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Presumptive C/15 Translocation and Familial Large Y Identified by Autoradiography

A. DE CAPOA,* F. H. ALLEN, Jr., A. P. GOLD, R. KOENIGSBERGER, and O. J. MILLER†

From the Departments of Obstetrics and Gynecology, Neurology (Division of Pediatric Neurology), and Pediatrics, College of Physicians and Surgeons, Columbia University; and The New York Blood Center, New York, N.Y., U.S.A.

Translocations between C- and D-group chromosomes are relatively uncommon. Only 4 cases of such a translocation have been reported up to now. In 2 of these, autoradiographic studies (Pitt *et al.*, 1967; Bloom and Gerald, 1967) identified the missing D-group chromosome as a No. 14 and a No. 13, respectively. The present paper presents a case of C/15 translocation in a child with psychomotor retardation and few other clinical stigmata.

Case Report

The patient (Fig. 1) is a 9-month-old boy, born in January 1967. The father was 27 and the mother 23 at the time of the proband's birth. Both parents are of Italian ancestry. An older brother, born in 1965, is normal. There is no history of abortions.

The child was born after a 38 weeks' pregnancy which was characterized by a paucity of foetal movements; his birthweight was 2325 g. Difficulties with sucking and a weak squeaky high-pitched cry were characteristic of the neonatal and early infancy periods. Examination showed microcephaly (head circumference 40·2 cm.), micrognathia, eyebrows that met in the midline, a very high arched palate, a small penis, and a poorly developed scrotum, with undescended testes. The right testis was in the inguinal canal, and the left could not be palpated. Psychomotor function was below the 3-month level, with a paucity of purposeful extremity movements and no interest in the immediate environment. Muscular hypotonia, hyperextensibility of joints, and hyperactive deep tendon reflexes were prominent clinical features. *X*-rays for bone age were 0–3 months, and a pneumoencephalogram showed mild lateral ventrical dilatation. Re-examination at 12 months confirmed the diagnosis of psychomotor retardation, and the child was functioning at a 5-month level.

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* Population Council Fellow.

† Career Scientist of the Health Research Council of the City of New York.

Material and Methods

Peripheral blood from the patient, his parents, and his normal brother was cultured using Eagle's Minimum Essential Medium, supplemented with 20% foetal calf serum, glutamine, and amino acids; ^{3}H-thymidine (Schwarz BioResearch, specific activity 1·9 Ci/mM) was added 7 hours before termination of the culture in the final concentration of 0·5 μCi/ml. Colcemid (Ciba) was added for the last 2 hours. After hypotonic treatment and fixation, slides were made, which were stained with Giemsa, and 39 metaphase spreads were photographed. After photography, the preparations were destained and then restained with 2% acetic orcein. The slides were then filmed with Kodak AR-10 stripping film, using the technique of Schmid (1965). After 9 days' exposure at 4° C., the preparations were developed and fixed, the metaphases located on the slide, and the grains counted under the microscope.

Chromosome Studies

All the cells examined from the proband have 45 chromosomes. One C- and one D-group chromosome are missing, and there is an additional large metacentric chromosome, similar in morphology to a No. 3 (Fig. 2). The Y chromosome is usually large, being almost equal in size to a chromosome No. 15. The same very large Y chromosome is present in the patient's father and brother, both of whom have normal D-group complements.

The karyotypes of the parents and of the brother otherwise are normal.

Buccal smears on the patient and the other members of the family show the sex chromatin pattern expected on the basis of each person's sex.

Labelling Studies. Of the 39 cells which were photographed before autoradiography because of their good chromosome morphology and lack of overlapping of the Y, A, and D-group chromosomes, 36 were labelled. Grain counts were possible over the Y and D-group chromosomes in 35 cells, and over chromosome 3 and the presumptive C/D chromosome in 30 cells.

