Chromosome 7 Long Arm Deletion in Myeloid Disorders: A Narrow Breakpoint Region in 7q22 Defined by Molecular Mapping

By J. Kere, T. Ruutu, K.A. Davies, I.B. Roninson, P.C. Watkins, R. Winqvist, and A. de la Chapelle

The involvement of the erythropoietin (EPO), plasminogen activator inhibitor type I (PAI1), and multi-drug resistance (MDR2) genes located in chromosomal region 7q21-22 was studied in patients with myeloid disorders and with or without a chromosome 7 abnormality. Separated blood mononuclear cells and granulocytes from 21 patients were used in restriction fragment length polymorphism (RFLP) studies with gene-specific DNA probes. A marked weakness of one of the allelic bands was observed in granulocyte-derived DNA from heterozygous patients with monosomy 7. In four patients with a partial deletion of chromosome 7 long arm (7q-), marked weakness of an allelic band was observed in granulocyte-derived DNA with PAI1 probe (four heterozygous patients) and MDR2 probe (one heterozygous patient), implying deletion of these genes. In contrast, the EPO gene was not deleted in these patients, as demonstrated by the presence of two allelic bands of equal strength in granulocyte-derived DNA (two patients) or by gene dosage estimation (two patients). Two allelic bands of equal strength were also observed in three heterozygous patients with an arbitrary probe (pKv13) located in 7cen-q21.3. Unexpected hemizygosity or hybridization bands were not observed in any patient. We conclude that PAI1 and MDR2 are located distally of EPO in 7q22, and that none of these genes is commonly rearranged in myeloid disorders. The chromosome 7 long arm deletion breakpoint is located in a relatively narrow segment between the PAI1 and EPO genes in different patients. The deletion may involve a specific site in DNA, since the genetic distance between the PAI1 and EPO genes is only 3 cM.

T HE LOSS of heterozygosity has been associated with many malignant tumors.1-7 In some cases the hemizygosity is related to a gross chromosomal abnormality (deletion or monosomy).6,7 In other cases the karyotype of the affected cells is apparently normal, and the abnormality can only be detected with molecular methods. A recurrent cancer-associated cytogenetic abnormality may offer a clue to the biology of a particular tumor type. The loss of a dominantly inherited protective gene may disclose a cancer-related gene behaving as a recessive on the cellular level, as in the case of retinoblastoma.1 Bone marrow monosomy 7 or the partial deletion of the long arm of chromosome 7 (7q-) are frequently observed in myelodysplastic syndromes (MDS) and acute nonlymphocytic leukemia (ANLL), and they are especially common in patients with previous exposure to mutagenic agents or radiation therapy.8,9 These chromosomal abnormalities have been associated with a granulocyte locomotor defect, a susceptibility to infections, a rapid progression of the disease, and a poor response to therapy.10-13 The biologic significance of the total or partial deletion of chromosome 7 and the molecular events leading to deletions are unknown.

Recently, the plasminogen activator inhibitor type 1 (PAI1), multi-drug resistance (MDR1 and 2), and erythropoietin (EPO) genes have been mapped to 7q22, the most common breakpoint region in 7q- chromosomes. P-glycoprotein encoded in humans by the MDR1 gene is a transmembrane protein expressed at high levels in cell lines resistant to a variety of anticancer drugs, and it probably counteracts the accumulation of drugs in the cells.14-18 Rearrangements have been detected in drug-resistant cell lines by probe MDR2.19 PAI1 codes for a glycoprotein involved in the regulation of fibrinolysis by inhibiting tissue plasminogen activator and urokinase. It is found in platelets, endothelial cells, and different malignant cell types.19,20 EPO codes for a glycoprotein hormone produced mainly in the kidneys, and it regulates the production of RBCs by promoting the proliferation and differentiation of erythroid precursor cells.21-23

Even if these genes may not be expressed in the hematopoietic cells they provide useful markers for studying deletions in the 7q22 region. This study was designed, first, to map in molecular terms the chromosome 7 breakpoints in patients with a myeloid disorder and a partial deletion of the long arm of chromosome 7 (7q-), and second, to detect any possible rearrangements of these genes or the loss of heterozygosity in patients with a myeloid disorder and two apparently normal chromosomes 7.

