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A Partial D-trisomy/Normal Mosaic Female*

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During a chromosome survey of mentally retarded children with epicanthic folds, an 8-year-old girl was found to have an extra medium-sized, satellited, acrocentric chromosome in a percentage of cells in peripheral blood, bone marrow, and skin. However, she did not show any of the clinical abnormalities associated with D-trisomy (Patau *et al*, 1960). Therefore clinical and cytogenetic studies together with special investigations performed on this child are described.

Case Report

The proposita J.M. was born on 21 June 1960 by a breech delivery, to a 38-year-old mother and a 46-year-old father, who were unrelated. Her birth weight was 3040 g. Apart from an early threatened miscarriage treated with progesterone, the pregnancy was normal.

Clinical examination of the proposita at the age of 8 years showed profound mental retardation, epicanthic folds, and a mild degree of microcephaly (Fig. 1). On psychological testing she was found to be hyperactive and to have an IQ below 20. Radiology of the skull was normal. The Guthrie test for phenylketonuria and urine amino-acid chromatography were negative. The red cell phosphohexokinase level was within the normal range, ie, 16·9–24·6 micromoles of substrate/min/3·2 g Hb (Baikie *et al*, 1965). Abnormal projections from the nuclei of the polymorphonuclear leucocytes as described in D-trisomy by Huehns, Lutzner, and Hecht (1964b) were not present.

Fetal haemoglobin, estimated when the proposita was aged 7 years, was found to be 9·3% by the alkali denaturation method (Singer, Chernoff, and Singer, 1951) and, at the same time using the acid-elution technique of Kleihauer, Braun, and Betke (1957), 55% of the erythrocytes were shown to contain some fetal haemoglobin. However, subsequent tests by alkali denaturation in the same laboratory over a period of 3 years showed wide variation even in replicates. The measurement had a mean of 3·56% but ranged from the 9·3% maximum to zero.

The proposita was the 6th child in a family of 7 (Fig. 2). The first child (II.1) died 12 hours after being delivered 2 months prematurely by Caesarean section for placenta praevia. This child was stated to have had no congenital abnormalities. The mother had two miscarriages, one at 3 months and the other at 6 weeks.

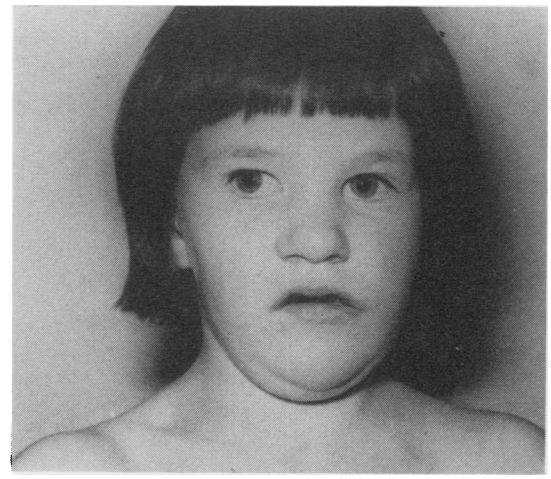

FIG. 1. The proposita (II.6).

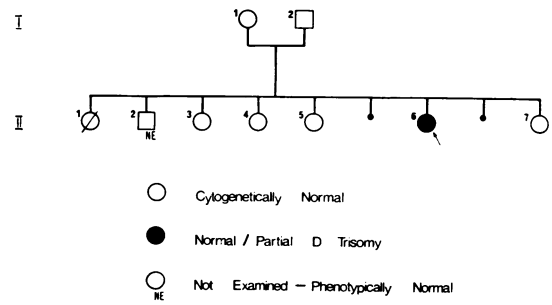

FIG. 2. Pedigree of the family.

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These occurred one year before and one year after the birth of the proposita.

Clinical examination of the parents and 4 female sibs showed no abnormality apart from one sib (II.5) who had had an eye removed at the age of 8 months for congenital glaucoma. There was no other family history of congenital abnormalities. The only male sib (II.2) was not available for examination but was stated to be normal.

