A Family with D/D Translocation

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Balanced D/D translocation carriers appear to be normal in most instances (Court-Brown *et al.*, 1966; Giannelli and Howlett, 1966). In some cases, however, a variety of congenital malformations has been reported, such as Klinefelter's syndrome (Lejeune, Turpin, and Decourt, 1960), little secondary sex development (Walker and Harris, 1962), malformation of the spine (de Grouchy *et al.*, 1963), arrested hydrocephalus accompanied by mental retardation and spastic diplegia (Jagiello, 1963), and hydrocele and azoospermia (Yunis *et al.*, 1964). Dekaban (1966) described a family with transmission of a D/D translocation and a high incidence of mental retardation. A D/D translocation was found in a patient with Down's syndrome; in that case, the patient had a modal number of 46 and a trisomy 21 (Zergollern *et al.*, 1964). It has also been suggested as a cause of familial non-disjunction (Hamerton, Giannelli, and Carter, 1963), and was found in association with chronic granulocytic leukaemia (Engel *et al.*, 1965).

When appearance and measurements of the D group chromosomes were used as criteria for identifying the chromosomes involved in reciprocal translocation, chromosomes 13 and 15 (Walker and Harris, 1962; Hamerton *et al.*, 1963), and 14 and 15 (Lejeune *et al.*, 1960; de Grouchy *et al.*, 1963; Zergollern *et al.*, 1964) were suggested as the chromosomes of origin. Using ³H-thymidine labelling and autoradiography, Yunis *et al.* (1964), Giannelli and Howlett (1966), Dekaban (1966), and Tiepolo *et al.* (1967) assumed that the chromosomes involved in reciprocal translocation were a late and an intermediate replicator, probably Nos. 13 and 14. The purpose of this paper is to describe a family in which a D/D translocation chromosome was

transmitted through three generations without signs of mental retardation or of physical abnormalities in any member of the family; identification of the D chromosomes involved in the reciprocal translocation was attempted with ³H-thymidine labelling and autoradiography.

Case History of Propositus

A 10-month-old Caucasian female infant was admitted to hospital for failure to thrive. Physical examination revealed that she was in the third centile for weight and height. The muscles were flaccid and the joints were hyperextensible. The laboratory tests, including complete blood count, urine analysis, blood sugar, urea nitrogen, serum protein electrophoresis, calcium, phosphorus, electrolytes, and pH, were within normal limits. Chest and skull x-rays and intravenous pyelogram were normal. While in hospital, the child ate well and gained 900 g. At the age of 20 months she was found to be in the 50th centile for height and over the 90th centile for weight.

Family Studies

The patient has an older sister who is apparently normal. The first pregnancy of the mother resulted in miscarriage. There was no history of mental retardation in the family through four generations. The pedigree of the family is shown in Fig. 1.

Physical examinations, blood grouping, haptoglobin typing, serum protein electrophoresis, and leucocyte alkaline phosphatase determinations were carried out on the propositus and 6 members of 4 generations of the family. No abnormalities were observed.

The result of the dermatoglyphic examinations are shown in Table I. A wide *aid* angle and *t'* were observed in several members of the family.

Chromosome Studies

Chromosome studies using the method of Moorhead *et al.* (1960) were carried out on short-term peripheral

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FIG. 1. Pedigree of Family G.

blood cultures obtained from the propositus and 6 members of the family. The selected metaphases were examined under oil immersion. 35 mm. photographs were taken with a Zeiss photomicroscope, and the films were projected with a 3M Filmac 100 Reader-Printer (3M—Minnesota Mining and Manufacturing Co., St. Paul, Minnesota) to facilitate exact counting of the chromosomes. The chromosomes were then cut out from enlarged prints and arranged according to the Denver nomenclature.

The propositus, her mother, and grandmother were found to have a balanced D/D translocation. Results of the karyotyping are summarized in Table II.

Autoradiographic Studies

The pattern of DNA synthesis in the chromosomes obtained from peripheral blood cultures was studied by ^3H -thymidine labelling and autoradiography; the labelled metaphases were followed through the W to Z intervals of the synthesis (S) period (German, 1964).

