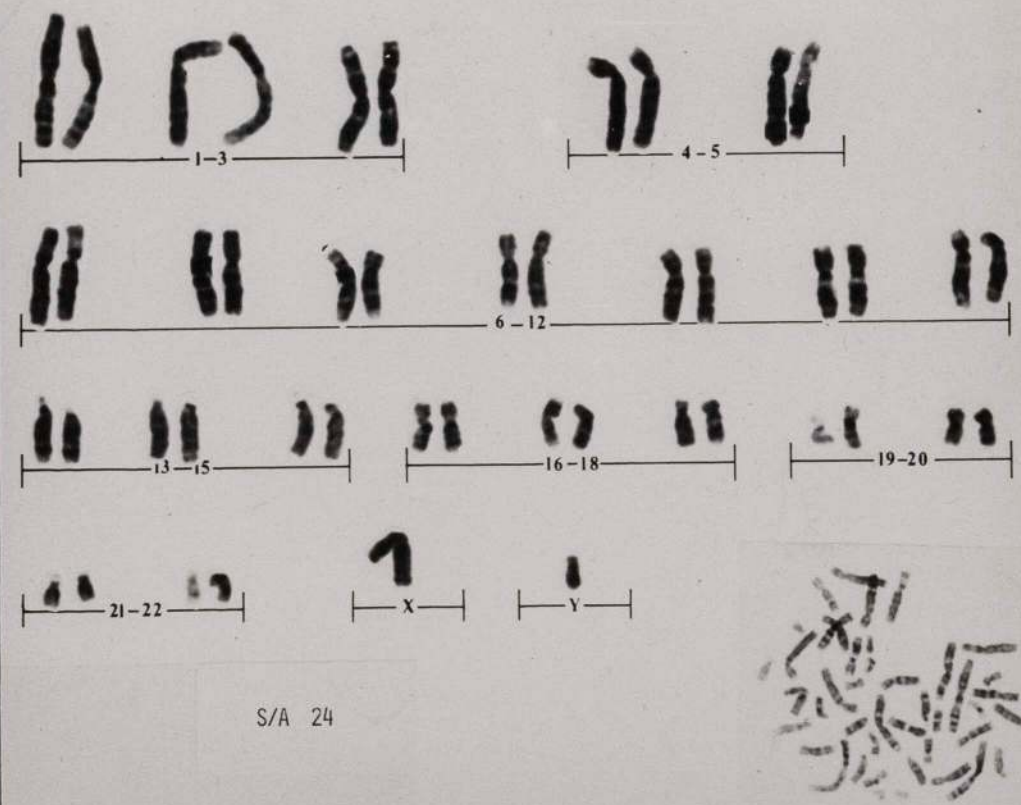
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22	2		2
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1		1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21		2	2
	21 - 21 - 22	1		1
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	2	3
	13 - 14	3	3	6
	13 - 15	2		2
	14 - 14			
	14 - 15		7	7
	15 - 15			
G/G	21 - 21	1		1
	21 - 22	3	9	12
	22 - 22			
D/G	13 - 21	2	2	4
	13 - 22		3	3
	14 - 21	1	3	4
	14 - 22		1	1
	15 - 21			
	15 - 22	2	1	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	2		2
	13 - 14 - 22	1		1
	13 - 15 - 21	1		1
	13 - 15 - 22	1		1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21		1	1
	14 - 15 - 22		1	1
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22		1	1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13	1		1
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 23


1-3
4-5
6-12
13-15
16-18
19-20
21-22
X
Y

S/A 24

S/A NUMBER	25	26
SEX	F	M
DATE OF BIRTH	21.7.47	2.2.45
NATIONALITY	Irish	British
CHILDREN Date of Birth Sex Details	1 26.1.74 M Downs syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Iron only Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	None relevant Not known	None relevant Not known
CHROMOSOME CONSTITUTION	46,XX	46,XY

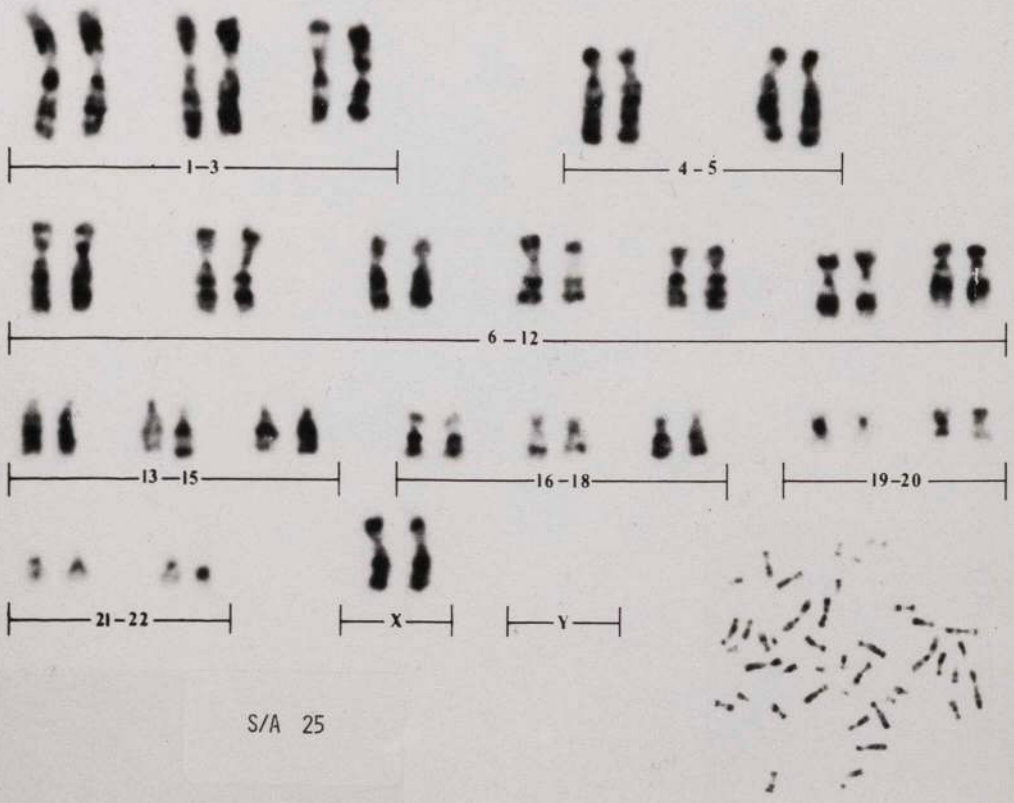
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1		1
	13 - 14	4	4	8
	13 - 15	1	1	2
	14 - 14	1	1	2
	14 - 15	5	5	10
	15 - 15	2		2
G/G	21 - 21			
	21 - 22	2		2
	22 - 22			
D/G	13 - 21	1	1	2
	13 - 22		1	1
	14 - 21	1	3	4
	14 - 22		3	3
	15 - 21	3	1	4
	15 - 22	2	1	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21		1	1
	13 - 14 - 22			
	13 - 15 - 21	1		1
	13 - 15 - 22	1		1
	14 - 14 - 21	1		1
	14 - 14 - 22			
	14 - 15 - 21	1	1	2
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

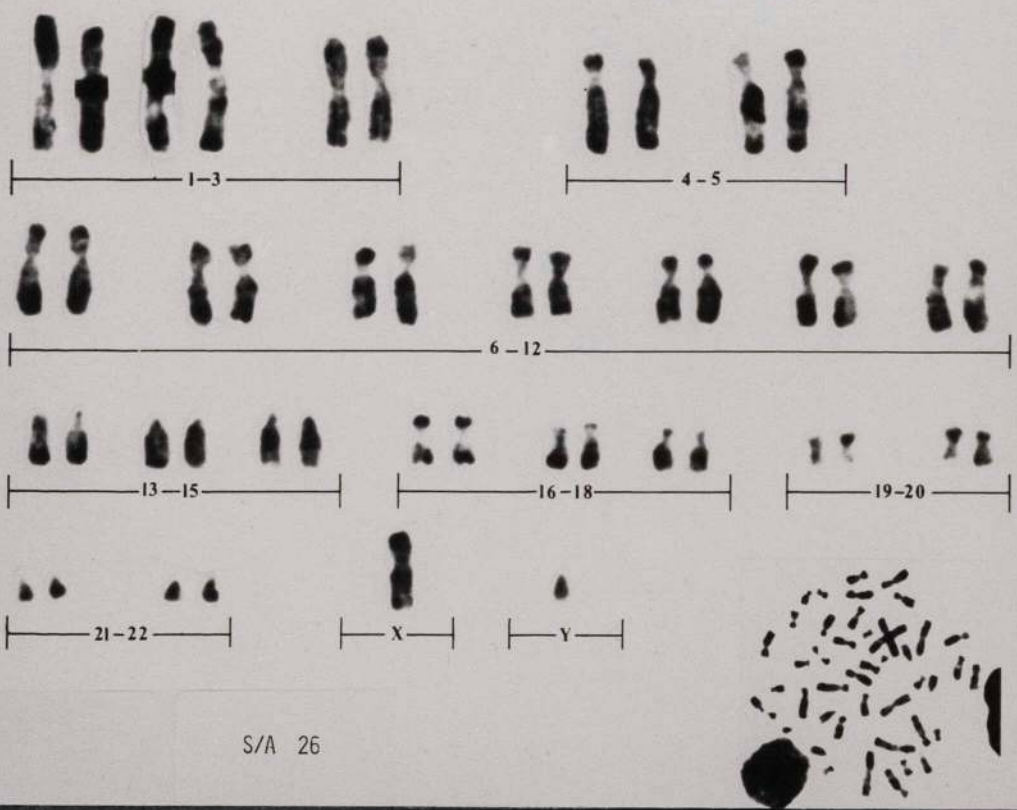
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15	2		2
	13 - 13 - 14	1		1
	13 - 14 - 14	1		1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	13-14-15-21	) 1		1
	13-14-15-21	) 1		1
	13-14-15-21-15	1		1
	13-14-14-22	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		1	1
	13 - 15		1	1
	14 - 14			
	14 - 15		2	2
	15 - 15	1		1
G/G	21 - 21			
	21 - 22	10	11	21
	22 - 22		2	2
D/G	13 - 21	1	1	2
	13 - 22	1	2	3
	14 - 21		8	8
	14 - 22	1	2	3
	15 - 21	1	2	3
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22	1		1
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21	1		1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22		1	1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	2		2
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22	1		1
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21	1	1	2
	21 - 21 - 22	1	1	2
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 25


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 26

S/A NUMBER	27	28
SEX	F	M
DATE OF BIRTH	12.1.45	3.5.45
NATIONALITY	British	British
CHILDREN	1	2
Date of Birth	6.10.67	20.6.69
Sex	F	M
Details	Normal	Downs syndrome
AFFECTED PREGNANCY		
Drugs	Nil	
Accidents	Nil	
Radiation	Nil	
Other	Nil	
FAMILY HISTORY		
Illness	None relevant	None relevant
Affected Members		
Other		
CHROMOSOME CONSTITUTION	46,XX	46,XY