MATERIALS AND METHODS

Patients and samples. Twenty-one patients with a myeloid disorder and a clonal chromosomal abnormality in bone marrow were included in the study (Table 1). The study was approved by the Ethics committee of the Department of Medical Genetics, University of Helsinki. Patients no. 1 through 4 had a 7q- chromosome (Fig 1). Patients no. 5 through 11 had monosomy 7, and patient no. 11 had monosomy 7 plus a translocation chromosome consisting of the short arm of chromosome 7 and the long arm of chromosome 1. In patients no. 12 through 21 both homologous chromosomes 7 were apparently normal, but other chromosomal abnormalities were

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Table 1. Clinical and Cytogenetic Characteristics of the Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex, Age at Study</th>
<th>Diagnosis, Age at Diagnosis</th>
<th>History of Exposure</th>
<th>Bone Marrow Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F, 77</td>
<td>RAEB, 72</td>
<td>CT, RP</td>
<td>46,XX,del(7)(q21q32-35),del(20)(q11) = 16</td>
</tr>
<tr>
<td>2</td>
<td>M, 82</td>
<td>RAEB, 80</td>
<td>None</td>
<td>46,XY,t(12;13)(p21;q12),del(7)(q22q33-34) = 20</td>
</tr>
<tr>
<td>3</td>
<td>M, 38</td>
<td>ANLL M2, 38</td>
<td>CT, RT</td>
<td>46,XY=5/46,XY,del(7)(q22) = 15/46,XY,del(7)(q22),del(16)(q22) = 2</td>
</tr>
<tr>
<td>4</td>
<td>F, 40</td>
<td>RAEB, 40</td>
<td>CT, RT</td>
<td>46,XX=2/44,XY, − 17 = 21,del(5)(q13q22),del(7)(q22),del(13)(q12q21),del(20)(q11) = 21</td>
</tr>
<tr>
<td>5</td>
<td>M, 71</td>
<td>RAEB, 71</td>
<td>None</td>
<td>46,XY=7/45,XY, − 4</td>
</tr>
<tr>
<td>6</td>
<td>M, 76</td>
<td>RA, 76</td>
<td>CT</td>
<td>45,XY, − 7 = 19/45,XY, − 7,del(20)(q11) = 2</td>
</tr>
<tr>
<td>7</td>
<td>F, 61</td>
<td>ANLL M2, 60</td>
<td>None</td>
<td>46,XX = 4/45,XX, − 7 = 8/44,XX, − 7 = 14</td>
</tr>
<tr>
<td>8</td>
<td>F, 66</td>
<td>RAEB, 66</td>
<td>CT</td>
<td>45,XX, − 7,del(5)(q12q33) = 12</td>
</tr>
<tr>
<td>9</td>
<td>F, 53</td>
<td>RAEB, 52</td>
<td>None</td>
<td>45,XX, − 7 = 17</td>
</tr>
<tr>
<td>10</td>
<td>M, 67</td>
<td>CMML, 67</td>
<td>None</td>
<td>46,XY=3/45,XY, − 7</td>
</tr>
<tr>
<td>11</td>
<td>F, 70</td>
<td>RAEB, 70</td>
<td>RP</td>
<td>46,XX, − 7, +t(1;7)(p11;q11),del(12)(p12p13) = 10</td>
</tr>
<tr>
<td>12</td>
<td>F, 51</td>
<td>RA, 43</td>
<td>None</td>
<td>46,XX = 7/46,XX,del(5)(q13q33) = 10</td>
</tr>
<tr>
<td>13</td>
<td>M, 61</td>
<td>RA, 60</td>
<td>None</td>
<td>46,XY = 11/46,XY,del(13)(q14q21) = 3</td>
</tr>
<tr>
<td>14</td>
<td>M, 39</td>
<td>RA, 31</td>
<td>None</td>
<td>46,XY = 7/46,XY,del(13)(q14q22) = 14</td>
</tr>
<tr>
<td>15</td>
<td>M, 61</td>
<td>ANLL M2, 61</td>
<td>None</td>
<td>46,XY = 1/47,XY, + 8</td>
</tr>
<tr>
<td>16</td>
<td>M, 32</td>
<td>CMML, 32</td>
<td>None</td>
<td>46,XY = 1/48,XY, + 8, + 21 = 1</td>
</tr>
<tr>
<td>17</td>
<td>M, 70</td>
<td>MDS*</td>
<td>None</td>
<td>46,XY,del(20)(q11) = 8</td>
</tr>
<tr>
<td>18</td>
<td>F, 51</td>
<td>RA, 51</td>
<td>None</td>
<td>47,XX, + 8 = 20</td>
</tr>
<tr>
<td>19</td>
<td>F, 77</td>
<td>RAEB, 77</td>
<td>None</td>
<td>46,XX = 2/46,XX,del(5)(q11q31) = 17</td>
</tr>
<tr>
<td>20</td>
<td>F, 78</td>
<td>RA, 73</td>
<td>None</td>
<td>46,XX = 6/46,XX,del(5)(q12q33) = 12</td>
</tr>
<tr>
<td>21</td>
<td>F, 59</td>
<td>ANLL M1, 59</td>
<td>CT</td>
<td>ND, chaotic karyotype in spontaneous blood mitoses</td>
</tr>
</tbody>
</table>

