"Masked" Ph' Chromosome Abnormalities in CML: A Report of Two Unique Cases

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Two patients with chronic myeloid leukemia (CML) showed previously undescribed variants of a "masked" Ph' abnormality. The first patient had the karyotype 46,XY,+21,−9,−22,+mar9,mar18 at presentation in the chronic phase. The dicentric marker 9 was interpreted as representing the usual translocation of 22q11 to 9q34, followed by translocation of the Ph1 chromosome (the deleted 22) to 9p and probable translocation of 9p to the distal long arm of the marker. The patient developed clones containing 2 and 3 copies of the "Ph'-containing" marker 9 concomitant with the metamorphosis of his disease to a more aggressive phase. The second case presented with the karyotype 46,XY,−9,−22,+two D-group markers. A complex rearrangement of chromosomes 9 and 22 is postulated, with interstitial insertion of either 9p or distal 9q into chromosome 22q11. This patient is still in the chronic phase of his disease 9 mo after presentation. The common denominator in these unusual "masked" cases is the 22q11 breakpoint. The paucity of published reports of duplication of 9q without concurrent duplication of the Ph1 chromosome, supported by the findings in our first case, leads us to conclude that the amplification of genes on the Ph1 chromosome are more important for the evolution of the abnormal stem cell in CML than the chromosome 9 derivative.

A VARIANT Philadelphia (Ph1) chromosome translocation was first reported in 1973, the same year that Rowley showed that the Ph1 chromosome abnormality was not a simple deletion of chromosome 22q, but a translocation of the deleted segment to chromosome 9q.

At least 100 variants, both simple and complex, have been reported. In the great majority of these variants, the deleted chromosome 22 (the Ph1 chromosome) is easily identified, but a chromosome other than 9 may be the recipient of the deleted 22 (simple variant), or more commonly, chromosomes in addition to chromosome 9 are involved in 3-way and even 4-way complex translocations. A variant Ph1 translocation is estimated to occur with a frequency of 4%–8%. Of the 1,027 Ph1-positive patients reviewed by Oshimura et al., only 1.3% had a simple translocation not involving chromosome 9, i.e., 98.7% of Ph1 translocations involved chromosome 9 as well as chromosome 22. Thirty percent of Oshimura et al.'s 42 variant Ph1 cases had a simple translocation, whereas 40% of the 104 variant Ph1 translocations reviewed by Mitelman and Levan were simple.

Rarely has a complex variant been described where the Ph1 chromosome itself is not recognizable as a discrete 22q− entity. The first such "masked" or "concealed" Ph1 abnormality was described in 1974. The significance of variant Ph1 abnormalities, including the "masked" Ph1, in the pathogenesis of chronic myeloid leukemia (CML) is presently unknown. It has been postulated that the critical event in the initiation of CML is the deletion and transposition of genes on chromosome 22 distal to region q11, rather than the specificity of chromosome 9 as the acceptor, because chromosomes other than 9 may be recipients, but chromosome 22 is always the donor.

The validity of the assumption that chromosome 9 is merely coincidently involved has, however, been questioned by postulating that simple variants are in fact complex ones that cannot be cytogenetically detected. The recent demonstration by recombinant DNA technology that the standard Ph1 translocation is in fact reciprocal supports the concept that it is the 22q11:9q34 junction on the 22q− component of the t(9;22) that is the critical initiating event in CML.

The frequent duplication of the Ph1 chromosome during the metamorphosis of CML to a more aggressive phase does suggest that the derivative, consisting of 22q− and 9q34−qter, is more important in the maintenance and promotion of the leukemic process than the reciprocal chromosome 9 component of the t(9;22), because 2 copies of the abnormal 9 have been reported very rarely.

We document two cases of CML with a "masked" Ph1 abnormality presenting with monosomy 22. One patient showed evolution of both 2 and 3 copies of a "Ph1-containing" aberrant chromosome 9. The karyotypic evolution noted in the latter patient provides further indirect evidence that the amplification of the 22q− component (containing the 22q11:9q34 junction) is a more significant phenomenon in the progression of the disease than the chromosome 9 derivative.

