Polycythemia Vera with Ph' Chromosomes in Two Brothers

By William C. Levin, Elsie W. Houston and Stephan E. Ritzmann

In a recent review of the clinical and epidemiologic aspects of polycythemia, Modan discussed familial polycythemia, which was first reported in 1907 and in several reviews subsequently. As more sophisticated diagnostic technics have become available, familial polycythemia has been recognized to be a clinical entity, distinct from polycythemia rubra vera (PRV) in its clinical, hematologic and prognostic aspects. It may be a subgroup of benign erythrocytosis. Although the erythrocyte count, hemoglobin, and blood volume are elevated and splenomegaly may be present in the familial variant, the total and differential white blood cell and platelet counts are normal and there are no adverse effects exhibited by the affected children. Modan accepts only two reports in the literature dealing with familial polycythemia as representing unequivocal examples of familial PRV.

This report concerns two brothers with clinically and hematologically typical PRV: one diagnosed 22 years ago and treated on several occasions with prior to the present study, and the other diagnosed recently with baseline studies, including the cytogenetic examinations, performed before therapy was started. Only phlebotomy had been performed prior to the cytogenetic studies in the latter. In contrast to previously reported variable and inconsistent chromosomal abnormalities in untreated and treated patients with PRV, both brothers presented the unusual combination of Philadelphia (Ph') chromosomes in marrow cells and elevated leukocyte alkaline phosphatase (LAP). Whether this represents a familial disease or the fortuitous occurrence of Ph'-positive polycythemia vera in two brothers cannot be ascertained from the available data.

Methods

Directly harvested cells from marrow aspirates, by the method of Tjio and Whang, were employed for cytogenetic studies. Air-dried preparations were made by blaze drying on slides rinsed in 70 per cent ethanol and were stained with Wright's stain. Microscopic analysis was made of all metaphases counted. Representative cells were photographed with phase microscopy on 35 mm. High Contrast Kodacopy Film and karyotypes were prepared from the enlarged prints.
Leukocyte alkaline phosphatase determinations were performed on peripheral blood smears stained by an azo-dye technic* and scored by the method of Kaplow.**

**CASE SUMMARIES**

A. P. is a 68-year-old white male who was admitted to John Sealy Hospital on March 20, 1965, with complaints of malaise, weakness and fullness in the head since November 1964. Suboccipital headaches and dyspnea were present. A dusky redness had been noticed a few months earlier. On March 15, 1965, he passed tarry stools on three or four occasions. A diagnosis of duodenal ulcer was made on the basis of x-ray examination. Increased susceptibility to bruising had been noted for the past few months.

The blood pressure was 200/100 mm. Hg but later decreased to 120/70 mm. Hg. The vital signs were normal. There was generalized plethora of the skin and mucous membranes. A systolic murmur was heard over apex and the aortic area. The tip of the spleen was barely palpable on deep inspiration.

**Laboratory Results**

A hemogram on 3/22/65 showed: erythrocytes 7,650,000/mm.³, Hb 20.4+ Gm. per cent, hematocrit 72 per cent, MCV 94 μ³, reticulocytes 2.6 per cent, platelets 293,000/mm.³, leukocytes 13,800/mm.³ with segmented PMN 77 per cent, basophils 2 per cent, eosinophils 2 per cent, lymphocytes 18 per cent, and monocytes 1 per cent. The marrow picture was compatible with the diagnosis of polycythemia rubra vera. The VDRL was negative. A BUN, fasting blood sugar, prothrombin time, urinalysis and serum uric acid were normal. The red cell mass was 62.5 ml./Kg. as determined with Cr²¹-labeled red cells. Pulmonary function studies revealed a normal arterial oxygen saturation, slightly augmented by hyperventilation. Chest x-ray, upper G.I. series, skull x-ray and barium enema were essentially normal. An ECG revealed evidence of myocardial ischemia.

The patient was treated with phlebotomies of 500 cc. each on five occasions and was given 6 mc. P³² intravenously on 3/24/65. When seen in February 1966 the patient had no complaints and the polycythemia was well controlled. Hemogram at that time revealed erythrocytes 4,740,000/mm.³, Hb 14.6 Gm. per cent, hematocrit 45.5 per cent, reticulocytes 0.2 per cent, platelets 183,000/mm.³, leukocytes 7100/mm.³ with segmented PMN 73 per cent, eosinophils 2 per cent, basophils 2 per cent, lymphocytes 18 per cent, and monocytes 5 per cent.

E. P., the brother of A. P., is a 55-year-old white male who was first seen as an out-patient on May 16, 1955; he complained of intense bone aches, easy bleeding and bruising, occasional aching pain over the precordium, and intermittent indigestion. A diagnosis of polycythemia rubra vera had been made in 1944, and the disease had been treated exclusively with 119 phlebotomies in the interim.

The blood pressure was 122/76 mm. Hg, and the pulse rate was 48 per minute and regular. A geographic tongue, moderate gingivitis, leukoplakia of the lower lip, moderate emphysema, and a barely palpable spleen were noted on physical examination. There were no cardiovascular symptoms, nor was lymphadenopathy, hepatomegaly or edema noted. An adequate funduscopic examination could not be done.

