CGG-Repeat Polymorphism of the BCR Gene Rules out Predisposing Alleles Leading to the Philadelphia Chromosome


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The Philadelphia chromosome (Ph) is associated with leukemia, most frequently of the chronic myelogenous variety. The Ph chromosome is a translocation chromosome which gains oncogenic potential through the fusion of the ABL oncogene of chromosome 9 with the BCR gene of chromosome 22. The Ph is believed to arise from random chromosome rearrangements with a subsequent selective advantage of the malignant cell line. However, alleles may be present in the population which predispose toward this specific rearrangement. We used a highly polymorphic CGG-repeat polymorphism within the first exon of the BCR gene to determine BCR allele frequencies among 26 leukemia patients with the Ph chromosome and 63 control individuals. Eight BCR alleles of variable CGG-repeat length were present in both groups at statistically similar frequencies and in Hardy-Weinberg equilibrium. We therefore concluded that there are no alleles of the BCR gene that have a major predisposing influence on the development of the Ph chromosome and subsequent leukemia. Genes Chrom Cancer 9:141-144 (1994). © 1994 Wiley-Liss, Inc.

INTRODUCTION

The Philadelphia chromosome (Ph) is a translocation chromosome involving chromosomes 9 and 22 at breakpoints 9q34 and 22q11 (Nowell and Hungerford, 1960; Rowley, 1973; Grifffen et al., 1984). More than 90% of patients with chronic myelogenous leukemia (CML) are positive for the Ph chromosome, as are 10% of acute lymphoblastic leukemia (ALL) patients and 5% of acute myelogenous leukemia (AML) patients (Kurzrock et al., 1988). The 9;22 translocation of the Ph chromosome joins the BCR gene with the ABL oncogene, resulting in a fusion protein with tyrosine kinase activity that is believed to be a major contributor to the malignant potential of the leukemic cell (Shivelman et al., 1985; Daley et al., 1990; Heisterkamp et al., 1990). The BCR gene is approximately 130 kb in size, consisting of 18 exons, 5 of which are found within a 5.8 kb breakpoint cluster region that participates in the Ph chromosome translocation in CML patients and in many Ph-positive ALL patients (Donay et al., 1989; Campbell and Arlinghaus, 1991). It is believed that the translocation forming the Ph chromosome is the initial somatic event that leads to the leukemic phenotype (Daley et al., 1990).

It is unclear whether the initial rearrangement leading to the Ph chromosome is random, occurring in a dynamic genome with subsequent selection, or whether there are predisposing influences which may modulate this specific translocation event. There is evidence that exposure to benzene and ionizing radiation increases the risk for CML, but these environmental insults probably increase genome instability in general (Court Brown and Dunn, 1965; Jacobs, 1989). However, at least one familial clustering of CML may indicate genetic influences upon the risk of Ph formation (Lillie and Standall, 1984). Because we have recently demonstrated a CGG-repeat polymorphism, with multiple-length alleles, in the 5' untranslated region of the BCR gene (Riggins et al., 1992), it was feasible to test whether or not there are BCR alleles that predispose to formation of the Ph chromosome and therefore to certain types of leukemia. Though the CGG repeat is a portion of the gene that is not adjacent to the breakpoint and unlikely to be directly involved, it is within a distance where linkage disequilibrium might be observed. Such studies have previously demonstrated an association between certain HRAS alleles and several types of cancer (Heighway et al., 1986; Carter et al., 1988; Diedrich et al., 1988). We therefore genotyped Ph chromosome-positive leukemia patients and cor-

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control individuals at the BCR CGG-repeat polymorphism to detect any evidence of linkage disequilibrium which could indicate BCR alleles predisposing to the rearrangement.

MATERIALS AND METHODS

Populations

Three populations were available for study. The first two groups, Ph-positive and diseased (Ph-negative with other hematologic disorders), were collected as follows. Bone marrow samples or, in a few cases, blood was collected originally for diagnostic cytogenetic analysis. Unused bone marrow samples were collected and tested blindly as described below for BCR CGG-repeat length. The individuals were divided into two groups based on the presence or complete absence of the (9;22)(q34;q11) translocation. Those individuals testing negative for the Ph were considered a control group. They are referred to as the diseased control group, because they had a variety of hematologic or malignant disorders other than the Ph.

The second control group consisted of roughly half DNA samples of unrelated normal individuals from the CEPH genetic mapping panel (n = 15). The other half were normal unrelated individuals collected at random from available DNA samples in the laboratory.

