Characterization of CEBPA Mutations in Acute Myeloid Leukemia: Most Patients with CEBPA Mutations Have Biallelic Mutations and Show a Distinct Immunophenotype of the Leukemic Cells

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ABSTRACT

Purpose: The transcription factor CCAAT/enhancer binding protein α, encoded by the CEBPA, is crucial for the differentiation of immature granulocytes. Mutation of the CEBPA may play an important role in leukemogenesis and prognosis. We sought to characterize the CEBPA mutation in acute myeloid leukemia (AML) and to clarify if there is a distinct immunophenotype for leukemic cells with the mutation.

Experiment Design: One hundred and four patients with de novo AML were evaluated for the CEBPA mutation and immunophenotype of the leukemic cells.

Results: Twenty-two distinct mutations were identified in 16 (15%) of 104 AML patients. Fourteen patients had biallelic mutations, mostly involving both the NH2-terminal TAD1 region and the COOH-terminal basic leucine zipper domain (bZIP). The mutations in the bZIP region were always tandem duplications and were located at hot-spot regions for topoisomerase II sites. Sequential study of the CEBPA mutations showed that the mutations disappeared at complete remission but the same mutations reappeared at relapse. None of the patients developed novel mutations during the follow-up period. Patients with CEBPA mutations had significantly higher incidences of CD7 (73%), CD15 (100%), CD34 (93%), and HLA-DR (93%) expression on the leukemic cells.

Conclusion: These data revealed that most AML with CEBPA mutations were associated with an immunophenotype of HLA-DR+CD7+CD13+CD14+CD15+CD33+CD34+. The close relationship of CEBPA mutations with the leukemia status of the patients and the concordance of mutation in presenting and relapse samples implicate the CEBPA mutation as a potential marker for monitoring minimal residue disease.

INTRODUCTION

CCAAT/enhancer binding protein α (CEBPa) is a 42-kDa transcription factor that possesses a DNA-binding basic leucine zipper domain (bZIP), composed of a basic region and a leucine zipper domain, in the COOH terminus and two transactivation domains TAD 1 and TAD 2 in the NH2 terminus (1). CEBPα dimersize via its leucine zipper domain and then binds DNA via the adjacent basic region. Once bound to DNA, CEBPα mediates transactivation via its NH2-terminal TAD (2). CEBPα expression is selectively maintained during granulocyte differentiation but is markedly down-regulated with monocytic differentiation (3, 4). Microarray analysis showed that CEBPα could up-regulate those genes important for regulation of hematopoietic stem cell homing and granulocytic differentiation but down-regulate those genes coding for signaling molecules and transcription factors that are implicated in regulation of proliferation of hematopoietic cells (5, 6). It could block progression from the G1 to S phase and induce terminal maturation of hematopoietic cells (7, 8).

Diminished C/EBPα activity is widely known to contribute to the transformation of myeloid progenitors via reduction of their differentiation potential (9). Recently, CEBPA mutations were detected in 7% to 15% of patients with acute myeloid leukemia (AML; refs. 10–14). These mutations largely fall into two major categories: one comprises those mutations that prevent C/EBPα DNA binding via alteration of its COOH-terminal bZIP, and the other comprises those that disrupt translation of the C/EBPα NH2 terminus, leading to reinitiation of translation at an alternative internal ATG codon located 351 nucleotides downstream of the main AUG initiation codon, and as a result, formation of a 30-kDa C/EBPα isoform. This 30-kDa isoform has the capacity to further reduce wild-type C/EBPα activity by inhibiting its DNA binding and transactivation of the target genes in a dominant-negative effect (10, 15). CEBPA mutations were most frequently found in AML M1 or M2 subtype and in those with intermediate-risk cytogenetics. However, the age distribution of the patients with the CEBPA mutations and the immunophenotype of their leukemic cells are not known. Sequential studies of these patients at remission and relapse are also limited (15). In the present study, we analyzed entire CEBPA sequences in 104 patients with de novo AML diagnosed during the period from 1995 to 2000 and 18 selected children diagnosed before the year 1995. We found that the AML patients in this area had a higher frequency of CEBPA mutations than those in the West. The children with AML had a probability to have CEBPA mutation similar to that of the adults.
In addition, we showed for the first time that most patients (73%) with CEBPA mutations showed expression of all CD7, CD15, CD34, and HLA-DR on the leukemic cells, compared with only 4% in those without the mutation. Most patients with CEBPA mutations had biallelic mutations involving both the TAD1 and bZIP regions. The mutations in the bZIP region were always internal tandem duplications and might relate to the presence of the potential topoisomerase II sites in the region.