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MATERIALS AND METHODS

The two patients described in this study were referred for chromosomal investigation during the course of a study of chromosomal patterns in 198 patients suffering from CML. They comprised 2 of 14 patients in the series, in whom a variant Ph1 chromosome abnormality was detected.

Hematologic Methods

The criteria for the diagnosis of CML, metamorphosis to a more aggressive phase of the disease, and acute blastic transformation were the same as previously described.6

Chromosome Studies

Initial chromosome analyses were performed in both cases on metaphases derived from peripheral blood cultures, prior to receipt of any chemotherapy. Three serial analyses were conducted on case I and two analyses on case II. The peripheral white cell count exceeded 15 x 10^9/liter, and immature myeloid cells capable of division comprised greater than 10% of the differential count at the time of the analyses in all instances, except on the repeat analysis of case II, where only 6% immature myeloid cells were present. Twenty-four-hour and 48-hr unstimulated cultures were used for the determination of the leukemic cell line and 48- and 72-hr phytohemagglutinin (PHA) stimulated cultures served as controls to establish the constitutional cell line.6

Various banding techniques were used to establish the identity of the abnormal chromosomes in both cases. Giemsa (GTG), quinacrine (QFQ), and centromeric (CBG) banding were performed by conventional techniques and Ag-AS staining, to demonstrate the nucleolar organizer regions (NOR) of acrocentric chromosomes, was done according to the method of Bloom and Goodpasture.16

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CASE REPORTS

Case I

A 36-yr-old man, D.P., was referred for the investigation of an elevated white cell count to the hematology clinic of the main teaching hospital of the University of the Witwatersrand Medical School.

The patient had a long-standing history of bullous lung disease and had undergone resection of bullae 10 yr and 8 yr previously. (He was previously a panel beater by trade, but had been a salesman for the past few years.)

Examination on admission revealed a cachectic man with mild dyspnea and central and peripheral cyanosis. Small, discrete cervical supraclavicular and inguinal lymph nodes were palpable, and both his liver and spleen were palpable 2 cm below the costal margins.

Hematologic investigations at presentation revealed a white cell count of 74.4 x 10^9/liter, red cell count 4.68 x 10^12/liter, hemoglobin 16.1 g/dl, and platelets (of normal morphology) 311 x 10^9/liter. The white cell differential count was 71% neutrophils, 2% lymphocytes, 3% monocytes, 2% basophils, 7% metamyelocytes, 9% myelocytes, 5% promyelocytes, and 1% blasts (Table I).

Marrow aspirate examination showed a hypercellular marrow with a myeloid:erythroid ratio of 50:1; granulopoiesis was markedly increased. Morphologically normal megakaryocytes were found in adequate numbers, and free-lying platelets were plentiful. Blasts comprised 4% of the marrow nucleated cell count. Macrophages and plasma cells were normal in number and morphology. Marrow trephine biopsy confirmed the hypercellularity due to increased granulopoiesis. A few single blasts were scattered throughout the specimen. There was a moderate, diffuse increase in marrow fibrosis. The aspirate and trephine findings were consistent with the diagnosis of CML.

Serum electrolytes, gases, and protein electrophoresis were normal. Microscopic examination of sputum and sputum culture were negative for tuberculosis, and no other infective agent was detected.

The neutrophil alkaline phosphatase was 40 U at presentation and again 40 U when retested 4 mo later; on both occasions, the patient was suffering from pneumonia. Vitamin B12 and B12 binders were elevated above 2,000 U. No terminal deoxynucleotidyl transferase (TdT) activity was detected in an assay performed 4 mo after presentation.

