A Survey of Chromosome Anomalies in Malta

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ABSTRACT

433 individuals referred for chromosome analysis between 1983 and 1987 were included in the survey. Among individuals with dysmorphic features or congenital anomalies 42% of babies referred in the neonatal period and 12 to 30% of individuals in older age groups had a chromosome abnormality. Chromosome abnormalities were also found in 10 or 11% of boys or girls with problems of pubertal development, in 14% of azoospermic or severly oligospermic men, in 8.3% of couples with repeated foetal loss and in 5% of couples with malformed children.

Whereas most cases of autosomal aneuploidies were diagnosed, a large proportion of sex chromosome anomalies, particularly in males, remained undetected presumbly because of under-referral in the pubertal period. The prevalence of chromosome anomalies in Malta was 2.20 per 1,000 births between 1984 and 1987. The incidence of Down Syndrome showed great annual fluctuation with a mean of 1.88 per 1,000 births of which 61% occured in mothers over 35 years of age. The lowest occurence risk for trisomy 21 appears to be in the 25 to 34 years maternal age group.

INTRODUCTION

Chrosome analysis is an important diagnostic procedure in a variety of conditions. It is especially important in the investigation of infants and children with congenital anomalies, dysmorphic features or psychomotor retardation. In such cases its usefulness extends beyond the affected infant since it is a prerequisite for genetic counselling of their parents and relatives. Chromosome analysis is also an essential part of the investigation of problems affecting sexual development including ambiguous genitalia, developmental problems at puberty and infertility in marital life. Chromosome anomalies are leading causes of embryonic and foetal loss, and although cytogenetic investigation of spontaneous abortions is not usually undertaken as a routine procedure, chromosome analysis holds an important place in the investigation of couples with repeated foetal loss.

This study reviews all the cases in which chromosome analysis was performed in Malta during the four year period from January 1984, when a regular cytogenetic and genetic counselling service was established, to December 1987. During that period an ongoing register of congenital anomalies detected at birth was also being held, and every effort was made to confirm cytogenetically all suspected cases of chromosome anomalies in neonates and infants. Thus, it has been possible to obtain a reliable estimate of the incidence of chromosome anomalies among newborn babies in the Maltese Islands.This survey also analyses the results and usefulness of chromosome analysis in various categories of patients.

MATERIALS AND METHODS

All the cases referred for chromosome analysis over the four year period January 1984 to December 1987 were classified according to the reason for referral into five main categories:

1. Individuals with congenital anomalies or dysmorphic features including also growth and psychomotor retardation. These were subdivided according to the age at which the patient was referred: (a) in the neonatal period up to one month; (b) in infancy between the ages of one month and two years; (c) during childhood, between the ages of two and thirteen years, and (d) in adults.

2. Individuals with anomalies of the genitalia including ambiguous external genitalia, perineoscrotal hypospadias and inguinal herniae in females; these were also subclassified according to the age at referral.

3. Individuals presenting with problems related to puberty, subdivided into two groups: (a) boys with hypogonadism, infantile genitalia and absence of secondary sexual characteristics at or after the expected time of puberty (all were 12 to 20 years old); (b) Girls with primary or secondary amenorrhoea.

4. Infertile men with oligospermia or azoospermia.

5. Couples presenting with recurrent foetal loss, defined as two or more consecutive spontaneous abortions or stillbirths.

6. Couples investigated because one or more of their children had a congenital, genetic or chromosomal anomaly.

Patients referred for investigation because of malignant and other conditions were excluded from this study.

In all cases chromosome analysis was performed on 72-hour cultures of peripheral blood lymphocytes in RPMI 1640 medium supplemented with 15% Foetal Calf Serum. Trypsin-Giemsa banding was used routinely on all patients. Other banding procedures including Quinacrine (Q) banding, reverse (R) banding, centromere (C) banding and others were used as necessary.