MATERIALS AND METHODS

Patients. The CEBPA mutation was studied in the bone marrow cells from 104 unselected patients with de novo AML who had had cells cryopreserved at the National Taiwan University Hospital from 1995 to 2000. Sixty-one were males and 43 were females. There were 96 adults and 8 children (≤15 years). The median age was 46 years, ranging from 1 to 85 years. According to the French-American-British classification (16), 25 were M1, 41 were M2, 17 were M3, 13 were M4, six were M5, and two were M7 subtypes. Excluding the 25 patients who did not receive any chemotherapy or were only treated with low-dose cytosine arabinoside because of old age and/or poor performance status, all other patients with non-M3 subtypes of AML received conventional induction chemotherapy with one of the anthracyclines (doxorubicin or idarubicin) for 3 days and cytosine arabinoside for 7 days. The patients with acute promyelocytic leukemia (M3 subtype) received all-trans retinoic acid with or without concurrent induction chemotherapy. After complete remission was achieved, the patients received consolidation chemotherapy with a conventional dose of cytosine arabinoside and one anthracycline or with high-dose cytosine arabinoside. Sixteen patients received hematopoietic stem cell transplantation.

Immunophenotyping and Cytogenetic Study. A panel of monoclonal antibodies to myeloid-associated antigens including CD13, CD33, CD11b, CD15, CD14, and CD41a, as well as lymphoid-associated antigens including CD2, CD5, CD7, CD19, CD10, and CD20, and lineage nonspecific antigens HLA-DR, CD34, and CD56 was used to characterize the phenotypes of the leukemic cells. Expression of surface antigens on the leukemic cells was shown by an indirect immunoalkaline phosphatase method as described before (17). The cutoff value for positive result of the markers was more than 20%. Chromosome analyses were carried out as described previously (18). Bone marrow cells were harvested directly or after 1 to 3 days of nonstimulated culture. Metaphase chromosomes were banded by the conventional trypsin-Giemsa banding technique.

Chromosome analyses were carried out as described previously (18). METAPHASE CHROMOSOMES WERE BANDED BY THE CONVENTIONAL TRYSIN-GIEMSA BANDING TECHNIQUE. Chromosomal abnormalities were grouped as poor-risk cytogenetics, and all other cytogenetic abnormalities were considered as good-risk cytogenetics (19). Chromosomal abnormalities 1(8;21), t(15;17), and inv(16) were considered as good-risk Cytogenetic Nomenclature (19). Chromosomal abnormalities banded by the conventional trypsin-Giemsa banding technique were evaluated in 50 metaphases for each patient. All patients had at least two metaphases meeting the criteria. The percentage of metaphases with abnormal chromosomal abnormalities was used to determine the type of cytogenetic abnormalities. The cutoff value for positive result of the markers was more than 20%. Chromosome analyses were carried out as described previously (18). Bone marrow cells were harvested directly or after 1 to 3 days of nonstimulated culture. Metaphase chromosomes were banded by the conventional trypsin-Giemsa banding technique and karyotyped according to the International System for Human Cytogenetic Nomenclature (19). Chromosomal abnormalities t(8;21), t(15;17), and inv(16) were considered as good-risk cytogenetics; those of −5/del(5q), −7/del(7q), 3q abnormality and complex karyotype with four or more unrelated abnormalities were grouped as poor-risk cytogenetics, and all other aberrations as intermediate-risk cytogenetics.