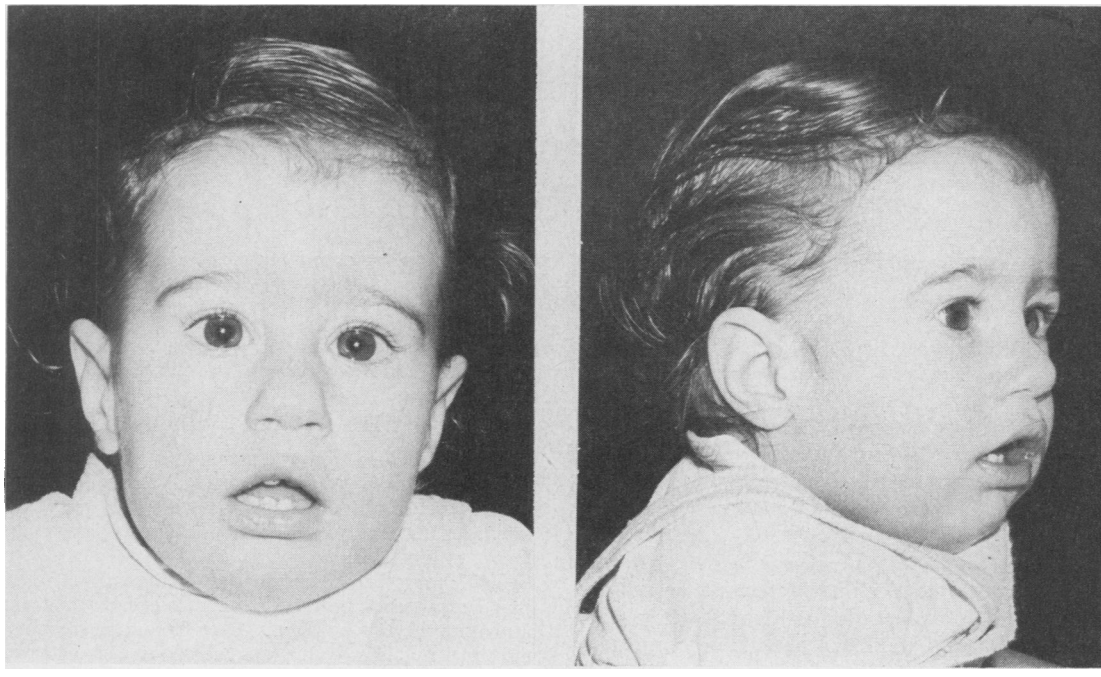

FIG. 1. The proband at 1 year of age.

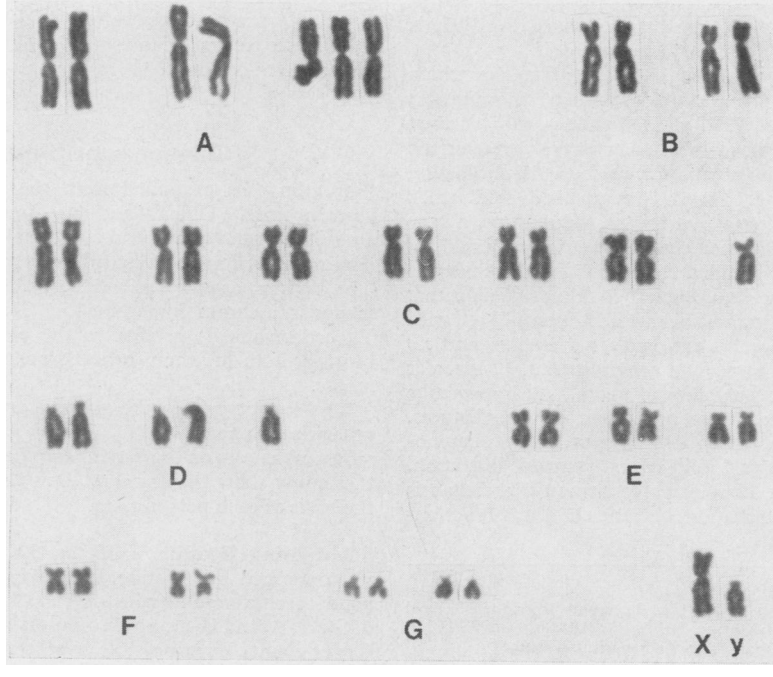

FIG. 2. The chromosomes of the proband.

