RAPID COMMUNICATION

Paternal Origin of the Rearranged Major Breakpoint Cluster Region in Chronic Myeloid Leukemia

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The Philadelphia chromosome, t(9;22), is present in virtually all cases of chronic myeloid leukemia (CML). It has previously been shown by cytogenetic studies that the rearranged chromosome 22 in patients with CML is exclusively maternal in origin. To address this issue at a molecular level, the major breakpoint cluster region (M-bcr) on chromosome 22 was examined using Southern blot assays and M-bcr Pvu II and Msp I restriction site polymorphisms in three CML patients. In all three cases, the rearranged allele was paternal in origin. These results indicate that the paternally derived M-bcr allele may also be involved in the M-bcr rearrangement.

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CHRONIC MYELOID leukemia (CML) is a hematologic malignancy that is virtually always associated with the t(9;22) by cytogenetic analysis. This translocation juxtaposes the BCR gene on chromosome 22 with the c-abl oncogene on chromosome 9, producing a hybrid BCR/c-abl gene believed essential in the pathogenesis of this disease.1

By using chromosome polymorphisms in families of patients with Philadelphia chromosome-positive CML, Haas et al2 demonstrated a marked "parent of origin" bias in the chromosomes involved in the t(9;22). Without exception, the derivation of chromosome 9 and chromosome 22 in this translocation was paternal and maternal, respectively. This has led to speculation that imprinting may have some role in the mechanism of the translocation.

To substantiate this finding at a molecular level, the present study used restriction fragment length polymorphisms of the major breakpoint cluster region (M-bcr) in families of CML patients to determine the parental origin of the rearranged M-bcr.

**MATERIALS AND METHODS**

**Patient and parent material.** To be included in this study the following criteria had to be met. (1) The patient had to have a rearrangement detectable by Southern blot in a Bgl II and Pvu II digest with probes 1 and 2 (intra vide). (2) The patient had to be heterozygous for the M-bcr Pvu II or Msp I restriction site polymorphism.3,4 (3) DNA samples had to be obtainable from both of the patient’s parents. (4) At least one of the parents had to be homozygous for the presence or absence of the M-bcr Msp I or Pvu II restriction site to produce an informative crossing.

All samples were obtained with approval from and in accordance with the rules governing the University of Minnesota Committee on the Use of Human Subjects in Research. Ten CML patients and their parents from the University of Minnesota were examined for the presence of informative Pvu II or Msp I polymorphisms. Of these, three met the above criteria. The clinical characteristics of these patients are shown in Table 1.
A restriction map of the M-bcr with relevant restriction enzyme sites is presented in Fig 1. The results of the three informative families are shown in Figs 2 through 4 and summarized in Table 1.

Family 1 (Fig 2). The absence and presence of the M-bcr Pvu II polymorphism manifests as a 4.8-kb and 2.5-kb restriction fragment, respectively, in Pvu II-digested DNA hybridized with probe 1 (Fig 1). The mother has both of these restriction fragments and is a heterozygote; the father has only the 4.8-kb fragment and is a homozygote for the absence of the Pvu II site. The child with CML has a 2.5-kb fragment indicating inheritance from the mother. The paternal restriction fragment is 2.9 kb in length and represents the rearranged M-bcr allele. A faint 4.8-kb germline band representing the unrearranged paternal allele in a small (<5%) population of Ph-negative cells is noted in the child.

Family 2 (Fig 3). The mother has 4.8-kb and 2.5-kb Pvu II M-bcr restriction fragments with probe 1 and is heterozygous for the Pvu II polymorphism. The father has only the 4.8-kb fragment and is homozygous for the absence of the Pvu II site. The child with CML has inherited the maternal 2.5-kb fragment; this allele is unrearranged. The paternal allele is rearranged with a length of 2.9 kb.