Analysis of the Mutation of the CEBPA. Mutation of the CEBPA was detected by genomic DNA PCR and direct sequencing according to the method described previously (17). The primer sets used were the same as those designed by Pabst et al. (10). Briefly, two overlapping primer pairs were used to amplify the entire coding region of human CEBPA: PP1 (550 bp) 5'-TCGCCATGCGGAGAATCTAAC-3' (sense) and 5'-CTGGTAAGGGAAGGCCGCGAG-3' (antisense), PP2 (680 bp) 5'-CCGCTGTTGATCAACGAGGAG-3' (sense) and 5'-CAGC GTCTGGCAAGCCCTCGAGAT-3' (antisense). Four alternative primer pairs were used in cases of abnormal or ambiguous results: PP3 (290 bp) 5'-TCG- CCATGCGCGGAGAATCTC A-C-3' and 5'-ACGGCGCGTCCT- GGCGTCTCCTCGAT-3', PP4 (279 bp) 5'-CTTCA ACA CGAGTCTCAGGCCGCA-3' and 5'-AGCCTGCTGGTTCATCT- CTCTC-3', PP5 (371 bp) 5'-CCGCTGTTGATCAACGAGGAG-3' and 5'-CCGGTCTCAGTTGCAGAT-3' and 5'-ACGGCTGGTTGG- CAAGCTCGAGAT-3'. PCR reactions were run in a final volume of 50 L containing genomic DNA (100 ng), KCI (50 mmol/L), Tris-HCl (20 mmol/L, pH 8.4), MgCl2 (2.5 mmol/L), 5 volume % DMSO, primers (2 mmol/L of each), nucleotides (0.1 mmol/L of each), and Taq DNA polymerase (1.25 units, Life Technologies, Gaithersburg, MD). The mixture was denatured at 94°C for 1 minute, annealed at 61°C for 40 seconds, and extended at 72°C for 90 seconds for 35 cycles, with a final step for 10 minutes at 72°C. PCR products were electrophoresed on 2% agarose gels, purified (Qiagen, Hilden, Germany) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit, which contained AmpliTaq DNA polymerase FS (Applied Biosystems, Foster City, CA), on an automated ABI-3100 Genetic Analyzer (Applied Biosystems). Abnormal sequencing results were confirmed by repeated analyses at least thrice in both directions, including performing different PCR and sequencing with an alternative pair of PCR primers. Because the number of children studied in this series was too low to compare the frequency of CEBPA mutation in this age group with that in adults, 18 additional children with AML diagnosed before the study period (1995-2000) were recruited for analysis. The DNA extracted from the peripheral blood mononuclear cells of 19 healthy persons were used as normal controls.

To determine whether double mutations in different regions of the CEBPA of these patients were in different alleles, the entire coding region was amplified with the primers PP1 sense and PP2 antisense shown above. The PCR products were then cloned into the TA-cloning vector pGEM-T Easy (Promega, Madison, WI) and 7 to 13 clones were sequenced in each patient with the primers used to amplify the corresponding regions of the CEBPA.

Analysis of the Internal Tandem Duplication of the FLT. Genomic DNA was amplified by PCR using the primer pairs of 5'-CAATTAGGTAGTAAAGGC-3' (forward) and 5'-GTACCCCTAGGATTGACA-3' (reverse), which covered the justamembrane domain through the TK1 domain (20). The PCR products were then electrophoresed on 4% agarose gel and visualized with ethidium bromide under an UV lamp. The abnormal PCR products were then purified and cloned into the TA-cloning vector pGEM-T Easy and sequenced.

Statistics. Continuous variables were compared by Wilcoxan rank-sum test, and discrete ones, by χ² or Fisher’s exact test. Curves of survival and complete remission (CR) duration were plotted by the Kaplan-Meier method; differences between curves were analyzed by the log-rank test. All statistical analyses were done using the SPSS 8.0 for Windows (SPSS, Chicago, IL). The significance of results was defined as a level of P < 0.005 at both tails.
RESULTS

Characterization of CEBPA Mutations. Twenty-eight mutations were identified in the 104 AML patients, which were mainly located in the TAD1 region or its upstream and in the bZIP domain (Table 1).

