Mutations in the Nijmegen Breakage Syndrome Gene (NBS1) in Childhood Acute Lymphoblastic Leukemia (ALL)¹

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Abstract

The Nijmegen Breakage Syndrome (NBS) is a rare autosomal recessive disorder associated with immune deficiency, chromosome instability, and increased susceptibility to lymphoid malignancies. The aim of the present study was to elucidate the potential role of the gene mutated in NBS (NBS1) in the pathogenesis and disease progression of childhood acute lymphoblastic leukemia (ALL). Samples from 47 children with first relapse of ALL were analyzed for mutations in all 16 exons of the NBS1 gene, and in 7 of them (14.9%), four novel amino acid substitutions were identified. Mutations S93L, D95N, and I171V occur in the two known domains of nibrin that are probably involved in protein-protein interactions. Germ-line origin of the I171V mutation was confirmed in three patients, whereas the D95N exchange was present only in leukemic cells. The R215W mutation was observed in one ALL but also in a population-based study and probably represents a rare sequence variant. No additional mutations were found on the second allele in any of these seven patients. The observed NBS1 gene mutations in ALL patients points to its possible involvement in the pathogenesis of this disease.

Introduction

ALL is the most common malignant disorder in childhood, with an overall excellent prognosis approaching 80% achieved by current multiagent treatment protocols. Twenty to 25% of children with ALL suffer a relapse. Genetically, leukemogenesis has been associated with specific chromosomal translocations such as t(9;22), t(1;19), and t(12;21) (1, 2) or with rearrangements involving antigen receptor gene loci (immunoglobulin or T-cell receptor; Ref. 3). Increasingly, inherited mutations of genes coding for proteins involved in cell-cycle control, DNA damage repair, and apoptosis have been associated with an elevated predisposition to the development of malignancies (3). Among these, tumor suppressor genes might also play a role in the pathogenesis of ALL (4).

NBS is a chromosomal instability disorder characterized by microcephaly, immunodeficiency, radiation sensitivity, and high susceptibility to lymphoid malignancy. The gene product, nibrin, is a member of the hMRE11/RAD50 protein complex involved in DNA double-strand break repair and recombination (5). Recently it has been shown that nibrin and ATM participate in a common pathway (6–8). Thus far, two domains have been found in the NH2-terminal region of the protein—a FHA and a BRCT, both spanning the first 200 amino acids of nibrin (9)—that are also present in a number of other proteins involved in the cell cycle control (10, 11). On the basis of epidemiological data, it has been suggested that NBS heterozygotes also have an elevated cancer risk (12) similar to AT or other syndromes associated with immune deficiencies (4). The findings that the ATM gene is involved in the pathogenesis of B-CLL (13, 14) and T-cell prolymphocytic leukemia (15) as well as in breast cancer (16) implicate its role as a tumor suppressor gene. The high predisposition of NBS patients to lymphoid malignancy and the fact that the NBS and AT are indistinguishable at the cellular level (17) stimulated us to initiate a study that purposes to answer whether the NBS1 gene is involved in the pathogenesis of ALL and whether it influences the course of the disease and so has its place among the tumor suppressor genes.

Materials and Methods

We analyzed bone marrow samples from 47 patients with first relapse of ALL (42 BCP-ALL and 5 T-ALL), mostly of German origin and unrelated to NBS families, for mutations in the NBS1 gene. All patients were enrolled in multicentric ALL relapse trials of the Berlin-Frankfurt-Münster study group (ALL-REZ BFM). Selection criteria were first relapse of ALL, bone marrow lymphoblasts >95%, and age <18 years. To disclose both the influence of NBS1 on outcome and the efficiency of frontline therapy, as well as its role in leukemogenesis and disease progression, the analysis of NBS1 mutations were primarily focused on relapsed childhood ALL. Informed written consent was obtained from either the parents or the guardians. DNA was extracted from bone marrow after Ficoll separation using a DNA extraction kit (Qiagen, GmbH, Hilden, Germany). All samples were analyzed by PCR–single strand conformation polymorphisms and partially by denaturing high pressure liquid chromatography analysis for all 16 exons of the NBS1 gene. The amplicons were electrophoresed on nondenaturing polyacrylamide gels as previously described (9) or analyzed on a denaturing high pressure liquid chromatography system (WAVE Transgenomic, Santa Clara, CA). The samples showing shifts with either method were directly sequenced. Mutations identified were also analyzed in germ-line DNA, when available, and in DNA samples of control individuals. In addition, we analyzed five highly polymorphic markers on chromosome 8q21, i.e., D8S271, D8S1800, D8S88, D8S1811, and D8S1724, by PCR with fluorescent-labeled primers and electrophoresis on sequencing gel (ALF; Pharmacia, Uppsala, Sweden).