RESULTS AND DISCUSSION

A total of 433 individuals had chromosome analysis. Of these, 123 (28%) were neonates, 44 (10%) were infants, 82 (19%) were children and 185 (43%) were adults. A total of 82 abnormalities were detected; 19% of the individuals examined had a chromosome anomaly. The distribution of normal and abnormal Karyotypes in the six categories of individuals are summarized in tables I, II and III.

Individuals with dysmorphic features formed not only the largest group, but also the one in which the largest proportion of abnormal karyotypes was encountered. In fact, in the neonatal period, 42% of babies with dysmorphic features or congenital anomlies had a chromosome abnormalty. As expec- ▶

ted, Trisomy 21 formed by far the largest group of chromosome abnormalities but a variety of other conditions including translocations, deletions, inversions, ring chromosomes and mosaicism were all encountered.

15% of the group of neonates were referred becauseof mild facial dysmorphia or "odd" features but were subsequently shown to be quite normal developmentally and phenotypically and had normal chromosomes. On the other hand, a small but important number of individuals with mildly dysmorphic features had a chromosome abnormality such as mosaic trisomy 21, 47, XXX syndrome, and deletions involving chromosomes no 1, 9 or 11 (Fig 1, 2, 3). The detection of such cases requires clinical alertness to the possibility of a chromosome abnormality in individuals showing even mild dysmorphic features. It is however, very difficult to draw a line between true dysmorphia and "odd" features which could pass as normal variations. In fact, chromosome analysis can be considered to be an important early step in the investigation of dysmorphic features and a preliminary to the arduous task of syndrome indentification.

Two cases of Turner Syndrome, one 45,X and one with 45,X/46 XXr mosaicism (Fig 4,) were identified in the neonatal period when they presented with oedema of the feet. This usually subsides in infancy when these children appear clinically normal. Two other cases of Turner Syndrome (one involving 45, X/47, XXX mosaicism) were encountered in childhood as part of the investigative procedure for short stature. Only one case of Turner Syndrome was encountered in an adult, presenting as primary amenorrhoea. Being a 45, X/46, XX mosaic, short stature and other phenotypic abnormalities were not prominent in this case.

Apart from sex chromosome anomalies notably 47, XXY, the vast majority of chromosome anomalies are detected at birth. Of the five cases detected in infancy (1 month to 2 years), only one case had been 'missed' in the neonatal period. This was the case of *de novo* translocation 46, XY, t(1;9) involving a minimal deletion of band 9p11 Fig 2. This child had delayed developmental milestones and mild facial dysmorphia. The other four cases detected in infancy were in fact born in 1983 and were referred for chromosome analysis when the cytogenetic service was established. At least some of the chromosome anomalies detected in children or adults (and certainly cases of trisomy 21) were probably detectable in the first month of life because of the obvious dysmorphic features and were not karyotyped earlier because a cytogenetic service was not then available. It is, therefore, expected that with increased surveillance and early detection, even fewer anomalies will be detected in infancy or childhood. Nevertheless, chromosome analysis remains an important procedure even in this age group for the detection of chromosome abnormalities which manifest very mild clinical features.

Anomalies of the external genitalia and the presence of inguinal herniae in phenotypic females, are important reasons for referral for chromosome analysis, especially in the neonatal period or in early infancy. Although no abnormalities were encountered in this group (Table II), chromosome analysis served to confirm the genetic sex of the individual and to exclude the possibility of sex chromosome mosaicism.

10% — 11% of individuals presenting with problems of pubertal development — amenorrhae in girls or infantile genitalia in boys — had a chromosome abnormality. Of these, one was the testicular feminization syndrome.

Three cases of 47,XXY Klinefelter syndrome were diagnosed over the five-year period. Two were in adolescent boys presenting with impaired sexual devlopment at the time of puberty and one presented after marriage as infertility. Klinefelter syndrome is not usually detected at birth, except incidentally, because clinical features become evident at puberty.

