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Cytological Studies of Tumors, XLV.
The Short Arm Deletion of a G Group Chromosome
in a Patient with Chronic Myelogenous
Leukemia and in his Family¹⁾

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(With 4 Text-figures and 2 Tables)

Finding by Nowell and Hungerford (1960) of the Ph¹ chromosome in blood cultures of patients with chronic myelogenous leukemia (CML), as well-known by the partial long arm deletion of a G chromosome, has been widely noted as one of striking contributions to current cytogenetics of human leukemia. Indeed, this provided essential criteria for the understanding of pathogenetic properties of leukemic diseases in general and also for the differential diagnosis of CML. Current literature, however, reports the occurrence of Ph¹ negative CML. Tjio *et al.* (1966) showed that the Ph¹ negative CML patients responded poorly to chemotherapy and had shorter survivals in contrast to the Ph¹ positive population. On the other hand, Gunz *et al.* (1962) found an abnormal chromosome called Christchurch or Ch¹ chromosome specific to chronic lymphocytic leukemia (CLL). The Ch¹ chromosome has been recognized as the short arm deletion of a G group chromosome. Detailed investigations, however revealed that the Ch¹ was existent in non-leukemic healthy individuals of the same pedigree. This suggests it to be non-specific to leukemic cells. The chromosome abnormality similar to the Ch¹ has been recorded in several cases by Shaw *et al.* (1962), Migeon (1965), Teasdale *et al.* (1966), Ito and Makino (1966), Subrt *et al.* (1966), Abott (1966). It is problem that the short arm deleted chromosome in those cases is not always associated with diseases. It seems likely however that the regulation of hematopoietic function may in some way be controlled by one of the G chromosomes, in the light of the facts that Ph¹ chromosome is associated with CML and that leukemias occur in high frequency in Down's syndrome.

Recently we found a case of Ph¹ negative CML having the short arm deletion of one of the G group chromosomes. We investigated in some detail the chromosome constitution of this case, especially the relationship between the cytogenetic

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features of blood or bone marrow and the disease state, the mode of a familial transmission of the abnormal chromosome and the origin of that chromosome by means of autoradiography.

Material and Method

Venous blood, about 10 ml, was drawn into a heparinized syringe from the patient, and left at room temperature for 1 hour. The resultant plasma (1 ml) containing leucocytes was incubated with TC-109 medium (9 ml), calf serum (2 ml), and phyto-hemagglutinin (PHA-M, 0.2 ml) at 37°C for 3 days. At the same time the culture containing no PHA was prepared under the same situation.

Cultures for chromosome study were made according to the Moorhead method with minor modifications. Colchicine treatment was made for a final 1.5 hour of incubation. Following 20 minutes' treatment with a KCl-heparin hypotonic solution at 37°C, cells were fixed with acetic alcohol (1:3). Chromosome slides were prepared according to the routine air-drying technique, and stained with carbol-fuchsin for 1 hour.

For the autoradiographic study Eagle's medium was used, since TC-109 contains thymidine which dilutes the radio active thymidine incorporation: ³H-thymidine with a specific activity of 5.0 c/mM was added to the culture at a final concentration of 1μc/ml, 7 hours before harvesting the cells. Sakura NR-M2 liquid emulsion was used for coating. Slides were developed with Minidol for 5 minutes at 20°C after being exposed for 1 week at 4°C.

In addition to the blood cultures, chromosome studies were undertaken with bone marrow cells by means of direct and culture methods, as well as with skin cultures. The marrow cells cultured in TC-109 medium with 25% calf serum (without PHA) for 3 days at 37°C were air-dried. For direct air-drying, the marrow aspirates were incubated in TC-109 with 25% calf serum and 10⁻⁷M colchicine for 2 hours at 37°C. A piece of the skin, about 5 mm² in size, was obtained from the right arm of the patient under sterilized conditions, thoroughly minced and cultivated in TC-109 supplemented with 15 percent calf serum according to the plasma-clot technique. At the end of *in vitro* growth, about 2 months after culture, cells were harvested with trypsin following colchicine treatment for 1.5 hour. Then the cells were treated with hypotonic phosphate buffer solution, fixed with methanol-acetic acid and air-dried. Chromosomes of 5 siblings of the patient were investigated with blood cultures.