Methods. Seven hours before termination of the short-term peripheral blood cultures, ^3H -thymidine

TABLE I
DERMATOGLYPHS OF FAMILY G

Patient and Family	Left and Right Hand	atd Angle	Interdigital Patterns		Finger Patterns					Thenar Pattern	Hypothenar Pattern
			3 IS	4 IS	1	2	3	4	5		
Mother	L	37° (t)	VL	VL	W	W	UL	UL	UL	O	O
	R	47° (t')	VL	O	W	RL	UL	UL	UL	O	O
Propositus	L	47° (t')	DL	DL	W	UL	UL	UL	W	O	O
	R	47° (t')	DL	O	W	UL	RL	W	UL	O	O
Sister	L	46° (t')	O	DL	W	W	W	W	W	O	O
	R	50° (t')	DL	O	W	W	UL	W	W	O	O
Maternal aunt	L	38° (t)	DL	VL	UL	A	UL	UL	UL	O	O
	R	34° (t)	DL	O	W	RL	UL	UL	UL	O	O
Grandmother	L	53° (t')	DL	O	UL	RL	UL	UL	VL	O	O
	R	56° (t')	DL	O	W	RL	A	UL	UL	O	O

atd, angle formed by line drawn from the most lateral triradius (a) to the most distal palmar triradius (t, t', or t'') to the most medial triradius (d); IS, interdigital space; DL, distal loop; VL, vestigial loop; O, open field; W, whorl; UL, ulnar loop; RL, radial loop; A, arch.

TABLE II
SUMMARY OF CHROMOSOME ANALYSIS OF THE PROPOSITUS AND HER FAMILY

Pedigree No.	Members of Family	Chromosome Counts			Karyotype Interpretation
		< 45*	45	46	
IV.1	Father	2		10	Normal
IV.2	Mother	2	11		Balanced translocation, D/D
V.3	Propositus	4	86		Balanced translocation, D/D
V.2	Sister	3		11	Normal
IV.3	Maternal aunt	1		10	Normal
III.2	Grandmother	5	24		Balanced translocation, D/D
II.2	Great-grandfather	4		21	Normal

* Random loss.

(obtained from Schwartz Bioresearch, Inc.,* as a sterile aqueous solution, specific activity 6.0 Ci/mmmole, conc. 1 Ci/ml.) was added to the medium for a final concentration of 1 μ Ci/ml. After 3 hours, the cultures were treated with colchicine.

The chromosomes were prepared by the technique described by Moorhead *et al.* (1960). The slides were coated with Kodak Nuclear Track Emulsion Type NTB-3, and after 9 days of incubation at 4°C. they were developed with Kodak D-19 developer. They were then stained with Giemsa's stain, and scored for metaphases containing readily distinguishable late-labelling X chromosomes. The location of each metaphase on the slide was recorded and photographed, and photographic prints were prepared. After removing the grains with Kodak Farmer's Reducer, the slides were restained, the metaphases relocated and rephotographed, and prints made with a 3M Filmac 100 Reader-Printer. The matching prints were then paired and examined.

Results. Of 103 labelled and photographed metaphases scored, 55 were selected for examination using the following criteria: (1) presence of a late-labelling X chromosome, indicating that this metaphase had been exposed to ^3H -thymidine during the W to Z interval of the S period; (2) a chromosome complement of 45; (3) absence of overlap between the chromosomes of groups A and D. The results are summarized in Tables III and IV.

Discussion

Though the combination of labelling patterns of the D chromosomes not involved in the translocation varies (Table IV), a classification of the 13-15 group chromosomes is possible, since the majority of metaphases out of a large number examined will follow a distinct DNA replication pattern (German, 1964; Giannelli, 1965; Giannelli and Howlett, 1966). The end of the DNA synthesis (S) period in which the chromosomes complete their replication was divided by German (1964) into six intervals: U, V, W, X, Y, and Z. In most instances, the No. 13 pair replicates the middle and distal portions of the long arms during the X interval and was referred to by German (1964) as late replicator; by Giannelli (1965) as chromosomes with labelling pattern A. The No. 14 pair replicates portions of its DNA (short arms and centromeric regions) also during the X interval (German: intermediate replicator; Giannelli: labelling pattern B). The No. 15 pair completes its synthesis during the U interval (German: early replicator; Giannelli: labelling pattern C).

