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Specific chromosome aberrations in ataxia telangiectasia

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Summary. Cytogenetic observations on seven cases of ataxia telangiectasia are presented. The aberration frequency was found to be increased in all of them with a specificity for the involvement of the D-group chromosomes in rearrangements. Clones of cytogenetically abnormal cells were observed in the lymphocytes of three cases and in the cultured skin fibroblasts of two cases, again with a specificity for D-group involvement. G-banding shows that chromosome 14 is frequently involved in rearrangements in clone cells and that the band 14q12 may be a highly specific exchange point. The significance of lymphocyte clones with a proliferative advantage *in vivo* is discussed. Cytogenetic studies of the parents and sibs of these cases are also reported.

The occurrence of spontaneous chromosome changes in the lymphocytes of patients with ataxia telangiectasia was first reported by Hecht and his colleagues in 1966. Since then many reports clearly show the emergence of clones of cytogenetically marked lymphocytes which may constitute a large proportion of the total lymphocytes of the patient (Gropp and Flatz, 1967; Goodman *et al*, 1969; Haerer *et al*, 1969; Hecht and Case, 1969; Lisker and Cobo, 1970; Pfeiffer, 1970; Schmid and Jerusalem, 1972; Schuler *et al*, 1972; Cohen *et al*, 1973; Hecht *et al*, 1973; Bochkov *et al*, 1974; Harnden, 1974a). More recently, it has become apparent that the involvement of chromosomes in the rearrangements found in these clones is not random (Cohen *et al*, 1973; Harnden *et al*, 1973; Hecht *et al*, 1973; Bochkov *et al*, 1974; Harnden, 1974a).

In addition to the progressive cerebellar ataxia and the oculocutaneous telangiectases, from which the disease derives its name, patients with ataxia telangiectasia may show a variety of other features which have aroused considerable interest (Harnden, 1974a). Defects of the immunological system usually include a diminished cellular immunity and in many cases IgA is often either completely absent or present only at a very low level. The common occurrence of serious respiratory tract infections, which often cause the death of the patient at an early age, may be a consequence of this immunological defect. Similarly, the high incidence of malignant disease, particularly, but not exclusively of the reticuloendothelial system (Kersey *et al*, 1973) may at least in part be a result of disturbed functioning of the immunological system. However, it also seems probable that the high incidence of malignant disease may be related in some way to the occurrence of spontaneous chromosome aberrations since in other instances of chromosomal instability these are also associated with a susceptibility to malignant disease (German, 1973).

In this report cytogenetic observations on seven patients with ataxia telangiectasia are presented. Cases 1, 2, 6, and 7 were briefly reported by Harnden (1974a). The clinical features of case 5 have been reported by Cunliffe *et al* (1974). The other two are completely new cases.

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Case histories

Case 1. The proposita, a girl of 16 years of age when her chromosomes were first studied by us in 1972, had a history of unsteadiness on her feet since she started walking. At the age of 8 years she was reported to have difficulty in coping at a normal school although she was considered to be 'quite intelligent'. Conjunctival telangiec-

tases were first recorded at the age of 9. Her IgA was first studied at age 11 and was noted to be towards the lower limits of normal. Subsequent studies confirmed her IgA level to be low normal (44% of standard normal serum).

Her ataxia progressed until at the age of 12 she required a wheelchair; she also developed involuntary movements of the face and early pes cavus. The diagnosis of ataxia telangiectasia was considered firmly established before the first cytogenetic study. Repeated observations have shown no evidence of an haematological abnormality. She has one affected sister (case 2) and two normal brothers.

Case 2. The sister of case 1 was aged 25 when her chromosomes were first studied by us in 1972. Like her sister she showed unsteadiness on her feet from the time she first started to walk. At the age of 7 her ataxia and her difficulty in writing were causing problems at normal school. Her IQ was assessed at 66 (Stanford-Binet) at age 9, and it was noted that she was small for her age. She deteriorated rapidly with development of nystagmus, tremor, and grimacing. Her ataxia was recognized to be of the cerebellar type and she soon required a wheelchair. At the age of 13, after two years at a special school she was regarded as ineducable.

Conjunctival telangiectases were not recorded until she was 18, at the same time that they were observed in her sister, but may have been present earlier. Also at this time her IgA level was found to be below the normal level (16% of standard normal serum). Her haematological picture has been tested routinely on several occasions and found to be normal although, when a very detailed study was carried out, very rare abnormal lymphocytes were noticed but these were not considered to be evidence of a leukaemic process.

Case 3. A girl aged 16 in 1972 was first brought to our attention because she attended the same special school and lived in the same small town as cases 1 and 2. No relationship was found between the two families but detailed investigation proved difficult. Cerebellar ataxia was diagnosed at the age of 2½ years and a marked development of conjunctival telangiectases was recorded at the age of 13, although the age at which they were first noticed is not known. She has never been independent and has always required help with activities such as dressing and bathing. Both the ataxia and the telangiectasia are progressing and she is now almost totally confined to a wheelchair. At the age of 10 she was said to be of normal intelligence, although her attainments were retarded due to physical handicap. Her IgA level is within normal limits. She has one older unaffected sister.