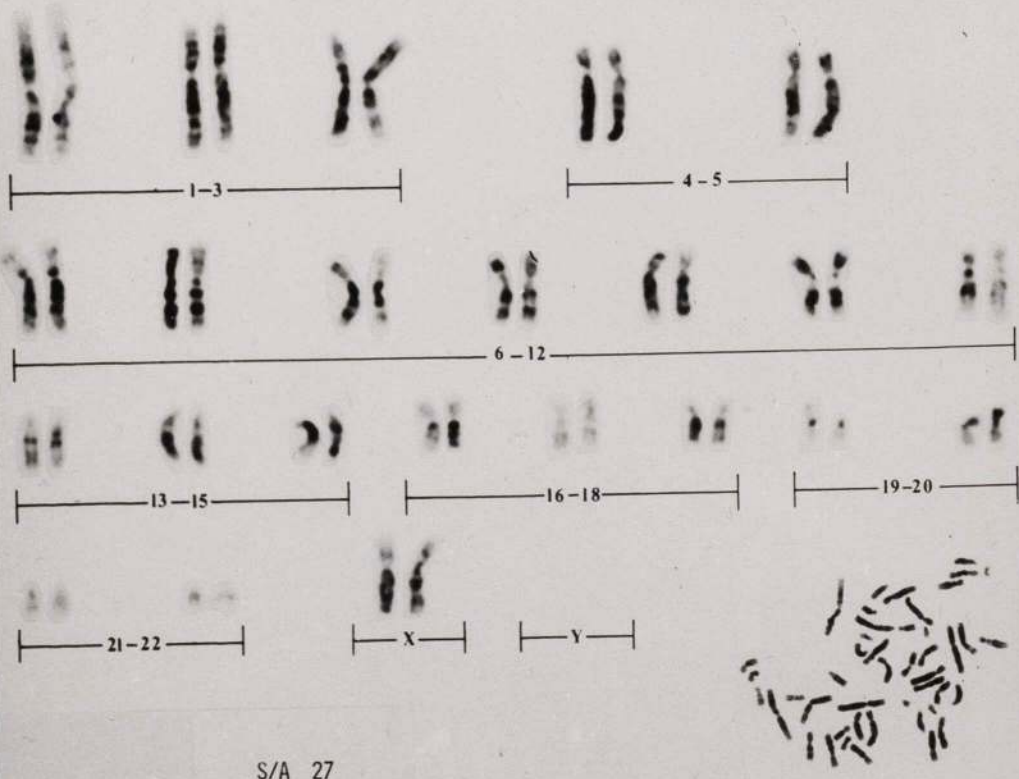
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1		1
	13 - 14	4	3	7
	13 - 15	1	2	3
	14 - 14		1	1
	14 - 15	5	3	8
	15 - 15	1		1
G/G	21 - 21	1		1
	21 - 22	5	3	8
	22 - 22		1	1
D/G	13 - 21	2	2	4
	13 - 22	1	2	3
	14 - 21	4	4	8
	14 - 22	2	3	5
	15 - 21	2	1	3
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21	1		1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

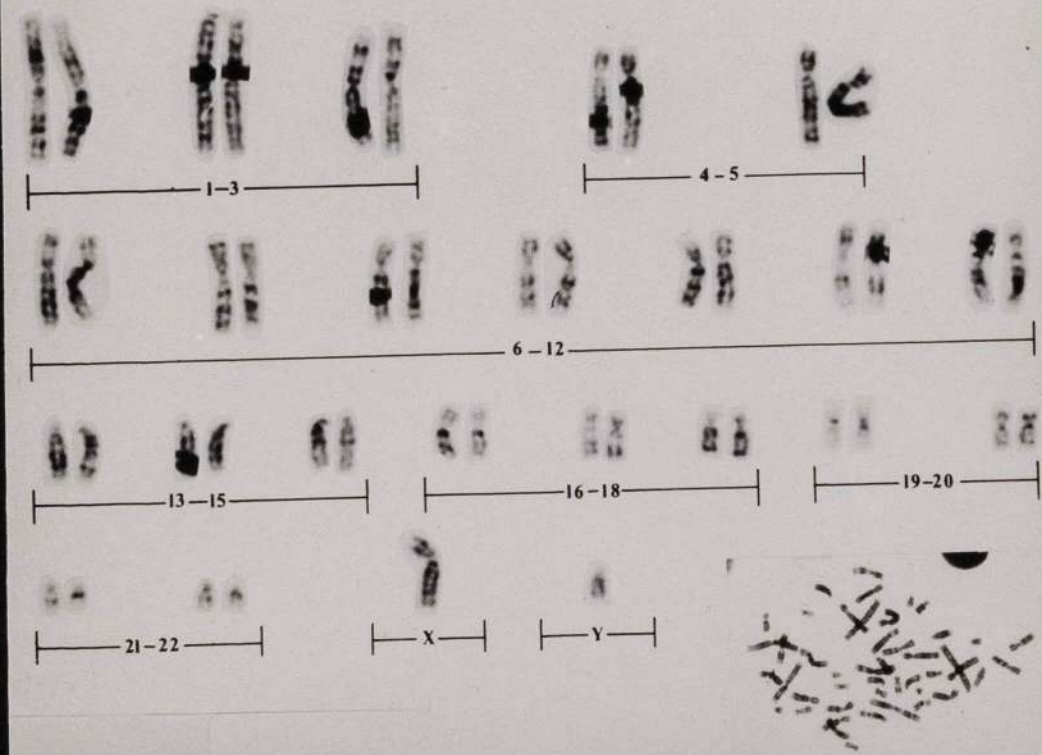
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21	1		1
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21	1		1
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14		1	1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	2		2
	13 - 14	2	2	4
	13 - 15			
	14 - 14			
	14 - 15	1	2	3
	15 - 15	1	1	2
G/G	21 - 21	1	1	2
	21 - 22	3	8	11
	22 - 22			
D/G	13 - 21		3	3
	13 - 22			
	14 - 21	2	3	5
	14 - 22	1	3	4
	15 - 21		2	2
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22	1		1
	14 - 21 - 21			
	14 - 21 - 22		1	1
	14 - 22 - 22	1		1
	15 - 21 - 21		1	1
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 27


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 28

S/A NUMBER	31	32
SEX	F	M
DATE OF BIRTH	21.6.46	20.12.43
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 6.11.70 M Downs syndrome	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	 Iron only Nil Nil Nil	
FAMILY HISTORY Illness Affected Members Other	 Thrombosis None relevant	 None relevant None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

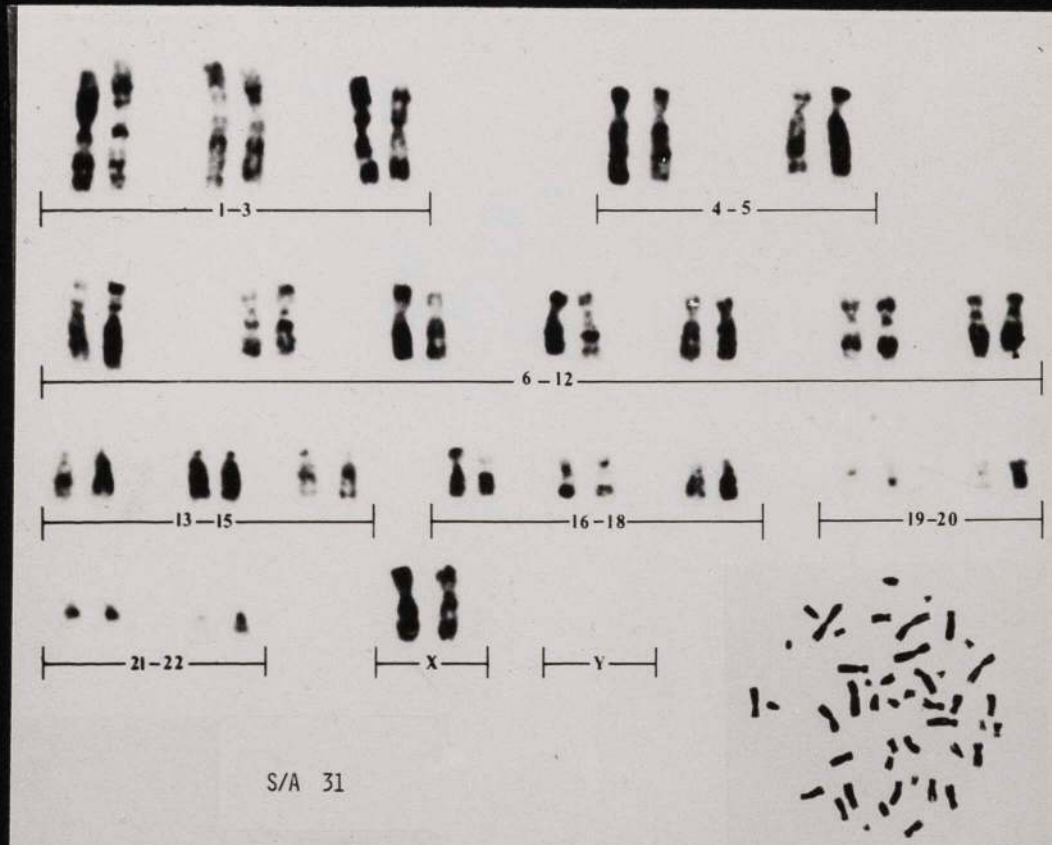
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	3	3	6
	13 - 15	4		4
	14 - 14			
	14 - 15	1	1	2
	15 - 15	1		1
G/G	21 - 21			
	21 - 22	2	3	5
	22 - 22			
D/G	13 - 21	3	2	5
	13 - 22	3	1	4
	14 - 21	1	4	5
	14 - 22	3	2	5
	15 - 21	2		2
	15 - 22		2	2
D/D/G	13 - 13 - 21	1		1
	13 - 13 - 22			
	13 - 14 - 21	2		2
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22	1		1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21	1		1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

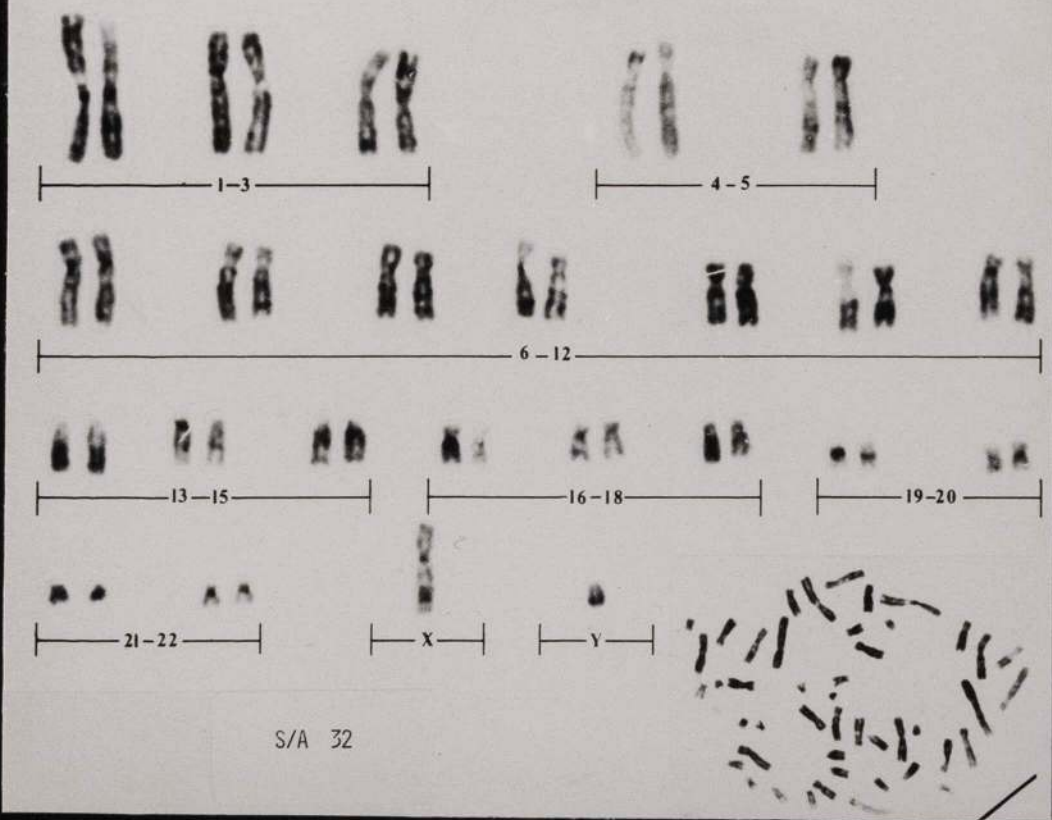
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22		1	1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14	1		1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	14-13-15-13-21-22	1		1
	13-14-21-13		1	1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	2		2
	13 - 14	2	2	4
	13 - 15	1	2	3
	14 - 14			
	14 - 15	1	2	3
	15 - 15			
G/G	21 - 21		1	1
	21 - 22	2	1	3
	22 - 22			
D/G	13 - 21	1	2	3
	13 - 22	1	1	2
	14 - 21	4	1	5
	14 - 22		3	3
	15 - 21	1	3	4
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22		1	1
	13 - 15 - 21	1		1
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22	1		1
	14 - 15 - 21	1		1
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	2	2	4
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14	1		1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 31


