Chronic Myelocytic Leukemia in Identical Twins and a Sibling

By George K. Tokuhata, Charles L. Neely and Dorothy L. Williams

The occurrence of multiple cases of chronic myelocytic leukemia in a family is rare and, to our knowledge, has not been reported in identical twins. An analysis of such data may provide evidence for the existence of genetic factors in human leukemia. This is a report of the development of chronic myelocytic leukemia in identical twins and in one of their brothers. (Figure 1). In addition, epidemiological, hematological and cytogenetic data are presented.

Case Reports

The proband twin (II-5) is a 64 year old white male who was admitted to Lexington-Henderson County Hospital, Tennessee on April 20, 1964 because of progressive weakness of 3 years' duration. His height was 69 inches and weight 144 pounds. WBC was 39,000/mm³ with a granulocytosis (Table 1) and bone marrow was hyperplastic. There was no lymphadenopathy. The liver was palpated 11 cm. below the right costal margin and the spleen 15 cm. below the left costal margin. These findings were consistent with chronic myelocytic leukemia. He developed pancytopenia six months after initiation of busulfan (168 mg total dose) and expired from gastrointestinal hemorrhage.

Co-twin (II-4) of the proband was admitted to the same hospital for acute pulmonary edema on April 23, 1964. He was 68 inches tall and weighed 157 pounds. WBC was 53,000/mm³ with a granulocytosis (Table 1) and bone marrow was hyperplastic. The liver was palpated 4 cm. below the right costal margin and the spleen 8 cm. below the left costal margin. These findings were also consistent with chronic myelocytic leukemia. He responded to digitalis and diuretics and was treated with busulfan (716 mg. total dose) with remission lasting 31 months. During remission, leukocyte alkaline phosphatase values were normal.

The oldest living brother (II-2) of the twins is 68 years of age and has never sought medical care except for a back injury. Hematological screening test in June, 1964 revealed a WBC of 20,000/mm³ with a granulocytosis. He was asymptomatic and had received no therapy. Fifteen months later his WBC had increased to 29,400/mm³ (Table 1). Serum LDH was 1,760 Wroblewski units and PMN alkaline phosphatase score was 218. Platelet count was 934,000/mm³. He was still asymptomatic, but, on the basis of these results including the finding of the Philadelphia chromosome, a diagnosis of chronic myelocytic leukemia was made.
MYELOCYTIC LEUKEMIA IN TWINS AND A SIBLING

FAMILY PEDIGREE

I

II

III

IV

SEX UNKNOWN

MONOZYGOTIC TWINS

CONSANGUINITY

PROBAND

CHRONIC MYELOCYTIC LEUKEMIA (MALE)

MISCARRIAGE (MALE)

DEAD (FEMALE)

BIRTH ORDER UNKNOWN

DEAD (MALE)

STILL BIRTH, SEX UNKNOWN

Fig. 1.—Pedigree of the family.

Table 1.—Hematocrit, WBC and Differential for the Twins, Their Siblings and Offspring

<table>
<thead>
<tr>
<th>Individual</th>
<th>II-2*</th>
<th>II-3</th>
<th>II-4*</th>
<th>II-5*</th>
<th>II-6</th>
<th>III-3</th>
<th>III-4</th>
<th>III-5</th>
<th>III-6</th>
<th>III-7</th>
</tr>
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<tbody>
<tr>
<td>Date</td>
<td>9-66</td>
<td>5-66</td>
<td>4-64</td>
<td>4-64</td>
<td>6-64</td>
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<td>6-64</td>
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<td>6-64</td>
<td>5-66</td>
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<tr>
<td>PCV</td>
<td>43</td>
<td>37</td>
<td>42</td>
<td>38</td>
<td>43</td>
<td>46</td>
<td>43</td>
<td>41</td>
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<tr>
<td>WBC</td>
<td>29,400</td>
<td>7,290</td>
<td>53,000</td>
<td>39,000</td>
<td>7,000</td>
<td>7,100</td>
<td>6,200</td>
<td>8,300</td>
<td>6,900</td>
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<td>Blast</td>
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<td>.5</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro. My.</td>
<td></td>
<td></td>
<td>5.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My.</td>
<td>1.5</td>
<td></td>
<td>Occ.</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Meta. My.</td>
<td>4</td>
<td></td>
<td>.5</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. Band</td>
<td>7.5</td>
<td></td>
<td>1</td>
<td>14.5</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Seg.</td>
<td>61.5</td>
<td></td>
<td>52</td>
<td>82</td>
<td>41.5</td>
<td>60</td>
<td>36</td>
<td>39</td>
<td>57</td>
<td>63</td>
</tr>
<tr>
<td>Eos.</td>
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<td>4</td>
<td>4.5</td>
<td>1.5</td>
<td>6</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Base.</td>
<td>1.5</td>
<td>1</td>
<td>2.5</td>
<td>2.5</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymph.</td>
<td>19</td>
<td>39</td>
<td>5.5</td>
<td>16.5</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>23</td>
<td>29</td>
<td>56</td>
</tr>
<tr>
<td>Mono.</td>
<td>2.5</td>
<td>4</td>
<td>3.5</td>
<td>6.5</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>14</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-RBC/100</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Chronic Myelocytic Leukemia.

