Double Autosomal Trisomy and Mosaicism for Three Cell Lines in Man

Coexisting Trisomy 13-15, Trisomy 17-18, and a Minority Cell Line Trisomic for a Chromosome of Both Groups

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There have been relatively few reports of individuals with double autosomal trisomy (Gagnon *et al.*, 1961; Becker, Burke, and Albert, 1963; Hsu *et al.*, 1965; Marks, Wiggins, and Spector, 1967). There have been rather more published accounts of individuals showing trisomy for one autosome and an additional chromosome in the sex chromosome complement as well. Of these, only those with the sex chromosome constitution XXX can properly be described as double trisomics (Uchida *et al.*, 1962b; Day *et al.*, 1963; Ricci and Borgatti, 1963; Yunis, Hook, and Alter, 1964; F. J. W. Lewis and L. Haas, 1965, personal communication; A. E. Mirkinson, 1966, personal communication), the others having the sex chromosome constitution XXY (Ford *et al.*, 1959; Lanman *et al.*, 1960; Lehmann and Forssman, 1960; Hustinx *et al.*, 1961; R. A. Pfeiffer, 1964, personal communication), or less commonly XYY (Gustavson *et al.*, 1962; Verresen and van den Berghe, 1965). With few exceptions, the reports of double aneuploidy have concerned individuals with only a single cell line. Hsu and her colleagues described a child who was a mosaic, with a normal cell line and a doubly trisomic line, trisomic for chromosomes 18 and 21. A cell line trisomic for chromosome 21 and a second line trisomic for chromosome 18 were demonstrated in a child reported by Marks *et al.* (1967). We earlier reported a child who was a mosaic for trisomy 13-15 and trisomy 17-18 (Baikie, Garson, and Birrell, 1965), and it is that case we now describe in detail.

Case Report

A female child was born in 1964, the first child of a mother aged 26 who had had no abortions, and has

since had a normal pregnancy resulting in the birth of a healthy female child. The father was aged 31 and had one reputedly normal male child of a previous marriage. There was no history of his first wife having any pregnancies which did not go to term. Three weeks before the birth of the proposita a breech presentation was detected and version attempted, but this was abandoned because of vaginal bleeding. The baby was delivered at term without difficulty as a breech with extended legs, after a four-hour labour. She was slightly cyanosed at birth but revived after tracheal aspiration and the administration of oxygen. On the same day she was admitted to the paediatric unit at St. Vincent's Hospital for investigation of multiple congenital abnormalities.

Physical examination. The child weighed 2475 g., was 50.8 cm. in length, and her head circumference was 35.6 cm. She had micrognathia, low-set malformed ears, with the helices adherent to the scalp posteriorly, and microphthalmia on the left side (Fig. 1). There was marked hypotonicity of the limb muscles, and redundant folds of the skin about the neck gave an impression of neck webbing (Fig. 2). Movements at both hip joints showed limitation of abduction, and congenital dislocation of the left hip was diagnosed later. The great toes were short, dorsiflexed at the metatarsophalangeal joint, and plantar-flexed at the interphalangeal joint, giving a hammer-toe deformity. In addition, there was partial syndactyly of the second and third toes on both feet, and the heels were unduly prominent posteriorly (Fig. 3). The hands were held in the so-called surrender position, with the index and little fingers overlapping the middle and ring fingers. The dermal ridges on the fingertips were poorly developed. Examination of the chest showed some intercostal retraction, and a systolic murmur (grade 2/5) was heard all over the praecordium. Ophthalmoscopic examination showed the right eye to be apparently normal while the left, as well as being unduly small, had a deep anterior chamber, a very small lens, and visible ciliary processes. In addition, appearances suggested much retinal gliosis as well as a persistent hyaloid membrane.

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FIG. 1. Unilateral microphthalmia and hands in the 'surrender position'.

FIG. 2. Malformed ear with helix adherent to the scalp, and folds of redundant skin on back.