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 32

S/A NUMBER	39	40
SEX	F	M
DATE OF BIRTH	15.8.40	9.2.40
NATIONALITY	British	British
CHILDREN	1	2
Date of Birth	14.8.70	25.6.74
Sex	F	M
Details	Normal	Downs syndrome
AFFECTED PREGNANCY		
Drugs	Iron only	
Accidents	Nil	
Radiation	Nil	
Other	Nil	
FAMILY HISTORY		
Illness	None relevant	None relevant
Affected Members		
Other		
CHROMOSOME CONSTITUTION	46,XX	46,XY

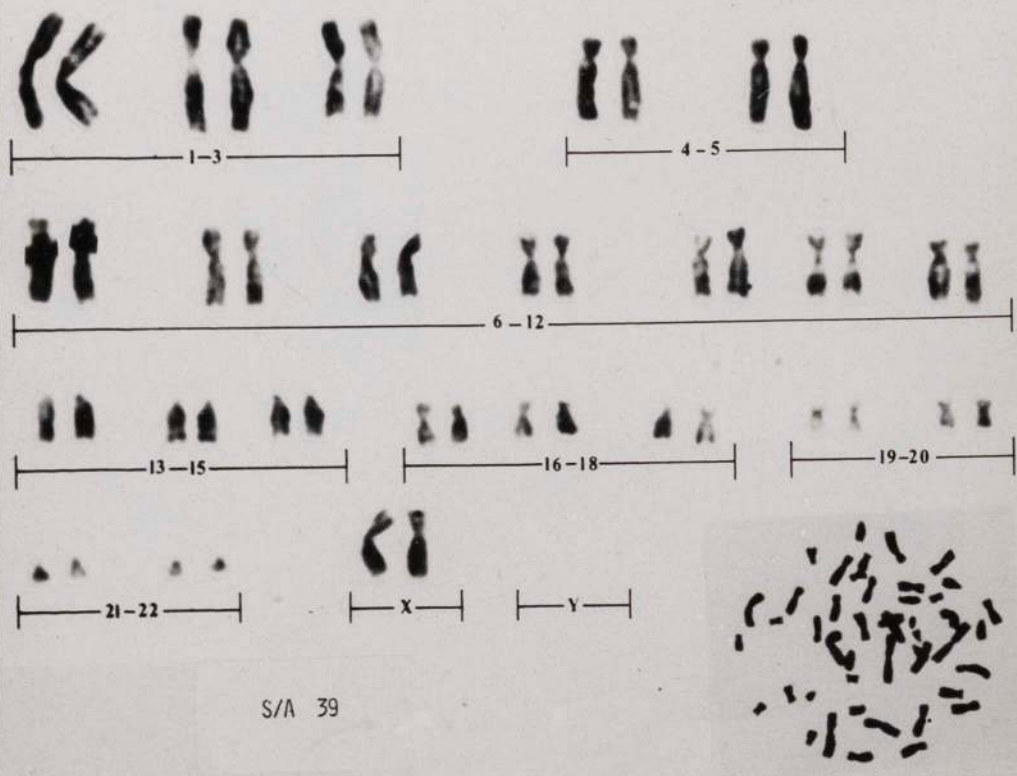
PARENTS OF A MONGOL CHILD

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	6	7
	13 - 15			
	14 - 14	2	2	4
	14 - 15	1		1
	15 - 15			
G/G	21 - 21			
	21 - 22	7	7	14
	22 - 22			
D/G	13 - 21	2	2	4
	13 - 22		4	4
	14 - 21	1	3	4
	14 - 22	1	4	5
	15 - 21		2	2
	15 - 22	1		1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21		1	1
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

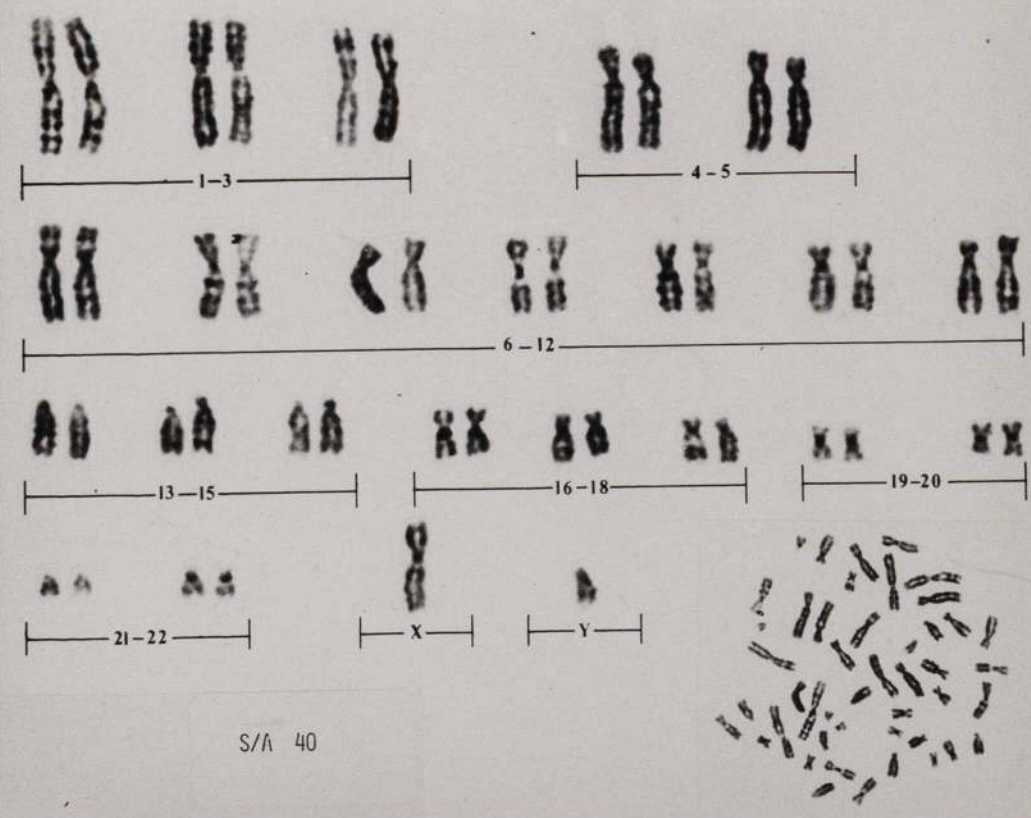
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21		1	1
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1	1	2
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14		1	1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22	1		1
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		8	8
	13 - 15	1		1
	14 - 14			
	14 - 15		1	1
	15 - 15			
G/G	21 - 21			
	21 - 22	3	2	5
	22 - 22			
D/G	13 - 21	2	2	4
	13 - 22	1	1	2
	14 - 21	3	1	4
	14 - 22	2	4	6
	15 - 21	1		1
	15 - 22		2	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22	1		1
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22		1	1
D/D/D	13 - 14 - 15			
	13 - 13 - 14	1		1
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 39


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 40

S/A NUMBER	13	14
SEX	F	M
DATE OF BIRTH	2.5.47	16.3.47
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 M Normal	2 M Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

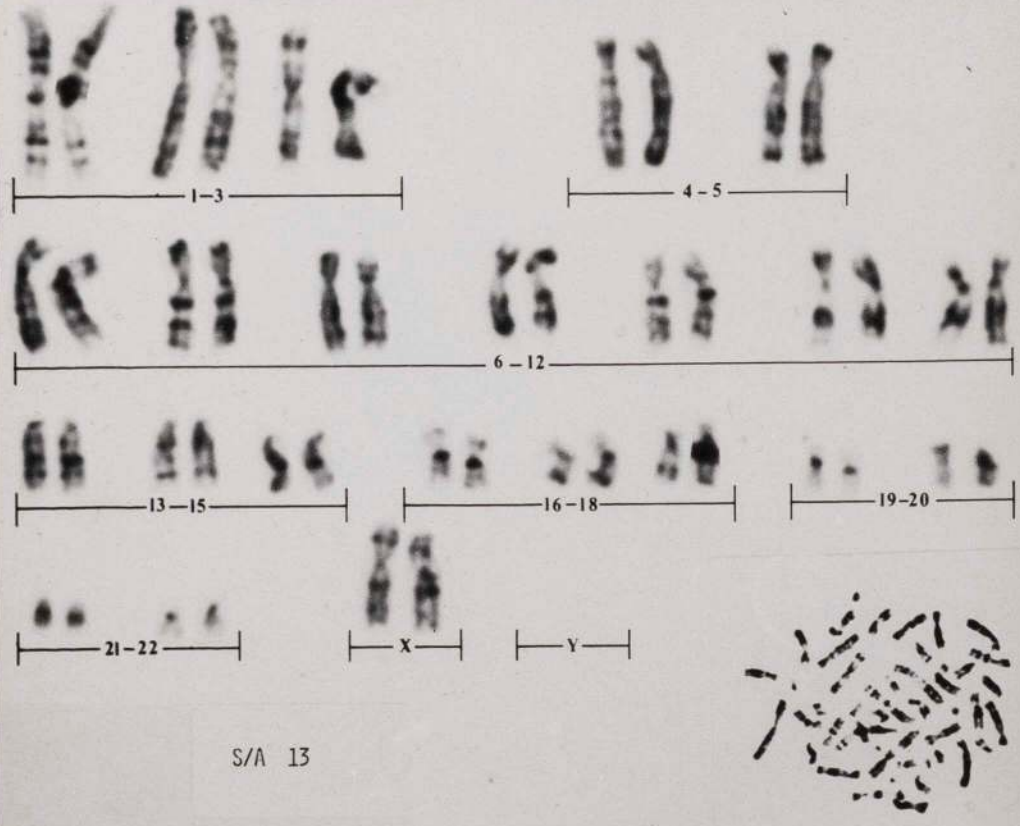
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	2	3
	13 - 14	2	5	7
	13 - 15		1	1
	14 - 14		1	1
	14 - 15		3	3
	15 - 15			
G/G	21 - 21	1	1	2
	21 - 22	2	4	6
	22 - 22		1	1
D/G	13 - 21		1	1
	13 - 22		2	2
	14 - 21		3	3
	14 - 22	1	4	5
	15 - 21	1	3	4
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22	1	1	2
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