Case 4. A boy, aged 4 when first studied by us in 1973, was noted to have muscular incoordination at the age of 1 year, and required a complete dental clearance at the age of 3 years. The ataxia is more evident in the lower limbs, although he has difficulty holding a pencil. Telangiectases were noted in both sclerotics at the age of 4 years. Immunoglobulin studies have shown a complete and specific absence of IgA on two separate occasions. He is considered to be of normal intelligence and is coping well at a normal school.

He has two sibs; an older sister who is normal and a younger brother who has a confirmed diagnosis of cystic fibrosis.

Case 5. This boy came to our attention in 1973 at the age of 7 years when a malignant lymphoma was removed from his right lung. He had a history of recurrent respiratory tract infections for which he had been treated over a period of two years. He had suffered from anorexia, weight loss, and weakness for four months immediately before our studies. He had ataxia of the upper limbs and his speech was slurred. His eyes showed oculomotor apraxia and nystagmus on lateral gaze. Telangiectatic vessels were seen both on the conjunctivae and on the ears. His serum IgA and IgG levels were both found to be low. (IgA 6.0 g/l, normal 0.6–2.2 g/l; IgG 3.6 g/l normal 6.0–15.0 g/l.)

He had an older sister who died in 1964 of recurrent pulmonary infections and has an older healthy brother. This patient subsequently died following radiotherapy.

Case 6. Case 6 and her sister, case 7, were originally brought to our attention in 1971, by Dr Luis Vassallo (St Julians, Malta), who referred them to the Galton Laboratory for chromosome studies. This patient, aged 12 in 1971, was normal as a baby but showed neurological signs of the disease during the fourth year of life. When seen in 1971 she had severe cerebellar ataxia, and marked ocular and cutaneous telangiectases. She suffered from recurrent severe respiratory tract infections and had no detectable IgA.

Case 7. The younger sister of case 6 was 9 years of age in 1971. She showed signs of the condition later than her sister; during the sixth year of life. Ocular telangiectases were apparent in 1969, but she did not appear to be susceptible to infections in the same way as her sister, although no IgA could be detected. The parents of cases 6 and 7 are both in good health and there is no evidence of consanguinity.

Materials and methods

Peripheral blood lymphocytes were cultured by incubating 0.4ml of whole heparinized blood with 4ml Ham's F10 (Flow Laboratories), 0.5ml bovine serum (Flow Laboratories), penicillin (100 μg/ml), streptomycin (100 μg/ml), and 0.05ml phytohaemagglutinin (Wellcome) in a water bath at 37° C. Chromosome preparations were made after two to three days. A poor response to phytohaemagglutinin has been reported in some cases of ataxia telangiectasia (Mcfarlin and Oppenheim, 1969) and estimates of blastic transformation and mitotic index were made on two of the patients using non-autologous serum and are given in Table I. While the number of dividing cells was reduced in all cases, it was not difficult to find an adequate number of mitoses to analyse.

Routinely, 0.5ml of 0.02% colchicine was added to the

TABLE I

Subject	Blastic Transformation (%)			Mitotic Index (%)		
	Mean	Group Mean	SD	Mean	Group Mean	SD
Case 6	55.3	55.0	±3.0	1.0	1.4	±1.1
Case 7	54.7			1.9		
Mother	65.2	69.7	±6.1	3.7	4.9	±1.6
Father	74.2			6.2		
Control	66.7	74.5	±8.9	3.5	4.0	±0.9
Control	82.4			4.6		

The difference between cases and parents, and cases and controls are significant at $P < 0.01$.

cultures for 60 min before harvesting and the cells treated with 0.075 mol/l KCl for 10 min before fixing in 3:1 methanol-acetic acid fixative. Air-dried preparations were routinely stained with orcein. G-banded preparations were made using the technique of Gallimore and Richardson (1973) which involves incubation in 2 × SSC at 60° C followed by a mild trypsin treatment before staining with Giemsa.

Skin fibroblasts were cultured by the method described by Harnden (1974b). Skin biopsies were cut into small pieces and immobilized under glass coverslips in 5 cm plastic Petri dishes (Nunclon). Cultures containing 70% Ham's F10 (Flow Laboratories) 10% tryptose phosphate broth (Difco) and 20% fetal calf serum (Flow Laboratories) with penicillin (100 μg/ml) and streptomycin (100 μg/ml) were incubated in a humidified incubator at 37° C. Subcultures were made using a trypsin-versene mixture and 1:2 dilutions. All cultures were examined at early passage levels. After the cultures had been pretreated for 60 min with colchicine, the cells were brought into suspension using trypsin-versene mixture and then handled in the same way as lymphocytes.

The orcein-stained cells were fully analysed down the microscope. In the case of lymphocytes, 100 cells were examined from each blood sample while in the case of cultured fibroblasts, 50 or more cells were examined from each of the patients except case 1, where poor growth allowed analysis of only 12 cells.

Aberrations were scored as (1) chromosome or chromatid gaps and breaks; (2) rings, dicentrics, and fragments; (3) abnormal monocentric chromosomes, and (4) clonal rearrangements. The results are expressed as the number of cells containing one or more such aberrations. A clone is defined as three or more cells with the same structural aberration. Where a cell contains aberrations of more than one type it is included in all appropriate categories.