Klinefelter syndrome occurs with a frequency of 1.2 per 1,000 live male births and thus it would be expected that in Malta about three cases would be born annually. The three cases detected over the past four years are far less than the expected number, implying that many cases remain clinically undectected. The only constant features of Klinefelter syndrome are testicular atrophy and gynaecomastia. Development of the penis, scrotum and pubic hair are usually normal and stature is not a reliable criterion. Chromosome anaylsis should be performed on all boys with abnormal pubertal development and men with azoospermia or severe oligospermia.

Of the two cases of male infertility with a chromosome abnormality one had a fragile site on chromosome no 16: 46, XY, fra(16)(q 23) (Figs 5). This was detected in over 10% of cells in routine culture. This unusual finding has been reported previously in childless couples and a distinct association with repeated abortions appears to exist. Its occurance in oligospermic males has not, however, been previously documented.

Four cases of balanced chromosome rearrangements were found in the present study, three in couples with repeated foetal loss (8.3%) and one in a couple with previously abnormal offspring (Fig 6, 7).

It is generally acknowledged that balanced chromosome rearrangements, particulary Robertsonian translocations, are increased in couples with recurrent spontaneous abortions, but their frequences varied considerably in different studies from 2.2% (Pantzar *et al*, 1984) to over 14% of couples (Kardon *et al*, 1980). The frequency is greater in couples who had a malformed live or stillborn child in addition to the recurrent abortions (Bortotto *et al*, 1980).

Balanced chromosome rearrangements occur with an exptected frequency of about 0.21% in the general population. Since there is not loss or gain of genetic material they do not produce any phenotypic effects but may results in unbalanced rearrangements in the offspring.

Fig 8 and 9 illustrate the possible rearrangements in the offspring resulting from the t(14; 15) and t(2;6) translocations. The nonviable rearrangements, shown in the shaded area, would end as spontaneous abortions or intrauterine deaths. Some abnormalities are compatible with intrauterine life and may result in children with recognized chromosome syndromes. The case of paracentric inversion involving the long arm of chromosome no 4 was detected by investigating the parents of a live-born child with multiple anomalies and a duplication of 4(q31-q35).

Chromosome variants encountered in G-banded preparations are shown in Table IV. In two cases the parents were karyotyped to confirm the polymophisms indentified in their child. One was a very large short arm of chromosome 15, originally thought to be a 21/15 translocation in a child referred because of suspected features of Down Syndrome but subsequently shown to be a familial variant. The other case was a rare combination of trisomy 21, pericentric inversion of chromosome 9 and percentric inversion of chromosome 17 (Fig 10). The inv 9 was also found in the father and the inv 17 was also found in the mother.

There have been several suggestions of an association between chromosome polymorphisms and congenital anomalies (Boue *et al*, 1975, Lubs, 1977) and of a high frequency of polymorphism among parents of children with chromosome anomalies (Nielsen, 1974; Halbricht and Shabty, 1976). However, the clinical implication of chromosome polymorphisms remain controversial and their biological significance is poorly understood.

In this study, the prevalence of chromosome anomalies in Malta, expressed per 1,000 births, was calculated from the number of affected individuals born in the 4 year period 1984-1987 (Table V). The overall frequency was 2.2 per 1,000 (1 in 485) births. The average prevalence reported in 17 registries from 9 European countries coordinated by EUROCAT (European Register of Congenital Anomalies and Twins) was 1.7 per 1,000 births with rates varying from 1 to 2.86 per 1,000 (de Wals & Le Chat, 1983).