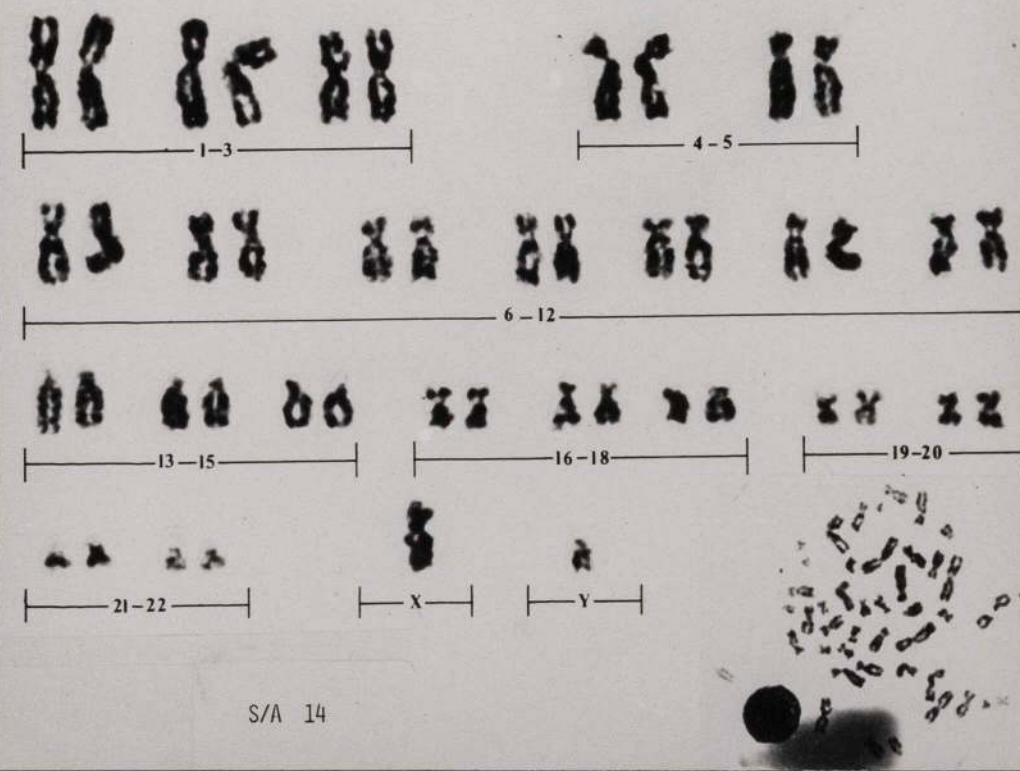
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14			
	13 - 14 - 14	1		1
	14 - 15 - 15			
	14 - 14 - 15	1		1
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	3	4
	13 - 15	1	1	2
	14 - 14			
	14 - 15	1	1	2
	15 - 15			
G/G	21 - 21			
	21 - 22		10	10
	22 - 22			
D/G	13 - 21	1	1	2
	13 - 22		1	1
	14 - 21	1	6	7
	14 - 22	3	1	4
	15 - 21	2	2	4
	15 - 22	2	1	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21		1	1
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22		1	1
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15	1		1
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21		1	1
	21 - 21 - 22	1		1
OTHER	13-14-21-21	1		1


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 13


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 14

S/A NUMBER	33	34
SEX	F	M
DATE OF BIRTH	3.10.47	30.2.46
NATIONALITY	West Indian	West Indian
CHILDREN Date of Birth Sex Details	1 6.10.70 M Normal	2 9.5.74 M Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

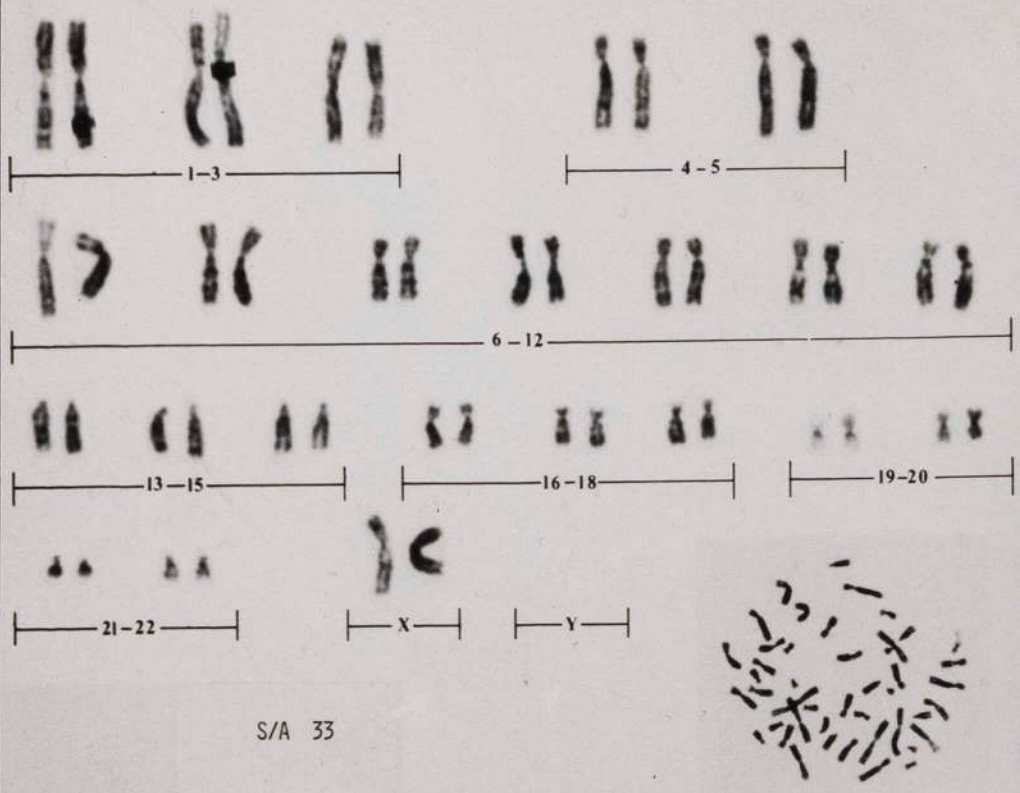
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	2	6	8
	13 - 15	2	1	3
	14 - 14			
	14 - 15	5	4	9
	15 - 15			
G/G	21 - 21			
	21 - 22		5	5
	22 - 22		1	1
D/G	13 - 21	1	2	3
	13 - 22			
	14 - 21	1	2	3
	14 - 22	1		1
	15 - 21	1	2	3
	15 - 22	1	1	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

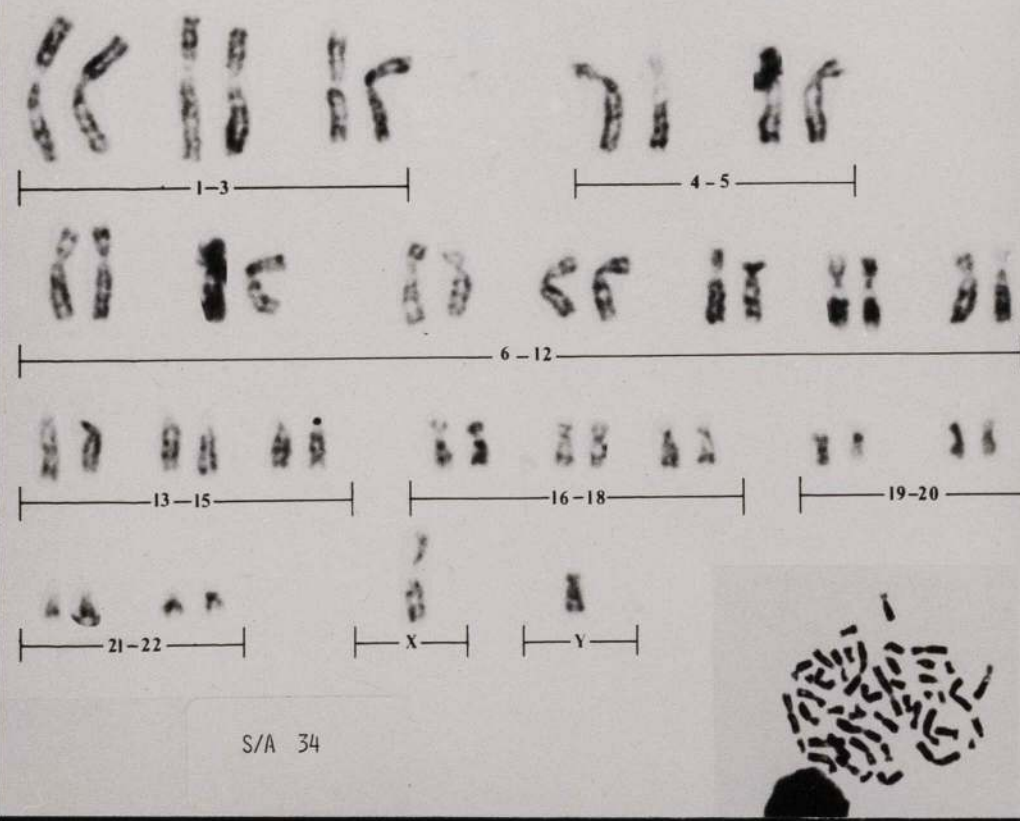
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	13-14-14-21	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	6	7
	13 - 15		2	2
	14 - 14			
	14 - 15	4	1	5
	15 - 15			
G/G	21 - 21			
	21 - 22	5	4	9
	22 - 22			
D/G	13 - 21	3	1	4
	13 - 22		1	1
	14 - 21	1	3	4
	14 - 22			
	15 - 21	1	1	2
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14	1		1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19 – 20
21 – 22
X
Y

S/A 33


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19 – 20
21 – 22
X
Y

S/A 34

S/A NUMBER	41	42
SEX	F	M
DATE OF BIRTH	7.8.45	10.6.47
NATIONALITY	British	British
CHILDREN	1	2	3
Date of Birth	23.11.66	31.10.68	19.3.72
Sex	M	F	M
Details	Normal	Normal	Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable		
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