The child was obviously jaundiced on the day of birth, and jaundice deepened over the next two days, the serum bilirubin rising to a maximum of 15.0 mg./100 ml., but returned to normal levels 7 days later. Hb was 11.3 g./100 ml. on the day of birth but fell to 7.9 g./100 ml. three days later, at which time she was given a transfusion of 100 ml. group A Rh-negative blood. Apart from the initial anaemia, haematological examination was negative. Blood group studies on the child and both parents were carried out by Dr. R. T. Simmons of the Common-

wealth Serum Laboratories, Melbourne, and the results are given later in Table II.

Radiological examination of the chest soon after birth showed cardiomegaly, and this was found to be increased at subsequent examination. There was no abnormality on radiological examination of the skull and long bones.

During the five weeks after birth the clinical course was punctuated by cyanotic attacks and recurrent respiratory infections, so that the child had to be nursed in a humidified incubator. She failed to gain weight despite

FIG. 3. Feet of child showing prominent heels, hammer toes, and partial syndactyly.

regular gavage, and feeding often precipitated cyanotic attacks. During the fifth week she developed severe bronchopneumonia which did not respond to antibiotic therapy, and she died aged 37 days.

Necropsy. This was performed 8 hours after death, with the following results. In the heart, there was a high ventricular septal defect and the ductus arteriosus was incompletely closed. There was a small Meckel's diverticulum, and several pieces of ectopic pancreatic tissue were found in the subserosal layer of the proximal jejunum. There were numerous tiny cysts scattered throughout the cortices of both kidneys. Microdissection of the kidney showed that while most of the nephrons were of normal morphology, a few were cystic, all parts of the nephron being affected. Cystic renal corpuscles were frequent as well as localized cystic dilations at the crest of Henle's loop, in the distal convolutions, and in the proximal region of the collecting tubules. A detailed report of the findings on renal microdissection will be published separately by Dr. T. J. Baxter of the Department of Pathology, University of Melbourne.

Particular attention was paid to the findings in the brain and left eye which were fixed and sectioned later. In the brain the only abnormality was an unduly small cerebellum. The optic nerves and the olfactory tracts were of normal size. There was no abnormality on histological examination of the brain. Histological examination of the left eye showed the major defect to be peripapillary coloboma completely surrounding the nerve head. In the colobomatous area choroid was absent and was replaced by dysplastic retinal tissue. Persistent hyperplastic primary vitreous containing persistent and patent hyaloid vessels extended as a thick stalk from the posterior surface of the lens into the substance of the nerve head.

Cytogenetic Studies. Buccal mucosal smears were examined for sex chromatin and found to be normally chromatin positive. A drumstick count carried out on a film of peripheral blood showed 10 drumsticks in 4000 polymorphonuclear leucocytes. These leucocytes were also examined for nuclear appendages as described by Huehns, Lutzner, and Hecht (1964b) in trisomy 13-15, but no excess was found.

Chromosome studies were carried out on cultures of peripheral blood lymphocytes using a modification of the method of Moorhead *et al.* (1960), first when the patient was 1 day old and again 8 days later. A total of 60 cells was counted (Table I). Fifty-seven of these cells were suitable for detailed graphic analysis. Of the 50 containing 47 chromosomes, 34 were trisomic for a chromosome of the group 17-18 (Fig. 4), and 16 were trisomic for a chromosome of the group 13-15 (Fig. 5). The three cells with 48 chromosomes had an extra chromosome in each of these two groups. The three cells with 46 chromosomes and the one with 45 were probably cells which had had 47 chromosomes *in vivo* but which had undergone random chromosomal loss during preparation: two of these cells were trisomic for the group 17-18 and the other was trisomic for the group 13-15.