**Laboratory Results**

A hemogram revealed erythrocytes 6,220,000/mm.³, Hb 12.4 Gm. per cent.

*Leukocyte alkaline phosphatase kit, Sigma Chemical Company, 3500 DeKalb Street, St. Louis.
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hematocrit 43.5 per cent, reticulocytes 1.0 per cent, platelets 951,000/mm³, leukocytes 22,250/mm³ with segmented PMN 78 per cent, stabs 4 per cent, lymphocytes 13 per cent, and monocytes 5 per cent. A marrow examination revealed intense hypercellularity which involved the myeloid, erythroid and megakaryocytic elements, and was considered to be compatible with polycythemia rubra vera. Chest x-ray, ECG and urinalysis were normal.

The patient received radiation treatment in the form of P³² administered according to the schedule in Table 1. This maintained the patient in clinical and hematologic remission. During the ensuing years, headaches, precordial pain, epigastric distress, and bone pain, particularly in the extremities, have been the most troublesome symptoms, and have been controlled by phlebotomies. The platelets have fluctuated between 246,000/mm³ and 1,500,000/mm³, the erythrocytes between 5,140,000/mm³ and 6,710,000/mm³, and the leukocytes between 9650/mm³ and 36,450/mm³. At no time has the differential count manifested a left shift in the granulocytes.

When the patient was last seen in February 1966, aching of the hands and arms with use was the only complaint. Hemogram revealed erythrocytes 5,300,000/mm³, Hb 9.4 Gm. per cent, hematocrit 38 per cent, MCV 72 p³, MCH 18 picograms, MCHC 25 per cent, reticulocytes 1.2 per cent, platelets 435,000/mm³, leukocytes 27,700/mm³ with segmented PMN 88 per cent, lymphocytes 8 per cent, and monocytes 4 per cent. The spleen was palpated 2 finger-breadths below the left costal margin.

RESULTS

Chromosome Studies

E.P. Cells directly harvested from marrow aspirated on 2/9/66 showed a modal count of 46 chromosomes. Of 22 cells counted and analyzed, 21 had 46 chromosomes and one had 45. All possessed a Ph¹ chromosome. No other significant abnormalities were found. The morphology was excellent and matching was relatively easy (Fig. 1A). Group G and Y chromosomes from five cells are shown in Figure 1B. Each cell possesses a Ph¹ chromosome. No mitoses were found in 48-hour cultures of peripheral leukocytes incubated without phytohemagglutinin.

A.P. Cells from a direct harvest of marrow aspirated on 3/22/65 showed a modal count of 46 chromosomes. Adequate spreading was difficult to achieve and the morphology was somewhat variable in quality. The Ph¹ chromosome was present in 19 of 21 cells evaluated and was not clearly absent in any cell examined. Four cells in the octoploid range of chromosome counts were encountered. No other significant abnormalities were noted (Fig. 2A). Group G and Y chromosomes from five cells are shown in Figure 2B. Each cell presents a Ph¹ chromosome.

LAP

Both brothers had moderately increased levels of leukocyte alkaline phosphatase. E.P. had an LAP score of 189 and A.P. a score of 157 in March 1966; the control score was 97.

A female sibling of the patients is apparently not affected. A blood count and chromosome study, kindly performed on the sister by Dr. William
Table 1.—Radiation Treatment Schedule of P

<table>
<thead>
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<th>Date</th>
<th>Dosage of P (mc.)</th>
<th>Mode of Administration</th>
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<tbody>
<tr>
<td>5/18/55</td>
<td>5.9</td>
<td>Intravenous</td>
</tr>
<tr>
<td>8/30/55</td>
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<td>4/10/56</td>
<td>4.64</td>
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</tr>
<tr>
<td>9/20/57</td>
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<td>6/17/59</td>
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<td>Intravenous</td>
</tr>
<tr>
<td>1/12/61</td>
<td>4.0</td>
<td>Intravenous</td>
</tr>
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<td>10/19/61</td>
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<tr>
<td>10/8/64</td>
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<td>Intravenous</td>
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<td><strong>Total</strong></td>
<td><strong>41.02</strong></td>
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Dameshek, then at Tufts-New England Medical Center, Boston, Massachusetts, revealed no abnormalities. Another male sibling has not been studied but he exhibits no clinical evidence of hematologic disease.