Table VI compares the number of chromosome abnormalities diagnosed with the expected incidence calculated from cumulative population studies from several countries (Hook and Hamerton, 1977). The total incidence for all chromosome abnormalities is expected to be approximately 6.2 per 1,000 but such a rate is unattainable unless whole

population screening is undertaken. The number of diagnosed autosomal aneuploidies, mainly trisomy 21, exceeds the expected number although the difference is not significant ($p > 0.1$) Ascertainment of aneuploidies appears to be complete. However, the number of unbalanced chromosome rearrangements diagnosed was only 38% of the expected number. The difference was significant ($p < 0.005$) and indicates underascertainment. Whereas the clinical pictures of autosomal trisomies are well-known and,in most cases, easily recognized, the rare duplications or deletions involving small segments may be more difficult to recognize. Some may present with relatively minor anomalies which might even be considered trivial, such as scalp defects, as in 4p-; colomboma of the iris as in 4p- or the cat-eye syndrome; or thumb anomalies as in trisomy 18, 13q- and 4p-. Others may present with major anomalies such as pyloric stenosis in 21q-, anal atresia in the cat eye syndrome , 13q- and tri-8, omphalocoele in the triploidy syndrome or cyclops in 18q-. Although such defects often occur as isolated disorders, it is important to realise that they may be part of a syndrome and are indications for chromosome analysis.

It is understandable that no balanced translocations were detected although these account for 34% of all chromosome abnormalities as they present no clinical features except reproductive loss and malformed offspring. As referred to earlier, most of the sex chromosome anomalies, especially in males, do not show any clinical features until the time of puberty. Underascertainment was also reported in a 10-year study in Denmark where only 10% of the expected number of cases wqere diagnosed (Nielsen and Videbach, 1984).

The incidence of Down Syndrome during the years 1984-1987 (Table VII) was 1.88 per 1,000 (1 in 552 births). There was considerable fluctuation in the annual incidence: it is remarkable that in 1987, 2.99 per 1,000 (1 in every 358) babies were born with Down Syndrome and 3.16 per 1,000 (1 in 316) babies had a chromosome abnormality detected at birth. These annual fluctuations may be attributable to statistical variations, given the relatively small numbers involved. The distribution of Down Syndrome according to maternal age is given in Table VIII. In Malta 61% of Down Syndrome babies were born to mothers over 35 years of age and 39% were born to mothers below 35 years of age. Similarly high proportions were reported in Ireland where the prevalence of Down Syndrome was also high (1.73 per 1,000) (de Wals & Le Chat, 1987). In most other countries where termination of pregnancy is practised, prevalence rates were 0.75 to 1.5 per 1,000 births and the percentage of Down Syndrome born to mothers above 35 ranged between 20 and 48%.Table VIII also gives the occurence of Down Syndrome in different age groups. The lowest risk of occurence appears to be in the 25-34 year age group; it is about 3 times higher in

TABLE I

Karyotypes of individuals referred because of dysmorphic features or congenital anomalies

Karyotype	No.	0-1 months	1-24 months	2-13 years	over 13 yrs Adults
Normal:					
46,XX	108	45	17	41	7
46,XY	62	21	18	18	5
Total	**170**	**64**	**35**	**59**	**12**
Abnormal					
47,XX+21	21	15	1	3	2
47,XX+21	34	22	3	6	3
47,XX+21/46,XX	2	1	-	1	1
46,XY,+21t(14; 21)	2	1	-		--
47,XY+18	1	1	-	-	-
47,XXX	2	1	-	1	-
47,XXX/45,X	1	-	-	1	-
45,X	2	1	-	-	-
45,X/46,XXr	1	1	-	-	-
46,XY del(1)(q42-qter)	1	1	-	-	-
46,XY del (11)(q23-qter)	1	1	-	-	-
46,XX del 18q(q21-qter)	1	-	-	1	-
46,XY t(1,9),del(9)(p11)	1	-	1	-	-
46.XX,inv dup(4)(q31-q35)	1	1	-	-	-
46,XY t(4;22)	1	-	-	-	1
Total abnormal Karyotypes	**72**	**46**	**5**	**14**	**7**
Total no.of cases	**244**	**110**	**40**	**73**	**21**
% abnormal Karyotypes	**30%**	**42%**	**12%**	**19%**	**30%**

TABLE II

Karyotypes of individuals with genital anomalies

	Total No.	0-1 months	1-24 months	2-13 years	over 13 years
46,XX	8	3	4	1	0
46,XY	15	10	-	3	2
Total	**23**	**13**	**4**	**4**	**2**