Twin Zygosity

Meaningful interpretation of twin data depends upon accurate diagnosis of zygosity. For like-sex twins, this is often difficult to ascertain and no standard criteria are established. The proband and his co-twin under study had identical blood groups: A1, C+, c+, D+, E+, MN and Kell+. The number of "arches," "loops" and "whorls" in their fingerprints
were identical and external gross physical characteristics were similar. The twins were considered to be monozygotic.

**Family Histories**

The father (I-5) of the proband died at age 54 with pneumonia. The mother (I-4) died at age 75 from heart disease. Five children, all males, were born to these parents. The first son (II-1) died at age 5 years from scarlet fever. The second son (II-2) is now 68 years old and has always lived in Henderson County, Tennessee. The youngest son (II-6) is 53 years old and has lived in Ohio for 20 years. He has symptoms suggesting emphysema, chronic lymphadenitis and otitis.

The proband twin (II-5) had 3 children: a healthy 30 year old daughter (III-8) who has had one miscarriage, a stillborn child (III-9), and a male blue baby (III-10) who died at age 15 days. The co-twin (II-4) married a maternal cousin's daughter (II-3) and had 2 female and 5 male offsprings. Both daughters are living: one (III-5) is 28 years old, married and apparently healthy; she has had one miscarriage. The other (III-7) is 17 years old, single and has myoclonic seizures with mental retardation. Of the five sons, two have died, one (III-1) at age 19 years from diabetes mellitus and the other (III-2) at age 16 years from influenza and myocarditis. The remaining three sons are living. One (III-3) is 36 years old and has benign prostatic hypertrophy. Another is 33 years old (III-4) and has a cardiorespiratory disorder. The youngest one (III-6) is 25 years old and has dermatitis.

**Epidemiological Data on Twins**

The twins were born and reared on a farm in Henderson County, Tennessee, and have spent their entire lives in this county. Birth weights were reported to be 3.5 pounds each. Both remembered having had measles, mumps, whooping cough, and chickenpox during childhood. They lived together until one (II-4) married at age 22. The proband (II-5) married at age 32. Both twins continued to farm, but 10 miles apart. During the past 15 years, the proband has worked as a "concrete mixer." There is no sewage system in the area and they have used well and cistern water all their lives. In the last 15 years, both have been exposed to insecticides, mostly powder types. They have chewed tobacco and used snuff since adolescence. There is no history of allergy, serious illness, X-ray exposure or hospitalization prior to the current illness.

**Blood Analyses from Siblings and Offsprings of the Twins**

Venous blood hemograms were obtained from one other brother (II-6), one maternal first cousin once-removed (II-3), three sons (III-3, III-4, III-6), and two daughters (III-5, III-7) of the twins (Table 1). There were no marked deviations of clinical significance among these relatives.

**CHROMOSOME STUDIES**

Chromosome studies were done on the twins (II-4, II-5) and the oldest living brother (II-2), each with chronic myelocytic leukemia, and a daughter (III-7) of one of the twins. She has had familial myoclonic seizures with mental retardation.