Case report: Male, age 43. In July, 1966, he had pains in the throat and the hypocondrium, and was diagnosed as a chronic myelogenous leukemia due to positive peroxidase reaction and a high white blood cell count (78,400). The count decreased gradually to 18,200 by May, '68 without any therapy. Serum alkaline phosphatase activity, as measured by a modification of the Kaplow's method, was 40%. On May 22, '68, he was hospitalized because of simultaneous occurrence of a stomach ulcer. On June 9 of the same year he gave rise to blastic crisis, and was immediately treated with daily administrations of 25 mg Prednisolone and 100 mg 6-mercaptopurine (6-MP). The patient died of cerebral hemorrhage on the frontal lobe on July 22, '68. Hematocrit was from 35 to 47 percent during a period from July, '66 to June, '68. At autopsy the spleen and the liver were extremely enlarged (spleen; 1180 g, liver; 2250g). Blood spots and small hemorrhage were recognized in the skin, epicardium, eye ball conjunctiva. In addition, cardiac lung, infiltration of leukemic cells into liver, spleen, lymph node around trachea and stomach erosion were recognized. The hematological data are shown in Table 1.

Table. 1 Hematological data of

	Peripheral blood			
	'66 7/13	'67 11/2	'68 2/1	'68 5/20
Leucocyte no.	78,400	21,200	17,400	18,200
Erythrocyte no. ($\times 10^4$)	425	366	480	460
Platelet no. ($\times 10^4$)	33		2	40
Myeloblast	1%	1	0	2
Promyelocyte	1	1	3	2
Myelocyte	17.5	25	17	12
Metamyelocyte	9.5	9	5	11
Staffcyte	28.5	11	14	9
Segmentocyte	40	38	47	55
Lymphocyte	3	5	10	5
Monocyte		2	2	3
Basophilic cell		7	2	
Eosinophilic cell		1		
Macroblast				
Normoblast				
Proerythroblast				
Mitosis				

Cytological findings

Chromosomes were studied in samples taken at 5 times in a period ranging from 11/2/67 to 6/17/68. In total, 211 metaphases coming from 55 bone marrow cells and 156 leucocytes were analyzed under microscope. Out of them 199 cells showed 46 chromosomes, while the remaining 12 had 45 chromosomes. The cells having 45 chromosomes showed random loss without specificity for cells. It seems that they may be artificially damaged cells in the course of treatments. Detailed karyotype analyses were made in 33 cells with 46 chromosomes and revealed that a short arm deletion occurred in one of the G group chromosomes, other chromosomes remaining unchanged (Fig. 1). Such an abnormal chromosome was detected in the leucocytes cultured either with or without PHA, as well as in the direct sample of bone marrow and cultured marrow cells without PHA. Further, 15 cells from skin cultures also showed the same chromosomal abnormality. Since this feature suggests this abnormality to be inheritable, a familial survey was undertaken (Fig. 3). As the parents of the propositus died, three non-leukemic healthy siblings were investigated chromosomally. The results indicated that younger brother and sister of the propositus were carriers of the abnormal chromosome, while the other younger sister was normal. The offspring of the younger brother, a carrier of the abnormal chromosome, had the same abnormality (Fig. 2), while that of the younger sister had a normal chromosome complement. Based on the above features it is most probable that the short arm deleted chromosome is not specific to the leukemic cells, or is not always associated with leukemia.

the patient under study

		Bone marrow		
'68 6/11	'68 6/17	'66 8/10	'67 10/23	'68 5/22
81,200	636,200			
377	392			
11				
64,5	80,5	1%	0,5	4,5
3,5	6	1	6	6,5
0,5	2	18,5	22	28,5
0,5	2,5	17	21,5	16,5
8	2	25,5	19	13,5
17	6	23,5	24,5	23,5
3				1
3				
1				
		0,5	5	2,5
		10,5	1	0,5
			0,5	2,5
				1