TABLE III

Abnormal karyotypes in individuals with problems relating to puberty, male infertility, foetal loss or abnormal offspring

Reason for referral	No. of cases	No.	%	Diagnosis
Primary amenorrhoea and associated disorders	20	2	10%	46,XY-Testicular feminization syndrome 45,X/46,XX
Male hypogonadism (presenting as pubertal problem)	18	2	11%	47,XXY 47,XXY
Male infertility (presenting after marriage)	14	2	14%	47,XXY 46,XY, frag 16
Couples with repeated foetal loss	36 couples	3	8.3% of couples	45,XY,t(14q 15q) 45,XX,t(14q 15q) 46,XX,t(2,6)
Couples with previous abnormal offspring	21	1	5%	46,XY inv 4q

mothers below 20 years old, and 25-30 times higher in the 40-45 year age group. No cases were reported in mother over 45 years, birth at this age being exceedingly rare.

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TABLE IV
CHROMOSOME POLYMORPHISMS

Reason for referral	15p+	9qh+	inv 9	inv 17	Tot. cases
Dysmorphic features or congenital anomaly	4	1	2*	1*	7* (2.9%)
Couples with repeated foetal loss	-	1	2	-	3 (4.1%)
Genital anomalies and infertility	-	-	1	-	1 (1.9%)
Parents of affected children	1	-	1	1	1

*include one case in which two heteromorphisms were present simultaneously

TABLE V
PREVALENCE OF CHROMOSOME ABNORMALITIES IN MALTA 1984-1987

Year	Total Births	No.	Rate/1000	Frequency
1984	5602	5	0.89	1/1123
1985	5522	15	2.72	1/376
1986	5310	11	2.07	1/483
1987	5374	17	3.16	1/316
Total	**21,808**	**48**	**2.20**	**1/434**

TABLE VI
Number and frequency per 1,000 births of chromosoms anomalies diagnosed between 1984 and 1987 compared to the expected numbers and frequencies

	Cases diagnosed		Expected*	
	Number	Frequency per 1,000	Number	Frequency per 1,000
Autosomal aneuploidies	40	1.83	31	1.43
Unbalanaced Structural anomalies	5	0.23	13	0.6
Balanced Structural anomalies	0	0	46	2.1
Sex chromosome anomalies	3	0.14	43	2.0
Total Chromosome Anomalies	**48**	**2.2**	**134**	**6.15**

*Calculated from collective data of various studies (Hook and Hamerton, 1977)

TABLE VII
Prevalence of Down Syndrome in Malta 1984-1987

Year	Total Births	No.	Rate/1,000	Frequency
1984	5602	4	0.71	1/1408
1985	5522	12	2.17	1/460
1986	5310	10	1.88	1/532
1987	5374	15*	2.79	1/358
Total	**21,808**	**41**	**1.88**	**1/532**

* includes one case of translocation Down Syndrome

TABLE VIII
MATERNAL AGE DISTRIBUTION FOR DOWN SYNDROME IN MALTA

Maternal Age	No. of Births	No.	Frequency /1,000	Rate
under 19 yrs	723	2	2.7	1/370
20 — 24 yrs	4,783	6	1.25	1/800
25 — 29 yrs	8,143	5*	0.61	1/1639
30 — 34 yrs	5,195	3	0.58	1/1724
35 — 39	2,522	12	4.76	1/210
40 — 44 yrs	426	13	30.52	1/33
over 45 yrs	16	—	—	—
Total	**21,808**	**41**	**1.88**	**1/532**

*Includes one case of translocation Down Syndrome, born in 1987

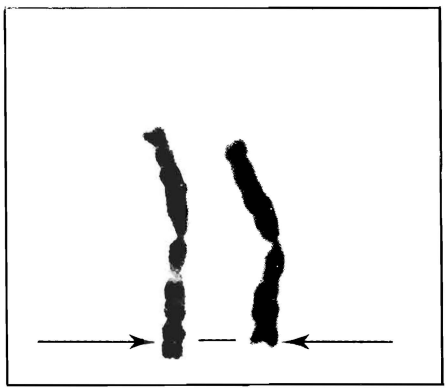

Fig 1. Terminal deletion of the long arm of chromosome 1. 46,XY, del(1)(q43). The break point is shown by arrows.