TABLE II

CHROMOSOMES OF LYMPHOCYTES IN CASES OF ATAXIA TELANGIECTASIA

Date of Examination	No. of Cells	Chromosome Count					Total Normal	Cells with:			Clone Cells	Total Abnormal
		<45	45	46	47	>47		Gaps and Breaks	Rings, Dicentrics, and Fragments	Non-clonal Rearrangements		
Case 1, F, born 11.7.56												
4.2.72	100	3	6	91	0	0	84	9	4	4	1	16
10.7.73	100	3	9	87	1	0	61	15	5	15	8	39
8.1.74	100	2	4	94	0	0	68	12	5	11	12	32
2.7.74	100	7	12	80	0	0	50	10	1	11	38*	50
Case 2, F, 24.6.47												
13.3.72	100	4	20	76	0	0	15	10	17	17	80	85
10.7.73	100	6	11	82	1	0	14	5	8	13	80†	86
8.1.74	100	8	23	69	0	0	17	13	20	30	71‡	83
2.7.74	100	27	24	47	2	0	24	6	17	24	69**	76
Case 3, F, 10.7.56												
29.9.72	100	3	6	90	1	0	78	5	4	18	0	22
10.7.73	100	4	4	92	0	0	85	8	1	6	0	15
Case 4, M, 5.5.68												
22.1.73	100	3	7	88	1	1	65	15	5	20	0	35
15.1.74	100	5	4	90	1	0	81	7	3	11	0	19
Case 5, M, 1.6.66												
6.7.73	100	5	3	92	0	0	46	12	5	16	29	54
Case 6, F, 12.7.59												
23.9.71	100	1	11	84	3	1	80	7	3	13	0	20
Case 7, F, 21.6.62												
23.9.71	100	2	11	87	0	0	75	15	1	11	0	25

* Sub-clone of three cells – G – D + dicentric.
† Sub-clone of 13 cells – B + C.
‡ Two sub-clones; five cells – G – mar2 + dicentric; three cells – G – C + dicentric.
** Sub-clone of two cells – G – mar2 + dicentric.

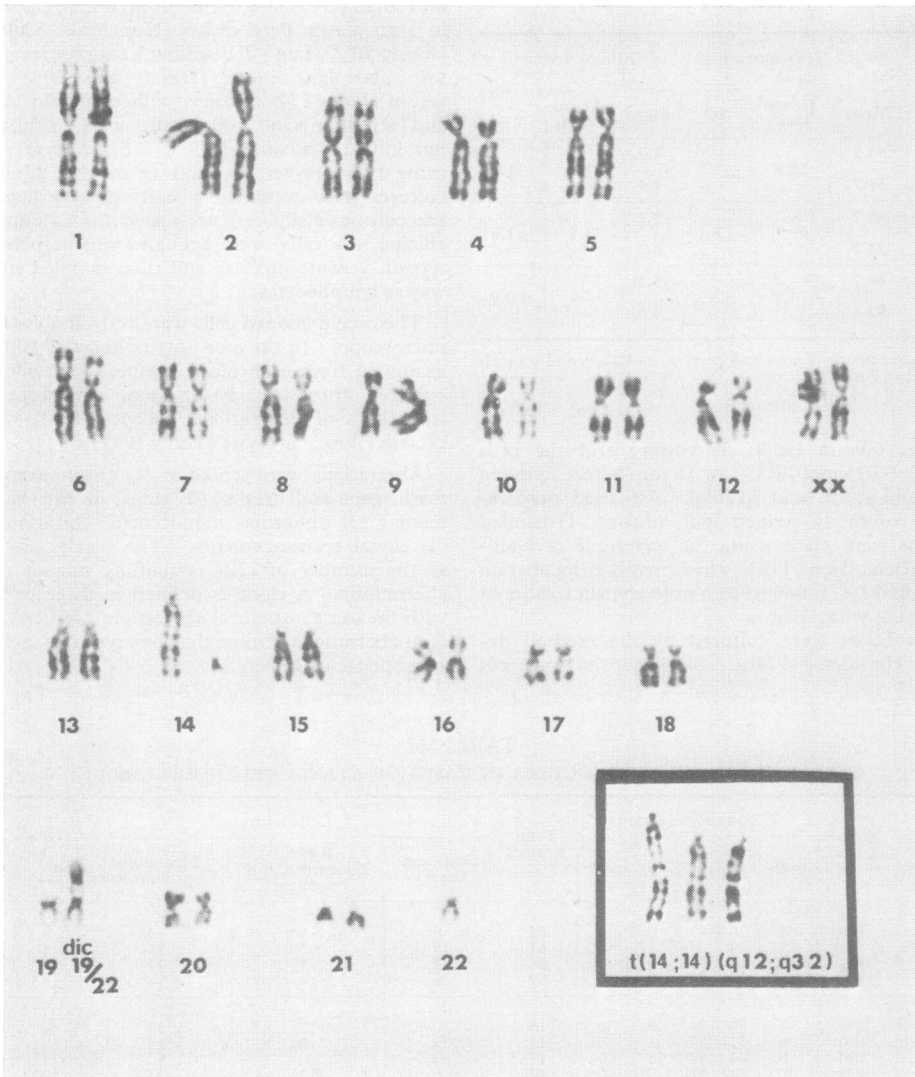

FIG. 1. G-banded karyotype of clone cell from case 1 with clonal translocation (14;14)(q12;q32). In addition, there is a dicentric chromosome formed from one chromosome 19 and one chromosome 22. This latter is a random change. The inset shows three clonal markers from other cells of the clone.