Identification of Y chromosome. The large Y chromosome was identified by morphology alone in 34 out of the 39 cells photographed before autoradiography, even though by length it could easily have been mistaken for one of the shortest chromosomes in the D-group. However, the chromatids of the Y were closer together than those of the D-group chromosomes, its short arms were distinctive and without fuzziness, and it was never involved in a satellite association.

This identification has been confirmed by the grain count, the Y chromosome being uniformly and heavily labelled (Table I). The average grain count on this chromosome is 24, as opposed to the average count of 16 per chromosome on No. 13.

TABLE I

GRAIN COUNTS OVER THE Y AND THE 5 D-GROUP CHROMOSOMES IN ORDER OF DECREASING COUNT OVER DISTAL HALF OF EACH CHROMOSOME IN 35 CELLS

Grain Count	*					
Total	840	589	513	438	342	260
Mean	24·0	16·8	14·7	12·5	9·8	7·4
Proximal half	310	171	189	240	221	199
Mean	8·9	5·0	5·4	6·9	6·3	5·7
Distal half	530	418	324	198	121	61
Mean	15·1	11·9	9·3	5·7	3·5	1·7

* Morphologically identified as the Y in 32 of these 35 cells.

The Y chromosome is the most heavily labelled of the large acrocentric chromosomes in 32 out of 35 cells, and, in almost all the cells analysed, it is also the most heavily labelled chromosome in the whole complement (see Fig. 6).

Identification of D-group Chromosomes. These chromosomes have been identified by means of both grain counts and labelling pattern. Chromosome 13 terminates DNA synthesis latest in the distal portion of its long arm, while chromosome 14 is terminally labelled more heavily in the proximal, or centromeric, portion (Giannelli and Howlett, 1966). Consequently, better discrimination between chromosomes 13 and 14 should be obtained by ranking chromosomes by the grain count over their distal half than by their total grain count. We have found this to be so, and therefore present the data in this form (Table I). The two most heavily labelled D-group chromosomes have much lower grain counts over their proximal halves than over their distal halves in almost every cell, whereas the remaining three D-group chromosomes are more heavily labelled in their centromeric portions than in their distal halves. This difference is so striking that the mean grain count over the proximal half of each of these three chromosomes is greater than that of the corresponding portions of the two most heavily labelled D-group chromosomes (Table I).

When the grain counts are standardized by multiplying each grain count by the ratio of the mean grain count over the D-group in all 35 cells to the grain count of the D-group in the individual cell, the frequency distributions of the adjusted grain counts appear to support the identification of the Y and the two most late replicating D-group chromosomes (presumptive No. 13). However, they do not permit a distinction between a missing medium-labelled and a missing lightly-labelled chromosome. On the other hand, the frequency distribution of the adjusted grain counts over the distal half of each chromosome (Fig. 4) shows clearly that the missing chromosome is probably one of the most lightly labelled pair.

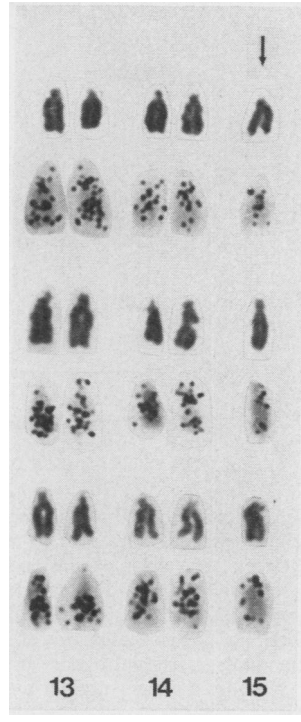

FIG. 3. The D-group chromosomes of the proband. The arrow marks the unpaired No. 15 chromosome.