Family 3 (Fig 4). The M-bcr Mae II polymorphism is approximately 100 bp 5' of the M-bcr Pvu II polymorphic site (Fig 1). This polymorphism manifests as a 2.5-kb Bgl II/Mae II fragment in Bgl II/Mae II double-digested genomic DNA hybridized to probes 1 or 2. When the Mae II site is absent, a 4.8-kb Bgl II/Bgl II fragment is identified.

The mother is homozygous for the absence of the Mae II site and has only 4.8-kb Bgl II/Bgl II fragments with probe 1. The child with CML and father are heterozygous for the absence of the Mae II site and have 2.9-kb Bgl II/Bgl II fragments with probe 1.
PATERNAL ORIGIN OF M-bcr IN CML

PATERNAL ORIGIN OF M-BCR IN CHRONIC MYELOGENOUS LEUKEMIA

FAMILY 3

Fig 4. Family 3. Autoradiograms of Southern blots of Bgl II/Mae II double digests of genomic DNA from the patient's father, the patient, and the patient's mother hybridized to the 5' M-bcr probe 1 and the 3' M-bcr probe 2 (see Fig 1). As seen with the 5' probe 1, the child inherited the 2.5-kb allele from the father. The 3' probe identifies this allele as the rearranged allele. The maternal 4.8-kb allele is intact with both probes.

Mae II polymorphism with both 4.8-kb Bgl II/Bgl II and 2.5-kb Bgl II/Mae II fragments with probe 1. When the same Southern blot was hybridized with probe 2, a 4.8-kb Bgl II/Bgl II germline fragment was present in the child's DNA. This fragment lacks the Mae II polymorphic site and was inherited from his mother. The rearranged fragment that is 4.0 kb in length is paternal in origin.

DISCUSSION

These data indicate that the paternal major breakpoint cluster region on chromosome 22 (M-bcr) may participate in the t(9;22) at the molecular level. The nature of the translocation did not allow for determination by molecular methods of the parental origin of the rearranged chromosome 9 in these cases.1

The results presented here do not necessarily contradict the results of Haas et al.2 Those cytogenetic studies were based on silver staining polymorphisms of the nucleolus organizing region (Ag-NOR) on the short arm of chromosome 22, some distance from the M-bcr. It is possible that a somatic recombinatorial event between the maternally and paternally derived chromosomes 22 in CML patients could be occurring before or during the Philadelphia chromosome translocation; the recombination would occur between the M-bcr on the long arm and the Ag-NOR on the short arm of chromosome 22. The exclusive paternal and maternal origin of the M-bcr and chromosome 22 Ag-NOR elements, respectively, on the Philadelphia chromosome suggests that this event may be a common occurrence. Such a finding may be biologically significant in the genesis of the BCR/c-abl hybrid gene in CML. Confirmation of this hypothesis will require combined cytogenetic and molecular analysis in individual cases.

Alternatively, the exceptional cases presented may indicate other factors besides parental origin in determining the participation of a given chromosome in the t(9;22). It is important to recognize that the cases in the present study represent a small and biased population. The selection criteria include the presence of the Mae II or Pvu II polymorphism; the latter polymorphism is found exclusively in a small percentage of sibships of patients with CML and other hematologic malignancies.4 It is possible that these polymorphisms or some property of the M-bcr associated with the polymorphisms may be a more important determinant in chromosomal participation in the t(9;22) than parental origin. Interestingly, the Pvu II polymorphism was maternally derived and on the nonrearranged M-bcr in both instances, indicating that whatever function, if any, the polymorphism may play in the translocation, it need not be present on the involved chromosome.

The biologic basis of the chromosomal bias described by Haas et al2 and the exceptions presented here are not currently understood. Those investigators allude to DNA methylation as a possible mechanism for the apparent imprinting of the chromosomes involved in the t(9;22). Supporting this concept, it has previously been shown that cell-specific hypomethylated sites are clustered in those regions of the M-bcr most frequently translocated.5,6 In addition, patients with CML have aberrant methylation patterns of the rearranged M-bcr.6 Whether this aberrancy is the cause or result of the translocation remains to be determined.

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