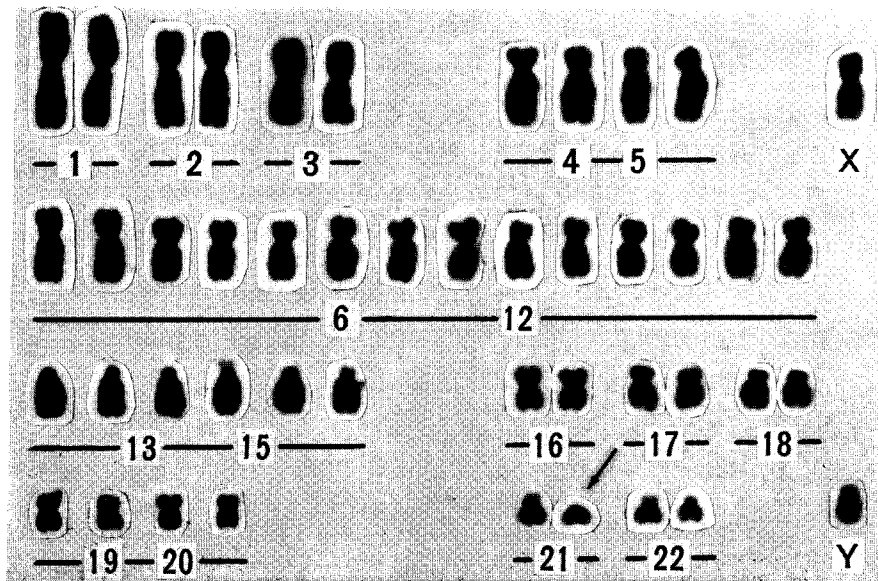


Fig. 1. Karyotype analysis from a cultured leucocyte of the propositus. The arrow indicates the abnormal chromosome.

DNA replication pattern was studied autoradiographically with blood cultures of the patient in a hope to identify this abnormal chromosome, no. 21 or no. 22 (Table. 2, Fig. 4). The investigation made it clear that the short arm

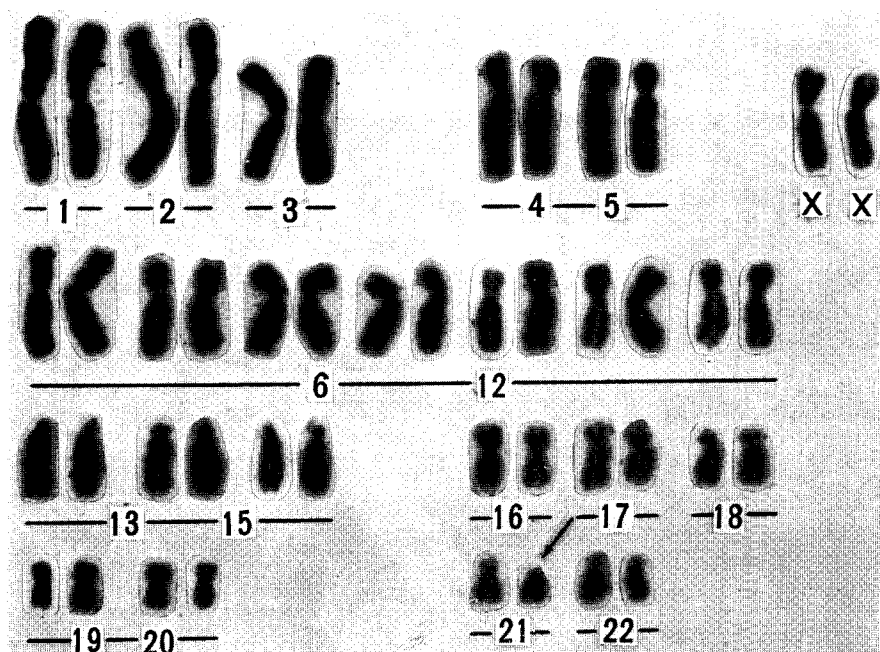


Fig. 2. Karyotype analysis from a cultured leucocyte of the patient's niece.