Results

Lymphocytes. Peripheral blood lymphocytes from two of the cases (1 and 2) were examined on four occasions, two (3 and 4) were examined twice, while case 5, who died, and cases 6 and 7 who returned to their home in Malta, were examined only once (Table II). In each case an excess of abnormal cells was found. In most instances there was an excess in all three classes of aberrations as compared with normal aberration rates for the two laboratories, and as reported from other laboratories (Court Brown *et al*, 1966).

In case 1 the number of abnormal cells increased from 16% to 50% during the two-year period of observation. A clone of cells with a reciprocal translocation between two D-group chromosomes was recognized for the first time in the second sample when it accounted for 8% of the cells

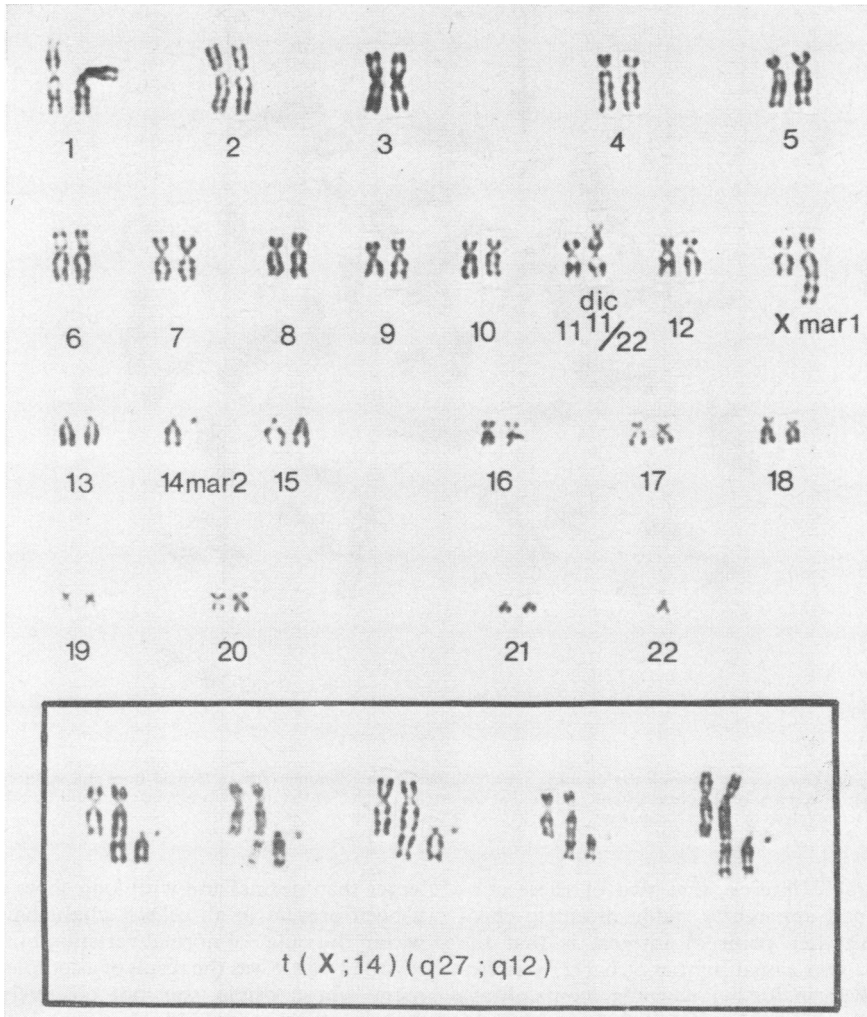

FIG. 2. G-banded karyotype of a clone cell from case 2 with clonal translocation (X;14)(q27;q12). In addition there is a dicentric chromosome formed from one chromosome 11 and one chromosome 22. This latter is a random change. The larger marker is referred to as mar 1 and the smaller as mar 2.

examined (Fig. 1). In retrospect, it was recognized that at the time of the first observation a year earlier, 1% of the cells already belonged to this clone. The clone progressed over the two-year period to reach 38% in the most recent observation. G-banding revealed that the clonal karyotype was 46,XX,t (14;14)(q12;q32).

In case 2, the total number of abnormal cells observed on the four occasions varied only slightly. On the first occasion the 85 abnormal cells included a clone of 80 cells with a reciprocal translocation between a D-group chromosome and a C-group chromosome (Fig. 2). This total declined slightly in the subsequent observations and there was a proportionate increase in the number of normal cells. G-banding showed that chromosome 14 was again involved and that the breakpoint on No. 14 was the same as one of those involved in the rearrangement in the clone in case 1. The clonal karyotype was found to be 46,X,t(X;14)(q27;q12). In addition, three different subclones-that is, groups of cells which contained a specific rearrangement additional to the clonal markers-were seen, though only one of these was observed on two different occasions (Fig. 3). It