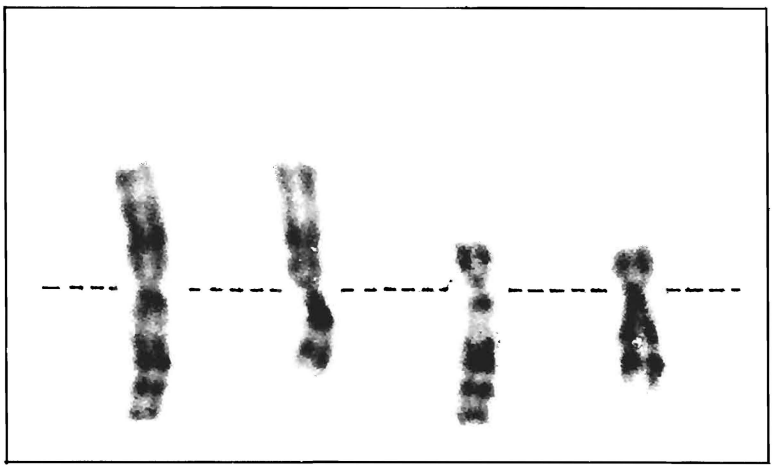

Fig 2. Reciprocal translocation between chromosomes 1 and 9; deletion of 9p11.

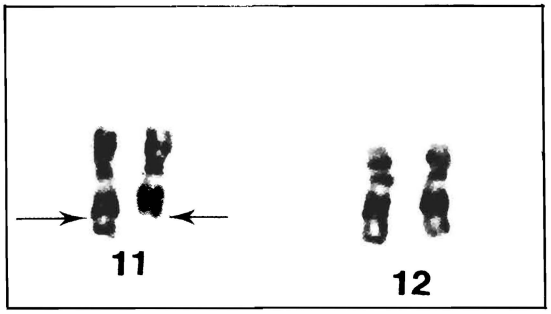

Fig 3. Deletion of the long arm of chromosome 11. 46, XY, del (11)(q23).

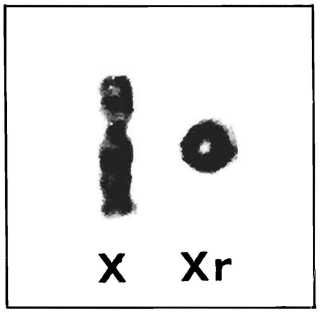

Fig 4. Ring chromosome X in a baby with mosaic Turner Syndrome, 45,X/46,XX.

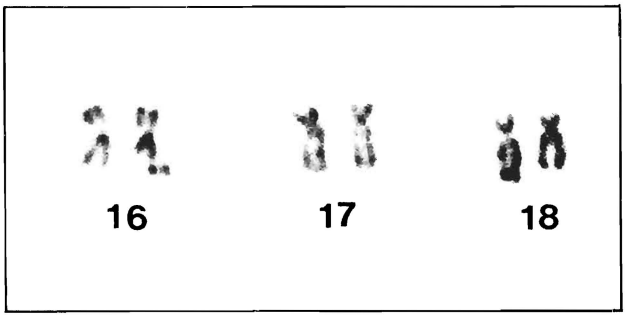

Fig 5. Fragile site on chromosome 16. 46,XY, Fra(16)(q22).