Subcutaneous fibroblasts were cultured by the method described by Ferguson (1962). Forty-one cells were examined and the results are shown in Table I. Twelve of these cells were analysed photographically. Three were trisomic for a chromosome of the group 13-15, one trisomic for the group 17-18, and two cells appeared to be of normal chromosome constitution. Six others had an additional chromosome but it was impossible to decide whether it was of the group 13-15 or the group 17-18.

Bone-marrow aspiration was attempted from the right tibia, with the object of obtaining direct chromosome preparations derived from a third tissue, but insufficient marrow was obtained.

In summary, the child was found to possess three chromosomally distinct cell lines, namely 47,XX, $\uparrow 18 + / 47,XX,D + / 48,XX,D + \uparrow 18 +$.

Chromosome studies were carried out on the cultured peripheral blood lymphocytes of both parents with normal results. The chromosome count distributions are shown in Table I.

Blood Group and Haemoglobin Studies. The results of blood group studies on the parents and child are shown in Table II.

Attention must be drawn to the results in the Duffy blood group system (Fy^a , Fy^b), since there was evidence of anomalous inheritance of its antigens. The father was Fy^a positive and the mother Fy^b positive so that the child's expected Duffy blood group was Fy^a positive,

TABLE I
CHROMOSOME STUDIES ON PROPOSITA AND PARENTS

Culture	Subject	Date	No. of Chromosomes per Cell					No. of Cells Examined
			44	45	46	47	48	
Peripheral blood	Proposita	29.8.64 6.9.64	—	—	2 1	16 37	1 2	19 41
Total			—	1	3	53	3	60
Subcutaneous fibroblasts	Proposita	3.9.64	—	—	2	39	—	41
Peripheral blood	Mother		1	1	18	—	—	20
	Father		—	1	19	—	—	20

FIG. 4. Karyotype showing an additional chromosome in group 17-18.

FIG. 5. Karyotype showing an additional chromosome in group 13-15.

TABLE II
BLOOD GROUP STUDIES ON PROPOSITA AND PARENTS

	ABO	MNSs	Rh	P ₁	Le ^A	Fy ^a	Fy ^b	K	Jk ^a	Lu ^a	Lu ^b
Father	A ₁	MNSs	rh	—	—	+	—	—	—	—	+
Mother	A ₂ B	Mss	Rh ₁	+	—	—	+	—	+	—	+
Proposita	A ₁	MNSs	Rh ₁ Rh ₁ rh	+	—	—	+	—	—	—	+

Fy^b positive. The significance of the child being Fy^b positive has been discussed elsewhere (Simmons, Baikie, and Garson, 1965).

Huehns *et al.* (1964a) have described the occurrence of abnormal haemoglobins including Hb Gower in cases of trisomy 13–15. Because of this report a blood specimen from the child was sent to Dr. Huehns at University College Hospital, London. Since the sample was not refrigerated the result must be accepted with reservation, but no abnormal haemoglobin was detected.

Discussion

A confident clinical diagnosis of trisomy 17–18 was made in this child because of the low-set malformed ears, micrognathia, the surrender position of the hands, clinical evidence of congenital heart disease, limitation of abduction of the hips, and abnormalities of the feet including prominent heels and short flexed great toes (Edwards *et al.*, 1960; German *et al.*, 1962; Uchida, Bowman, and Wang, 1962a; Hecht *et al.*, 1963). In addition, it was noted that the child had unilateral microphthalmia. We know of only one report of abnormally small eyes in trisomy 17–18 (Weber *et al.*, 1964). In that case there was said to be unilateral microcornea but the ocular pathology was not described. On the other hand, microphthalmia is a common feature of trisomy 13–15 (Smith *et al.*, 1963). In the present case the combined histological findings of coloboma, persistent hyperplastic primary vitreous with persistent hyaloid vessels and retinal dysplasia in the unduly small eye, were in accordance with findings commonly described in trisomy 13–15 (Sergovich *et al.*, 1963; Miller *et al.*, 1963; Ginsberg and Perrin, 1965). Though coloboma alone has been described in trisomy 17–18 (Townes *et al.*, 1963; Hecht *et al.*, 1963; Weber *et al.*, 1964), the other findings in our case seem to be peculiar to trisomy 13–15 among the constitutional chromosomal abnormalities. Of the other findings at necropsy, ventricular septal defect and patent ductus arteriosus have been described in both syndromes; a small cerebellum may occur in trisomy 13–15 (Smith *et al.*, 1963) but not in trisomy 17–18; and the occurrence of renal cortical cysts, especially with normal and pathological