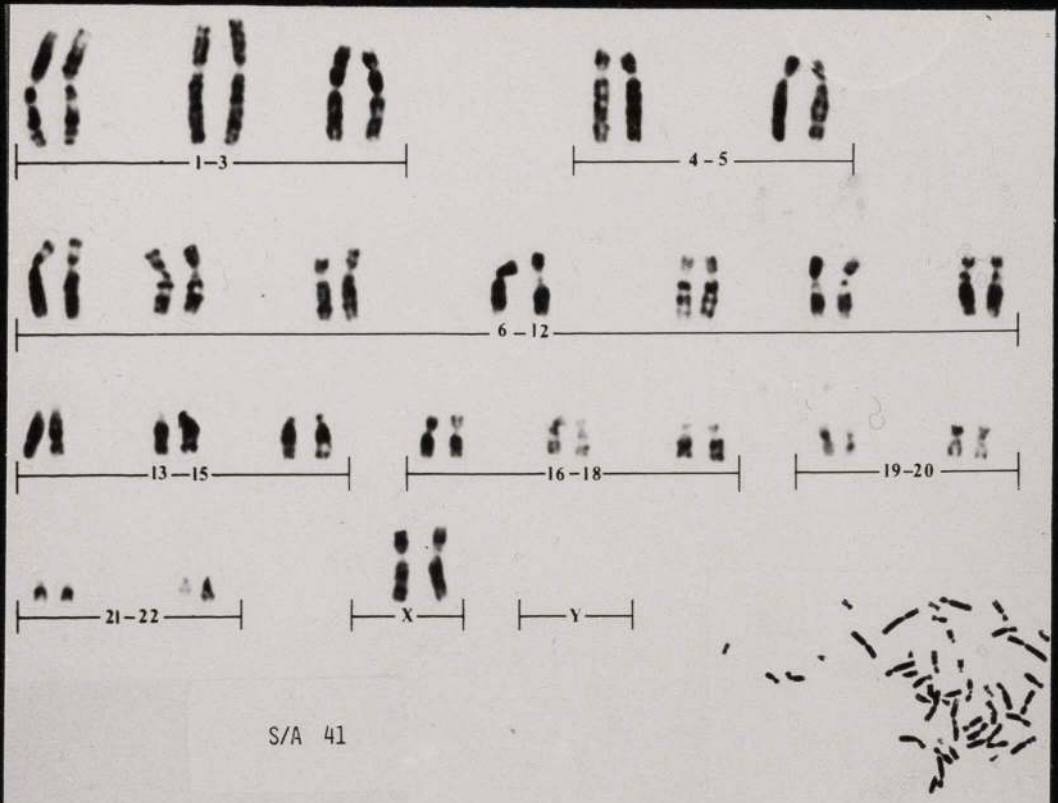
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	1	2
	13 - 14	1	4	5
	13 - 15	2	3	5
	14 - 14		1	1
	14 - 15	1	3	4
	15 - 15			
G/G	21 - 21			
	21 - 22		3	3
	22 - 22		1	1
D/G	13 - 21		3	3
	13 - 22		3	3
	14 - 21	1	2	3
	14 - 22	2		2
	15 - 21	1	4	5
	15 - 22			
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1	1	2
	13 - 14 - 22			
	13 - 15 - 21	1		1
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

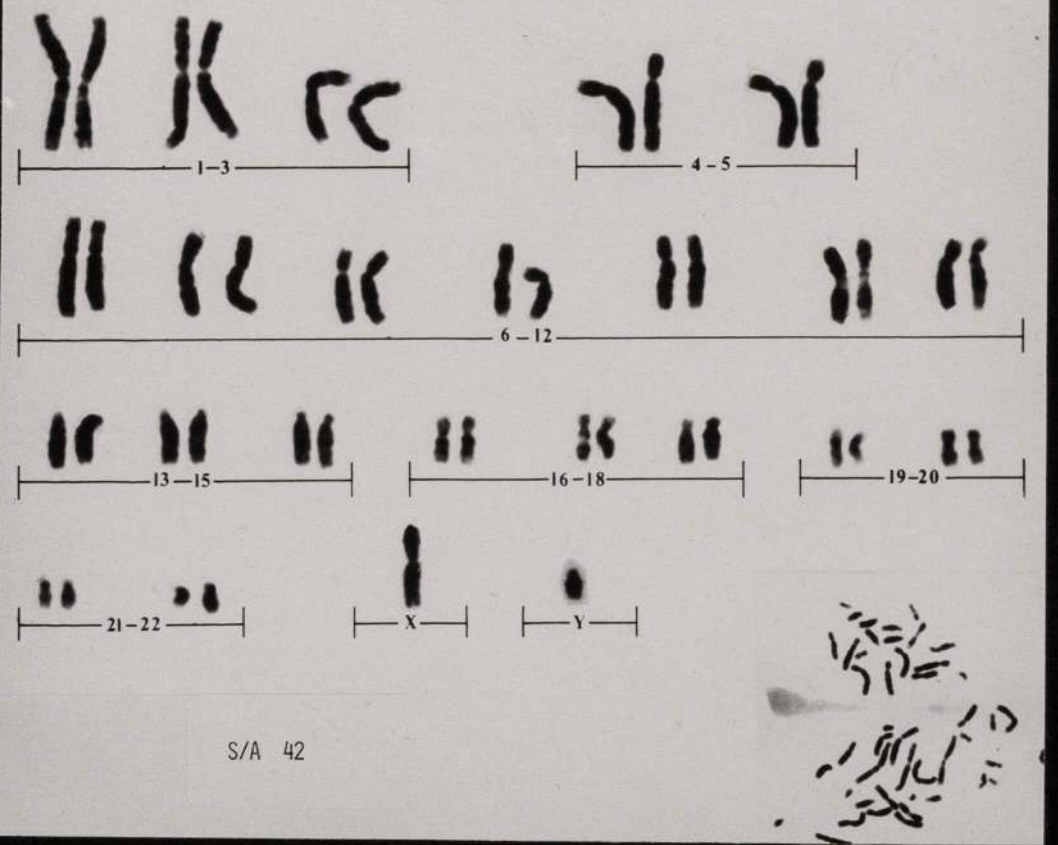
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1		1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15	1		1
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	14-14-15-21	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	2	7	9
	13 - 15	3	2	5
	14 - 14		1	1
	14 - 15	3	3	6
	15 - 15		1	1
G/G	21 - 21			
	21 - 22	1	1	2
	22 - 22			
D/G	13 - 21		1	1
	13 - 22	1	1	2
	14 - 21	1	1	2
	14 - 22	2	2	4
	15 - 21	1	2	3
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1	1	2
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21	1		1
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1	1	2
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14	1		1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	14-14-15-21		1	1


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 41


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 42

S/A NUMBER	43	44
SEX	F	M
DATE OF BIRTH	28.10.52	22.7.50
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 22.6.70 F Normal	2 7.5.72 M Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XX	46,XY

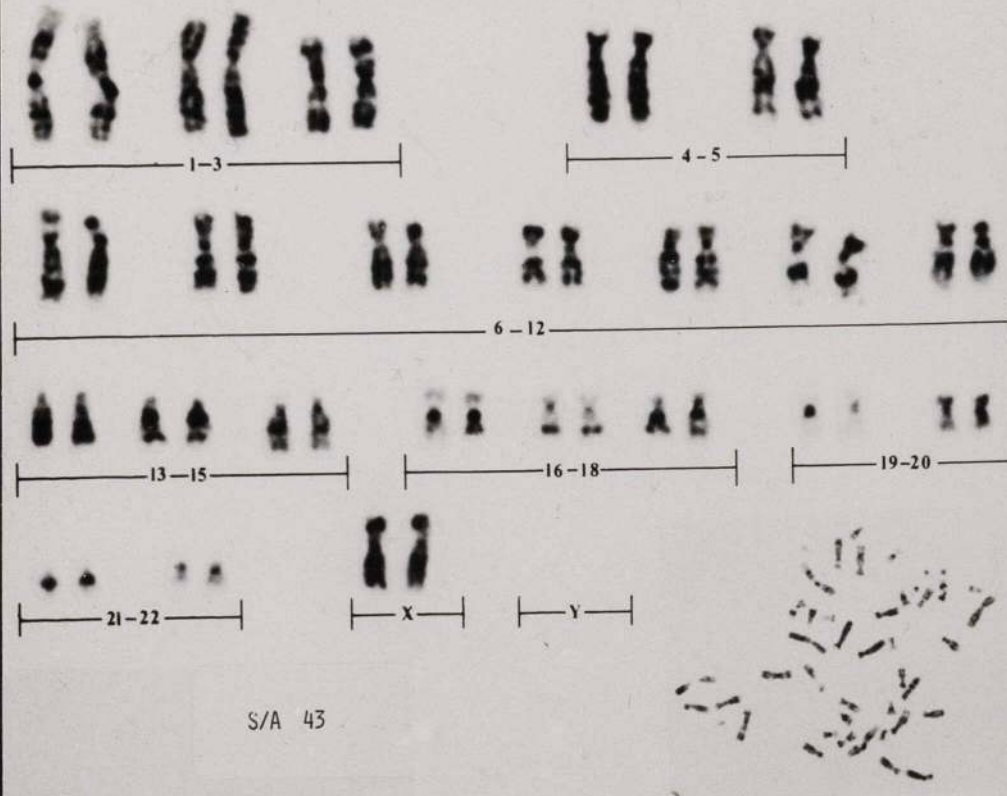
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13		1	1
	13 - 14		3	3
	13 - 15	1	3	4
	14 - 14		1	1
	14 - 15	1	2	3
	15 - 15		1	1
G/G	21 - 21			
	21 - 22		5	5
	22 - 22			
D/G	13 - 21	1	4	5
	13 - 22		1	1
	14 - 21		3	3
	14 - 22		2	2
	15 - 21		4	4
	15 - 22	1		1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21	1		1
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

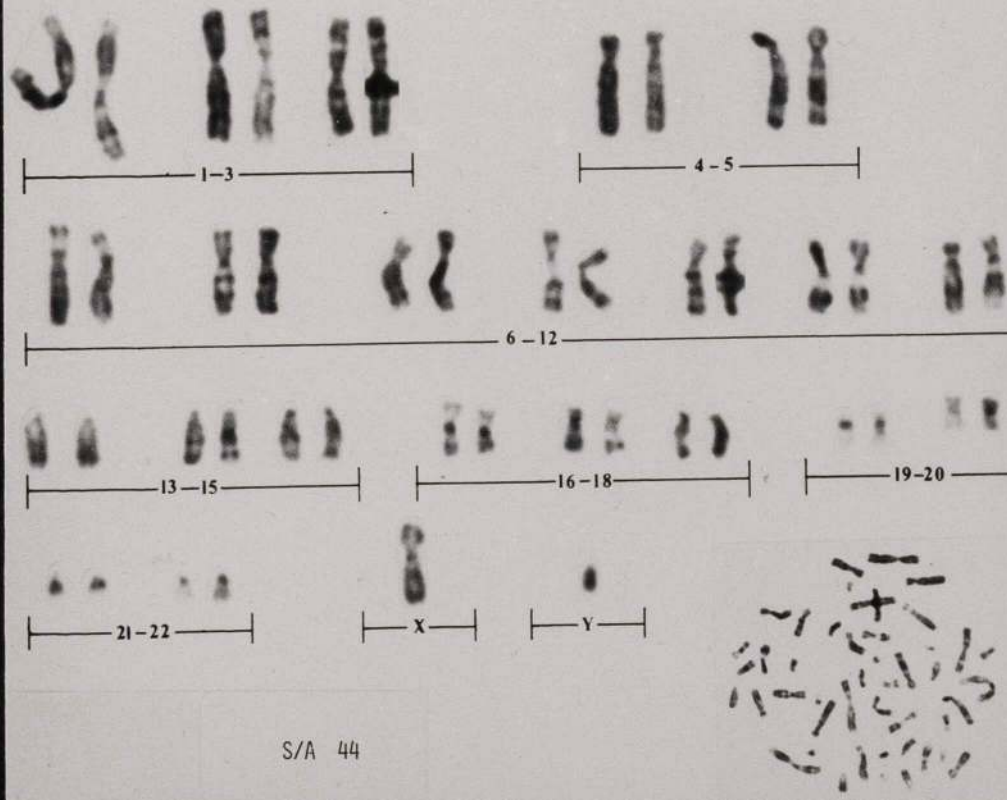
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1		1
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13	1		1
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	3	4
	13 - 15		1	1
	14 - 14		2	2
	14 - 15		3	3
	15 - 15	1		1
G/G	21 - 21			
	21 - 22		5	5
	22 - 22			
D/G	13 - 21	2	1	3
	13 - 22			
	14 - 21			
	14 - 22	2	6	8
	15 - 21	1	4	5
	15 - 22	1	2	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21		1	1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22		1	1
	14 - 14 - 21			
	14 - 14 - 22		1	1
	14 - 15 - 21			
	14 - 15 - 22		1	1
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22	1		1
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 43


