Chromosome Studies in Familial Leukaemia*

P. H. FITZGERALD, P. E. CROSSEN, A. C. ADAMS, C. V. SHARMAN, and F. W. GUNZ

From the Cytogenetics Unit†, Christchurch Hospital, Christchurch, New Zealand

The finding of an inherited chromosome abnormality (Ch¹) in several members of a family, including two who had developed chronic lymphocytic leukaemia (Gunz, Fitzgerald, and Adams, 1962), led to the suggestion that this abnormality predisposed its carriers to the development of the disease, and that inherited cytogenetic abnormalities of this type might determine some instances of familial leukaemia. In an attempt to test this hypothesis we have now made chromosome investigations of a further 11 families with leukaemia, or leukaemia and a related neoplastic disorder, in two or more first-degree relatives.

Methods

In families showing a multiple occurrence of leukaemia and related disorders, we examined the chromosomes of all surviving individuals with leukaemia and sometimes of their normal relatives. If no chromosome abnormality was found in the leukaemic individuals, no further members of the family were examined, the justification of this approach being that an inherited chromosome abnormality causing increased risk of leukaemia in a family should normally be present in the leukaemic members.

The chromosome examinations were made on blood leucocytes cultured with phytohaemagglutinin according to the method of Moorhead, Nowell, Mellman, Battips, and Hungerford (1960), with minor modifications. Although in some individuals the cultured cells may have consisted of a mixture of normal and leukaemic lymphocytes, this was not considered to detract from the usefulness of the method because the inherited cytogenetic abnormalities being investigated would be expected to occur in all cells of a carrier.

The chromosomes were examined in approximately 30 metaphase figures from each individual, particular attention being given to gross abnormalities in chromo-

some structure. Detailed karyotype analyses were made of several cells from each patient.

Family Studies

Cw. Family. This is the initial family in which the abnormal Ch¹ chromosome was reported to occur (Gunz *et al.*, 1962). Two of the sibs developed chronic lymphocytic leukaemia: the second-born, a female aged 73, and the fourth born, a male who died recently, aged 67. The third sib died from carcinoma of the uterus in 1947 and was said to have pernicious anaemia. The father of these sibs died from lymphosarcoma when aged 60 in 1926.

The Ch¹ chromosome, an abnormal G group acrocentric in which apparently all of the short arm was missing (Fig. 1), was present in the 2 sibs with leukaemia and in 2 other sibs, a male now aged 75 and a female aged 63, neither of whom show any symptoms of leukaemia. The abnormal chromosome was also present in 2 of the 8 members of the succeeding generation who were examined. Ch¹ is clearly an inherited cytogenetic abnormality, as was further indicated by its occurrence in all blood cells examined and its presence in all skin cells from the leukaemic patients.

Te. Family. In this family 3 of 6 sibs developed leukaemia. Chronic lymphocytic leukaemia was diagnosed in the first-born, a female, when aged 64, and in the third-born, a male, at the age of 57. The second sib, a male, died of chronic granulocytic leukaemia when aged 47 in 1937. The mother died at the age of 56 from carcinoma of the uterus.

The chromosomes of the 2 sibs with chronic lymphocytic leukaemia and of the 2 surviving sibs without leukaemia were normal, apart from the presence of a dicentric chromosome and an acentric fragment in one cell from a leukaemic sib who had been treated with chlorambucil, and an acentric fragment in one cell from a normal sib. These unstable abnormalities are probably of secondary importance and there was no evidence of any inherited cytogenetic abnormality in this family.

F. Family. This sibship of 16 individuals included 3 sets of twins. Two male sibs developed chronic lymphocytic leukaemia: the sixth-born who died at 74 and the eleventh who was 67 when the disease was diagnosed. Other reports of malignant disorder in this family are uncertain.

Received September 20, 1965.

* The present investigation was supported in part by grant CA-3707 from the U.S. National Cancer Institute, National Institutes of Health.

† Supported by the Canterbury and Westland Division of the Cancer Society of New Zealand.

FIG. 1. Karyotype of a leucocyte metaphase from a male member of the Cw. family. The abnormal Ch^1 chromosome, from which apparently all of the short arm is absent, is indicated by an arrow.

The chromosomes of the surviving sib with leukaemia were examined. The first attempt to culture blood cells from this patient yielded few mitoses. The short arms of the small acrocentric chromosomes were not always clearly defined, and it appeared that an abnormality similar to the one in the Cw. family was present. However, better quality metaphases from two further cultures clearly showed 5 complete small acrocentric chromosomes. The suspected abnormality in the first preparation was due to misinterpretation of the relatively small size of the short arm of an unusually long Y chromosome present in the cells of this patient. Apart from this Y chromosome, the chromosomes were normal.