Dermatoglyphic Studies. Dermatoglyphs of the proposita (II.6), showed generally normal features in the palms and soles, without any of the peculiarities usually associated with D-trisomy (Penrose, 1966). However the total ridge counts of the proposita, her mother and 4 female sibs were higher than normal, some significantly higher (Table I). Of additional interest were patterns at the base of the thumbs of the female sib (II.4) and the 4 radial digital loops on the mother's fingers.

TABLE I
DERMATOGLYPHIC STUDIES

Patient	Total Ridge Count	Total Triradius Count	Maximal *atd* angle	
			L	R
I.1	125	13*	32°	30°
II.3	123	15*	36°	35°
II.4	253*	20*	35°	37°
II.5	199*	20*	36°	36°
II.6†	212*	19*	33°	36°
II.7	198*	17*	45°	43°
Normal Melbourne Caucasians (Parsons, 1964)	115·4 ± 51·3	11·2 ± 0·8		

* Significantly raised ($p < 0.05$; 1 tail t test).
† The proposita.

Cytogenetic Studies. A buccal smear of the proposita stained by the method of Ross (1960) was normally chromatin positive (25/100). Chromosome counts are shown in Table II. Peripheral blood lymphocyte cultures were performed on four occasions using a modification of the method of Moorhead *et al* (1960). There were two cell lines present, the major cell line contained 47 chromosomes and the other a normal female set of 46 chromosomes per cell. The extra chromosome present in the 47 chromosome line was a satellited acrocentric chromosome, intermediate in size between groups D and G (Fig. 3). It was seen in satellite association with other acrocentric chromosomes and in most spreads there was a negatively heteropycnotic area at the distal end of the long arms. This chromosome was thought to be a D group chromosome with deletion of part of the long arms.

The same abnormal cell line, designated 47,XX, (Dq−)+ using the Chicago nomenclature, was also seen in direct bone marrow preparations based on the method of Kiossoglou, Mitus, and Dameshek (1964). Two skin cultures contained the extra chromosome but differed significantly in the proportion of normal to abnormal cells. Only a few cells were abnormal in the first culture but two years later the second culture, yielded results similar to those of the peripheral blood culture having 75% abnormal cells.

Lymphocyte cultures were performed on the mother, father, and 4 sibs and all showed normal karyotypes.

Autoradiographic Studies. Slides for the investigation of the pattern of late replication in mitotic cells were prepared by a technique based on that of Frøland (1965) using Ilford K2 liquid emulsion. The chromosomes were stained with Giemsa stain before and after grain removal. Forty-three well-spread metaphase figures with a late labelling X chromosome and a suitable number of grains over the autosomes, were photographed and the grains on the chromosomes relevant to this study were drawn on a print of each cell and counted. After removal of the grains, the identification of the chromosomes was checked and the metaphase figures were rephotographed using either brightfield or phase optics on a Zeiss Ultraphot microscope. Chromosome lengths were measured to the nearest 0·5 mm from prints standardized to a magnification of 3000.

TABLE II
CHROMOSOME COUNTS

Institution	Tissue	Date	46,XX Line					47,XX,(Dq−)+ Line					Total Cells
			45	46	92	Total	%	46	47	48	Total	%	
A*	Peripheral blood	5/65	—	5	—	5	20	—	20	—	20	80	25
A*	Peripheral blood	10/66	5	34	0	39	39	2	58	1	61	61	100
B†	Peripheral blood	6/67	1	9	1	11	25	2	30	1	33	75	44
B†	Peripheral blood	6/67	1	8	0	9	21	2	31	1	34	79	43
D‡	Peripheral blood	1/68	—	18	—	18	50	—	18	—	18	50	36
Total			7	74	1	82	33	6	157	3	166	67	248
B	Bone marrow	11/66	0	1	0	1	3	3	29	0	32	97	33
C	Skin**	12/65	—	61	—	61	94	—	3	1	4	6	65
D‡	Skin††	1/68	—	9	—	9	25	—	27	—	27	75	36

* Different cultures and observers.
† Same culture, different observers; second score from autoradiographic studies.
‡ Skin and blood taken on same occasion.
** 7th week, 4th passage 26 (46), 2 (47), 1 (48); 9th week, 7th passage 35 (46), 1 (47). Method of Ferguson (1962).
†† 7th week, 3rd passage. Method of Hsu and Kellogg (1960).