Excluding the mutations stated above, 16 AML patients (15%) were found to have 22 distinct mutations (Table 3). Fourteen patients were shown to have biallelic mutations by cloning and subsequent nucleotide sequence analyses. In 11 of them, the mutations were heterogeneous, with one allele containing a mutation in the TAD1 region and the other allele, a mutation in the bZIP regions, although one or two cloned alleles from two patients showed both mutations on the same alleles. In one patient (patient 52), the two alleles had different bZIP mutations; in another (patient 78), the two alleles carried a TAD1 mutation and a TAD2 mutation, respectively; and in the remaining one (patient 23) the mutation was homozgyous in the bZIP region (Table 3). The nine NH2-terminal mutations comprised of TAD1-A to TAD1-I (Table 1), were all frame-shift mutations and were predicted to generate a shortened dominant-negative 30-kDa (CEBPap30) isoform instead of the 42-kDa wild form by using alternative internal initiation codon (10, 15). Most of the COOH-terminal mutations (9 of 12), comprising bZIP-B to bZIP-H, bZIP-J, and bZIP-K, were in-frame mutations with internal tandem duplications clustered in the junction between the basic region and the leucine zipper (Table 1). The remaining TAD2-A mutation was a frame-shift mutation located in TAD2, downstream of the alternative initiation site for C/EBPap30.

### Table 1. Characterization of the mutations of the CEBPA coding region

<table>
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<tr>
<th>Mutation</th>
<th>Nucleotide feature</th>
<th>Protein feature</th>
</tr>
</thead>
<tbody>
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<td>TAD1-A nt641-647 (-7 bp)</td>
<td>GCCACCT</td>
<td>H186X157</td>
</tr>
<tr>
<td>TAD1-B nt655 (-CC)</td>
<td>6C → 4C</td>
<td>P236X159</td>
</tr>
<tr>
<td>P1 nt654 (C → A)</td>
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<td></td>
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<tr>
<td>TAD1-C nt715 (-C)</td>
<td>2C → 1C</td>
<td>P426X159</td>
</tr>
<tr>
<td>TAD1-D nt773 (-C)</td>
<td>2C → 1C</td>
<td>I626X159</td>
</tr>
<tr>
<td>TAD1-E nt775/776 (+A)</td>
<td>1A → 2A</td>
<td>I626X159</td>
</tr>
<tr>
<td>TAD1-F* nt800/801 (+C)</td>
<td>3C → 4C</td>
<td>A716X159</td>
</tr>
<tr>
<td>TAD1-G nt838 (-C)</td>
<td>2C → 1C</td>
<td>Q836X159</td>
</tr>
<tr>
<td>TAD1-H nt855/861 (-5 bp)</td>
<td>GGAGAAG, +CA</td>
<td>Q885H, S896X100</td>
</tr>
<tr>
<td>TAD1-I nt872-877 (-6 bp)</td>
<td>CCCTGC</td>
<td>normal, P129P</td>
</tr>
<tr>
<td>TAD1-J nt896-904 (-9 bp)</td>
<td>6GCC ← 3GCC</td>
<td>R1526X159</td>
</tr>
<tr>
<td>TAD1-K nt875/876 (+10 bp)</td>
<td>+T, nt868-875 duplication</td>
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</tr>
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<td>TAD2 P2 nt978 (C → T)</td>
<td>4G → 3G</td>
<td>P189-190ins, 7P → 8P</td>
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<td>TAD2-A nt1045 (-G)</td>
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<td></td>
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<tr>
<td>TAD2-B nt1146 (G → T)</td>
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<td></td>
</tr>
<tr>
<td>TAD2-C nt1157/1158 (+3 bp)</td>
<td>7GCC ← 8GCC</td>
<td>normal, P185P</td>
</tr>
<tr>
<td>TAD2-D nt1178/1179 (+6 bp)</td>
<td>3GCC/CC ← 4GCC/CC</td>
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<td>Upstream to bZIP P4* nt1281 (G → T)</td>
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<td>Q305-306ins, 10 → 2Q</td>
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<td>bZIP B nt1511/1512 (+9 bp)</td>
<td>nt1501-1511 duplication</td>
<td>QRN307-308ins</td>
</tr>
<tr>
<td>bZIP C nt1518/1519 (+3 bp)</td>
<td>nt1516-1518 duplication</td>
<td>E309-310ins</td>
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<td>nt1507-1530 duplication</td>
<td>RNVIETQ313-314ins</td>
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<td>bZIP G* nt1533/1534 (+3 bp)</td>
<td>nt1531-1533 duplication</td>
<td>V314-315ins</td>
</tr>
<tr>
<td>bZIP H nt1538/1539 (+36 bp)</td>
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<td>DQRVNETQK315-316ins</td>
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<tr>
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<td>+A, +TCAT</td>
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</tr>
<tr>
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<td>TQQKVLLET320-321ins</td>
</tr>
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<td>bZIP K nt1568/1569 (+57 bp)</td>
<td>nt1512-1568 duplication</td>
<td>VETQQKVLLET327-328ins</td>
</tr>
<tr>
<td>bZIP L nt1595 (T → A)</td>
<td>335 L → Q</td>
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Note. Sequence numbering is according to Genbank accession no. U340700.