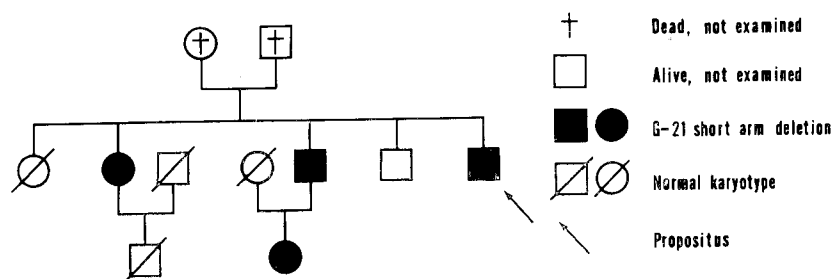


Fig. 3. Pedigree of the family under study.

deleted chromosome showed a relatively late DNA replication in the G group chromosomes. In addition, it is apparent that 2 chromosomes including a short arm deleted chromosome give a relatively later replication pattern, whereas the other 2 chromosomes early, perhaps the former being no. 21, and the latter no. 22.

On the fifth day after blastic crisis, the chromosomes were examined in direct and cultured bone marrow cells, and in cultured leucocytes. In all cases under examination, endoreduplication of the chromosomes was observed with about three percent mitotic rate.

Table. 2 The results of grain counts of G group chromosomes of the propositus

No. of cells	No. of G chromosomes with grains of	
	0~2	≥ 3
16	2	2 (D)
7	3	1 (D)
6	1	3 (D)
1	3 (D)	3
1	4 (D)	1

31 cells in total.

(D) indicates the involvement of a G chromosome with short arm deletion.

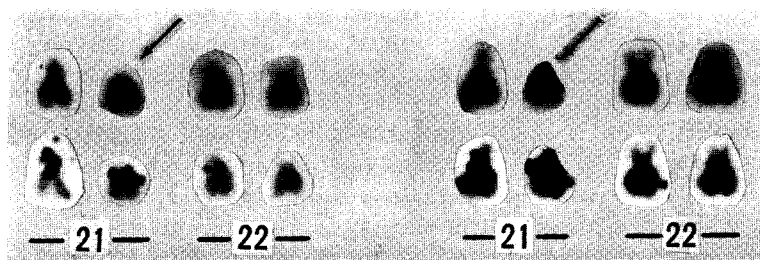


Fig. 4. Partial karyotypes showing G group chromosomes with the autoradiograms placed underneath, from two different cells of the propositus.

Discussion

Recent studies have demonstrated that there are apparently normal individuals showing a variation of the size and centromere position in one homologue of pair 16, and in the short arm of a member of pairs 13-15, or pairs 21-22, and with varying length of the Y chromosome. Court Brown *et al.* (1966) are of opinion that, because these changes appear unassociated with any harmful effects, it may be more proper to regard them as normal variations rather than as aberrations. The short arm deletion of a G chromosome observed in the patient studied here may be of a similar character to the above, on the basis of that a familial transmission of the abnormal chromosome has occurred without association with the disease in the same pedigree. It is also evident that this chromosome abnormality is a heritable character and was transmitted from one of the parents.

Ch¹ chromosome was first reported by Gunz *et al.* (1962) in a patient with CLL as well as in some healthy members of his family; it was designated by them as chromosome no. 21 by its morphological feature. A similar chromosome abnormality was found in patients with different diseases: Teasdale *et al.* (1966) in a CGL case, Subrt *et al.* (1966) in a case of Albright's syndrome, Migeon (1965) in a case with anomalous pulmonary venous return, Abott (1966) in healthy familial members, Ito and Makino (1966) and Shaw (1962) in 2 cases of Down's syndrome and some others. Particularly interesting is the fact that this type of the chromosome abnormality was found in 3 leukemic patients including the present case, together with 2 cases of Down's syndrome. A survey of such an abnormal chromosome in the natural population, and the association of this chromosome abnormality with leukemia remain as an important subject for future study.

Indeed, an exact identification of the chromosome with a short arm deletion in the G group is a matter of considerable difficulty with current technical procedures. Our DNA replication study has revealed that the abnormal chromosome here concerned synthesizes DNA relatively later among the G group chromosomes (Table 2). Gey (1966) and Back *et al.* (1967) showed that Ph¹ chromosome and the extra G chromosome of Down's syndrome synthesize DNA relatively later than the no. 22 chromosomes, and also than the other chromosomes of no. 21. Though this feature is not generally accepted, it is likely to see from the above bases that the short arm deleted chromosome may correspond to no. 21 as so in the Ph¹ of leukemia and the extra chromosome of Down's syndrome.