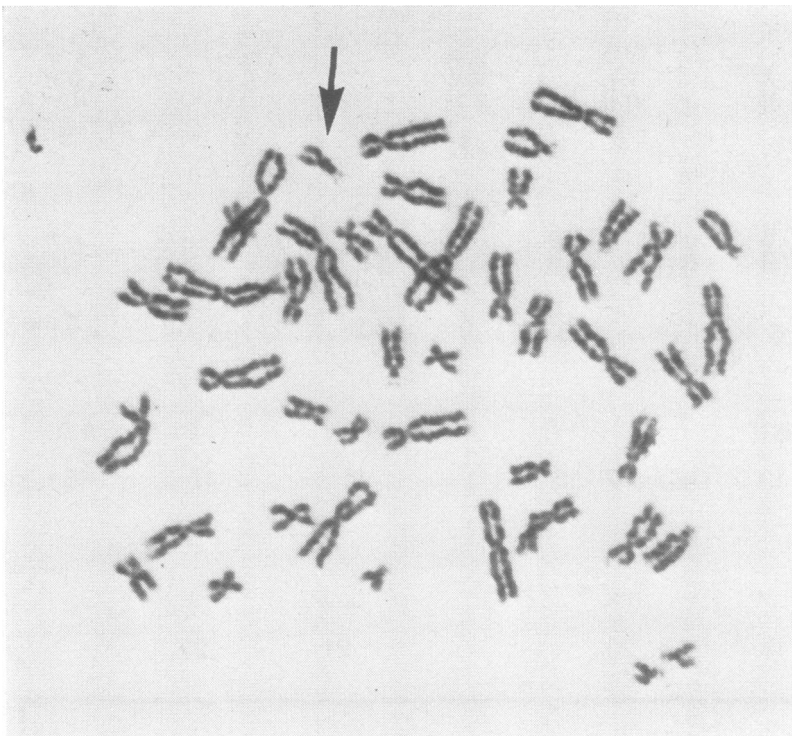

FIG. 3. Subclone cell from case 2 showing the dicentric subclonal marker chromosome (arrow) formed from the smaller of the original clonal markers (mar 2) and a G-group chromosome. Note that the satellite region of the marker is not involved and that the region 14q12 must again have been involved in the rearrangement.

is of considerable interest that two of these subclones contained apparently stable dicentric chromosomes. Another point of interest is that the smaller of the two clonal markers (mar 2) was frequently involved in further rearrangements always involving the broken end. Figure 3 shows that the dicentric in one of the subclones is derived from mar 2 and a G-group chromosome, while Fig. 4 illustrates a dicentric formed from mar 2 and a chromosome No. 11.

Case 3 showed an overall excess of aberrations on each occasion, though there were fewer cells with unstable and stable structural rearrangements on the second occasion. No evidence of clone formation was found. Case 4 was similar but, though no clone was positively recognized, two cells out of 100 in the first sample showed a D/D exchange of the type found in case 1. In case 5 all classes of aberrations were again increased and a clone involving 29 out of 100 cells was observed. In orcein-stained preparations this was characterized by the presence of an abnormal D-like chromosome slightly larger than normal and with long short arms. Had it been present in all cells it might have passed as within the range of normal variation but G-banding revealed that it was the result of a complex rearrangement whose origin was not positively identified, though it seems probable that one chromosome 14 was involved (Fig. 5). Since this patient died, it was not possible to make further preparations to confirm the nature of the rearrangement. Cases 6 and 7 showed an increase particularly of aberrations involving rearrangements, though no clone formation was observed.

The lymphocytes of all five sets of parents were examined as were those of the two brothers of cases 1 and 2, a sister of case 3, and a brother and sister of case 4. The overall incidence of cells with abnormalities is slightly higher than expected; this is particularly true of the families of cases 3 and 4 (Table III).

Fibroblasts. Fibroblast cultures were grown from six of the cases (Table IV). The cells of cases