M. Family. In this family 2 members of the sibship of 12 had chronic lymphocytic leukaemia. The first-born, who died at 69 from this disease, had been successfully treated with radiotherapy for carcinoma of the cervix 12 years previously. A brother, the eighth-born sib, died at the age of 55 from chronic lymphocytic leukaemia. The fourth sib died at 29 from carcinoma of the uterus, and the sixth sib, also a female, died at 58 from malignant melanoma. The chromosomes of the female sib with leukaemia were found to be normal.

Bo. Family. A sibship of 15 comprised 8 males and 7 females whose birth order was not determined. Chronic lymphocytic leukaemia was diagnosed in 2 brothers both in their eighth decade. The mother, 1 brother, and 3 sisters were reported to have died from unspecified forms of cancer.

The chromosomes of one of the male sibs with leukaemia were examined and found to be normal apart from an unduly long Y chromosome.

Cr. Family. A sibship of 8 in which the second-born, a female, died from chronic lymphocytic leukaemia at the age of 68. A sister, the third sib, who developed a local reticulosarcoma in the right groin at the age of 60, and 4 years later was diagnosed as having polycythaemia vera, died at the age of 70 in 1964. Another sister, the seventh sib, developed lupus erythematosus in 1958 and large cell lymphosarcoma with leukaemic changes in 1963.

The chromosomes of cultured blood cells from the sib with polycythaemia and from the sib with lymphosarcoma were normal. Some marrow cells from the sib with polycythaemia vera showed an abnormally large C

group chromosome, while others were lacking a C group chromosome but had an additional minute. These chromosome abnormalities were clearly in the abnormal myeloid cells only, and were not inherited cytogenetic abnormalities.

W. Family. The third of 5 sibs, a male, was diagnosed as having chronic lymphocytic leukaemia at the age of 63, and a sister, the fourth sib, aged 62, has benign follicular lymphoma (Brill-Symmers disease). Their mother died at 70 from carcinoma of the stomach. The chromosomes of the sib with chronic lymphocytic leukaemia were found to be normal.

H. Family. A sibship of 6 in which the fourth-born, a male, developed chronic lymphocytic leukaemia at the age of 74, and the third sib, a female aged 76, has benign follicular lymphoma (Brill-Symmers disease). The chromosomes of the sib with chronic lymphocytic leukaemia were found to be normal apart from the presence of an abnormally large Y chromosome.

S. Family. The last born of 7 sibs, a male, was diagnosed as having chronic lymphocytic leukaemia at the age of 70. A brother, the fourth sib, died in 1909, aged 24, of leukaemia, probably the chronic granulocytic form. No chromosome abnormalities were evident in cultured leucocytes from the sib with chronic lymphocytic leukaemia.

Br. Family. A sibship of 11 including 5 males and 6 females of undetermined birth order. A female sib was diagnosed as having chronic lymphocytic leukaemia at the age of 67, and a brother died at 74 from chronic granulocytic leukaemia. The chromosomes of the sib with lymphocytic leukaemia showed no evidence of abnormality.

Ba. Family. A woman, one of 10 sibs, and her son, one of 2 sibs, developed chronic lymphocytic leukaemia at the age of 84 and 40 years, respectively. No abnormalities were found in the chromosomes of cultured leucocytes from both mother and son.

Tu. Family. In this Maori sibship of 9, including one half-sib, 4 definite cases and 1 possible case of acute leukaemia occurred during a five-year period. The children, 2 of whom were fraternal twins, developed symptoms at ages between 11 months and 6 years. A detailed report of this family has been presented elsewhere (Gunz, Fitzgerald, Crossen, Mackenzie, Powles, and Jensen, 1966). Chromosome examination of both parents and 3 of the affected sibs showed no evidence of inherited cytogenetic abnormalities.

Discussion

Twelve families with a multiple occurrence of leukaemia and related disorders have been investigated, and in only one, that reported originally

(Gunz *et al.*, 1962), was there any evidence of an inherited cytogenetic abnormality. To these may be added 3 further families in which no inherited chromosomal abnormality was found: one with chronic lymphocytic leukaemia in 2 brothers (Court Brown, 1964), another with 2 confirmed and probable cases of neonatal leukaemia in sibs (Campbell, Macafee, and Wade, 1962), and the third with 5 cases of acute leukaemia in 3 consecutive generations (Heath and Moloney, 1965). It is clear from these investigations that inherited cytogenetic abnormalities of a visible size are not a general factor determining familial leukaemia, especially those instances involving the chronic lymphocytic form. Likewise, the Ch^1 chromosome, in either an inherited or acquired form, is not a factor of general significance in the aetiology of chronic lymphocytic leukaemia as a whole, as is evident by the presence of this chromosome in only 2 of the 66 cases of chronic lymphocytic leukaemia and lymphosarcoma examined in this Unit (Fitzgerald and Adams, 1965, and unpublished data).