Another approach, which is not without possible bias, is to select cells in which the five D-group chromosomes can be readily classified as having an 'A', 'B', or 'C' pattern, as described by Giannelli and Howlett (1966) and by Yunis, Hook, and Mayer (1964). In 20 of these 35 cells a chromosome with a 'C' pattern is missing (Fig. 3), while in only one is a 'B' pattern missing. In no cell is a chromosome with an 'A' pattern missing. On the basis of both lines of evidence, we conclude that the D-group chromosome involved in the translocation is an early replicator, presumably a No. 15.

Identification of C/D translocation chromosome. The two chromosomes No. 1 are morphologically distinctive in the cells used for this analysis. Of the other three nearly metacentric chromosomes in the A-group, two, which have about the same grain count in each cell (average 22·6 grains per chromosome), have been identified as homologues by autoradiography. Their average maximum difference is 3·3 grains per chromosome. The third one, which has a much lower total grain count

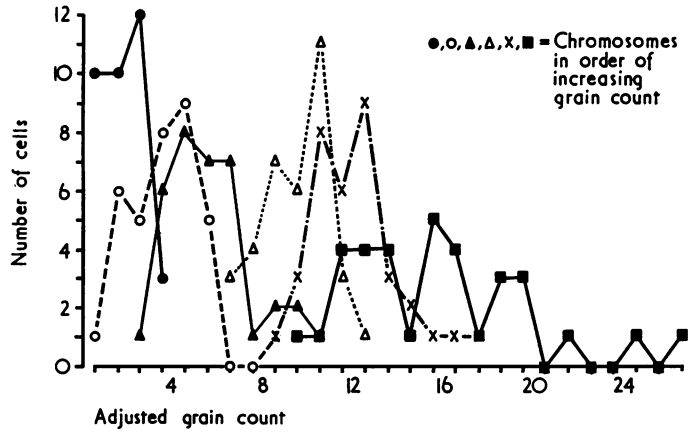

FIG. 4. Distribution of the 5 D-group chromosomes and the Y in order of adjusted grain count.

(average 14 grains per chromosome), appears to be the translocation chromosome. The average difference in grain count between this chromosome and the lighter No. 3 chromosome is 7 grains. The arms of this translocation chromosome are more unequally labelled than those of the other two chromosomes, one of its arms having about half as many grains as the other (Fig. 5). This is true of the 30 cells in which grain counts could be made (Table II).

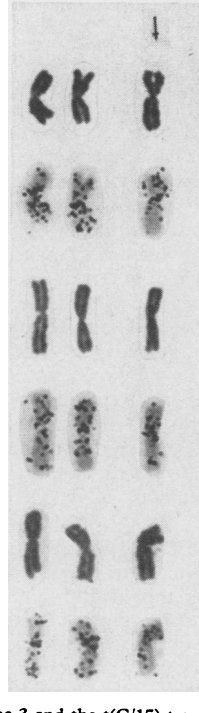

FIG. 5. Chromosome 3 and the t(C/15) translocation chromosome (marked by an arrow) in three cells.

TABLE II

GRAIN COUNTS ON TWO No. 3 CHROMOSOMES AND TRANSLOCATION CHROMOSOME

Heaviest	Next Heaviest	Lightest ?t(D,C)
9	9	4
25	22	17
18	18	8
26	20	8
40	33	22
28	23	15
28	22	11
31	30	22
19	15	15
24	19	12
25	22	18
27	27	7
27	21	2
60	60	35
21	20	14
11	11	6
8	6	4
21	15	10
32	26	16
15	14	7
38	34	25
21	18	15
30	25	20
36	31	30
21	18	15
4	4	1
15	12	11
26	25	22
16	10	10
27	20	17
720	630	419
$\bar{x}$ =	24·3	21·0	14·0
difference =		3·3 grains	7·0 grains

n = 30

Genetic Marker Studies. Genetic marker studies (Table III) performed on the proband and his family have indicated that the Rh, MN, Gc, and probably the Ag loci are not on the deleted segments since the proband was heterozygous at these loci. The results on other red cell, serum, and enzyme groups were not