Results and Discussion

Analysis of leukemic cell samples from 47 children with ALL revealed four novel single-base changes causing amino acid substitutions in bone marrow samples from 7 patients with first ALL relapse (5 of 42 BCP-ALL and 2 of 5 (pre-)T-ALL; Table 1). A transition C→T in exon 3 at nucleotide position 278 leading to mutation S93L was detected in patient 2897 with BCP-ALL. Another transition G→A in exon 3 at nucleotide position 283 leading to D95N was identified in patient 976 with BCP-ALL. A transition A→G in exon 3 at nucleotide position 283 leading to D95N was identified in patient 2897 with BCP-ALL. Another transition G→A in exon 3 at nucleotide position 283 leading to D95N was identified in patient 976 with BCP-ALL. Surprisingly, we found that four additional leukemic cell samples from unrelated ALL patients were heterozygous for the A→G at position 511 leading to I171V substitution in exon 5. These three newly identified mutations were
not detected among 110 (S93L and D95N) and among 220 (I171) control chromosomes analyzed. In one additional leukemia cell sample, a transition C→T at position 643 in exon 6 leading to amino acid change R215W was identified. This amino acid substitution was also found in nine probands of Slavic origin from a population-based study performed to estimate the prevalence of the major NBS1 mutation 657del5 (18). However, despite the fact that this exchange results in a substitution of a basic to a nonpolar amino acid, it remains unclear whether this is a causative mutation or a rare sequence variant.

Subsequently, we analyzed germ-line DNA samples to investigate whether the mutations found here were inherited or of somatic origin. Germ-line DNA was available for four of the patients. The constitutional genotype of patients 2796, 2253, and 2924 with the I171V mutation revealed that they were heterozygote carriers of this NBS1 mutation. In contrast, mutation D95N identified in patient 976 was not found in his normal cells, indicating that it arose de novo in the leukemic cell.

In comparison with patients with wt NBS1, children with mutated NBS1 tend to have late relapses (71.4% versus 45%) and an adverse outcome. Only 3 of 7 children with mutated NBS1 (43%) are in second complete remission in contrast to 23 of 40 with wt NBS1 (58%). No significant differences could be detected between patients with mutated and wt NBS1 regarding sex, age at initial disease, combined with the putative nibrin function, suggest that the NBS1 gene might represent a susceptibility factor and be involved in the pathogenesis of ALL. A possible mechanisms leading to the inactivation of the NBS1 gene in ALL could be a dominant negative effect of the missense mutation (21). Consequently, carriers of mutant NBS1 alleles possibly should avoid ionizing radiation and have their treatment protocols for malignant disease modified. However, to assess further the causative nature of the amino acid substitutions found here, functional experiments are in progress.

Acknowledgments

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References

4. Unpublished data.

Table 1 NBS1 gene mutations in ALL and patients’ characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>NBS1 exon</th>
<th>Amino acid exchange</th>
<th>Germ-line mutation</th>
<th>Immune phenotype</th>
<th>Age at diagnosis*</th>
<th>relapse*</th>
<th>Present clinical status</th>
</tr>
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<td>3</td>
<td>S93L</td>
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<td>76</td>
<td>111</td>
<td>2nd CCR</td>
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<tr>
<td>976</td>
<td>3</td>
<td>D95N</td>
<td></td>
<td>Common</td>
<td>94</td>
<td>131</td>
<td>2nd CCR</td>
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<tr>
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<td>5</td>
<td>I171V</td>
<td></td>
<td>Common</td>
<td>60</td>
<td>97</td>
<td>2nd CCR</td>
</tr>
<tr>
<td>2253</td>
<td>5</td>
<td>I171V</td>
<td></td>
<td>Common</td>
<td>43</td>
<td>72</td>
<td>Relapse</td>
</tr>
<tr>
<td>2924</td>
<td>5</td>
<td>I171V</td>
<td></td>
<td>Pre-B</td>
<td>145</td>
<td>176</td>
<td>Relapse</td>
</tr>
<tr>
<td>1958</td>
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<td>Pre-T</td>
<td>100</td>
<td>124</td>
<td>Relapse</td>
</tr>
<tr>
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<td>R215W</td>
<td>NA</td>
<td>T</td>
<td>98</td>
<td>108</td>
<td>Non-response</td>
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</tbody>
</table>

* Age in months.
NA, Germ-line DNA not available.
CCR, continuous complete remission.

Table 2 Analysis of loss of heterozygosity at 8q21

<table>
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<th>Patient no./Marker</th>
<th>DSS1271</th>
<th>DSS1800</th>
<th>DSS588</th>
<th>DSS1811</th>
<th>DSS1724</th>
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<td>2-3</td>
<td>3-7</td>
<td>1-7</td>
<td>3-3</td>
</tr>
<tr>
<td>2253/ALL</td>
<td>6-6</td>
<td>2-3</td>
<td>4-7</td>
<td>1-8</td>
<td>2-2</td>
</tr>
<tr>
<td>2255/ALL</td>
<td>6-6</td>
<td>2-2</td>
<td>4-5</td>
<td>1-7</td>
<td>2-2</td>
</tr>
<tr>
<td>2924/ALL</td>
<td>3-4</td>
<td>1-1</td>
<td>1-7</td>
<td>1-1</td>
<td>5-5</td>
</tr>
<tr>
<td>Germ line</td>
<td>3-4</td>
<td>1-1</td>
<td>1-7</td>
<td>1-1</td>
<td>5-5</td>
</tr>
<tr>
<td>2796/ALL</td>
<td>3-6</td>
<td>2-4</td>
<td>4-7</td>
<td>1-9</td>
<td>2-2</td>
</tr>
<tr>
<td>976/ALL</td>
<td>3-6</td>
<td>2-4</td>
<td>4-7</td>
<td>1-9</td>
<td>2-2</td>
</tr>
</tbody>
</table>

Genotypes for different closely linked polymorphic markers are given for tumor and germ-line DNA (where available) for all patients with NBS1 mutations. Alleles were renamed according to size and compared with an internal reference sample.

3571
3572

NBS1 MUTATIONS IN CHILDHOOD ALL


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