Recurrent attacks of pneumonia necessitated two further hospital admissions during the next few months. The infective episodes were always accompanied by a sharp rise in the white cell count, which subsequently dropped on recovery from the infections, which were treated with gentamicin and cephradine. He did not receive any therapy for his progressively rising white cell count during the next 4 mo, at which stage his hemoglobin began to drop (10.6 g/dl), and a rise in the percentage of myelocytes and metamyelocytes was noted (Table I).

Five months after presentation, D.P. decided to leave Johannesburg for the coastal city of Durban, and his subsequent course was kindly documented by Prof. H. B. W. Greig. Soon after his arrival in Durban, the patient again developed pneumonia, mouth ulcers, nausea, and vomiting. At this stage, he was very ill with fever, wasting, dyspnea, and pallor. He had generalized lymphadenopathy with lymph nodes up to 2 cm in diameter, and the spleen was

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Table 1. Serial Peripheral Blood Indices in Case I From Presentation to 1 wk Prior to His Death

| Date         | Hb (g/dl) | RCC (x 10^9/Liter) | Plasma (x 10^9/Liter) | WCC (x 10^9/Liter) | N | M | L | E | B | Band | Meta. | Myel. | Pro. | Blasts |
|--------------|-----------|--------------------|-----------------------|--------------------|---|---|---|---|---|------|-------|-------|------|-------|-------|
| Johannesburg |           |                    |                       |                    |   |   |   |   |   |      |       |       |      |       |
| 1/22/80*     | 16.1      | 4.68               | 311                   | 744                | 71| 3 | 2 | 0 | 2 | ?    | 7     | 9     | 5    | 1     |
| 2/28/80      | 15.7      | 4.82               | N                     | 39.2               | 43| 13| 22| 2 | 5 | ?    | 5     | 5     | 5    | 0     |
| 3/27/80*     | 16.2      | 4.79               | 358                   | 113.4              | 35| 8 | 5 | 3 | 0 | 24   | 8     | 8     | 8    | 1     |
| 5/19/80*     | 10.6      | ?                  | 381                   | 138.0              | 27| 1 | 0 | 0 | 1 | 30   | 17    | 17    | 20   | 3     |
| Durban       |           |                    |                       |                    |   |   |   |   |   |      |       |       |      |       |
| 7/2/80†      | 8.1       | 2.96               | 208                   | 270.0              | 41| 2 | 4 | 1 | 3 | 6    | 8     | 9     | 12   | 14    |
| 7/25/80†     | ↓         | ↓                  | 19.9                  | Not stated         |   |   |   |   |   |      |       |       |      |       |
| 8/15/80†     | 10.5      | 3.55               | 70.0                  | Not stated         |   |   |   |   |   |      |       |       |      |       |
| 8/22/80      | ?         | ?                  |                       | Not stated         |   |   |   |   |   |      |       |       |      |       |

*Dates of chromosome analyses.
†8 cm splenomegaly noted; transfusion and commencement of chemotherapy.
‡Further transfusions and cessation of chemotherapy.

RCC, red cell count; WCC, white cell count; N, neutrophils; M, monocytes; L, lymphocytes; E, erythrocytes; B, basophils.
enlarged to 8 cm below the costal margin. His hemoglobin concentration was 8.1 g/dl, and peripheral blood blasts had risen to 14% of a total white count of 279 x 10^9/liter. He was transfused with 2 U of fresh blood and received antibiotics for the infection. Therapy with busulphan, 6 mg daily, was begun but was changed to 4 mg daily 2 wk later, and in addition, 6-mercaptopurine, 50 mg daily, was simultaneously prescribed. The hemoglobin level continued to drop, and 3 wk after beginning chemotherapy, this was stopped and he received a further 4 U of blood. Four weeks later, he was seen for the last time; on this occasion he was in early congestive cardiac failure, the spleen was enlarged to 10 cm and the liver to 5 cm below the costal margin. The white cell count had again risen to 70 x 10^9/liter, but no differential count is available. He refused hospitalization and was therefore given 6-mercaptopurine orally (50 mg daily).