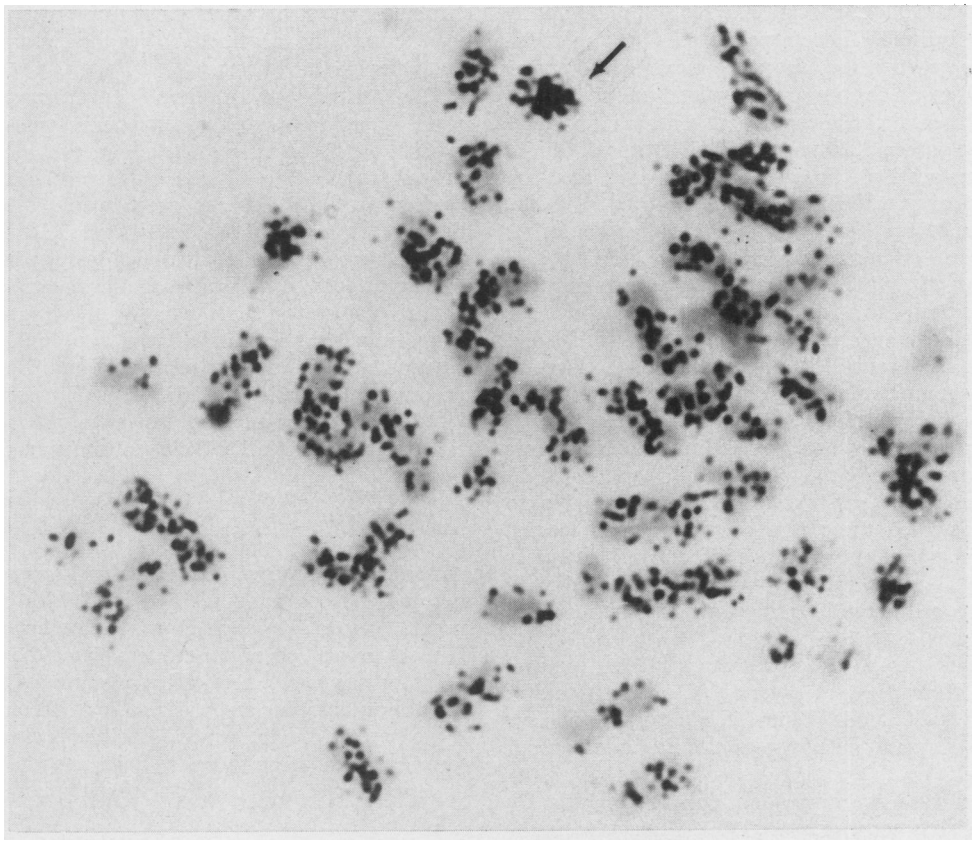

FIG. 6. A labelled metaphase from the proband. The late-replicating Y chromosome is indicated by the arrow.

TABLE III

BLOOD GROUP PHENOTYPES OF FAMILY

		Father	Mother	Proband	Brother
	ABO	O	A	O	O
	Rh	Rh_1 rh	rh	Rh_1 rh	rh
	MNS	MNs	MNs	MNs	Ms
	Lu^a	−	−	−	−
	Fy^a	−	+	+	+
	Xg^a	+*	+	+	+
	Hp	2-1	2-1	2-2	2-1
	Hb	AA_2	AA_2	AFA_2†	AA_2
	Gc	2-1	1-1	2-1	1-1
Ag‡	MDA	+	+	+	+
Ag‡	CM	−	+	+	−
Ag‡	GR	+	+	+	−
Ag‡	DG	−	+	+	−
	Gm 1	+	+	−	+
	2	−	+	−	+
	4	+	+	+	+
	5	+	+	+	+
	6	−	−	−	−
	13	+	+	+	+
	14	+	+	+	+
	17	+	+	−	+
	21	−	+	−	+
	22	+	+	+	+

Note: All four subjects are P+, K−, k+, Kp(a−b−), Jk(a+), TfC, PGM 1-1, AK 1-1, G6PD type B, and INV(1−, 2−).