*Mutations which have been reported before (10–14, 21).
Sequential Studies of CEBPA Mutations in Acute Myeloid Leukemia Patients. CEBPA mutations were serially studied in 27 patients (Table 2), including 11 patients with distinct CEBPA mutations at diagnosis and another 16 patients with no mutation (10 patients) or with insignificant mutations (6 patients). None developed a new mutation during the follow-up period. Among the 11 CEBPA-mutated patients, all mutations disappeared at CR. The same mutations as those at diagnosis were detected at first relapse in all four patients who relapsed (patients 2, 18, 52, and 93) and also at second relapse in patient 93, although cytogenetic evolution was found in three of them (patients 2, 18, and 93). Serial studies of allelic frequency and distribution of the mutations at diagnosis and relapse by cloning and sequencing were done in three patients with biallelic mutations (Table 4). One cloned allele with double mutations emerged at relapse in patient 18. On the contrary, double mutations detected at diagnosis were not found at relapse in patient 93. In the six patients with insignificant mutations, including one with TAD2-B, four with TAD2-C, and one with P2 silent mutation, the mutations remained the same at CR in all of them and also at relapse in the three patients studied. One patient (patient 95) showed chromosomal abnormalities at relapse that were different from those at diagnosis. In the 10 patients without mutation at diagnosis, there was also an

**Table 2** Sequential follow up of CEBPA mutations and karyotypic changes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Status</th>
<th>Karyotype</th>
<th>NH₂-terminal*</th>
<th>COOH-terminal*</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2</td>
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<tr>
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<td>N</td>
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NOTE. The data of the serial studies in another 10 patients without CEBPA mutation were not shown. There was also an absence of mutation at relapse in these patients after a median interval of 15 months (range, 4-58 months). Three of them had karyotypic evolution at relapse.

Abbreviations: CR, complete remission; N, normal karyotype or normal CEBPA; ND, not determined.

*The details of the mutations were shown in Table 1.

†Silent mutation.
absence of mutation at relapse after a median interval of 15 months (range, 4-58 months). Three of them had karyotypic evolution at relapse.

**Correlation of CEBPA Mutations with Biological and Clinical Features.** Comparison of the clinical and laboratory features between the 16 patients with and 88 without CEBPA mutation is summarized in Table 5. All but one patient with CEBPA mutations had M1 or M2 subtypes of AML. Overall, 15 (23%) of 66 patients with M1 or M2 subtypes of AML showed CEBPA mutations, compared with 1 (3%) of 38 patients with other subtypes of AML who did so \( (P = 0.001) \). One hundred and two patients had cytogenetic data. Fifteen (25%) of 61 patients with intermediate-risk cytogenetics and 11 (35%) of 31 patients with normal karyotype showed CEBPA mutations; however, none of those with good- and poor-risk cytogenetics showed these. Immunophenotyping of the leukemic cells was done in 97 patients. The patients with CEBPA mutations had significantly higher incidences of CD7 (73%), CD15 (100%), CD34 (93%), and HLA-DR (93%) expression than other patients (Tables 3 and 5). Eleven (73%) of 15 patients with CEBPA mutations showed expression of all these four antigens, compared with only 3 (4%) of 82 patients without CEBPA mutation \( (P < 0.001) \). The expression of other antigens in the CEBPA-mutated patients was similar to that of the patients without CEBPA mutations.