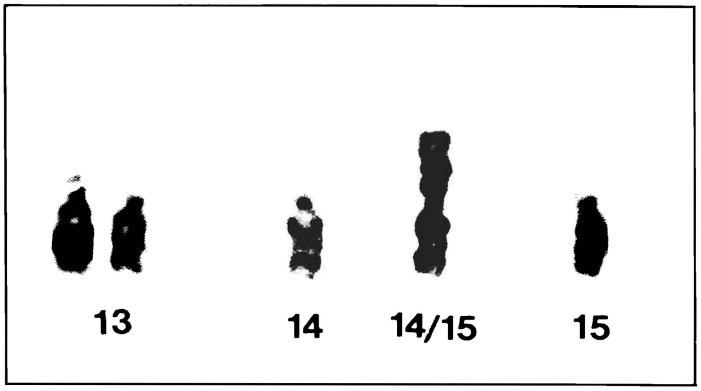

Fig 6. Balanced Robertsonian translocation between chromosomes 14 and 15. 46,XY,t(14;15).

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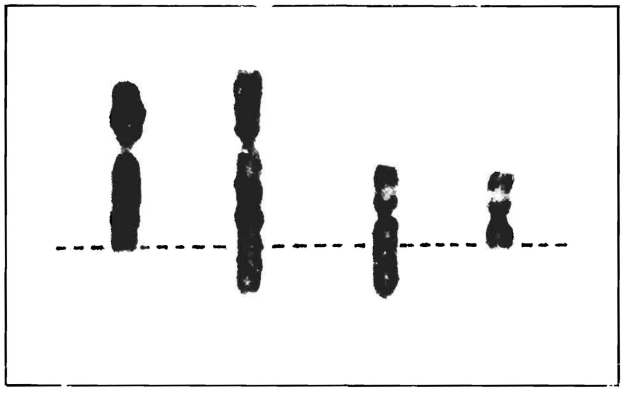

Fig 7. Balanced reciprocal translocation between chromosomes 2 and 6. 46,XY,t(2;6)(q36;q16). The break points are indicated by the dotted line.

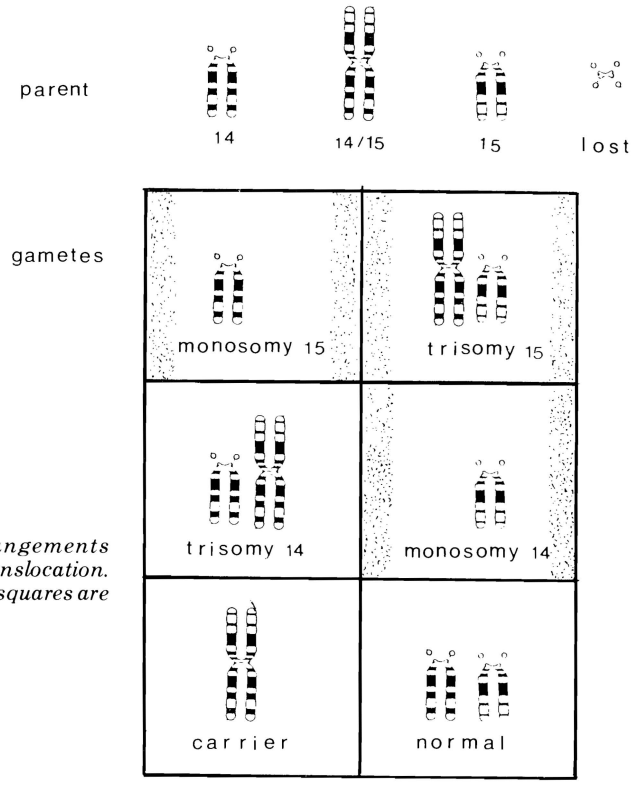

Fig 8. Possible chromosome rearrangements resulting from a parent with 14/15 translocation. The arrangements shown in the shaded squares are lethal.

