

BRIEF REPORT

Mutation of *CEBPA* in Familial Acute Myeloid Leukemia

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SUMMARY

We describe a family in whom three members affected by acute myeloid leukemia (AML) had an identical, 212delC mutation in *CEBPA*, the gene encoding the granulocytic differentiation factor C/EBP α . Unaffected family members did not have this mutation. Latent periods of 10, 18, and 30 years elapsed before the onset of overt leukemia in the three patients. One of them had a second *CEBPA* mutation, but only at the time of diagnosis. All three patients are currently well, with no abnormalities in the bone marrow. *CEBPA* mutation is apparently the primary event in the development of AML in this family.

INHERITED ACUTE MYELOID LEUKEMIA (AML) HAS BEEN REPORTED IN ONLY a few families outside of a syndromic setting such as trisomy 21 or a disorder involving defective DNA repair. True nonsyndromic familial AML, a heterogeneous group of disorders, includes autosomal recessive forms that become manifest during childhood in association with myelodysplasia and monosomy 7, as well as autosomal dominant forms that are preceded by various types of a dysplastic phase and that vary in morphologic subtype. The only genetic abnormality that has been identified in these disorders is one identified in the syndrome called familial platelet disorder with predisposition to AML: mutations in the gene encoding runt-related transcription factor 1 (*RUNX1*) have been found in 11 kindreds with this syndrome.¹ Acquired mutations have been identified both in *RUNX1* and in *CEBPA*, the gene that encodes CCAAT enhancer binding protein α (C/EBP α) in sporadic, nonfamilial AML.^{2,3}

CEBPA is a single-exon gene in the chromosomal region 19q13.1. C/EBP α , a granulocytic differentiation factor and a member of the bZIP family, consists of N-terminal transactivating domains, a basic region necessary for specific DNA sequence binding, and a leucine-zipper region necessary for dimerization at the C-terminal end (Fig. 1A). C/EBP α is important in the regulation of myeloid differentiation. Its presence is a characteristic feature of early myeloid cells, and it regulates a number of granulocyte-specific genes by activating promoters for myeloid-specific growth-factor receptors, such as the granulocyte colony-stimulating factor receptor, and neutrophil granule proteins.⁴ C/EBP α synergizes with other proteins necessary for the regulation of myelopoiesis, such as *RUNX1*.⁵ *CEBPA*-knockout mice have an early block in myeloid maturation⁶ and a phenotype similar to AML with differentiation (French–American–British [FAB] subtype M2).⁷

In this report, we describe a family in which multiple members were affected by AML associated with an identical mutation in *CEBPA*. A somatic mutation on the second allele of *CEBPA* was also found in one of the patients.

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CASE REPORTS

Two siblings were referred to St. Bartholomew's Hospital, London, within an interval of two weeks, for treatment of AML. Their father had had AML during childhood but was currently in remission. All the other family members were alive and well, with no medical history of note (Fig. 2).

The father, Patient II-3, had received the diagnosis of AML in September 1963, at the age of 10 years. The marrow aspirate was reported to be hypercellular, with 95 percent myeloperoxidase-positive blast cells — a finding that confirmed the diagnosis of AML without maturation (FAB subtype M1). Karyotypic evaluation was not performed. He was treated with prednisolone and mercaptopurine and entered a complete remission. He had a relapse in January 1965 and was treated with prednisolone and methotrexate; the result was a short-lived second remission. He had another relapse, in July 1965, and was treated with cyclophosphamide and prednisolone. This treatment was stopped in October 1965 because of hemorrhagic cystitis. He then entered a lasting remission despite having received inadequate therapy by current standards.

A son, Patient III-1, presented in February 2003, at the age of 30 years. His marrow was hypercellular, with 30 percent myeloperoxidase-positive blast cells containing Auer rods and aberrantly expressed CD7. There were increased numbers of eosinophil precursors — a finding consistent with a diagnosis of AML, subtype M2 with eosinophilia (M2Eo). Cytogenetic evaluation and fluorescence in situ hybridization for the detection of the t(8;21)(q22;q22) translocation both revealed no abnormalities.