One week later (7 mo after the diagnosis of CML), he suddenly collapsed at home and died within a few minutes. A pulmonary embolism was suspected, but permission for autopsy was refused.

Case II

V.N., aged 60 yr, presented on June 1, 1982, with abdominal discomfort, pain in the left hypochondrium, and anorexia of several months duration. He was an accountant by profession. On examination, his spleen was enlarged to 10 cm below the costal margin, but he did not have any lymphadenopathy or hepatomegaly.

Peripheral blood indices revealed a hemoglobin level of 113 g/dl, 175 x 10^9/liter platelets, and a total white cell count of 140 x 10^9/liter. The differential white cell count was 48% neutrophils, 1% monocytes, 1% lymphocytes, 2% basophils, 22% band forms, 15% metamyelocytes, 5% myelocytes, and 6% promyelocytes. The neutrophil alkaline phosphatase value was zero.

Treatment was begun with allopurinol, followed by busulphan. Therapy was discontinued 3.5 mo after diagnosis, at which stage he was feeling well, the spleen size had been reduced to about 4 cm, and his peripheral blood white cell count had been reduced to 8.6 x 10^9/liter, with a differential count of 80% neutrophils, 2% monocytes, 16% lymphocytes, and 2% eosinophils. An abdominal ultrasound scan 5 mo after presentation showed the spleen to be 18 cm in length, compared to the normal ultrasound measurement of 10 cm; there was no evidence of hepatomegaly or paraaortic lymphadenopathy. When last seen 9 mo after presentation, he was still feeling well, the spleen size had been reduced to about 4 cm, and his peripheral blood white cell count had risen to 30 x 10^9/liter, and the differential white cell count included 1% promyelocytes, 5% myelocytes, 9% metamyelocytes, and 16% atypical lymphocytes. The hemoglobin level was stable at 14.1 g/dl, but the platelet count had dropped to 101 x 10^9/liter. Busulphan therapy was again prescribed.

RESULTS OF CHROMOSOME ANALYSIS

Case I

At presentation, a pseudodiploid karyotype with a constant clonal abnormality was found in all 23 unstimulated metaphases analyzed (Table 2). The karyotype was 46,XY,+21,−9,−22,+,mar,9,mar,18 (clone I). This karyotype was interpreted as representing a complex "masked" Philadelphia chromosome abnormality (Fig. 1). The chromosome 9 marker had both abnormal short and long arms (Fig. 2A). The pale quinacrine fluorescent pattern of 9p was quite different from that of its normal homologue. Centromeric banding demonstrated the dicentric nature of the aberrant chromosome by showing two separate C-bands, the smaller C-band representing the chromosome 22 centromere and the larger C-band showing the variant 9q21 region (Fig. 2B). The abnormal 9p was thought to represent the missing deleted chromosome 22, i.e., the Ph' chromosome. Unfortunately attempts at silver staining to demonstrate the nucleolar organizer regions (NOR) of the acrocentric chromosome 22 were unsuccessful. The distal long arm of marker 9 showed a negatively staining Giemsa band that was presumed to represent the usual 22q11—qter deleted segment of chromosome 22, followed by a wide, darkly stained band. In some of the better metaphases, two distinct dark bands on distal 9q were visible (Fig. 2A, i and iii). The origin of this distal region is speculative, but it could represent the missing 9p which has two

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<th>Table 2. Results of Chromosome Analysis in Case I</th>
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<td><strong>Dates of Analyses</strong></td>
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<td><strong>and Distribution of NN:AA Cells</strong></td>
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<td>1. 1/22/80 — At diagnosis</td>
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Clone I: 46,XY,+21,—9,—22,+,mar,9,mar,18 (Figs. 1 and 2).
Clone II: 47,XY,+21,—9,—22,+,mar,9,mar,18.
Cell III: 48,XY,+21,—9,—22,+,mar,9,+,mar,9,mar,18 (Fig. 3).
Clone IV: 45,XY,—9,—22,+,mar,9,mar,18.