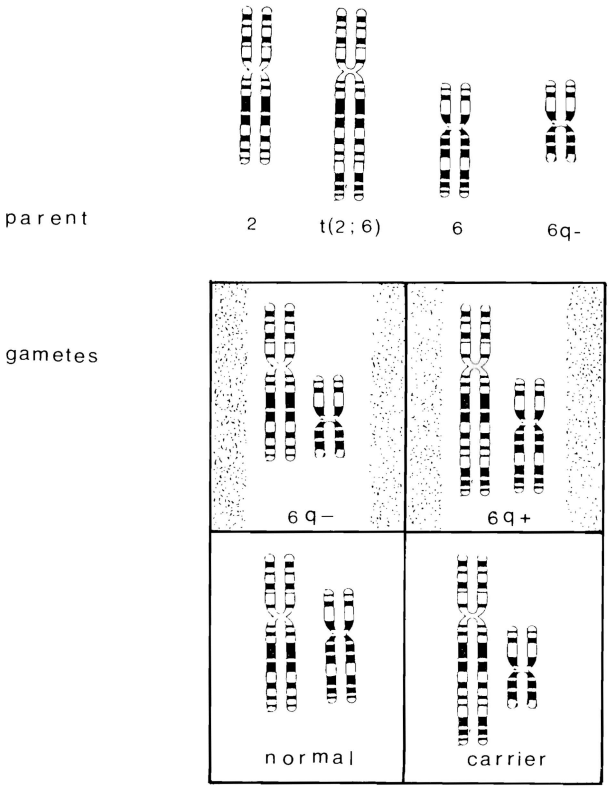

Fig 9. Possible chromosome rearrangements resulting from a parent with a 2/6 chromosome translocation. The arrangements shown in the shaded squares are lethal.

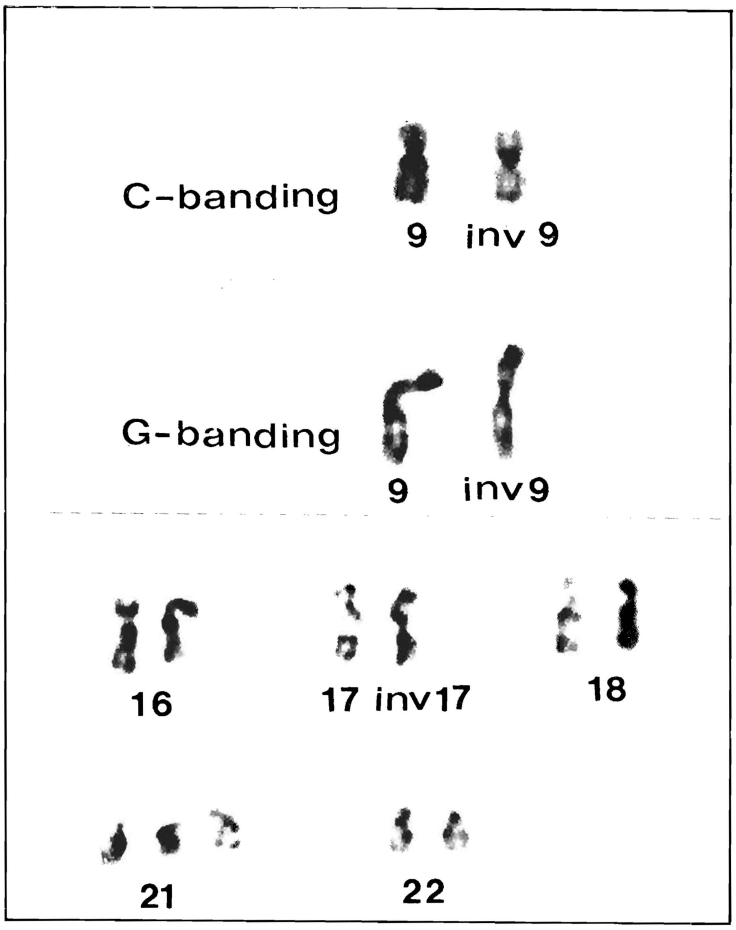

Fig 10. Partial karyotype showing pericentric inverson of chromosome 9, pericentric inversion of chromosome 17 and trisomy 21 in the same individual.