A daughter, Patient III-5, who was 18 years of age, saw her general practitioner the week after her brother's admission to the hospital. Her marrow was normocellular but contained 35 percent myeloperoxidase-positive blast cells with Auer rods and aberrantly expressed CD7. The finding that eosinophil precursors were present in increased numbers indicated, as it had in her brother, a diagnosis of AML, subtype M2Eo. Cytogenetic evaluation revealed no abnormalities.

Both Patient III-1 and Patient III-5 received four courses of combination chemotherapy. Both entered a complete remission after the first course of therapy. Shortly after the final course of therapy and discharge, both patients presented with arthralgias, fever, and malaise. Their white-cell counts were high (28,200 per cubic millimeter in Patient III-1 and 20,400 per cubic millimeter in Patient III-5); neither was still receiving granulocyte colony-stimulating factor, and neither had overt sepsis. In both patients the increase in leukocytes was primarily a neutrophilia, with small numbers of myelocytes and

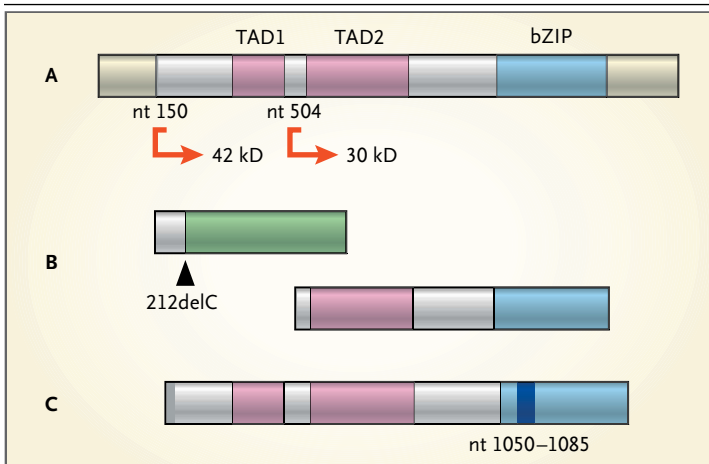


Figure 1. Schematic Representation of CEBPA.

Panel A shows the wild-type 42-kD CEBPA. Transactivating domains 1 and 2 (TAD1 and TAD2 [pink]) and the basic region and leucine zipper (bZIP [blue]) are indicated. Untranslated portions of the gene (gold) are also indicated. ATG start codons are located at nucleotides 150 and 504. The 212delC mutation results in a truncated N-terminal product (green) with an altered amino-acid sequence (Panel B). The 30-kD product that is then generated from the alternative start codon lacks the first transactivating domain. As shown in Panel C, duplication of nucleotides (nt) 1050 through 1085 occurs within the leucine-zipper domain.

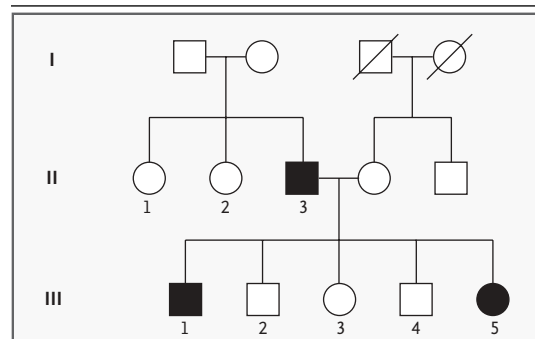


Figure 2. The Patients' Family Tree.

Black symbols indicate family members in whom AML developed, numbered symbols family members who were tested, and symbols with a slash deceased family members.

promyelocytes in Patient III-5 and mild eosinophilia in Patient III-1. The abnormalities in both patients resolved without medical intervention. Currently, more than 20 months since diagnosis, both patients are well and have normal blood counts.