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 44

S/A NUMBER	45	46
SEX	M	F
DATE OF BIRTH	17.1.50	7.12.49
NATIONALITY	Irish	Irish
CHILDREN Date of Birth Sex Details	1 7.2.69 M Normal	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

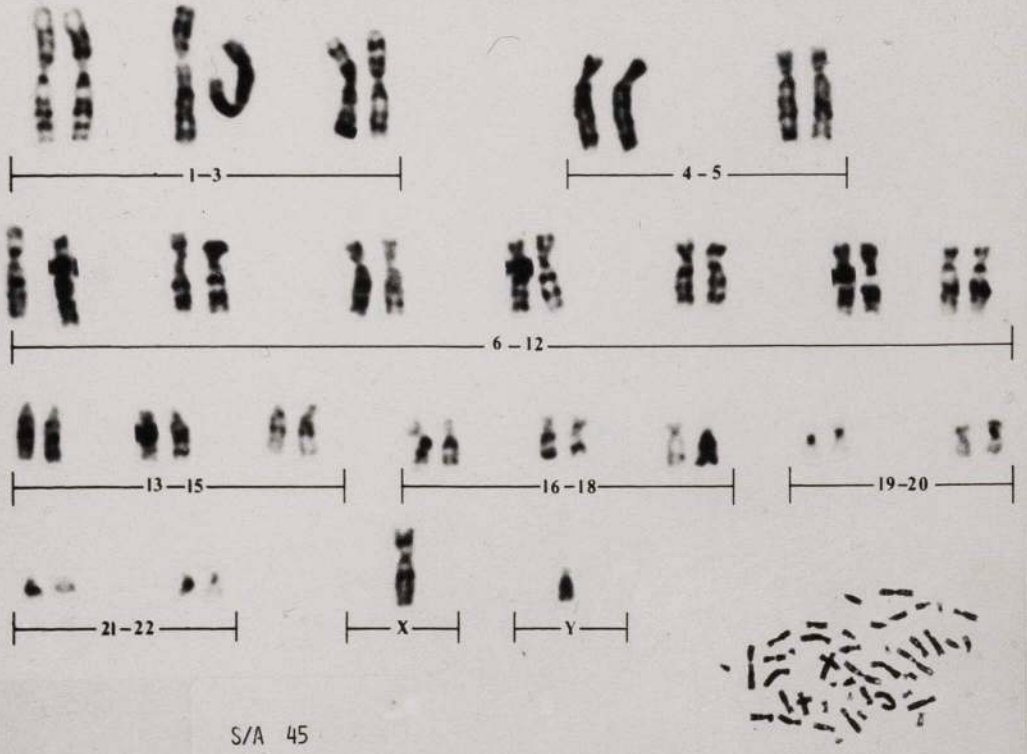
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	1	2
	13 - 14		7	7
	13 - 15		2	2
	14 - 14			
	14 - 15		3	3
	15 - 15			
G/G	21 - 21			
	21 - 22		2	2
	22 - 22			
D/G	13 - 21	1	2	3
	13 - 22		1	1
	14 - 21	1	3	4
	14 - 22	1	4	5
	15 - 21	1	2	3
	15 - 22		2	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	3	2	5
	13 - 14 - 22		1	1
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

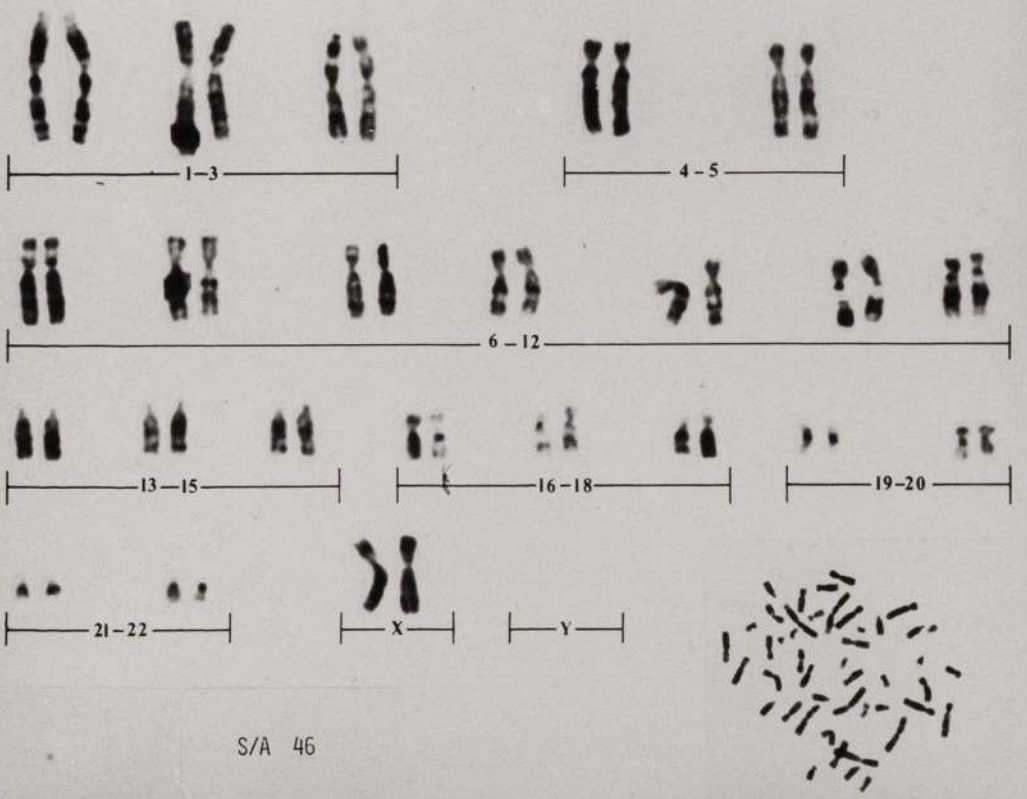
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	2	3
	13 - 15	2	2	4
	14 - 14			
	14 - 15	1	2	3
	15 - 15			
G/G	21 - 21			
	21 - 22	2	8	10
	22 - 22		1	1
D/G	13 - 21	3	1	4
	13 - 22		1	1
	14 - 21	3	1	4
	14 - 22		3	3
	15 - 21	1	3	4
	15 - 22	2		2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15	1		1
	14 - 14 - 15		1	1
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 45


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 46

S/A NUMBER	47	48
SEX	M	F
DATE OF BIRTH	1.8.39	15.6.40
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 14.2.65 F Normal	2 1.3.67 F Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

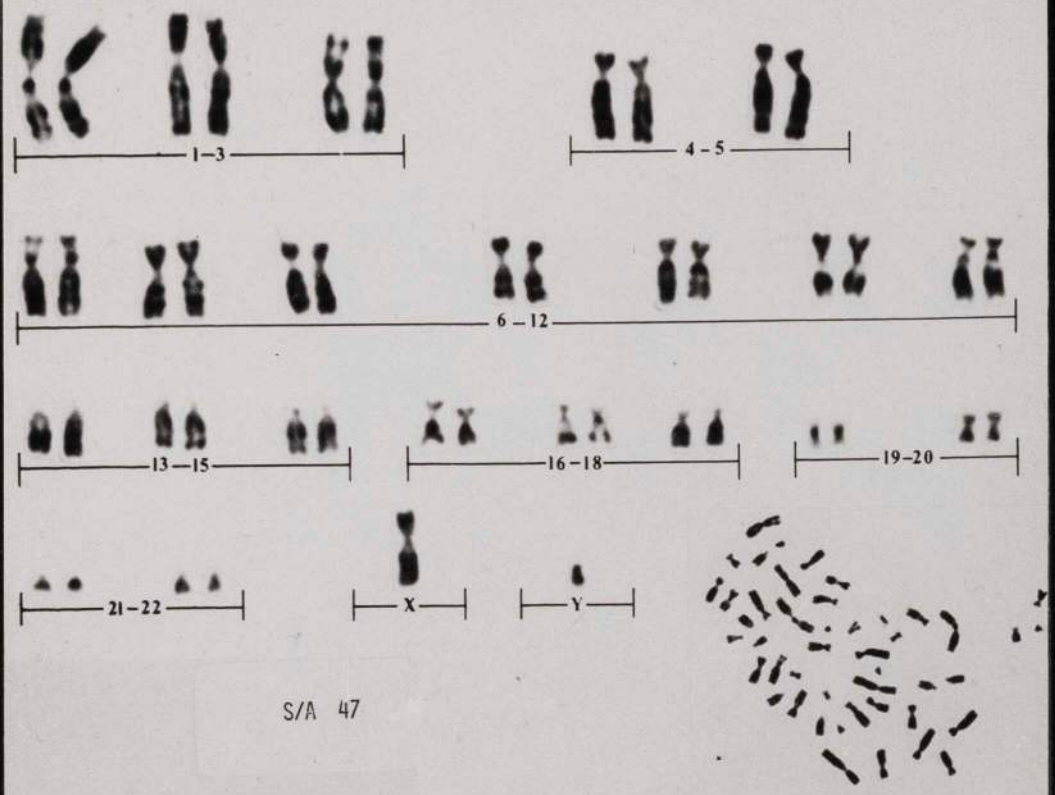
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13		2	2
	13 - 14		2	2
	13 - 15			
	14 - 14			
	14 - 15		1	1
	15 - 15			
G/G	21 - 21			
	21 - 22	7	7	14
	22 - 22		1	1
D/G	13 - 21	1	3	4
	13 - 22	2	3	5
	14 - 21	1	1	2
	14 - 22	1	5	6
	15 - 21	1	1	2
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22		1	1
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

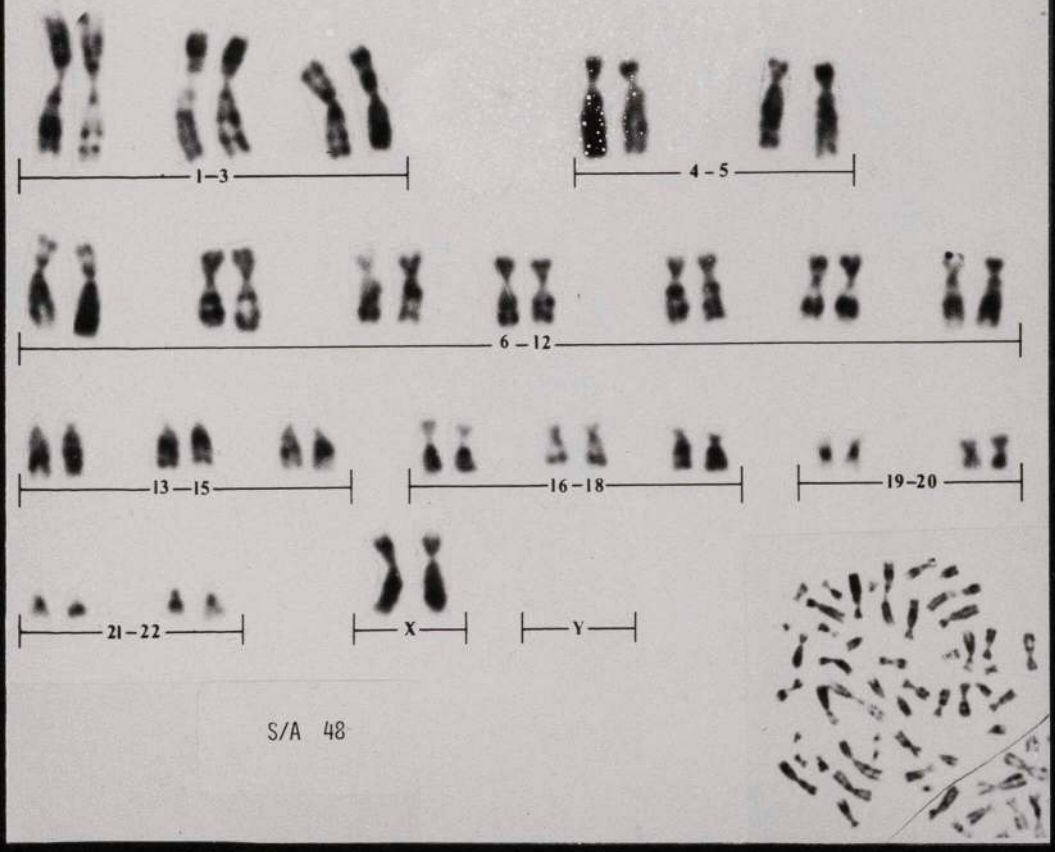
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21	1		1
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22	1	1	2
	14 - 22 - 22	1		1
	15 - 21 - 21			
	15 - 21 - 22		1	1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
	15 - 13 - 13			
G/G/G	22 - 22 - 21	1	1	2
	21 - 21 - 22	1		1
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	2	3	5
	13 - 15	1	4	5
	14 - 14			
	14 - 15	1	2	3
	15 - 15			
G/G	21 - 21	1		1
	21 - 22	3	1	4
	22 - 22			
D/G	13 - 21	3	2	5
	13 - 22			
	14 - 21	3	6	9
	14 - 22		2	2
	15 - 21		4	4
	15 - 22		2	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19 – 20
21 – 22
X
Y