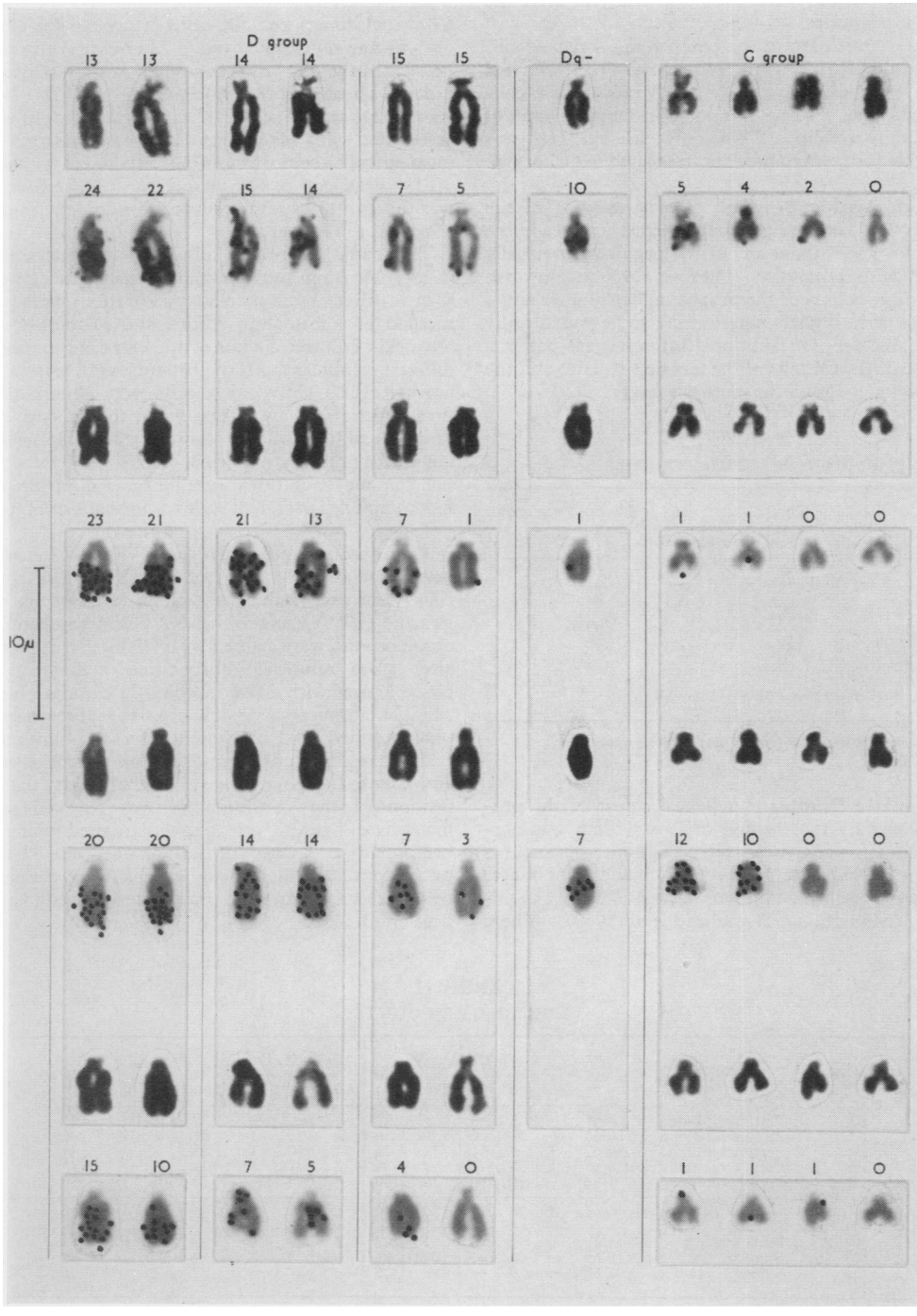

FIG. 3. ^{3}H-thymidine autoradiography of some of the D and G group chromosomes of the proposita. In all but the first cell the grains have been drawn onto the prints as in the investigation. The grain score is shown for each chromosome. All cells were 47,XX (Dq−)+ except the last which was 46,XX.