Sample Testing

Cells from bone marrow or blood were prepared and lysed for PCR with proximate K as described (Britten et al., 1988), or with a thermostable protease (Pretaq, Gibco BRL) as specified by the supplier. The following primers flanked the CGG-repeat and were used for amplification of the repeat by the polymerase chain reaction (PCR): forward, 5′-TGAGCTAGCCGAGAAG, reverse, 5′-GCGCGGCTATTTGTCG. The forward primer was end-labeled to a specific activity of 0.1 Ci/μmol of primer. The PCR amplification for CGG repeats had the following reaction components: 2 μl of the cell lysate, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.0 mM MgCl₂, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 150 μM 7-deaza-GTP, 0.2 μM primers, 10% DMSO, 1.5 units of Taq polymerase, and water to 30 μl. Thermal cycling was done for 35 cycles of PCR under conditions of denaturing: 95°C for 1 minute; annealing for 1 minute at 62°C, and extension at 72°C for 30 seconds. Initial denaturation was done for 5 minutes and final extension for 7 minutes. A volume of 4 μl of PCR products mixed with 50% sequencing dye [formamide with 0.1% (g per ml) xylene cyanol FF, 0.1% bromophenol blue, and 2% 0.5 M EDTA, pH 8] was loaded on a 0.4 mm thick, 30 cm × 40 cm, 6% polyacrylamide urea gel. Length determination was made by comparison to a labeled HpaII digest of pBR322 and BCR CGG repeat PCR products amplified from a mixture of templates of known size, providing a standard with the same gel mobility. The gel was electrophoresed at 80 W for approximately 2.5 hours, dried without fixing, and exposed to photographic film.

Statistics

Both the Pearson chi-square and the likelihood-ratio chi-square methods were used as tests for a general association. We set up the statistical test both by comparing the individual allele frequencies and, because of the small expected frequencies, by comparing groups of alleles (less than six repeats, six repeats, and more than six repeats). We tested Hardy-Weinberg equilibrium by using χ² analysis to compare the frequency of heterozygotes to that of homozygotes for both the Ph-positive and the control group.

RESULTS AND DISCUSSION

The patient group consisted of 26 unrelated individuals, all positive cytogenetically for the Ph chromosome. Thirteen individuals were diagnosed with ALL, eight with CML, two with undifferentiated acute leukemia, and one each with AML, lymphoma, and a diagnosis of myeloproliferative disorder. In order to test whether or not there were BCR allelic predispositions toward the Ph chromosome, we determined allele frequencies of triplet repeat polymorphisms found in the BCR gene in this patient group and in two control groups. The first control group (diseased control), which consisted of 63 individuals ascertained at the same time as the patient group, had various hematologic or malignant disorders, but no evidence of the Ph chromosome. Some had disorders which, in other patients, occasionally exhibit the Ph chromosome, whereas others had diagnoses unassociated with this rearrangement. A second control group consisted of 33 clinically normal individuals of similarly mixed ethnic background (American urban population). Individuals in all three groups were unrelated.

The CGG-repeat length of the BCR polymorphisms was determined by PCR on a total of 244 chromosomes from all three groups. A total of eight alleles were observed, ranging from two to ten trip-
BCR POLYMORPHISM RULES OUT Ph PREDISPOSITION

![Figure 1](image)

Figure 1. BCR CGG repeat alleles for eight patients from the study with Ph. The CGG-repeat lengths shown on the left of the gel correspond to PCR product lengths shown in parentheses. Lanes 1, 2, 4, and 6 are amplified from CML bone marrow. Lanes 3, 5, 7, and 8 are, respectively, undifferentiated leukemia, AML or ALL, lymphomas, and myeloproliferative disorder. Four different allele sizes are shown: six repeats in all lanes with eight repeats (lanes 3, 6, 8), and two repeats (lane 5), and three repeats (lane 7).

let repeats. An example of these data is shown in Figure 1. Allele frequencies for all three groups were determined and are shown in Table 1. In all three populations, the frequencies were in Hardy-Weinberg equilibrium, suggesting that ascertainment was without major bias with respect to the BCR locus. Allele frequencies of the patient (Ph chromosome) group were compared to those of the normal control group, the diseased control group, or both control groups combined (Table 1). Because certain allele-size classes are sometimes represented by a single individual, we made statistical comparisons by grouping the alleles as less than six repeats, six repeats, and more than six repeats, where six repeats is the overall mean repeat size for all groups. The data were analyzed both by chi-square analysis and by the likelihood-ratio test. No significant differences were apparent among the three groups, showing probabilities in excess of 50% in all cases in favor of similarity.