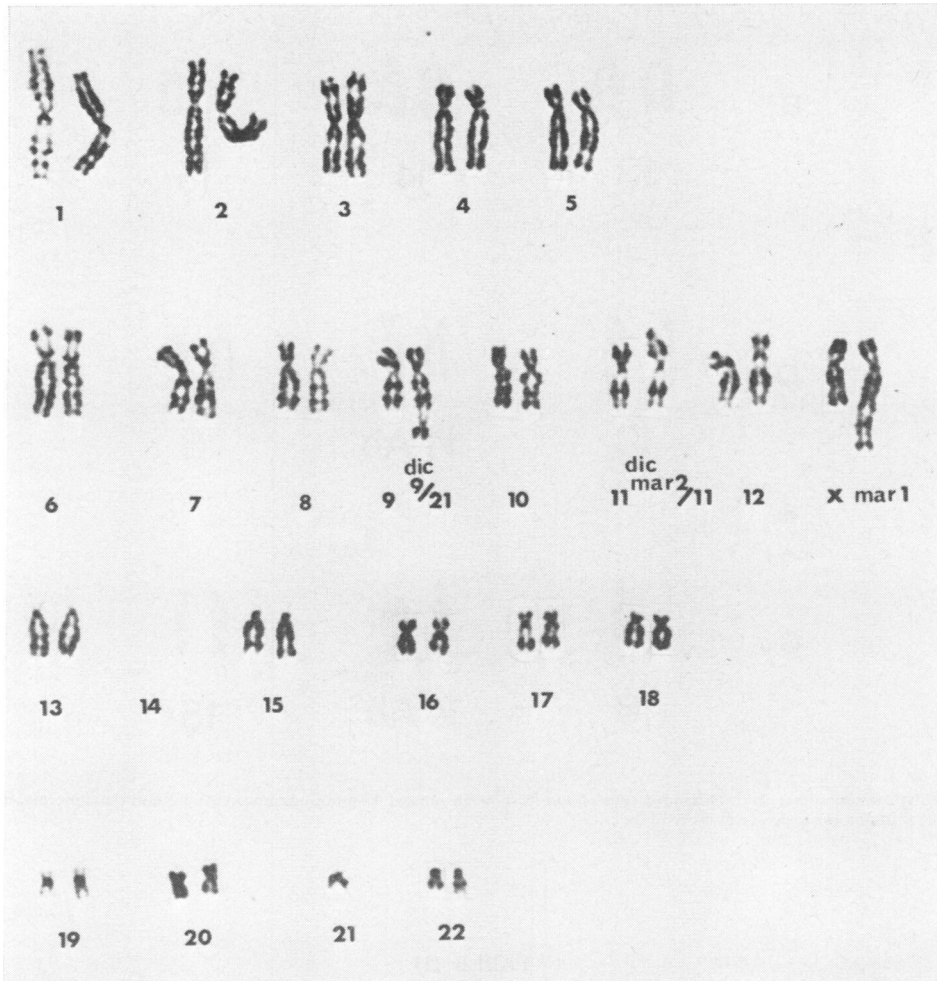

FIG. 4. G-banded karyotype of a clone cell of case 2 showing clonal marker (mar 1) and further rearrangements. One dicentric is derived from a chromosome 9 and a chromosome 21, and the other from the other clonal marker (mar 2) and a chromosome 11. The other chromosome 14 is missing but there are only 45 centromeres.

1, 2, 6, and 7 grew very slowly. Cells of case 6 did not progress beyond the primary culture and no chromosome preparations were obtained, and in case 1 no cells grew past the second subculture although the cultures were uncontaminated and were treated in the normal way. The cultures from cases 3, 4, and 5 grew normally. In case 1, only a few cells could be examined but these were all grossly abnormal. All the cells from case 2 were also abnormal. All but one of the cells clearly belonged to a clone of cells with two abnormal chromosomes apparently derived from an F/D translocation. There were in addition an excess of non-clonal rearrangements and of gaps and breaks. In case 7 a small clone (five out of 100 cells) was recognized with long short arms on a C-group chromosome. In the other three cases there was an excess of abnormal cells but no specific aberrations were noted at the first observation. Subsequent extensive studies on cells cultured from case 3 showed the development of large clones with a complex karyotype and a proliferative advantage in culture. Further studies of these fibroblasts including banding studies are in progress and will be reported elsewhere.

Fibroblast studies on six of the parents also yielded surprising results (Table V). In five out of

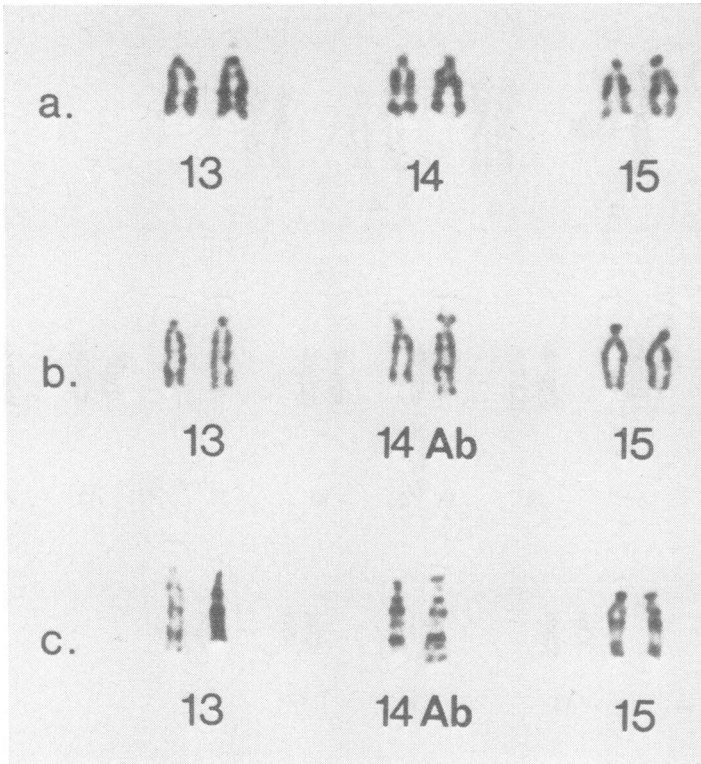

FIG. 5. D-group chromosomes from G-banded cells of case 5 showing normal D-group chromosomes (a) and the abnormal chromosome probably derived from a chromosome number 14 (b, c).