TABLE III

MEAN GRAIN COUNTS ON CHROMOSOMES RELEVANT TO THIS STUDY

The table is arranged so that the significance of mean grains is additive across the table. Thus the chromosomes identified as Dq − in the first row are significantly less heavily labelled than the No. 14 chromosomes and therefore significantly less labelled than the No. 13 chromosomes but the Dq − and No. 15 chromosomes are not significantly different.

Diploid Number	Parameters	D Group plus Dq − Chromosomes								G Group	
		Complete Chromosomes				Centromere plus Dq − Length				High	Low
		13	14	Dq −	15	13	14	Dq −	15		
47 (20 cells)	$\bar{x}$	16·17*	11·20*	4·25 NS	3·88	9·58 NS	9·23*	4·25 NS	3·23	4·08*	0·50
	n	40	40	20	40	40	40	20	40	40	40
	sd	7·31	5·90	3·86	3·36	5·24	4·88	3·86	2·94	2·80	0·75
46 (6 cells)	$\bar{x}$	13·50*	9·17	*	3·00					3·09*	0·92
	n	12	12		12					11	12
	sd	2·78	3·30		2·30					1·92	0·79

* Significant at 1% level.
$\bar{x}$ = mean; n = number of observations; sd = standard deviation; NS = no significant difference between means.

The criteria for classification of the group D chromosomes were a combination of qualitative and quantitative observations as described by Giannelli (1965). In cells labelled late in S phase the No. 13 chromosome was characterized by heavy labelling on the distal third of the long arm; all but two of the No. 13 chromosomes could be identified in this study. In most cells the No. 14 pair was late labelling around the centromere region and the No. 15 pair had little or no label, but in more lightly labelled cells these two chromosome pairs could not always be distinguished. We found, as did Giannelli, that the difference between these two chromosome pairs was more quantitative than qualitative.

The results of the autoradiographic analysis of 26 cells in which all D chromosomes could be identified are summarized in Table III. The D chromosomes which could be identified in the remaining cells were analysed and gave results very similar to those shown in Table III. The significance of the differences between adjacent pairs of means was tested by use of t tests allowing for significantly different variances where necessary (Bailey, 1959). The grain counts are expressed for the whole chromosome and for the length equivalent to the Dq−. The Dq− chromosome can be seen to have significantly lower label than the No. 13 or 14 chromosomes but not less than the No. 15 chromosome. The only effect of comparing grain counts over the proximal portion of the long arms of D chromosomes taking lengths the same as the Dq− chromosome, is to remove the significant difference in label between the 13 and 14 chromosomes, as would be anticipated from the usual labelling pattern of these two chromosomes. The apparent significant difference between the G group chromosome pairs shown in Table III is probably largely an effect of ranking them into pairs with high and low label.

The lengths of the chromosomes identified by autoradiography, shown in Table IV, show an expected decrease in length from chromosome 13 to 15. The length of the Dq− chromosome was about 83% of the length of an average group D chromosome.

TABLE IV

LENGTHS OF THE Dq− CHROMOSOME AND THE D GROUP CHROMOSOMES AS IDENTIFIED BY AUTORADIOGRAPHY MEASURED AT 3000 MAGNIFICATIONS

Diploid Number	Parameters and Test Significance	Chromosomes			
		13	14	15	Dq −
47 (20 cells)	$\bar{x}$	11·69	10·69	10·03	9·03
	n	40	40	40	20
	sd	1·51	1·50	1·30	1·43
	Significance	*		†	*
46 (6 cells)	$\bar{x}$	10·81	9·61	9·49	
	n	12	12	12	
	sd	1·91	2·13	1·93	
	Significance	NS	NS	NS	

* Significant at 1% level.
† Significant at 5% level.

Discussion

Singer's alkali-denaturation method is known to be unreliable for the determination of fetal haemoglobin levels of less than 10% (Beaven, Ellis, and White, 1960) whereas the acid-elution test is considered to be a much more reliable qualitative measure of slightly elevated fetal haemoglobin levels (Lie *et al*, 1968). The results in our patient support these findings, as although the levels of fetal haemoglobin as determined by the alkali denaturation method were equivocal, the acid elution technique showed a definite rise.