S/A 47


1–3
4 – 5
6 – 12
13 – 15
16 – 18
19 – 20
21 – 22
X
Y

S/A 48

S/A NUMBER	51	52
SEX	M	F
DATE OF BIRTH	20.3.46	5.8.47
NATIONALITY	British	British
CHILDREN Date of Birth Sex Details	1 4.1.72 M Normal	
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable	
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

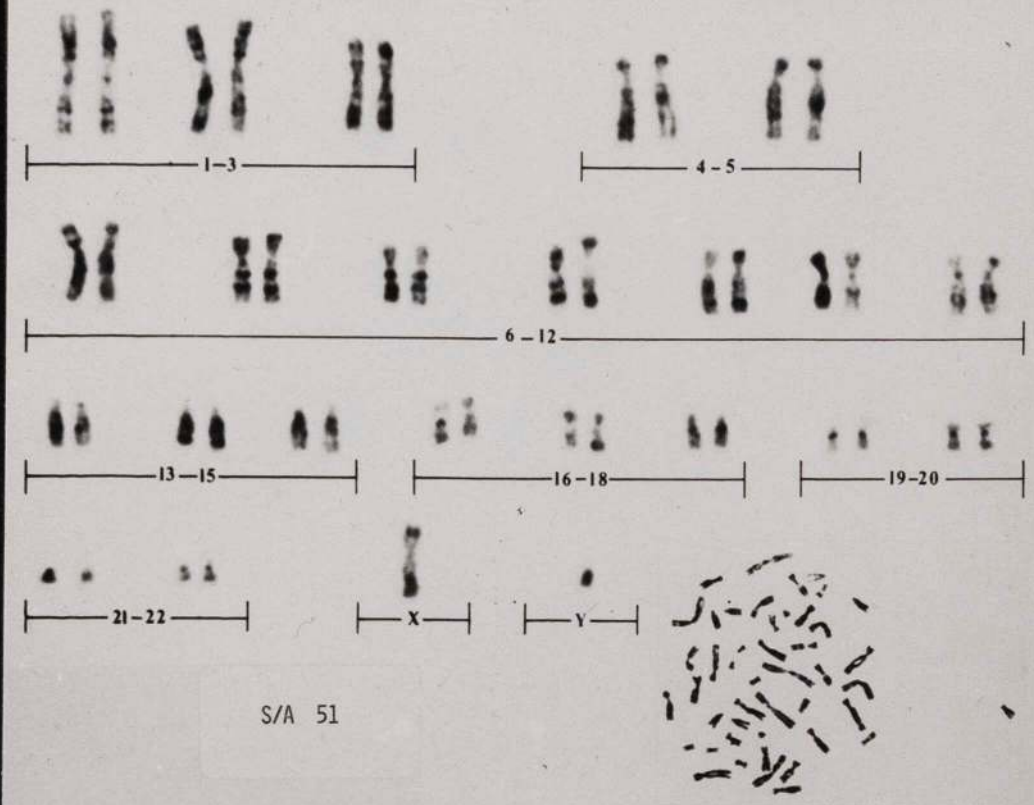
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1		1
	13 - 14	1	1	2
	13 - 15	1		1
	14 - 14		3	3
	14 - 15		3	3
	15 - 15			
G/G	21 - 21		1	1
	21 - 22		6	6
	22 - 22		2	2
D/G	13 - 21		3	3
	13 - 22		3	3
	14 - 21			
	14 - 22		5	5
	15 - 21	1		1
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21	1		1
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

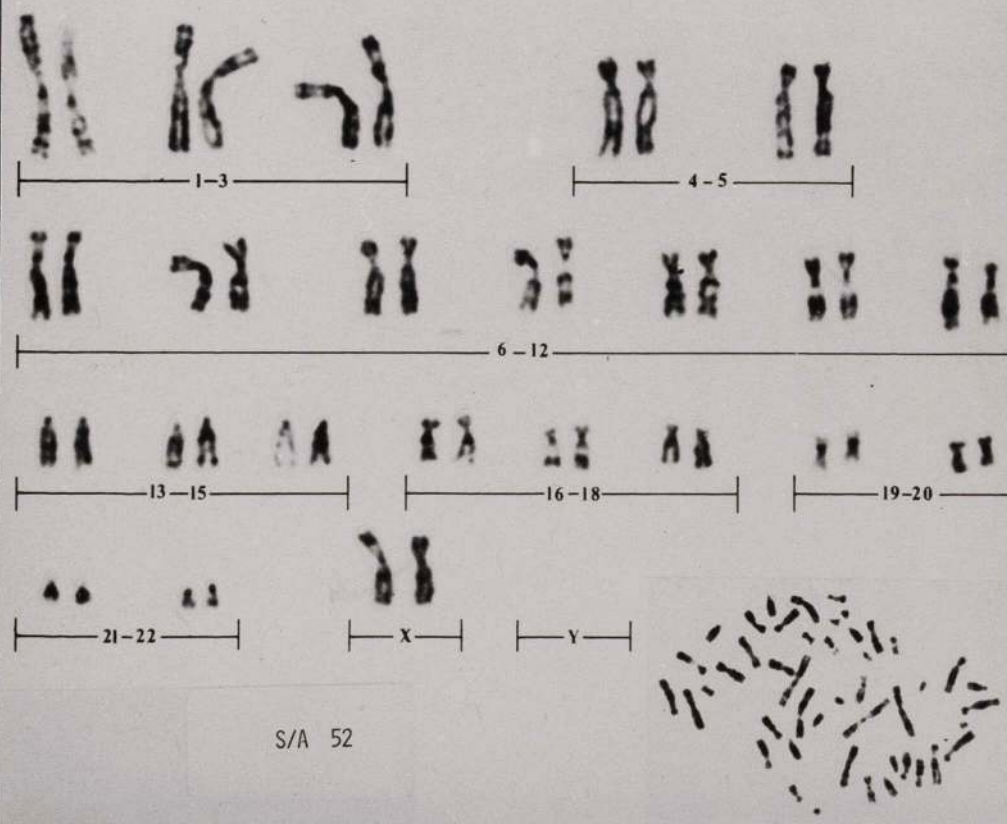
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14		1	1
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21		1	1
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	1	1	2
	13 - 15			
	14 - 14		1	1
	14 - 15	3	4	7
	15 - 15	1		1
G/G	21 - 21			
	21 - 22	2	4	6
	22 - 22			
D/G	13 - 21		3	3
	13 - 22	1	2	3
	14 - 21		1	1
	14 - 22		3	3
	15 - 21	1	3	4
	15 - 22	5	2	7
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21	1		1
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22	1		1
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 51


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 52

S/A NUMBER	59	60
SEX	M	F
DATE OF BIRTH	25.12.43	19.3.45
NATIONALITY	British	British
CHILDREN	1	2	3
Date of Birth	11.7.66	14.12.67	13.2.74
Sex	F	F	M
Details	Normal	Normal	Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable		
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

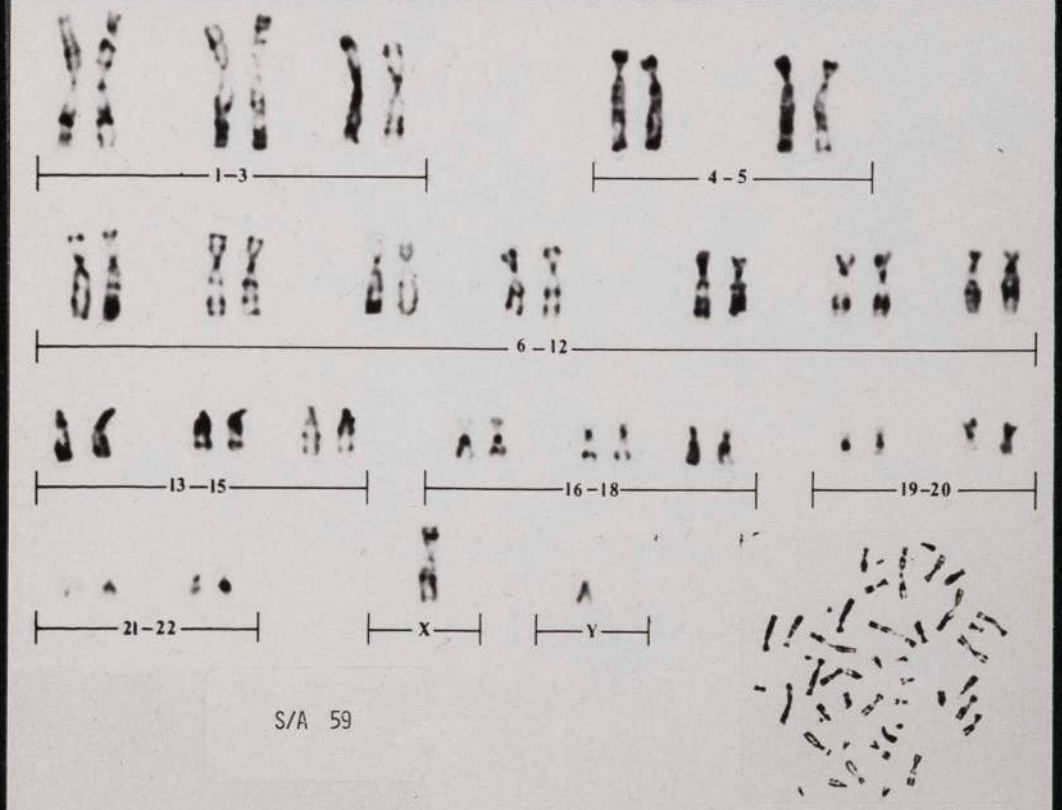
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14	2	3	5
	13 - 15	1	1	2
	14 - 14			
	14 - 15	1	3	4
	15 - 15			
G/G	21 - 21			
	21 - 22	3	4	7
	22 - 22		1	1
D/G	13 - 21		2	2
	13 - 22		2	2
	14 - 21		1	1
	14 - 22	2	2	4
	15 - 21		2	2
	15 - 22		2	2
D/D/G	13 - 13 - 21	1		1
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