The absence of gross cytogenetic abnormalities in these families does not lessen the possibility that Ch^1 may predispose its carriers to chronic lymphocytic leukaemia. The association of two rare events, the Ch^1 chromosome and familial leukaemia, while not enough to indicate a relation between them, does at least raise this possibility, and to dismiss the association as being merely fortuitous (Court Brown, 1964) is as unjustified as any claim to an established relationship. The significance of the association will only be decided by the finding, or otherwise, of lymphatic disorder in other carriers of the Ch^1 chromosome or similar cytogenetic abnormalities. Thus, while inherited cytogenetic abnormalities of a visible size are not of general significance in familial leukaemia, the possibility cannot be excluded that inherited chromosome abnormality might be an important predisposing factor to leukaemia in some families.

The chromosomes of the patients with chronic lymphocytic leukaemia were normal in the sense that no gross abnormality could be detected. However, recent investigations of the lengths of small acrocentric chromosomes have shown a small but significant difference between normal persons and patients with chronic lymphocytic leukaemia, the chromosomes of the leukaemic patients being shorter (Fitzgerald, 1965). While the nature of this variation in length of the small acrocentric chromosomes is unknown, it is possible that some of it may represent inherited or acquired chromosome abnormality which is significant in determining the onset of chronic lymphocytic leukaemia. However, this

type of variation has been found in other conditions (Kallen and Levan, 1962), and must be investigated more fully.

There have been two other reports of abnormal small acrocentric chromosomes similar to Ch¹. Migeon (1965) described an apparent deletion of the short arm of a G group chromosome in a normal male and in his infant daughter with congenital cardiac abnormality, and Shaw (1962) reported finding an abnormal small acrocentric chromosome, which was closely similar to Ch¹, in a sibship of 5, 3 of whom were trisomy-21 mongols. The 2 normal sibs and one of the mongols in this second family carried the abnormal chromosome which they had inherited from their mother. The relationship, if any, of this abnormal chromosome to the trisomy-21 was not clear, but an effect on the disjunction of some of the small acrocentric chromosomes is not unlikely. While the multiple occurrence of trisomy-21 and absence of leukaemia in this family contrasts strikingly with the multiple leukaemia and absence of mongolism in the Cw. family, it should be noted that the precise identification of the abnormal chromosomes is unknown, and there is no evidence that the same chromosome pair is involved in the two families. At the same time a connexion between the two events is not beyond the limits of possibility, as is indicated by reports of associations between leukaemia and disjunctional errors, both multiple (Buckton, Harnden, Baikie, and Woods, 1961; Miller, Breg, Schmickel, and Tretter, 1961; Baikie, Buckton, Court Brown, and Harnden, 1961; Stewart, 1961; Thompson, Bell, and Little, 1963), and repeated (Lejeune, Berger, Haines, Lafourcade, Vialatte, Satge, and Turpin, 1963; Kiosoglou, Rosenbaum, Mitus, and Dameshek, 1964). However, the significance of these associations is not clear, and in this connexion it should be noted that none of the 66 patients with chronic lymphocytic leukaemia or lymphosarcoma examined in this Unit showed any congenital chromosome abnormality due to non-disjunction, nor was there any evidence of a tendency to chromosome non-disjunction in the families with multiple leukaemia.

An interesting finding in the present investigation was the abnormally long Y chromosome in male leukaemic patients from 3 of the families with multiple occurrences of leukaemia. It is difficult to see any significance in this, because many of the patients in the leukaemic families were females, and also the Y chromosome shows considerable variability in size. Unusually long Y chromosomes have been reported in normal persons (Bender and Gooch, 1961; and one unpublished observation in

this Unit), in Marfan's syndrome (Kallen and Levan, 1962), in a mongol man, and 4 normal relatives (Bishop, Blank, and Hunter, 1962), and in a variety of other abnormal conditions and in the normal relatives of some of them (Muldal and Ockey, 1961; Conen, Bailey, Allemang, Thompson, and Ezrin, 1961; van Wijck, Tjink, and Stolte, 1962).