**Method**

The chromosome preparations were made from peripheral blood leukocytes cultured according to the method of Moorhead et al. with minor modifications. Metaphases were studied by a direct microscopic technique. In part I, a complete analysis of each spread was made. The second part consisted of a special study of the G group and Y chromosomes in additional spreads. In both parts, spreads appearing to be diploid or near diploid were chosen for study. To avoid possible bias, selections were made using a low-power objective. Photographs representing both normal and abnormal spreads were made in both parts of the study. In one patient (II-2), leukocytes were cultured both
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Fig. 2.—Leukocyte metaphase with 45 chromosomes showing a Ph¹ chromosome. The G group chromosomes are shown separately at the lower right in the order: normal autosome, Ph¹, 2 normal autosomes, and the Y chromosome (II-5).

with and without phytohemagglutinin. There were fewer spreads observed in the latter culture, but no significant differences were noted.

Results

II-5 (Proband twin). Chromosome preparations were made in June, 1964 by another laboratory. The patient was in remission with a WBC of 4,200/mm³. Analysis of nine metaphase spreads revealed that eight contained 46 chromosomes with an XY sex chromosome complex, while one contained 45 chromosomes with an XY sex chromosome complex. Six of the nine showed no abnormalities. The other three spreads, including the one with 45 chromosomes (Figure 2) contained an abnormally small G chromosome, appearing to have a deletion of the long arm; this was interpreted as a Ph¹ chromosome. Figure 2 shows at least one break and only 5 group E chromosomes. In another spread
Table 2(A).—Frequencies of Ph' Chromosome Observed in Complete Analysis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Karyotype</th>
<th>Cells Analyzed</th>
<th>No. Containing Ph' Chromosome</th>
<th>Percent with Ph' Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-5</td>
<td>44-XY/43-XY</td>
<td>9</td>
<td>3</td>
<td>33%</td>
</tr>
<tr>
<td>II-4</td>
<td>44-XY</td>
<td>30</td>
<td>3</td>
<td>10%</td>
</tr>
<tr>
<td>II-2 (With PHA*)</td>
<td>44-XY</td>
<td>38</td>
<td>3</td>
<td>7%</td>
</tr>
<tr>
<td>(Without PHA*)</td>
<td>44-XY</td>
<td>32</td>
<td>2</td>
<td>6%</td>
</tr>
</tbody>
</table>

*Phytohemagglutinin

Table 2(B).—Frequencies of Ph' Chromosome Observed in Additional Spreads in Which Only the G Group and Y Chromosomes Were Studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of Spreads Observed</th>
<th>No. Containing Ph' Chromosome</th>
<th>Percent with Ph' Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-5</td>
<td>10</td>
<td>2</td>
<td>20%</td>
</tr>
<tr>
<td>II-4</td>
<td>40</td>
<td>4</td>
<td>10%</td>
</tr>
<tr>
<td>II-2</td>
<td>350</td>
<td>15</td>
<td>4%</td>
</tr>
</tbody>
</table>

Table 3.—Summary of All Chromosome Abnormalities

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>No. of cells in which abnormality appeared</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II-5</td>
</tr>
<tr>
<td>Ph' + 3 Normal G</td>
<td>5 (26%)</td>
</tr>
<tr>
<td>Ph' + 3 Normal G + acentric Fragment</td>
<td>1</td>
</tr>
<tr>
<td>Ph' + 4 Normal G + other autosomal abn.</td>
<td></td>
</tr>
<tr>
<td>Acentric fragment + 3 Normal G</td>
<td>1</td>
</tr>
<tr>
<td>Acentric fragment + 4 Normal G</td>
<td>1</td>
</tr>
<tr>
<td>Dicentric chromosomes</td>
<td></td>
</tr>
<tr>
<td>Polyplody</td>
<td></td>
</tr>
<tr>
<td>Breaks</td>
<td>1</td>
</tr>
</tbody>
</table>

* From culture with PHA.
† Not evaluated.

A small acentric fragment was seen, which was extra chromosome material.