renal tubules coexisting, is relatively common in trisomy 13–15, and uncommon in trisomy 17–18 (Osathanondh and Potter, 1964; Mottet and Jensen, 1965). Meckel's diverticulum has been reported in both syndromes (Smith *et al.*, 1963), but ectopic pancreatic tissue has been found only in trisomy 17–18 (Lewis, 1964; Warkany, Passarge, and Smith, 1966).

The phenotypes associated with trisomy 13–15 and trisomy 17–18 have a number of features in common (Rosenfield *et al.*, 1962), but in the present case the features peculiar to trisomy 17–18 were more conspicuous than those peculiar to trisomy 13–15. This may be associated with the presence, at least among the peripheral blood lymphocytes, of twice as many cells with trisomy for group 17–18 compared with the number of cells with trisomy 13–15. If this finding was representative of the chromosome constitution of other tissues, the absence of the severe brain deformities commonly found in trisomy 13–15 may be explained in the same way.

Our findings suggest that both parents were of normal chromosomal constitution, but the possibility of mosaicism with gonadal chromosomal abnormality can hardly be excluded. The more recent birth of a normal sib must weigh against this possibility. There was no family history of congenital abnormalities or abortions, and no history of excessive radiation exposure of either parent. It seems likely that chance meiotic abnormality led to the formation of a zygote with double autosomal trisomy. Subsequent mitotic errors must have given rise to the two separate trisomic lines. Since only 3 cells with 48 chromosomes were found, compared with 53 cells with 47 chromosomes, it is likely that selection has operated against the doubly trisomic cells during the course of intrauterine development. Such selection has been invoked by Hecht *et al.* (1966) to explain their finding of 1% of 47,XXY cells in a phenotypic male whose main cell line had the chromosome constitution 46,XX. Similarly, in our case the cell line with trisomy 13–15 may have been at a disadvantage compared with cells trisomic for the group 17–18, since they were outnumbered two-to-one in the tissues we sampled. A

recent review of the occurrence of both these autosomal trisomies has suggested that the frequency of trisomy 17-18 at birth is approximately 1 in 4500, whereas the frequency of trisomy 13-15 is of the order of 1 in 14,500 (Conen and Erkman, 1966a, b). It also appears that there is a female preponderance among cases of both syndromes (Uchida *et al.*, 1962a), and that females with trisomy 17-18 survive longer than do males (Conen and Erkman, 1966b). These findings may be an indication of the relatively greater viability of female cells trisomic for group 17-18 compared with male cells similarly trisomic, and more especially by comparison with both male and female cells trisomic for the group 13-15. This selective advantage may account for the preponderance of cells trisomic for the group 17-18 and so for the more numerous clinical features associated with that trisomic state in the child we have described.

Summary

A female child with multiple congenital abnormalities was found to be doubly trisomic with mosaicism for three cell lines namely, 47,XX,D+/47,XX,?18+/48,XX,D+,?18+. The 47,XX,?18+ cell line was preponderant, and the cells of the 48,XX,D+,?18+ line were least numerous. The clinical and necropsy findings, including the ocular pathology, are described. Most of the abnormalities present were those commonly associated with trisomy 18, but some peculiar to trisomy 13-15 were also present. It seems likely that of the three cell lines of distinct chromosome constitution found in this child, the cell line trisomic for group 17-18 probably had a selective advantage over the others.

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