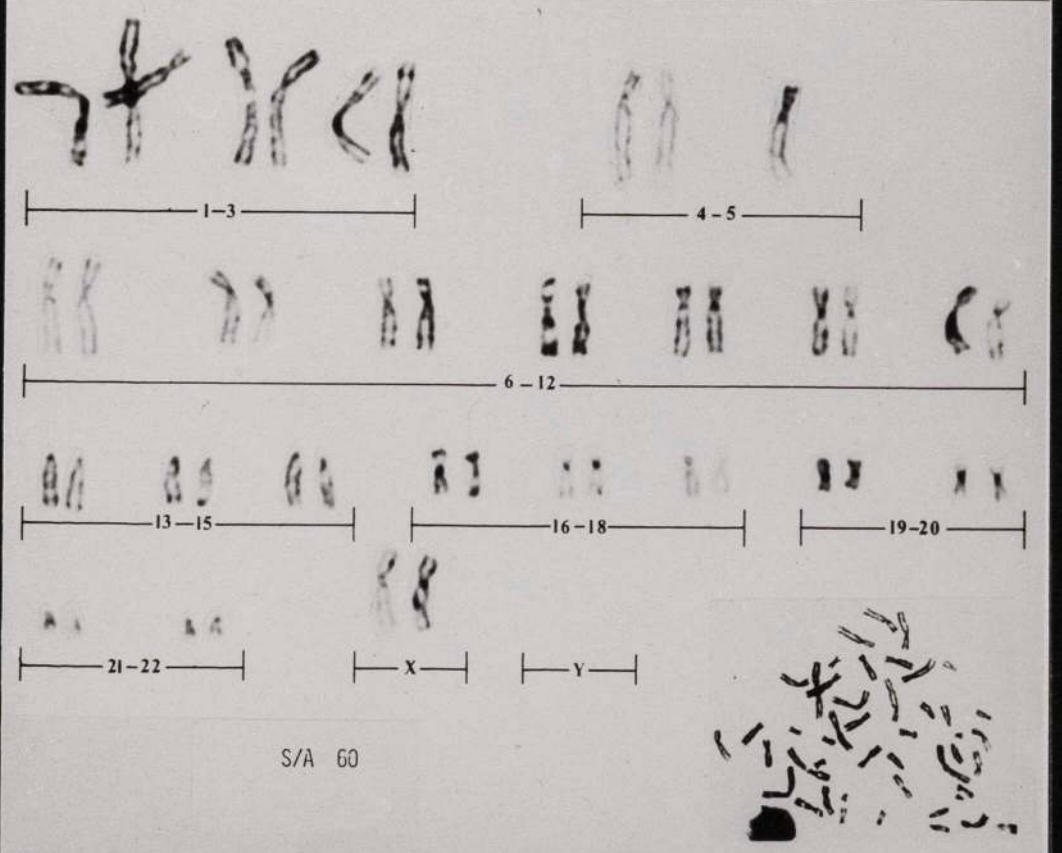
SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22		1	1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14		1	1
	14 - 15 - 15			
	14 - 14 - 15		1	1
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER	13-15-22-22	1		1

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13	1	1	2
	13 - 14		4	4
	13 - 15	1	2	3
	14 - 14	2		2
	14 - 15		3	3
	15 - 15			
G/G	21 - 21		1	1
	21 - 22	2	3	5
	22 - 22		1	1
D/G	13 - 21	1	2	3
	13 - 22		2	2
	14 - 21		4	4
	14 - 22	1		1
	15 - 21		2	2
	15 - 22		3	3
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 59


1–3
4–5
6–12
13–15
16–18
19–20
21–22
X
Y

S/A 60

S/A NUMBER	61	62
SEX	M	F
DATE OF BIRTH	30.6.42	27.10.44
NATIONALITY	British	British
CHILDREN	1	2	3
Date of Birth	3.12.63	17.11.65	8.1.70
Sex	M	F	F
Details	Normal	Normal	Normal
AFFECTED PREGNANCY Drugs Accidents Radiation Other	Not applicable		
FAMILY HISTORY Illness Affected Members Other	None relevant	None relevant
CHROMOSOME CONSTITUTION	46,XY	46,XX

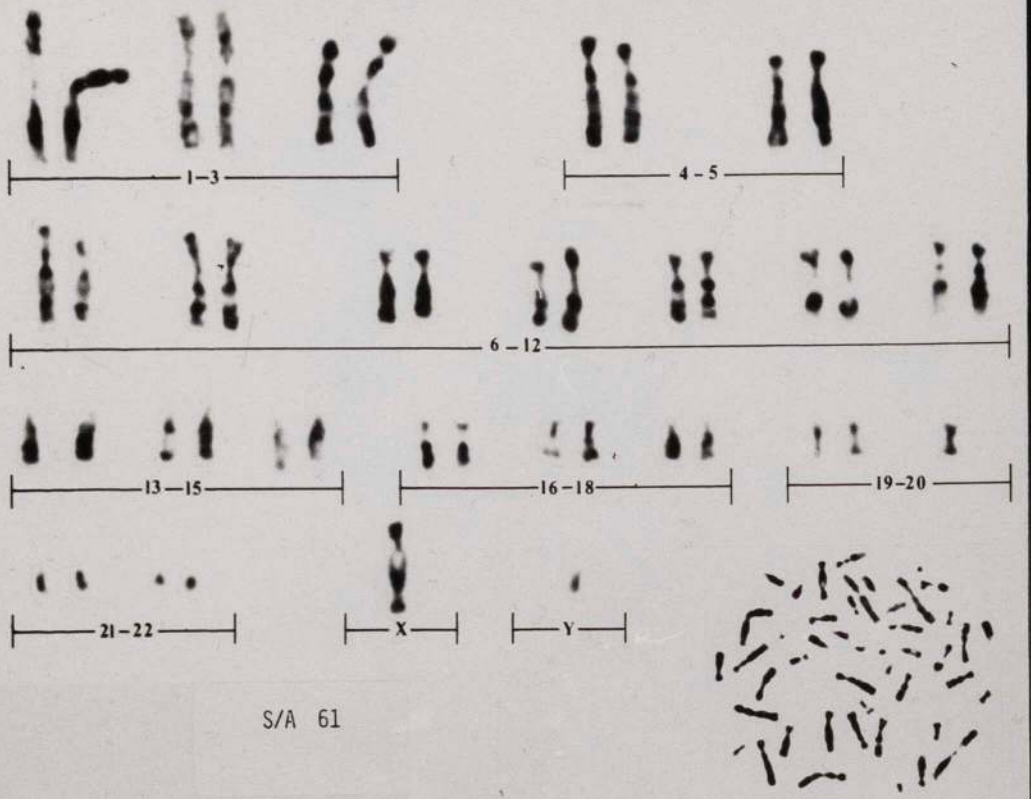
PARENTS OF NORMAL CHILDREN

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14			
	13 - 15			
	14 - 14			
	14 - 15		2	2
	15 - 15			
G/G	21 - 21			
	21 - 22	6	12	18
	22 - 22		2	2
D/G	13 - 21		1	1
	13 - 22	1	3	4
	14 - 21	2	8	10
	14 - 22		2	2
	15 - 21	1	5	6
	15 - 22		1	1
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21		2	2
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22			
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/D	13 - 13			
	13 - 14		2	2
	13 - 15		3	3
	14 - 14			
	14 - 15		1	1
	15 - 15	1		1
G/G	21 - 21			
	21 - 22	3	8	11
	22 - 22			
D/G	13 - 21		2	2
	13 - 22		3	3
	14 - 21	1	3	4
	14 - 22		4	4
	15 - 21		1	1
	15 - 22		2	2
D/D/G	13 - 13 - 21			
	13 - 13 - 22			
	13 - 14 - 21			
	13 - 14 - 22			
	13 - 15 - 21			
	13 - 15 - 22			
	14 - 14 - 21			
	14 - 14 - 22			
	14 - 15 - 21			
	14 - 15 - 22			
	15 - 15 - 21			
	15 - 15 - 22			

SPECIFIC ASSOCIATIONS		TYPE A	TYPE B	TOTAL
D/G/G	13 - 21 - 21			
	13 - 21 - 22			
	13 - 22 - 22			
	14 - 21 - 21			
	14 - 21 - 22			
	14 - 22 - 22			
	15 - 21 - 21			
	15 - 21 - 22	1		1
	15 - 22 - 22			
D/D/D	13 - 14 - 15			
	13 - 13 - 14			
	13 - 14 - 14			
	14 - 15 - 15			
	14 - 14 - 15			
	15 - 15 - 13			
G/G/G	15 - 13 - 13			
	22 - 22 - 21			
	21 - 21 - 22			
OTHER				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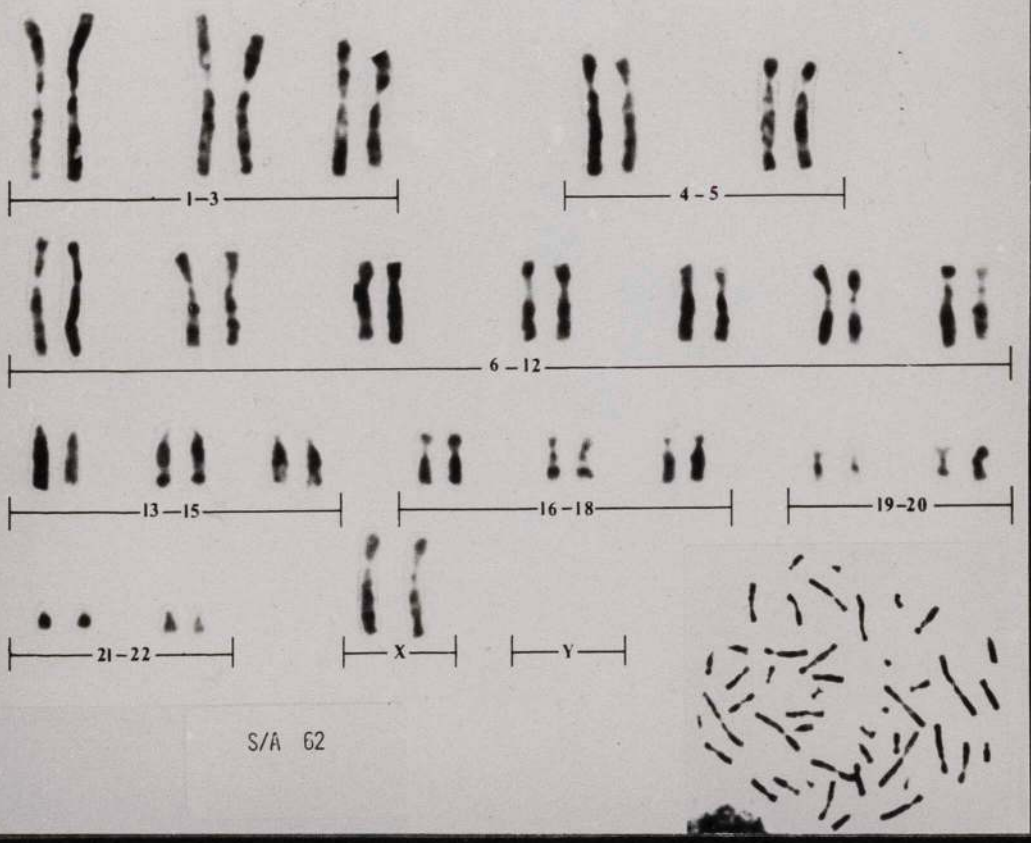
S/A 61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S/A 62

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