In 10 additional spreads, two contained an abnormally small G group chromosome. The Y chromosome appeared to be of normal size and configuration in all spreads. No polyploidy was seen and no other abnormalities were observed. The results of the chromosome studies are summarized in Tables 2 and 3.

II-4 (Co-twin). Chromosome preparations were made in June, 1966. Busulfan had been discontinued three months before this, but the patient was still in remission with a WBC of 5,100 mm³. Analysis of 30 spreads showed that 27 contained 46 chromosomes; two of these 27 spreads had a Ph' chromosome. The other three contained 46 chromosomes plus a small acentric fragment, only one of which had a Ph' chromosome (Figure 3). Figure 3 shows a dicentric chromosome. All spreads contained an XY sex chromosome complement with a normal Y chromosome.

In 40 additional spreads, four contained a Ph' chromosome and two a small
Fig. 3.—Leukocyte metaphase with 46 chromosomes showing a Ph1 chromosome and an acentric fragment. The G group chromosomes are shown separately at the lower right in the order: normal autosome, Ph1, 2 normal autosomes, acentric fragment, and the Y chromosome (II-4).

acentric fragment without a Ph1 chromosome. The Y chromosome appeared to be normal. Frequent polyploidy was observed with many tetraploid cells, some pentaploid and an occasional cell with even higher polyploidy. Frequent breaks were observed (Tables 2 and 3).

II-2 (Brother). Chromosome preparations were made in July, 1966. Thirty-eight spreads were analyzed from the culture grown with phytohemagglutinin. Thirty-six contained 46 chromosomes, two of which had a Ph1 chromosome (Figure 4). The two other spreads contained 47 chromosomes, one containing an extra C group chromosome and the other a Ph1 chromosome as an extra group G chromosome. In the latter, there are either only 5 group E or only 5 group D chromosomes. All spreads contained an XY sex chromosome complex, but the Y chromosome appeared to be unusually long. One spread contained a
dicentric chromosome. Some polyploidy was noted, with endore duplication seen in two cells. Occasional breaks were seen.

In the study without phytohemagglutinin 32 cells were analyzed, 2 containing a Ph1 chromosome. Twenty-nine spreads contained 46 chromosomes, two had 46 plus an acentric fragment, and one had 45 plus a "fragment." The "fragment" shown in the spread with 45 chromosomes is too dense and even to be convincing as chromosome material, rather than dust or stain. The fact that there are only 45 chromosomes and that this is the only cell observed, may indicate a technical accident. Most spreads contained an unusually long Y chromosome. An occasional tetraploid cell was observed, and a few breaks were also seen.

Because of the abnormal spreads containing 47 chromosomes described
earlier, a larger number of cells were studied in this patient. The purpose was to determine if a different cell line was present with the Ph\(^1\) chromosome as an extra G group chromosome. Of 350 additional spreads observed, 15 had a Ph\(^1\) chromosome, all appearing to be a part of the G group. Although additional breaks, polyplody and occasional large fragments were seen, no indication of the existence of a separate cell line was found. (Tables 2 and 3).

III-7 (Daughter). The chromosome preparations were made in May, 1966. She had no evidence of leukemia. Analysis of 30 metaphase spreads showed each spread to have a total of 46 chromosomes with an XX sex chromosome complex. No abnormalities were seen. Observations of 150 additional spreads showed no evidence of the Philadelphia chromosome.

**DISCUSSION**

There has been considerable controversy as to whether familial incidence of human leukemia indicates the presence of a hereditary factor, a common environmental factor or both. The importance of genetic factors in the etiology of leukemia was emphasized by Videback, but his argument was not supported by Steinberg. The number of familial cases reported and the information provided in such studies have been limited. Systematic ascertainment of a large series of familial cases of leukemia and detailed analysis of the cell type, age at onset, acute/chronic manifestation and sex segregation would help clarify this problem.