*See text for interpretation of abnormalities and clones.

NN, all normal cells; AA, only abnormal cells.
dark G-bands. The presumed formation of the abnormal chromosome 9 is diagrammatically represented in Fig. 2C. PHA-stimulated lymphocytes showed a normal karyotype.

Repeat analysis 2 mo later revealed the same abnormal clone as above, but an analysis 4 mo after diagnosis showed a very interesting pattern of clonal evolution. Two copies of the “Philadelphia chromosome” containing marker 9 were noted (clone II), and one cell showed three copies of the abnormal chromosome 9 (Fig. 3). This latter cell could not be called a clone, because by the definition of the First International Workshop on Chromosomes in Leukemia, a minimum of two cells with the same abnormality should be
THE "MASKED" Ph' CHROMOSOME IN CML

Fig. 3. Giemsa-banded karyotype of case I, showing triplication of the abnormal 9 marker, karyotype 48, XY, +21, −9, −22, +mar9, +mar9, +mar9,mar18. (Only one such cell was detected.)

demonstrated. However, this cell was obviously derived from clones I and II by further nondisjunction of marker 9.

The detection of 5 cells with the karyotype 45, XY, −9, −22, +mar9,mar18, labeled clone IV in Table 2, was puzzling, because it would be logical to suppose that this clone was the primary one, preceding clone I that showed trisomy 21, but this karyotype was not detected in the two prior analyses.

Case II

Chromosome analysis of unstimulated metaphases at presentation, revealed a karyotype of 46, XY, −9, −22, +two D-group markers in all 8 cells available for analysis (Figs. 4 and 5A). The loss of chromosomes 9 and 22, the two chromosomes involved in the usual Ph' translocation, and their substitution by two distinctive markers, led us to suspect a "masked" Ph' abnormality. The combined length of chromosomes 9 and 22 approximated closely that of the two marker chromosomes.

Chromosome studies were repeated 9 mo after presentation, when the patient was still in the chronic phase, but his white cell count had risen to 30 × 10⁹/liter.

Unstimulated metaphases again showed a karyotype of 46, XY, −9, −22, +two D-group markers in 39 cells. There was no evidence of any karyotypic evolu-
Fig. 5. (A) Partial Giemsa-banded karyotypes of the abnormal D-group markers and the normal 9 and 22 homologues from 4 cells in case II. (B) Partial karyotype of a serially Q-C-banded cell, showing the C-banded constitutive heterochromatin on the normal chromosome 9 and the Di marker (arrowed). (C) Partial karyotype of a serially Q-Ag-AS stained cell, showing NOR on the Dii marker (arrowed). (D) Diagram of the second interpretation of the 9;22 interchange, whereby 9q is inserted into 22q11. (E) Diagram of the first interpretation of the 9;22 interchange, whereby 9p is inserted into 22q11. (F) Diagram of the second interpretation of the 9;22 interchange, whereby 9q is inserted into 22q11 (see text for detailed interpretation).

At least 10 “masked” Ph' abnormalities have been described, but only two previous cases presented with a monosomy 22, due to the incorporation of the Ph' chromosome into another chromosome. All the other “masked” Ph' abnormalities manifested as a 22q+ abnormality, which arose from the translocation of chromosome material onto 22q11.

Both reported patients with monosomy 22 were described by Engel et al. In both instances, the Ph' chromosome was translocated to the short arm of chromosome 17, but the translocation of distal 22q differed, being translocated to terminal 17q in their first patient and to the usual position on 9q34 in their second case. Engel et al.'s first case bears a strong similarity to the findings in our first patient, except that chromosome 17 was dicentric in their case and chromosome 9 was dicentric in ours.