The diagnoses are according to the FAB classification.24Abbreviations: RA, refractory anemia; RAEB, RA with excess of blasts; CMML, chronic myelomonocytic leukemia; CML, chronic myelogenous leukemia; CT, chemotherapy; RT, radiotherapy; RP, radioactive phosphorus; ND, not determined. *FAB classification not applicable.
present. Patients no. 1 through 4, 6, 8, 9, 13, 15, and 18 have also been subjects in previous studies.22

Heparinized blood (20 to 30 mL) and bone marrow samples were taken for molecular and cytogenetic studies and processed as described.22 In brief, blood cultures with and without phytohemagglutinin, and bone marrow cultures without mitogens were done to karyotype lymphocytes, spontaneously dividing blood cells and bone marrow cells, respectively, using G-banded preparations.

Mononuclear cells were isolated from blood by Ficoll gradient centrifugation, and granulocytes were separated from RBCs by dextran sedimentation. The purity of the cell fractions was determined from cytologic specimens stained with May-Grünewald-Giemsa or Sudan Black B and Giemsa. The mononuclear cell fractions contained mainly lymphocytes. High molecular weight DNA was extracted from blood, mononuclear cells, and granulocytes.23

Gene probes and molecular studies. The properties of the probes used in molecular studies are summarized in Table 2.

The restriction fragment length polymorphism (RFLP) analyses were performed with standard techniques. For each experiment 3 to 5 μg samples of DNA were digested with restriction enzymes, electrophoresed in agarose, and transferred to nylon (Hybond N; Amersham, Buckinghamshire, UK) or nitrocellulose (Schleicher & Schuell, Dassel, FRG) filters. Blood cell-derived DNA samples from healthy subjects were included as controls. The probes were labeled with 32P by nick-translation (N.5000 nick-translation kit, Amersham) or by oligolabeling (Oligolabelling kit, Pharmacia, Uppsala, Sweden). The hybridization to filters was at 65°C, or at 42°C in the presence of 50% formamide. After high-stringency washes the filters were autoradiographed with intensifying screens at −70°C. The densitometric measurement of the strength of hybridization signals was performed on x-ray films by an LKB 2202 Ultrascan laser densitometer connected to a recording integrator (LKB Wallac, Bromma, Sweden).

RESULTS

Patients with monosomy 7. Heterozygosity for the HindIII/EPO polymorphism was observed by the study of mononuclear cell-derived DNA in patients no. 6 and 8, for the HindIII/PAI1 polymorphism in patients no. 6 and 7, and for the EcoRI and MspI/MDR2 polymorphisms in patient

Fig 1. The normal and partially deleted chromosomes 7 of patients no. 1 through 4 (Trypsin-Giemsa banding). Cytogenetically, the deletion appears interstitial in patients no. 1 and 2; molecularly, the 7q− chromosome of patient no. 1 contains the 7 cell receptor beta chain gene located at 7q32-35.24
no. 11. A marked weakness of an allelic band was detected in the granulocytic DNA in these patients, suggesting loss of a chromosome 7 in the majority of mature granulocytes. No unexpected hybridization bands were seen in these patients.