TABLE III

CHROMOSOMES OF LYMPHOCYTES IN RELATIVES OF ATAXIA TELANGIECTASIA PATIENTS

Subject	No. of Cells	Chromosome Count					Normal	Cells with			Total Abnormal
		<45	45	46	47	>47		Gaps and Breaks	Rings, Dicentrics, and Fragments	Non-clonal Rearrangements	
Father of 1 and 2	100	2	3	95	0	0	94	1	2	4	6
Mother of 1 and 2	100	2	4	94	0	0	96	3	1	0	4
Brother of 1 and 2	100	2	3	94	1	0	92	7	0	1	8
Brother of 1 and 2	100	1	2	97	0	0	92	8	0	0	8
Father of 3	100	3	3	92	2	0	86	7	2	5	14
Mother of 3	100	6	6	85	3	0	88	8	0	5	12
Sister of 3	100	0	2	97	1	0	88	6	2	4	12
Father of 4	100	2	5	92	1	0	87	11	0	2	13
Mother of 4	100	2	6	91	1	0	90	7	1	3	10
Sister of 4	100	8	8	84	0	0	93	6	0	1	7
Brother of 4*	100	2	3	94	1	0	89	8	0	5	11
Father of 5	100	1	3	96	0	0	95	3	1	1	5
Mother of 5	100	2	6	92	0	0	94	4	0	2	6
Father of 6 and 7	100	0	3	94	2	1	98	1	0	1	2
Mother of 6 and 7	100	0	7	91	2	0	95	3	0	2	5

* Cystic fibrosis.

TABLE IV

CHROMOSOMES OF FIBROBLASTS IN PATIENTS WITH ATAXIA TELANGIECTASIA

Case (sex)	Age at Examination (yr)	No. of Cells	Chromosome Count					Total Normal	Cells with:			Clone Cells	Total Abnormal
			<45	45	46	47	>47		Gaps and Breaks	Rings, Dicentrics, and Fragments	Non-clonal Rearrangements		
1 (F)	16	12	8	2	2	0	0	0	6	1	12	0	12
2 (F)	25	50	5	4	41	0	0	0	8	1	8	49	50
3 (F)	16	50	1	3	45	1	0	26	11	3	15	0	24
4 (M)	5	50	4	7	34	3	2	18	17	13	14	0	32
5 (M)	7	50	0	1	47	2	0	37	6	1	6	0	13
7 (F)	9	100	3	13	82	1	1	69	17	2	14	5	31

TABLE V

CHROMOSOMES OF FIBROBLASTS IN RELATIVES OF ATAXIA TELANGIECTASIA PATIENTS

Subject	No. of Cells	Chromosome Count					Total Normal	Cells with:			Clone Cells	Total Abnormal
		<45	45	46	47	>47		Gaps and Breaks	Rings, Dicentrics, and Fragments	Non-clonal Rearrangements		
Father of 1 and 2	50	3	0	46	1	0	45	2	0	3	0	5
Mother of 1 and 2	50	7	7	36	0	0	24	3	0	11	7/5/3*	26
Father of 5	50	10	8	31	1	0	30	7	1	2	16/3†	20
Mother of 5	50	1	4	45	0	0	14	6	1	1	32	36
Father of 6 and 7	100	0	3	97	0	0	91	1	0	2	6	9
Mother of 6 and 7	100	0	4	71	24	1	69	1	1	2	25/3†	31

* Three clones.
† Two clones.

TABLE VI

CHROMOSOMES INVOLVED IN NON-CLONAL REARRANGEMENTS*

Chromosome Group	Number of Chromosomes															χ^2
	Case 1				Case 2				Case 3		Case 4		Case 5	Total in Group		
	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	1st	2nd	1st	Obs.	Exp.†	
1	0	2	1	0	1	2	3	1	1	0	3	1	2	17	28	4.32
2	2	0	3	1	1	1	2	1	3	0	1	1	5	21	26	0.96
3	1	1	0	1	4	4	6	1	2	0	3	3	1	27	22	1.14
B	0	1	4	2	5	0	8	4	3	2	3	3	3	38	40	0.10
C	0	8	7	5	8	8	14	7	3	5	8	3	3	79	128	18.75
D	3	3	3	2	5	5	9	6	8	2	7	5	6	64	33	29.12
16	0	0	1	1	0	0	0	2	1	0	1	1	0	7	10	0.90
E	2	1	0	0	1	1	4	3	3	0	4	0	1	20	19	0.05
F	0	0	1	0	2	2	1	1	2	0	0	0	4	13	16	0.56
G	1	3	3	1	7	9	20	3	1	1	0	0	1	50	12	120.33
Total	9	19	23	13	34	32	67	29	27	10	30	17	26	336		176.25

Total $\chi^2 = 176.25$; $p < 0.001$.
* Total number of cells analysed was 100 in each case but only cells with 46 centromeres were included in this analysis.
† Expected in proportion to chromosome length as given in the report of the Paris Conference (1971).

the six cultures a relatively high incidence of aberrations was observed and in each of these one or more clones of cells were recognized. These were, most often, apparently the result of a rearrangement but, in the mother of cases 6 and 7, 25 cells out of 100 were found to have an extra chromosome similar to an F-group chromosome. Further studies are being carried out on these cells and on controls.

Discussion

It seems quite clear from these results that a high frequency of chromosome aberrations and rearrangements is a characteristic finding in cultured lymphocytes from cases of ataxia telangiestasia. The level of chromatid aberrations may be slightly elevated but not markedly so. The recognition of such chromosome changes in lymphocytes may be taken as an aid to the differential diagnosis in cases of ataxia.