These data indicate no deviation from expected BCR allele frequencies among individuals with the Ph chromosome and those without. These findings are consistent with the absence of linkage disequilibrium between the Ph chromosome and the BCR allele and another BCR sequence variation which leads to a major Ph chromosome predisposition. Therefore, our data support the alternative hypothesis that the rearrangement resulting in the Ph chromosome is random and, due to its unique malignant potential, has a selective advantage which leads to clonal expansion in the marrow. This possibility is, of course, widely accepted, although, prior to this study, with little direct scientific evidence. These conclusions, however, are limited to a major predisposing influence of the BCR locus alone for Ph chromosome formation as a group. Allelic predispositions at the ABL locus are not addressed here, nor would minor allelic predispositions of the BCR locus necessarily be detected. A study of a much larger scale would be necessary to detect allelic predisposition that might exist within a particular Ph-positive group (i.e., grouping based on breakpoint location or clinical disease type). In addition, detection of linkage disequilibrium using this CGG repeat is partially limited by the high prevalence of the six allele repeat.

The CGG-repeat polymorphism of the BCR gene used in this study may be extended to other analyses of the Ph chromosome or other BCR-related malignancies. For example, a recent report by Hau et al. (1992) suggested, by analysis of chromosome heterochromatin, an imprinting phenomenon leading to the Ph chromosome. The CGG-repeat of the BCR locus, because it is present in the BCR transcript, can rapidly distinguish imprinting leading to uniparental gene expression and therefore could be used directly as a test of this phenomenon. Further, loss of heterozygosity at the BCR locus can readily be ascertained with this marker. Because the BCR gene product appears to be a GTPase-activating protein for p21^{Ras}, a member of the RAS superfamily (Dickmann et al., 1991), it is possible that BCR is involved in other malignancies not involving the Ph chromosome.

In summary, we report the results of a study in
TABLE I. Frequencies of BCR Alleles in Control and Ph-Positive Individuals

<table>
<thead>
<tr>
<th>Allele size (CGG-repsec number)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph*-positive</td>
<td>1 (1.9)</td>
<td>2 (2.9)</td>
<td>0</td>
<td>0</td>
<td>35 (67.3)</td>
<td>1 (1.9)</td>
<td>12 (22.1)</td>
<td>1 (1.9)</td>
<td>0</td>
</tr>
<tr>
<td>Ph*-negative diseased</td>
<td>4 (3.2)</td>
<td>5 (4.0)</td>
<td>0</td>
<td>0</td>
<td>3 (2.4)</td>
<td>87 (69.05)</td>
<td>4 (3.2)</td>
<td>21 (16.7)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Normals</td>
<td>3 (4.6)</td>
<td>0</td>
<td>0</td>
<td>4 (6.1)</td>
<td>43 (65.2)</td>
<td>0</td>
<td>16 (24.2)</td>
<td>0</td>
<td>0</td>
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Chi square

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<tr>
<th>D.F.</th>
<th>Value</th>
<th>Probability</th>
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<tbody>
<tr>
<td>Ph*+ vs. diseased</td>
<td>2</td>
<td>1.11</td>
</tr>
<tr>
<td>Ph*+ vs. normal</td>
<td>2</td>
<td>0.91</td>
</tr>
<tr>
<td>Ph*+ vs. both</td>
<td>2</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Likelihood ratio

<table>
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<th>D.F.</th>
<th>Value</th>
<th>Probability</th>
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<tbody>
<tr>
<td>Ph*+ vs. diseased</td>
<td>2</td>
<td>1.15</td>
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<tr>
<td>Ph*+ vs. normal</td>
<td>2</td>
<td>0.94</td>
</tr>
<tr>
<td>Ph*+ vs. both</td>
<td>2</td>
<td>1.21</td>
</tr>
</tbody>
</table>

*Number of individuals given with percentages in parentheses.
*Degrees of freedom.

which we used a highly polymorphic marker of the BCR locus. The data are consistent with the absence of BCR alleles that confer a major predisposition toward development of the Ph chromosome and subsequent leukemia. These data are, then, most compatible with the notion of random rearrangement, with subsequent selection of the emerging leukemic clone.

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REFERENCES