Patients with a 7q− chromosome. In all four patients hybridization bands were of expected size. Heterozygosity for the HindIII/PAI1 polymorphism was detected in patients 1 through 4. One of the allelic bands visible in the mononuclear cell-derived DNA samples was much less intense or almost invisible in the granulocyte-derived samples, suggesting deletion of the gene (Fig 2). Patient no. 4 was heterozygous for the EcoRI and MspI/MDR2 polymorphisms, and one of the allelic bands was very weak in the granulocyte-derived sample. Patients no. 1, 2, and 4 were heterozygous for the MspI/KV13 polymorphism, and the allelic bands in mononuclear cell and granulocyte-derived samples were of equal strength. Patients no. 2 and 3 were heterozygous for the HindIII/EPO polymorphism and the allelic bands were of equal strength, implying that the sequence was not deleted. As the same blot was used for hybridizations to both PAI1 and EPO, the densitometric evaluation of the hybridization signals to estimate the gene dosage in patients no. 1 and 4 homozygous for the EPO polymorphism was attempted.

Comparison of the undeleted allelic PAI1 bands in mononuclear cell and granulocyte lanes disclosed that the amount of DNA in the granulocyte lane was 1.2 times greater than in the mononuclear cell lane in patient no. 1, and in patient no. 4 the ratio was 1.9. Comparison of the single EPO bands between mononuclear cell and granulocyte lanes revealed a ratio of 1.1 in patient no. 1 and 1.9 in patient no. 4. As these figures correspond closely to the estimated amount of DNA in the lanes, we conclude that two copies rather than one copy of the EPO gene were present in the granulocyte-derived DNA from patients no. 1 and 4.

Patients without chromosome 7 abnormalities. Patients no. 12-14, 17, and 21 were heterozygous for the HindIII/PAI1 polymorphism, patient no. 17 for the HindIII/EPO polymorphism, and patients no. 14 and 15 for the EcoRI and MspI/MDR2 polymorphisms. Thus, six of ten patients without a chromosome 7 abnormality were heterozygous for at least one polymorphism. In all heterozygous patients both allelic bands were of equal strength in the mononuclear cell and granulocyte fractions. All hybridizing DNA fragments were of expected size.

DISCUSSION

We detected differences between separated blood mononuclear cells and granulocytes using chromosome 7-specific probes in RFLP studies of patients with myeloid disorders. Weakness of an allelic band was observed in granulocyte-derived DNA in heterozygous subjects with monosomy 7 in the bone marrow. In granulocyte-derived DNA from heterozygous patients with a 7q− chromosome the EPO and KV13 probes showed two allelic bands of equal intensity, whereas the MDR2 and PAI1 probes showed weakness of an allelic band. The weakness of an allelic band is most probably related to the chromosome abnormality in these patients, and the results are in agreement with our previous results disclosing monosomy in granulocytes and monocytes but not in lymphocytes in myeloid disorders by chromosome-specific DNA probes. Cross-contamination of the cell fractions and a putative concomitant occurrence of cytogenetically affected and unaffected cells probably account for the presence of weak bands rather than their total absence.

No evidence for rearrangements was found in this or

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Table 2. The Properties of the DNA Probes

<table>
<thead>
<tr>
<th>Probe</th>
<th>Insert Size (kb)</th>
<th>Gene and Type of Probe</th>
<th>RFLP Enzyme</th>
<th>Size of Alleles (kb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KV13</td>
<td>0.6</td>
<td>Unassigned genomic DNA</td>
<td>MspI</td>
<td>6.4, 3.7</td>
<td>27</td>
</tr>
<tr>
<td>EPO</td>
<td>0.78</td>
<td>Erythropoietin cDNA</td>
<td>HindIII</td>
<td>16, 9</td>
<td>21</td>
</tr>
<tr>
<td>PAI1</td>
<td>2.0</td>
<td>Plasminogen activator inhibitor type 1 cDNA</td>
<td>HindIII</td>
<td>22, 18</td>
<td>19</td>
</tr>
<tr>
<td>MDR2</td>
<td>1.0</td>
<td>Multi-drug resistance 2 genomic DNA</td>
<td>EcoRI</td>
<td>4.5, 3.1</td>
<td>15</td>
</tr>
</tbody>
</table>

All probes are of human origin and have previously been mapped to 7q21.1-q22 except pKV13, which was mapped to 7cen-q21.3.