Summary

An investigation of 12 families with a multiple occurrence of leukaemia and related disorders in first degree relatives showed that inherited cytogenetic abnormalities of a visible size were not a general factor determining familial leukaemia, especially those instances involving the chronic lymphocytic form. Nevertheless, the presence of an inherited abnormality in one of the families raises the possibility that inherited chromosome abnormality might be an important predisposing factor to leukaemia in some families.

REFERENCES

- Baikie, A. G., Buckton, K. E., Court Brown, W. M., and Harnden, D. G. (1961). Two cases of leukaemia and a case of sex-chromosome abnormality in the same sibship. *Lancet*, **2**, 1003.
- Bender, M. A., and Gooch, P. C. (1961). An unusually long human Y chromosome. *ibid.*, **2**, 463.
- Bishop, A., Blank, C. E., and Hunter, H. (1962). Heritable variation in the length of the Y chromosome. *ibid.*, **2**, 18.
- Buckton, K. E., Harnden, D. G., Baikie, A. G., and Woods, G. E. (1961). Mongolism and leukaemia in the same sibship. *ibid.*, **1**, 171.
- Campbell, W. A. B., Macafee, A. L., and Wade, W. G. (1962). Familial neonatal leukaemia. *Arch. Dis. Childh.*, **37**, 93.
- Conen, P. E., Bailey, J. D., Allemang, W. H., Thompson, D. W., and Ezrin, C. (1961). A probable partial deletion of the Y chromosome in an intersex patient. *Lancet*, **2**, 294.
- Court Brown, W. M. (1964). Chromosomal abnormality and chronic lymphatic leukaemia. *ibid.*, **1**, 986.
- Fitzgerald, P. H. (1965). Abnormal length of the small acrocentric chromosomes in chronic lymphocytic leukemia. *Cancer Res.*, **25**, 1904.
- , and Adams, A. (1965). Chromosome studies in chronic lymphocytic leukemia and lymphosarcoma. *J. nat. Cancer Inst.*, **34**, 827.
- Gunz, F. W., Fitzgerald, P. H., and Adams, A. (1962). An abnormal chromosome in chronic lymphocytic leukaemia. *Brit. med. J.*, **2**, 1097.
- , Crossen, P. E., Mackenzie, I. S., Powles, C. P., and Jensen, G. R. (1966). Multiple cases of leukemia in a sibship. *Blood*. In the press.
- Heath, C. W., Jr., and Moloney, W. C. (1965). Familial leukemia: five cases of acute leukemia in three generations. *New Engl. J. Med.*, **272**, 882.
- Kallen, B., and Levan, A. (1962). Abnormal length of chromosomes 21 and 22 in four patients with Marfan's syndrome. *Cytogenetics*, **1**, 5.
- Kiosoglou, K. A., Rosenbaum, E. H., Mitus, W. J., and Dameshek, W. (1964). Multiple chromosomal aberrations in a patient with acute granulocytic leukemia associated with Down's syndrome and twinning. *Blood*, **24**, 134.
- Lejeune, J., Berger, R., Haines, M., Lafourcade, J., Vialatte, J., Satge, F., and Turpin, R. (1963). Constitution d'un clone à 54

- chromosomes au cours d'une leucoblastose congénitale chez une enfant mongolienne. *C.R. Acad. Sci. (Paris)*, **256**, 1195.
- Migeon, B. R. (1965). Familial variant autosomes: new human cytogenetic markers. *Bull. Johns Hopk. Hosp.*, **116**, 396.
- Miller, O. J., Breg, W. R., Schmickel, R. D., and Tretter, W. (1961). A family with an XXXXY male, a leukaemic male, and two 21-trisomic mongoloid females. *Lancet*, **2**, 78.
- Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A. (1960). Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.*, **20**, 613.
- Muldal, S., and Ockey, C. H. (1961). Muscular dystrophy and deletion of Y chromosome. *Lancet*, **2**, 601.
- Shaw, M. W. (1962). Familial mongolism. *Cytogenetics*, **1**, 141.
- Stewart, A. (1961). Aetiology of childhood malignancies: congenitally determined leukaemias. *Brit. med. J.*, **1**, 452.
- Thompson, M. W., Bell, R. E., and Little, A. S. (1963). Familial 21-trisomic mongolism coexistent with leukemia. *Canad. med. Ass. J.*, **88**, 893.
- van Wijck, J. A. M., Tjldink, G. A. J., and Stolte, L. A. M. (1962). Anomalies in the Y chromosome. *Lancet*, **1**, 218.