In 1968, Bloom and Gerald reported finding a 47,XX,(Dq−)+ chromosome constitution in a child who had elevated fetal haemoglobin (2·5% and 3·5%) and abnormal neutrophil projections (13% and 7%) when aged 6 and 7 years respectively.

The deleted chromosome was 65% as long as a normal D and showed strong distal late replication. The Dq− chromosome was identified autoradiographically as a No. 13 using the chromosome constitution of the mother: 46,XX,t(Cp+;Dq−). Yunis and Hook (1966) described a 47,XY,(Dq−)+ child whose Dq− chromosome was satellited at each end, smaller then a group G chromosome, and although early replicating was considered to be a proximal fragment of chromosome 13. The child had a normal fetal haemoglobin level and 30% abnormal neutrophil projections. These two cases were used to support the suggestion that the loci responsible for neutrophil abnormalities are closer to the centromere of chromosome 13 than the loci controlling fetal haemoglobin persistence. The apparent elevation of fetal haemoglobin, and absence of abnormal nuclear projections in the neutrophils in our case are at variance with this interpretation, in that the order of loci would be reversed if the deleted chromosome was a No. 13. However, our autoradiographic studies suggest this Dq− chromosome is derived from a No. 15.

The proposita has relatively few physical abnormalities; this may be largely attributed to mosaicism. Several reports of D trisomy/normal mosaics have shown no specific pattern of mental or physical abnormalities and fetal haemoglobin levels have been described as normal or slightly elevated (Warkany *et al*, 1962; Huehns *et al*, 1964a). In our patient the high proportion of abnormal 47,XX,(Dq−)+ cells in the bone marrow compared with other tissues indicates that the mosaicism varies between the tissues. The differences in cell line percentages in peripheral blood cultures probably reflects differences in procedure in the 4 institutions in which they were made and scored. Different conditions in the two skin cultures might have allowed the percentage of normal cells to increase with increasing time in the first culture or alternatively might be based on a low number of viable cells with an atypical ratio of normal to abnormal cells in the first skin biopsy.

In our opinion autoradiographic studies designed to investigate a novel chromosome should be quantitative whereas investigations involving more easily interpreted rearrangements such as reciprocal translocations can usually be dealt with by qualitative methods (Pitt *et al*, 1967; Fredga, 1968). In the present case, a simple quantitative study of the relative degree of late replication and the length of the Dq− chromosome was made, rather than a repeat of more exacting work done on the late replication and lengths of the D group chromosomes (Giannelli and Howlett, 1966).

By autoradiography the proximally late replicating chromosome 14 is almost certainly excluded. The No. 13 chromosome could have been deleted to form the Dq− only if the negatively heteropycnotic distal ends of the Dq− long arms represent some structural alteration of the distal region of a chromosome 13 leading to a change in the time of replication of the region. In comparison with other cases the relatively long Dq− chromosome of the proposita would be expected to be distally late replicating if it was a No. 13 chromosome. However the whole of the Dq− chromosome was early replicating so it must be assumed that it was derived from a No. 15 chromosome.

Summary

A mentally retarded girl with a mosaic chromosome constitution is described as a 46,XX/47,XX,(Dq−)+. The few physical abnormalities of the proposita include epicanthic folds, mild microcephaly, slightly elevated fetal haemoglobin and familial abnormal dermatoglyphics. By autoradiographic analysis chromosome 15 is the most likely D chromosome deleted to form the Dq−.

We are grateful to a number of Melbourne workers for their help. Skin cultures were grown and analysed by Miss Jean Ferguson and Miss Margaret Fitzgerald; Miss Fitzgerald was also responsible for one blood culture. Haemoglobin determinations were carried out by the Biochemistry Department of the Queen Victoria Hospital. Mr Grant Sutherland and Dr Saul Weiner generously allowed us to use the photomicroscope. Miss Wendy Milligan and Miss Lorraine Stanley gave technical assistance and Miss Jenny Meffet and Mr Robert Shakleton helped to prepare the publication. Dr Brian Turner and Professor Albert Baikie gave valuable advice.

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