In our study, most of the metaphases showing the translocation chromosome contained one No. 13,

one No. 14, and two No. 15 chromosomes (Tables III and IV; Fig. 2 and 3). The translocation chromosome resembled morphologically a No. 3 chromosome, but the labelling differed from that of the two chromosomes of pair No. 3, and resembled that of the No. 13 and 14 chromosomes. It was a large metacentric chromosome, in most metaphases heavily labelled over the distal and middle portion of one arm, which is consistent with the labelling pattern of the No. 13 chromosomes. The other arm was labelled in most metaphases around the centromeric region, suggesting a No. 14 chromosome. These observations indicate that the translocation chromosome was a t(13q14q) chromosome (Chicago Conference, 1966), i.e. the result of a translocation of the long arms of a No. 13 and No. 14 chromosome.

Concurrent translocation and non-disjunction were observed in some families, and a causal relation between them was suggested (Hamerton *et al.*, 1963; Yunis *et al.*, 1964). Richards and Stewart (1965), however, found only one balanced D/D translocation carrier in about 900 mongols, and suggested that the occurrence was fortuitous. In our family, studied through 4 generations, non-disjunction was not observed.

In cases in which the D/D translocation is accompanied by clinical manifestations, their variety may be explained by the fact that either different chromosomes are involved in the translocation or that the deleted portions of the short arms vary in size; or the D/D translocation may have no aetiological connexion with the clinical signs, since the minute deletions of the short arm may have been buffered by the individual genetic constitution.

In most reports, it was not established which chromosomes of the 13-15 group were involved in the D/D translocation or whether these chromosomes were identical in all cases. Identification has been attempted by chromosome measurements and by autoradiographic studies. The five cases examined so far by autoradiography (Yunis *et al.*, 1964; Giannelli and Howlett, 1966; Dekaban, 1966; Tiepolo *et al.*, 1967) and our case, suggest that the No. 13 and No. 14 chromosomes were involved in the translocation, though there might have been differences in the size of the deletions.

In the family described by Yunis *et al.* (1964), neither phenotypic abnormalities nor mental retardation were present, but diminished fertility and repeated miscarriages were reported. Giannelli and Howlett (1966) analysed the D group chromosomes in a phenotypically normal woman by autoradiography as well as chromosome measurements. In the family reported by Dekaban (1966), a high

* Orangeburg, New York.