Significantly, in our first patient, evidence of chromosomal clonal evolution was detected at the time that his disease became more aggressive, about 4 mo after diagnosis. There is no doubt that a gradual metamorphosis of his disease was occurring, as manifested by
the increasing spleen size, an increasing total white cell count, and decreasing hemoglobin and platelet levels. Five months after presentation, his white cell count had reached a peak of 270 x 10^9/liter, the percentage of blasts and promyelocytes in peripheral blood were increasing, and his low hemoglobin level necessitated blood transfusion. He failed to respond to therapy, as evidenced by a rising white cell count shortly before his sudden death.

The nature of the chromosomal changes at this stage are of great interest. The nondisjunctional events producing two and three copies of the aberrant "Ph'-containing" chromosome 9 are presumed to have arisen selectively by conferring a growth advantage to the leukemic cell line. Mitelman19 has emphasized the distinction between primary chromosomal changes initiating a malignant process and the secondary changes that confer a selective advantage to the already malignant cell. One of the secondary mechanisms for promotion of a tumor is by amplification of selected genes through duplication of the chromosomes containing these genes.19 We postulate that the duplication of the abnormal 9 occurred because it contained the 22q- derivative (composed of the 22q- joined to 9q34→9qter), equivalent to two copies of the Ph1 chromosome commonly evolving in CML patients with a standard Ph1.

Duplication of the chromosome 9 in the standard t(9;22) is very rare. We investigated a patient who presented in the acute phase of CML, in whom duplication of both the 9 and 22 derivatives of a standard Ph1 translocation was observed (unpublished). Sandberg has documented one such case in blast crisis (see his Table 44, case 6)13 and, in both his and our patient, 2 copies of the Ph1 chromosome were observed alone, but the derivative 9 duplication was never seen on its own—it was always associated with duplication of the Ph1 chromosome. The net effect of the duplication of both Ph1 components is thus a double "balanced" Ph1 translocation.

Prigogina et al.20 reported a patient in blast crisis with a single discrete Ph1 chromosome, i(9q+), -22, +marker. The interpretation of the small marker and i(9q+), chromosome is difficult to assess from the published photographs. Lyall and Garson's case21 had the karyotype 46,XX,Ph1,i(Xq) or i(9q), but here again, the exact origin of the isochromosome is in doubt, and if it were an i(9q), it is unclear as to whether it was the normal or abnormal 9 that was involved. Our finding of only one such case showing duplication of both 9 and 22 components in 180 cases of Ph1-positive CML identified by banding,6 and the paucity of such cases in the literature, leads us to conclude that the evolution of two copies of the aberrant 9q+ alone does not occur during the course of the disease in CML.

Our second patient, although he showed a unique type of "masked" Ph1 translocation, does not at the moment assist in elucidating the relative roles of chromosomes 9 and 22 in the evolution of CML. The patient is now in the chronic phase of his disease and does not show any further karyotypic changes at this stage. We will of course make every effort to study his chromosomes again, should his condition deteriorate. It would obviously be of interest to observe which, if either, of his two D-group markers (representing chromosomes 9 and 22) becomes duplicated later in the course of his disease.

Discussion as to which component of the apparently balanced reciprocal t(9;22) Philadelphia translocation is the more important in the pathogenesis of CML is not likely to be conclusively resolved until the actual gene loci at the 9q and 22q translocation sites are functionally identified at a molecular level.22,23 Progress in this direction has already been achieved by the identification of two cellular oncogenes within the translocated regions of the reciprocal 9;22 translocation. The oncogene c-abl has been localized to the distal 9q34 segment translocated to 22q1111 and c-sis has been assigned to the 22q11→22qter region,12 which is translocated to 9q34 in standard Ph1 translocations. The significance of these (and possibly other) oncogenes in activating genes adjacent to the breakpoints involved in standard and variant Ph1 translocation remains to be elucidated.

NOTE IN PROOF

Since the submission of this paper, Groffen et al. have shown that c-sis, localized to 22q11→qter, is in fact translocated to 9q34 in standard Ph1 translocations.

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