* Weak reaction with anti-Xg^a.

† 90% A.

‡ Specificities not certain.

directly informative in so far as no exception to the expected inheritance of these groups was observed.

Discussion

This is the first case in which a chromosome No. 15 has been identified in a C/D translocation.

Stalder *et al* (1964) described a case with such a translocation, leading to a partial trisomy for a D-group chromosome in a retarded malformed child and her sister. The father, who was carrying the translocation, had 45 chromosomes with a C- and D-group chromosome missing, and an extra large metacentric chromosome, resembling a No. 3. Though no labelling studies were carried out, the translocated D-group chromosome was defined as a D_1, presumably on the basis of the clinical findings, while the C-group chromosome was thought to be a No. 6 or 7.

Weller, Apley, and Raper (1966) described a case of congenital malformations associated with precocious synthesis of adult haemoglobin in a male infant. Chromosomal studies showed that the

child had a C/D translocation. No autoradiography was performed. The translocated C-group chromosome was identified morphologically as a No. 6 or 7, the D-group chromosome involved was not identified. Parental chromosomes were normal.

In two other published cases of C/D translocation, the translocation chromosome was identified by autoradiography. In one it was reported as a No. 14 chromosome (Pitt *et al.*, 1967), and in the other as a No. 13 (Bloom and Gerald, 1967). In the case of Pitt *et al.*, where the mother was carrying the translocation, the affected child was monosomic for part of the long arm of chromosome No. 14 and for the C-group chromosome. The C-group chromosome involved was identified by measure ments as being a No. 6 to 8 chromosome.

All these cases, which involve individuals trisomic or monosomic for large portions of C- and D-group chromosomes, hardly seem to be comparable to ours, in which there is a relatively small loss of genetic material. Also, since in none of these cases can the C-group chromosomes be identified with certainty, a different chromosome may be involved in each case.

Clinically, these cases present a different picture, varying from the very severely affected patients of Stalder *et al.*, and Weller *et al.*, to the relative paucity of symptoms of the case of Pitt *et al.*, and of our case.

In our case, we have identified the missing D-group chromosome as a No. 15. We have been unable to determine which chromosome was missing in the C-group, since at present there is no way, either by measurements or by labelling studies, to identify the individual autosomes in this group. Nor was it possible to establish which one of the two chromosomes involved in the translocation provided the centromere to the translocation chromosome.

If the centromere came from the C-group chromosome, which might have given it a part of its short arm too, we do not know how much, if any, of the No. 15's long arm is missing.

If instead the centromere came from the No. 15 chromosome, the patient is then monosomic for the short arm and the centromeric region of a C-group chromosome. The translocation chromosome has been tentatively identified by its total grain count and by its labelling pattern.

The possibility of a minimal loss of chromosomal material, an acentric fragment being translocated on some other chromosome where its presence is not obvious, cannot be excluded; this hypothesis would presuppose a three-break mechanism. Since no dicentric chromosomes were observed, it is clear that a centromere must have been lost, presumably with some loss of adjacent genetic material. The clinical picture, with its paucity of symptoms, is compatible with the possibility of a minimal loss of genetic material. A chance association of the chromosomal abnormality with the clinical findings, cannot, of course, be ruled out, unless more cases with the same cytogenetical and clinical abnormalities are found.

Summary

A translocation involving C- and D-group chromosomes has been found in a child with psychomotor retardation and multiple malformations. The D-group chromosome involved has been identified as an early replicator, presumably No. 15, by autoradiographic analysis. The Y chromosome in the patient, his father, and brother is almost the size of a chromosome 15, and is quite late replicating. Genetic marker studies are essentially uninformative.

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