TABLE III
DISTRIBUTION OF COUNTED GRAINS IN 55 METAPHASES

Metaphase No.	Number of Chromosomes	Number of Grains					
		X Chromosomes	Arms of D/D Chromosome	No. 3 Chromosome*	D Chromosomes†		
					No. 13	No. 14	No. 15
1	45	10	10 2	7 15	5	2	2 0
2	45	65‡	13 4	22 27	30‡	10	3 1
3	45	37	15 4	14 15	12		1 2 2
4	45	37	12 5	18 28	11	7 6	0
5	45	39‡	16 5	13 16	18	13	4 3
6	45	31‡	10 3	6 6	10	7‡	0 1
7	45	16	13 5	12 8	15	5	4 3
8	45	47‡	17 9	28‡ 26‡	20‡	11	0 3
9	45	45‡	12 5	24‡ 32‡	19‡	12	0 2
10	45	11	5 2	5 6	5	5	0 0
11	45	28‡	9 3	9 11	12	7	1 5
12	45	31‡	15 4	11 14	14	7	0 0
13	45	16‡	9 3	12 7	8 7	6 3	
14	45	27‡	13 3	5 2		3 7	1 0
15	45	28‡	11 3	2 1	9	6	1 0
16	45	24‡	12 2	4 7	10	3	1 0
17	45	5	2 1	6 0	1		0 0 0
18	45	32‡	8 2	12 13	7	6	1 0
19	45	14	4 1	1 5	12		2 1 0
20	45	28‡	7 3	7 8	5 7	2 3	
21	45	20‡	14 5	11 13	11		0 0
22	45	43‡	10 2	2 5	13	17	2 0
23	45	37‡	17 6	6 8	13	6	2 0
24	45	8‡	7‡ 3‡	7‡ 6‡	12		0 0 0
25	45	35‡	13 5	7 8	12	8	1 0
26	45	24‡	10 0	8‡ 8‡	9	2	1 0
27	45	30‡	12 3	18‡ 22‡	9‡	5	0 0
28	45	34‡	8 3	2 3		5	2 0 0
29	45	28‡	3 0	7 3	3		0 0 0
30	45	34‡	7 3	14‡ 9	14‡	5	1 0
31	45	25‡	10 4	5 8	9	5	0 0
32	45	28‡	11 4	8 5	12	5	0 0
33	45	22‡	7 4	6 7	6	3	0 0
34	45	28‡	12 1	5 3	12	6	1 0
35	45	31	8 4	1 3	11	3	1 1
36	45	27‡	11 5	6 8		2 8	1 1
37	45	35‡	14 8	7 9	8		0 0 0
38	45	40‡	13 4	5 7	8	5	0 0
39	45	34‡	6 5	11 12	7 6	5	0 0
40	45	40	15 2	8 12	8	7	0 0
41	45	90‡	20‡ 9‡	20‡ 29‡	15‡	16‡	0 0
42	45	48‡	23 11	12 15	17	5	2 0
43	45	30	14 2	1 4	9		1 1 0
44	45	17	7 1	3 5		4 6	1 0
45	45	48‡	8 3	12 13	7	5	0 0
46	45	10	5 0	8 3	7	2	0 0
47	45	60‡	3 1	2 3		1	0 0 0
48	45	13	6 3	4 6	5	3	0 0
49	45	12	4 2	9 17	8		0 0 0
50	45	32‡	8 2	6 13	7	5	2 0
51	45	7	7 2	3 4		2	0 0 0
52	45	13	4 1	1 1		2 3	0 0
53	45	15	3 0	3 6		2	1 1 0
54	45	14	7 4	9 8	7	6 6	0 0
55	45	41‡	5 2	4 4	7	2	0 0

* Grain count was not divided between the two arms because labelling occurred mostly in the centromeric region.

† Classified according to their labelling patterns (see text).

‡ Approximate number; exact count not possible because of overlap of grains.

TABLE IV
LABELLING PATTERNS OF FOUR D GROUP CHROMOSOMES NOT INVOLVED IN TRANSLOCATION

Combination of Labelling Patterns	Number of Metaphases
1 No. 13 + 1 No. 14 + 2 No. 15	34
1 No. 13 + 3 No. 15	8
1 No. 14 + 3 No. 15	4
2 No. 14 + 2 No. 15	4
1 No. 13 + 2 No. 14 + 1 No. 15	2
2 No. 13 + 2 No. 14	2
2 No. 13 + 1 No. 14 + 1 No. 15	1
Total	55

incidence of mental retardation was observed and family studies revealed repeated miscarriages. In the case studied by Tiepolo *et al.* (1967), the D/D translocation carrier had Klinefelter's syndrome. In our family, there were neither phenotypic abnormalities nor mental retardation; there was one miscarriage.

We believe that there are no characteristic clinical signs for the t(13q14q) translocation, but additional autoradiographic studies of D/D translocation carriers will be necessary to come to a conclusion.

FIG. 2. Autoradiographic labelling of chromosomes from a karyotype of the propositus (No. 10 in Table III). Upper row: labelled chromosomes. Lower row: the same chromosomes after grain reduction.

Summary

A D/D translocation chromosome transmitted through 3 generations was studied by ^3H -thymidine labelling and autoradiography. The results suggested that it was a $t(13q14q)$ chromosome.

Clinical, laboratory, cytogenetic, and dermatoglyphic data of the family were presented. Neither congenital abnormalities nor mental retardation were observed. Non-disjunction did not occur in any member of the family.