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Fig 2. The RFLP analysis results of 7q− patients no. 1 through 4 with probes KV13, EPO, PAI1, and MDR2. In each panel, the left lane contains mononuclear cell-derived and right lane granulocyte-derived DNA. The allelic bands are of equal strength in the granulocytic DNA hybridized to KV13 and EPO. One of the allelic bands in the granulocyte lanes is absent or much weaker than the other in panels showing hybridization with PAI1 and MDR2.
previous studies using chromosome 7 specific probes. The loss of heterozygosity unrelated to a chromosomal abnormality as observed in many tumors was not detected by any of the several chromosome 7 specific probes used in our present or previous studies. Our results do not exclude such events in myeloid disorders but suggest that other probes should be studied.

DNA rearrangements but no mRNA can be detected in drug-resistant cell lines using probe MDR2, whereas probe MDR1 detects an mRNA, and the two sequences are less than 350 kb apart. P-glycoprotein expression was detected in a recent study by an antibody in ANLL patients. We confirmed the deletion of MDR2 sequences in one informative patient with 7q- and in one patient with monosomy 7. Two patients with normal chromosomes 7 were heterozygous for MDR2 polymorphisms and had two allelic bands of equal strength in each cell fraction. The loss of one MDR2 allele thus seems to be associated with MDS or ANLL both in partial and total deletion of a chromosome 7, but we cannot exclude submicroscopic deletions in at least some patients with two normal chromosomes 7. The same also applies to PAI1, the deletion of which was confirmed in two patients with monosomy 7 and in four patients with a 7q- chromosome. The biologic significance of these associations remains open.

In four patients with 7q- the PAI1 sequences were deleted, whereas the EPO sequences were not. PAI1 and EPO are closely linked with a recombination fraction of 0.03 (LOD 10.08, confidence interval 0.001 to 0.11), and EPO is likely to be located centromeric of PAI1. Our results support this order assuming that no inversions or translocations took place in the 7q- chromosomes; the cytogenetic studies suggest no such rearrangements. The MDR2 sequence was deleted in patient no. 4, suggesting that MDR2 is located distally of EPO. This interpretation is not directly supported by the results of molecular in situ hybridization studies, which place the MDR genes at 7q21.1-21.3.6,17 Localization of MDR2 in relation to EPO and PAI1 by linkage and physical mapping is needed to determine the correct order.

The simplest interpretation of our data is that the patients with a 7q- chromosome had a deletion breakpoint in a narrow region (3 cM) between the EPO and PAI1 genes (Fig 3). The entire genetic length of chromosome 7 has been estimated to be 170 cM in males and 250 cM in females. Thus, in different patients the deletion breakpoint occurred within a region comprising approximately 1.2% to 1.8% of the whole chromosome. Furthermore, our results indicate the same molecular limits for the proximal breakpoint in patients with cytogenetically different 7q- chromosomes (Table 1, Fig 1). The nature of the sequences adjoining the deletion breakpoints in chromosome 7 is unknown at present. The relatively short distance in which deletions occurred in the different patients may suggest a specific site involved in the deletion. On the other hand, it is uncertain in which way, if any, the deletion is related to the leukemic process.

The region around the cystic fibrosis locus has been mapped in detail by linkage studies as well as physically. In this region a recombination distance of 1 cM corresponds to approximately 1,000 kb physical distance. Assuming that the recombination frequency is similar in the region between the EPO and PAI1 genes, an approximate distance of 3,000 kb (range, 100 to 11,000 kb) between these can be estimated. These data justify attempting to determine the breakpoints and distances by physical means using pulsed field gel electrophoresis (PFGE). Our preliminary results show that isolated mononuclear cells and granulocytes can be used as a source of very high molecular weight DNA for PFGE analysis, and experiments on patient material are in progress. Strategies for cloning the breakpoint region can be developed when accurate data on physical distances between known probes and breakpoints are available.

REFERENCES


Chromosome 7 long arm deletion in myeloid disorders: a narrow breakpoint region in 7q22 defined by molecular mapping

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