**Discussion**

During the 3-year period following Nowell and Hungerford's report of a minute chromosome in leukocytes from patients with CML, more than 70 additional cases have been reported confirming this association. The Ph chromosome is considered to be derived from a chromosome 21 through the loss of a portion of the long arm. The occurrence of this chromosomal anomaly in brothers may be fortuitous or may be the result of an inherited submicroscopic chromosomal defect which renders the affected chromosome more liable to mitotic accidents. The rarity of the familial occurrence of this chromosomal abnormality favors the former explanation. Cohen and Swisher described a 23-year-old male with typical Ph-positive CML, whose identical twin was clinically normal, without evidence of Ph chromosomes in either blood or marrow cells. Hirschhorn has described a family in which the Ph chromosome was found in 100 per cent of the marrow cells of a grandfather with CML and also in 10–15 per cent of the marrow cells of 3 healthy descendants. In addition, CML is believed to have been the cause of the deaths of four close relatives of the index case. Hirschhorn postulated that a cancer-causing agent rather than a genetic factor was operative in the causation of the chromosomal defect in this family. Previous studies of familial PRV which Modan accepts as unequivocal were reported before chromosomal studies on human material became available; thus, it is not possible to assess from the available data the relationship of the Ph chromosome to the familial occurrence of PRV in earlier reports.

There is good evidence that the Ph chromosome is present in both the erythroid and myeloid cells and probably also in the megakaryocytes of patients with CML, but not in lymphocytes or skin fibroblasts. Whang et al. found the abnormal chromosome in 80–100 per cent of metaphases in direct preparations of marrow from 24 patients with CML. They proposed that this chromosomal defect usually arises in a stem cell common to erythro-
Fig. 1A.—Metaphase plate and karyotype of a representative cell from directly harvested marrow of E.P.

cytes, granulocytes, and megakaryocytes, and confers some proliferative advantage to cells possessing it. Hence, it is conceivable that it may be associated with any of the myeloproliferative diseases. A chromosomal defect morphologically indistinguishable from a Ph chromosome has been described in patients with polycythemia vera, myelofibrosis, atypical granulocytic leukemia presenting with marked thrombocytopenia with high LAP levels and no splenomegaly, eosinophilic leukemia and acute granulocytic leukemia. The factors governing its association with "CML in most cases, and conversely, the rarity of its association with other forms of myeloproliferative disease, are unknown.

In 1961, Nowell and Hungerford postulated that the chromosomal abnormality and the long recognized low LAP values in CML may be related. Subsequent reports and this report do not support this concept. Teplitz et al. in 1964 suggested that, at best, modifiers of LAP may be located on chromosome 21; the evidence in their case failed to support the concept of a simple gene-dose relationship.

Few reports of cytogenetic studies of patients with PRV are available. Normal karyotypes and karyotypic abnormalities in untreated and radiation-treated patients with PRV or other forms of myeloproliferative disease which developed during the course of PRV have been described. Levan et al. found a minute chromosome in the Y-21-22 group in karyotypes of seven cells from blood cultures of a patient with PRV. These authors concluded
that the minute chromosomes were not morphologically typical of Ph¹ chromosomes and submitted the karyotypes to Dr. D. Hungerford, who concurred in this opinion. The only report found in the literature describing the occurrence of a Ph¹ chromosome was by Anstey et al.⁸ in cultured marrow cells from a patient with clinically typical PRV. This patient had been treated previously with P³². Cytogenetic studies were performed because the patient exhibited a very low LAP, discovered in the course of studies to determine possible correlation between LAP levels and some aspects of the clinical course of PRV. Five months later the patient exhibited the clinical and hematologic findings of classical CML. The low LAP and the development of CML within a few months cast doubt upon the validity of the diagnosis of PRV at the time that the cytogenetic studies were performed. Indeed, in a subsequent publication,⁴¹ the same patient reported by Anstey et al.⁸ is described as having chronic myeloid leukemia.

In contrast to the extensive cytogenetic studies in patients with CML, similar analyses in patients with other forms of the myeloproliferative syn-
Fig. 2A.—Metaphase plate and karyotype of a representative cell from directly harvested marrow of A.P.

The coincidence of polycythemia rubra vera and Ph' chromosomes in two brothers has not been previously reported. It is tempting to suggest that genetic factors may be responsible, but environmental influences cannot be excluded. It is quite possible that the interaction of genetic characteristics and environmental factors has been required to produce identical disease and identical chromosomal anomalies in these two brothers.

These observations indicate the need for more intensive investigation of the kindred of patients with myeloproliferative syndromes clinically, cytogenetically and with any other pertinent methodology which may become available.

**Summary**

Two brothers with polycythemia rubra vera have been studied: one before treatment and one after many years of treatment. Direct harvests of marrow aspirates from both patients exhibited the presence of the Ph' chromosome. Moderately increased LAP levels were present in both. Available data do not
Fig. 2B.—Group G and Y chromosomes from five cells from directly harvested marrow of A.P.

indicate whether the disease and the chromosomal aberrations in these two brothers are familial or fortuitous.

SUMMARIO IN INTERLINGUA

Duo fratres con polycythemia ver esseva studiate, le un ante le therapia e le altere multe annos post le initiation de mesuras de therapia. Directe collectas de aspiratos de medulla ossee ab ambe patientes exhibiva le presentia del chromosoma Ph. Moderatemente augmentate nivellos del leucocytic phosphatase alcalin esseva presente in ambes. Le datos currentemente disponibile non indica si le morbo e le aberrationes chromosomal in iste duo fratres es familial o coincidental.

REFERENCES

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5. Lawrence, J. H., and Goetsch, A. T.:
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