The patients with CEBPA mutations had significantly higher hemoglobin levels and a trend of lower platelet counts than did those without the mutation (Table 5). The adults with CEBPA mutations had trends of longer median CR duration and survival time than those without the mutation (19 versus 9 months, \( P = 0.212 \) and not reached versus 32 months, \( P = 0.225 \), respectively). If only the patients with intermediate-risk cytogenetics were analyzed, the median CR duration and survival time were 17 months and not reached respectively in the patients with CEBPA mutations, compared with 7 and 26 months, respectively, in those without the mutation \( (P = 0.071 \) and 0.265, respectively).

**Internal Tandem Duplication of the FLT3.** Internal tandem duplication of the FLT3 was detected in 18 (17%) of 104 AML patients. Only two patients with CEBPA mutations showed FLT3-internal tandem duplication.

### DISCUSSION

In this study, CEBPA mutations were detected in 15% (16 of 104) of the total AML patients, 25% (15 of 61) of the

<table>
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<th>Mutation 2*</th>
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</table>

NOTE. No new mutation was detected at relapse.

*Mutation 1 and Mutation 2 stand for leftward and rightward mutation, respectively.
AML patients with intermediate-risk cytogenetics, and 35% (11 of 31) of those with normal karyotype. These frequencies were higher than those reported in western countries: around 7% in total AML and 15% in those with intermediate-risk cytogenetics or those with normal karyotype (10, 11, 14, 21). Uneven geographic distribution of nonrandom chromosome abnormalities in malignant disorders has been reported before (17, 22, 23). It is suggested that the heterogeneity in the incidence of nonrandom cytogenetic abnormalities in various areas may reflect differences in racial or environmental factors (24). The distribution of the genetic changes in AML that can not be detected in cytogenetic studies, such as CEBPA mutations, may also show geographic differences. The studies of the CEBPA mutation in the literature were all done on adults, and the gene mutations in pediatric AML have not been characterized yet. Because the number of AML children in this study was very limited, the incidence of the CEBPA mutation in these children (0 of 8) could not reflect the true condition. To clarify the occurrence of the CEBPA mutation in pediatric AML, 18 additional children with AML diagnosed before the year 1995 were recruited for analysis. Three (12%) of a total of 26 children were found to have CEBPA mutations (data not shown), an incidence similar to that in the adults (16 of 96, 17%), indicating there is no discrepancy in CEBPA aberration in children and in adults.

AML patients with some specific chromosomal abnormalities showed distinct immunophenotypes (17, 25, 26). However, the immunophenotype of the CEBPA-mutated AML has not been reported before. In the present study, the leukemic cells from 11 of 15 CEBPA-mutated patients expressed all the HLA-DR, CD7, CD15, and CD34 antigens; in addition, the majority of CEBPA-mutated leukemic cells expressed CD13 (87%) and CD33 (87%), but not CD14 (13%), delineated an immunophenotype of HLA-DR+CD7+CD13+CD14−CD15−CD33+CD34+. The CEBPA mutations occur most frequently in AML M1 or M2 subtypes (10–12, 14). CD15 is usually detected in the more differentiated subtype M2 of AML, and can also be present in other AML, including M0 and M1 subtypes (27, 28). Interestingly, all patients with t(8;21), a chromosome translocation associated with down regulation of C/EBPx expression and function (29), showed CD15 expression on the leukemic cells, as shown in our previous study (17) and in this study (data not shown). CD7 is a T cell–associated antigen, the frequency (73%) of CD7 expression was found in the CEBPA-mutated leukemic cells. Recently, Valk et al., using Affymetrix U133A GeneChips analysis, also showed clustering of up-regulated CD7 with CEBPA mutations in AML cells (34). Whether expression of one of the dominant-negative CEBPA mutations induces CD7 expression in a myeloid cell line is a subject that requires further study.