Fitzgerald *et al.* (1965) has mentioned that endoreduplication in leukemic cells may occur as a consequence of chemotherapy. Recently Fitzgerald (1966) made it clear that abnormal chromosomes except the Ph¹ chromosome were frequent in the blastic crisis of CML. In the course of blastic crisis of the present case, endoreduplication was observed in about three percent mitosis. It is questionable however in our case whether the endoreduplication is related to the blastic crisis or chemotherapy.

According to Tjio *et al.* (1966), Ph¹ negative CML cases are infrequent in occurrence and different in disease state from that of Ph¹ positive cases. Further they stated that Ph¹ positive patients were more sensitive for chemotherapy than Ph¹ negative patients, and the survival of Ph¹ positive CML cases was much longer than that of Ph¹ negative cases. The case here under study showed no Ph¹ chromosome at all, and the platelets number was 215 thousand/mm³. He died 23 months after an attack of the disease under no effect of chemotherapy.

Summary

The short arm deletion was observed in one of the G group chromosomes in cells from 3-day leucocyte cultures with and without PHA, and/or in bone marrow cells from direct specimens as well as after cultivation without PHA for 3 days. The same abnormality was found to occur in the somatic cells from skin cultures of the

patient. It was also found in his non-leukemic brother and sister. Autoradiographically, the short arm deleted chromosome was identified as no. 21 chromosome. Endoreduplication was observed in the course of blastic crisis.

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References

- Abott, C.R. 1966. The Christchurch chromosome. *Lancet* i: 1155.
- Back, F., P. Dörner, P. Baumann, and E. Olbrich 1967. Problems of chromosome autoradiography. Autoradiographic investigations of G-chromosomes in mongolism. *Humangenetik* 4: 305-319.
- Court Brown, W.M. 1964. Chromosomal abnormality and chronic lymphatic leukemia. *Lancet* i: 986-987.
- Fitzgerald, P.H. 1966. A complex pattern of chromosome abnormalities in the acute phase of chronic granulocytic leukemia. *J. Med. Genet.* 3: 258-264.
- Fitzgerald, P.H., and A. Adams 1965. Chromosome studies in chronic lymphocytic leukemia and lymphosarcoma. *J. Nat. Cancer Inst.* 34: 827-839.
- Gey, W. 1966. Untersuchungen über die DNS-Replikationsmuster der Chromosomengruppen 4-5, 13-15 und 21-22 an *in vitro* getzüchteten menschlichen Lymphocyten. *Humangenetik* 2: 246-261.
- Gunz, F.W., P.H. Fitzgerald, and A. Adams 1962. An anomalous chromosome in chronic lymphocytic leukemia. *Brit. Med. J.* ii: 1097-1099.
- Ito, N., and S. Makino 1966. A preliminary note on a case of trisomic Down's syndrome with a short arm deletion of a G group (21-22) chromosome. *Proc. Jap. Acad.* 42: 1207-1210.
- Moorhead, P.S., P.C. Nowell, W.J. Mellman, D.M. Battips, and D.A. Fitzgerald 1960. Chromosome preparations of leucocytes cultured from human peripheral blood. *Exp. Cell Res.* 20: 613-616.
- Migeon, B.R. 1965. Familial variant autosome: New human cytogenetic markers. *Bull Johns Hopkins Hosp.* 116: 396-402.
- Nowell, P.C., and D.A. Hungerford 1960. Minute chromosome in human chronic granulocytic leukemia. *Science* 132: 1497.
- Neu, R. L., J.C. Leao, and L.I. Gardner 1966. Short arm deletion in G-group chromosomes. *Lancet* ii: 390-391.
- Shaw, M.W. 1962. Familial mongolism. *Cytogenetics* 1: 141-179.
- Subrt, I., and V. Brychnač 1966. Chromosomes in Albright's syndrome. *Lancet* i: 549.
- Teasdale, J.M., J.R. Bensimon, and J.R. Miller 1966. Read at 36th Annual Meeting, Society for Pediatric Research, April 29, 1966, Atlantic City, New Jersey.
- Tjio, J.H., P.P. Carbone, J. Whang, and E. Frei 1966. The Philadelphia chromosome and chronic myelogenous leukemia. *J. Nat. Cancer Inst.* 36: 576-584.