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REFERENCES

- Chicago Conference (1966). Standardization in Human Cytogenetics. Birth Defects, Original Article Series, II: 2. The National Foundation March of Dimes, New York.
- Court-Brown, W. M., Buckton, K. E., Jacobs, P. A., Tough, I. M., Kuenssberg, E. V., and Knox, J. D. E. (1966). Chromosome studies on adults. Eugenics Laboratory Memoirs No. XLII, p. 23. Cambridge University Press, London.
- Dekaban, A. S. (1966). Transmission of a D/D reciprocal translocation in a family with high incidence of mental retardation. *Amer. J. hum. Genet.*, **18**, 288.
- Engel, E., McGee, B. J., Hartmann, R. C., and Engel-de Montmollin, M. (1965). Two leukemic peripheral blood stemlines during acute transformation of chronic myelogenous leukemia in a D/D translocation carrier. *Cytogenetics*, **4**, 157.
- German, J. L. (1964). The pattern of DNA synthesis in the chromosomes of human blood cells. *J. Cell Biol.*, **20**, 37.
- Giannelli, F. (1965). Autoradiographic identification of the D (13-15) chromosome responsible for D_1 trisomic Patau's syndrome. *Nature (Lond.)*, **208**, 669.
- , and Howlett, R. M. (1966). The identification of the chromosomes of the D group (13-15) Denver: An autoradiographic and measurement study. *Cytogenetics*, **5**, 186.
- de Grouchy, J., Mlynarski, J. C., Matoteaux, P., Lamy, M., Deshaies, G., Benichou, C., and Salmon, C. (1963). Syndrome polydyspondylique par translocation 14-15 et dyschondroostéose, chez un même sujet. Ségrégation familiale. *C. R. Acad. Sci. (Paris)*, **256**, 1614.
- Hamerton, J. L., Giannelli, F., and Carter, C. O. (1963). A family showing transmission of a D/D reciprocal translocation and a case of regular 21-trisomic Down's syndrome. *Cytogenetics*, **2**, 194.
- Jagiello, G. M. (1963). Familial 13-15 translocation abnormality (Denver Classification) associated with one case of cerebral palsy. Preliminary report. *New Engl. J. Med.*, **269**, 66.

FIG. 3. Autoradiographic labelling of groups 1-3, 13-15, and X chromosomes from 4 karyotypes (Nos. 10, 17, 29, and 41 in Table III). Upper row: labelled chromosomes. Lower row: the same chromosomes after grain reduction. Asterisk points to translocation chromosome.

- Lejeune, J., Turpin, R., and Decourt, J. (1960). Aberrations chromosomiques et maladies humaines. Syndrome de Klinefelter XXY à 46 chromosomes par fusion centromérique T-T. *C. R. Acad. Sci. (Paris)*, **250**, 2468.
- Moorhead, P. S., Nowell, P. C., Mellman, W. M., Battips, D. M., and Hungerford, D. A. (1960). Chromosome preparations of leukocyte cultures from human peripheral blood. *Exp. Cell Res.*, **20**, 613.
- Richards, B. W., and Stewart, A. (1965). D/D translocation. *Lancet*, **1**, 1076.
- Tiepolo, L., Fraccaro, M., Hultén, M., and Lindsten, J. (1967). Double aneuploidy [46, XXY, D-, D-, t(DqDq)+]. *Ann. Génét.*, **10**, 114.
- Walker, S., and Harris, R. (1962). Familial transmission of a translocation between two chromosomes of the 13-15 group (Denver Classification). *Ann. hum. Genet.*, **26**, 151.
- Yunis, J. J., Alter, M., Hook, E. B., and Mayer, M. (1964). Familial D~D translocation. Report of pedigree and DNA replication analysis. *New Engl. J. Med.*, **271**, 1133.
- Zergollern, L., Hoefnagel, D., Benirschke, K., and Corcoran, P. A. (1964). A patient with trisomy 21 and a reciprocal translocation in the 13-15 group. *Cytogenetics*, **3**, 148.