Several reports have linked CEBPA mutations with a favorable outcome in AML (11, 13, 14). In this study, whereas among the patients with intermediate-risk cytogenetics, the patients with CEBPA–mutations tended to have a longer remission duration and survival time than those without the mutation, the difference did not reach statistical significance. Snaddon et al. also found that CEBPA mutation was not of prognostic importance in their AML patients (12). Furthermore, although the patients in this study did not receive the same consolidation treatment, the percentage of the patients treated...
with conventional doses of cytosine arabinoside, high doses of cytosine arabinoside, or hematopoietic stem cell transplantation was similar between the patients with and without CEBPA mutations (data not shown). Therefore, the treatment heterogeneity might not influence the results of prognostic analysis. Moreover, other genomic abnormalities, such as internal tandem duplication of FLT3 and MLL and other genetic alteration, may accompany CEBPA mutations and have a prognostic impact in the CEBPA-mutated patients (35). Further prospective studies of the genetic alterations in more patients are needed to clarify this point. Functional studies of the C/EBPa protein affected by various mutations, alone, or in combination, may also be helpful to evaluate their influence on clinical outcome.

There were 22 distinct mutations identified in this study. From further evaluation of these CEBPA mutant sequences, two major types of mutants were revealed. One was repeat-number changes of simple sequence repeats, such as TAD1-B to TAD1-H, TAD2-A to TAD2-C, and bZIP-A mutations; the other was internal tandem duplications, such as TAD1-H, TAD1-I, bZIP-B to bZIP-H, bZIP-J, and bZIP-K mutations. The former was supposed to correlate with mismatch repair defect or insufficiency (36); the latter was supposed to correlate with topoisomerase II–induced double strand breaks and subsequent errors in DNA repair through overactive nonhomologous end joining systems (37). After the use of FUZZNUC nucleic acid pattern searches in the EMBoss program, several potential topoisomerase II sites were found within bZIP regions in the CEBPA (Fig. 1), supporting this hypothesis. The significance of topoisomerase II sites for other AML-related mutations, PML-RARA, FLT3-internal tandem duplication, and MLL duplication, has been revealed (39–41). Further topoisomerase II binding activity to these topoisomerase II sites in the CEBPA should be clarified to ensure the significance of topoisomerase II in leukemogenesis of patients with AML.

Most patients (14 of 16) with CEBPA mutations in this study showed genetic alterations in both alleles, most frequently (11 patients) with one allele containing a mutation in bZIP DNA-binding domain and the other, a TAD1 frameshift mutation. A similar finding has been reported before (13). Sequential analyses in this study showed that the CEBPA mutations detected at diagnosis disappeared at CR, but the same mutations reappeared at relapse. No novel mutation was detected during the follow-up period. Cloning and sequencing studies showed that the patients with biallelic mutations at presentation also disclosed the same pattern of mutations at relapse, although a few cloned alleles might have double mutations either at diagnosis or at relapse. Another serial analysis of the CEBPA has been reported (42), one in which no new mutation was detected at relapse, either. These findings suggest that the CEBPA mutation is probably a primary change in the development of AML, but does not play a role in the progression of the disease. Because of the close relationship between the CEBPA mutation and the disease status of the leukemia and the concordance of mutations in presentation and relapse samples, examination of CEBPA mutation may be a useful marker for monitoring minimal residual disease. Furthermore, since all but one CEBPA-mutated patient had bZIP mutants, the detection of minimal residual disease can be simplified by examining the bZIP region using PCR coupled with a DNA fragment analyzer.

![Fig. 1](image-url) Potential topoisomerase II sites in bZIP region of CEBPA gene. Top, consensus topoisomerase II recognition sequence (38, 39). Middle, partial bZIP sequence from nt1492 to nt1527, numbering according to Genbank accession no. U34070. Percentages of homologous rate at right side of potential topoisomerase II sites, showing hot spot at this area. Homologous nucleotides (underlined). Bottom, duplicated fragments of various bZIP mutants (→→).
REFERENCES


Characterization of CEBPA Mutations in Acute Myeloid Leukemia: Most Patients with CEBPA Mutations Have Biallelic Mutations and Show a Distinct Immunophenotype of the Leukemic Cells

Liang-In Lin, Chien-Yuan Chen, Dong-Tsamn Lin, et al.