Studies of twins, one or both of each pair with leukemia, are of greater importance particularly in regard to possible genetic factors in the etiology of this disease. However, scarcity of affected twins and technical difficulty in determining zygosity have been detrimental to such studies. In a study of 4,679 twin children, all with leukemia, MacMahon found that the concordance rate of monozygous pairs was as high as 25 percent. This finding is consistent with the hypothesis of a prenatal origin for childhood leukemia. There have been no reports in which a large series of adult twins, one or both of each pair with leukemia, have been systematically studied. However, there have been a number of individual case reports of leukemia in adult twins. Cases in which lymphocytic leukemia was involved have been described by Dameshek, Gunz, Cooke, Siegel and Pearson. A twin with chronic myelocytic leukemia was reported by Dougan, Goh and Jacobs.

The siblings presented in this report include identical twins and their older brother, all of whom were diagnosed as having chronic myelocytic leukemia within a span of 3 months. No similar observations of chronic myelocytic leukemia have ever been reported. It is of further interest that the third case (brother) was accidentally discovered through screening tests on relatives of the affected twins.

The Ph\(^1\) chromosome is a distinct abnormality which is found in the marrow or blood cells of nearly all patients with chronic myelocytic leukemia. In twins, the Ph\(^1\) chromosome has been found only in the affected individual. However, the role of the Ph\(^1\) chromosome, particularly in terms of the etiology of the disease, has not been clearly defined. It has been reported that during the chronic phase of this disease, Ph\(^1\) is the only detectable chromosome aberration.
in the majority of cases, but that in the acute terminal stages, Ph' is commonly associated with other chromosome abnormalities which vary considerably between patients. These secondary abnormalities include a second Ph'-like chromosome, a minute chromosome (or fragment) and complete loss of a G chromosome.

The chromosome constitution of acute leukemia in a pair of infant fraternal twins has been studied by Sandberg and co-workers. The variations found in the karyotypic findings of the twins were interpreted as being influenced by their different genetic constitution. If this assumption is correct, any karyotypic aberrations present in monozygous twins with simultaneous leukemia should be identical.

In the present study, chromosome analyses were made on peripheral blood cells. The Ph' chromosome was present in each of the three siblings with chronic myelocytic leukemia. The frequency of the Ph' chromosome ranged from 4 percent in II-2 to 33 percent in II-5. The percentage frequencies of the Ph' chromosome found in this study are probably somewhat less than would be expected in bone marrow preparations which were not available for the chromosome studies.

Several other chromosome abnormalities were observed in one or more of the three individuals; these were a small acentric fragment, dicentric chromosome, breaks, polyploidy, complete loss of a G chromosome, an extra C group chromosome and an extra G group chromosome which was satellited. Some of these abnormalities, particularly those found in the 68 year old brother, are similar to what has been described in the acute terminal phase of chronic myelocytic leukemia. It is of special interest that, while such abnormalities have often been attributed to chemotherapy, the patient (II-2) in the present study had never been treated for leukemia.

These observations, along with the absence of environmental exposures, which are known to cause karyotypic aberrations, suggest that the tendency to develop a Ph' chromosome may be inherited at least in some families, and may predispose its carriers to chronic myelocytic leukemia. Despite the hematological findings characteristic of chronic myelocytic leukemia, the presence of elevated alkaline phosphatase activity in the older brother suggests that this may be a variant of the usual chronic myelocytic leukemia.

**SUMMARY**

Identical twins and their older brother have been studied: all diagnosed within a span of three months as having chronic myelocytic leukemia; both twins were symptomatic and the brother asymptomatic. Chromosome analyses were made on peripheral blood cells. The asymptomatic brother had never been treated. The Ph' chromosome was present in each of the three siblings.

A number of other chromosome abnormalities were found. Results were interpreted in terms of a probable genetic factor in the Philadelphia chromosome and susceptibility to chronic myelocytic leukemia.

**SUMMARIO IN INTERLINGUA**

Esseva studiate un par de geminos identic e lor fratre senior. In omne le tres le diagnose de chronic leucemia myelocytic esseva establite intra un intervallo de tres menses. Le
MYELOCYTIC LEUKEMIA IN TWINS AND A SIBLING

The authors wish to thank Dr. J. C. Stripling and Dr. M. N. Lowry of Lexington, Tennessee, and Dr. J. H. Dickson of Lorain, Ohio, for their excellent cooperation in this study.

REFERENCES

Chronic Myelocytic Leukemia in Identical Twins and a Sibling
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