A preliminary study of the chromosomes involved in rearrangements has been made using orcein stained peparations. An estimate was made of the number of instances in which chromosomes of particular groups were involved in rearrangement by totalling the number of occasions on which a chromosome of a particular group was apparently absent from a cell with 46 centromeres. Clonal rearrangements were excluded from the analysis. Table VI shows that the distribution of chromosomes involved in rearrangements, using this rather crude analysis, was not random ($\chi^2 = 176.25$; $P < 0.001$). The greatest contribution to the χ^2 comes from an excess of D-group and G-group chromosomes involved in rearrangements. The excess involvement of G group chromosomes is attributable apparently to one patient (case 2), suggesting some unusual feature of this patient. In the case of the D-group chromosomes, however, the excess is attributable to all the cases, suggesting that in this disease there is some predisposition for D-group chromosomes to be involved in rearrangements. Further studies using banded preparations may help to clarify this point. It should be noted, however, that in a consideration of 129 subjects with reciprocal translocations, Ford and Clegg (1969) found that there was an excess of exchanges involving D- and G-group chromosomes, but they also found an excess involvement of B- and E-group chromosomes. Jacobs *et al* (1974), on a smaller number of cases mostly ascertained through balanced carriers, concluded that the distribution of breakpoints was random.

There is, however, clear evidence from a study of the clones that have been recognized by different workers that the D group is preferentially involved in clone formation (Goodman *et al*, 1969; Hecht and Case, 1969; Pfeiffer, 1970; Schmid and Jerusalem, 1972; Cohen *et al*, 1973; Hecht *et al*, 1973; Bochkov *et al*, 1974; Harnden, 1974a) and, moreover that the band 14q12 or 3 is involved in most clones where adequate banding studies have been carried out (McCaw *et al*, 1975). This could suggest than an alteration at a specific locus or loci in this region confers on the cells some sort of advantage so that this particular clone of lymphocytes proliferates at an advantage over the other lymphocytes in the patient. Since there is no haematological disorder in those cases where clones have been found, it seems clear that there must be a decrease in the number of lymphocytes which do not have the clonal karyotype and also that the lymphocyte population as a whole is still responding to normal regulatory mechanisms. It is important to stress that these results do reflect the *in vivo* situation, whereas the fibroblast results could be affected by culture conditions. Patients with this syndrome have an increased susceptibility to lymphoid neoplasms and one case has now been reported of a chronic lymphatic leukaemia in which the leukaemic cells had the same karyotype as cells of a pre-existing clone (Hecht and McCaw, 1974). Further information on this point is, however, required before we can conclude that the clones in any way represent a premalignant population.

If, as is suggested by these results, a particular locus is involved preferentially in translocations it is important to consider mechanisms which could bring this about. It is possible, of course, that translocations occur randomly and that certain specific rearrangements are selected out. At the other extreme it is possible, though unlikely, that the observed high frequency of certain rearrangements is not the result of clonal proliferation but of an accumulation of the products of frequent highly specific translocations. This latter possibility is made less likely because of the presence of subclones. Specific rearrangements could be due to the action of a virus which causes specific breakages to occur (*cf*, adenovirus 12; McDougall, 1971) or it could be that the particular loci have an abnormal level of metabolic activity which predisposes to breakage. There is, however, no evidence to support either hypothesis at present.

The susceptibility of patients with ataxia telangiectasia to develop malignant disease is now well documented (Kersey *et al*, 1973). While the immunological deficiencies of these patients may play an aetiological role in the development of tumours, it now seems probable that the susceptibility may also be determined by either spontaneous or induced

damage to the genetic material of the cells. Patients with other syndromes which have an increased incidence of spontaneous chromosome aberrations (Bloom's syndrome and Fanconi's anaemia) also have an increased risk of developing malignant disease (German, 1973).

There is clear evidence that these patients are unusually sensitive to the harmful effects of ionizing radiation and our case 5 died with evidence of severe local radiation damage after post-operative radiation, following removal of a malignant lymphoma from the right lung (Cunliffe *et al*, 1974). There are two reports of unusual sensitivity to radiation induced chromosome damage *in vitro* (Higurashi and Conen, 1973; Rary *et al*, 1974).

The chromosome changes observed in fibroblasts are also difficult to interpret at present. Similar abnormalities have been observed in fibroblasts cultured from skin biopsies of patients with Fanconi's anaemia (German and Crippa, 1966), porokeratosis of Mibelli (Taylor *et al*, 1973), basal cell naevus syndrome (Happle and Hoehn, 1973), and xeroderma pigmentosum (German *et al*, 1973). However, in our own laboratory we are now finding that clones of cells may be found in approximately 25% of cell lines established from normal individuals. Moreover, some, at least, of these clones have a proliferative advantage. While it seems clear that they occur more commonly in individuals susceptible to cancer and that the cells from which the clones originated may be present *in vivo*, at least in some instances there may also be some interplay with medium factors which influence the growth of these cells. Further work on the clones observed in the ataxia telangiectasia patients and particularly the significance of clones in fibroblasts cultured from the parents is at present being carried out.

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