METHODS

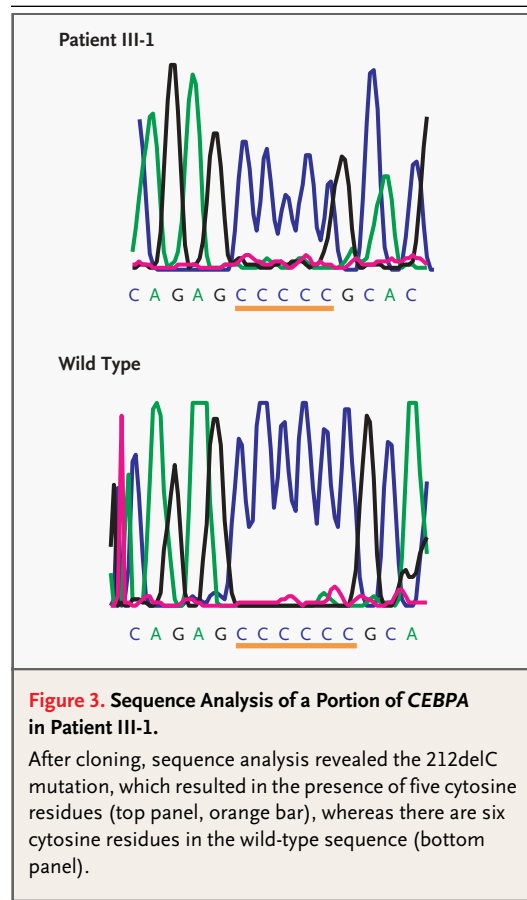
We received approval from the local research ethics committee to study the three patients, and written informed consent was obtained from the patients before evaluation. Samples of mononuclear-cell-enriched peripheral blood obtained at diagnosis were available from Patients III-1 and III-5, and peripheral-blood samples and buccal mucosal swab specimens obtained during remission were available from Patients II-3, III-1, and III-5. DNA was extracted with phenol–chloroform.

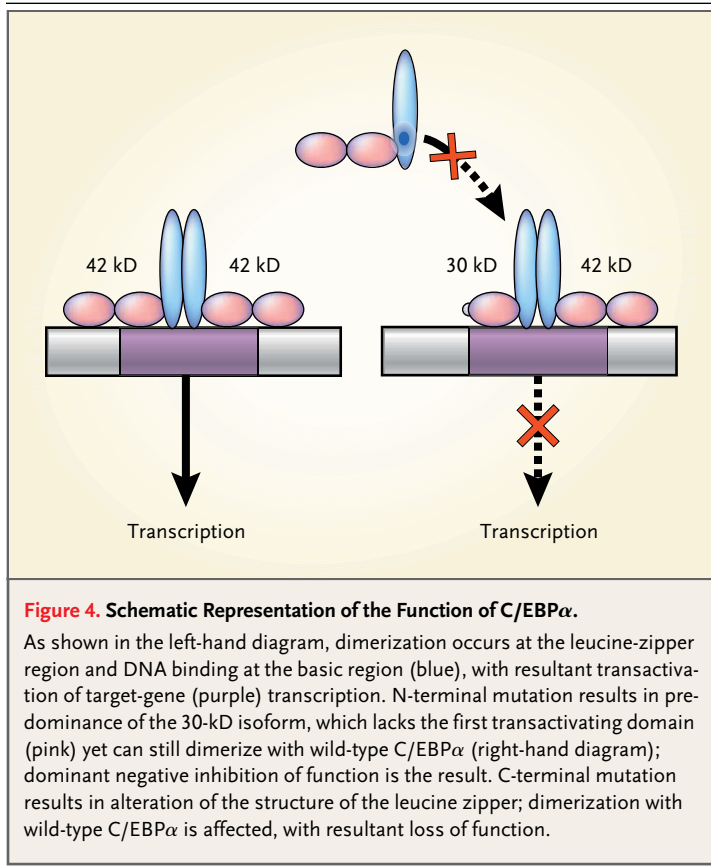
The entire coding region of *CEBPA* in all three patients was screened in four overlapping fragments with the use of overlapping sets of primers and under conditions described elsewhere.⁸ All polymerase-chain-reaction (PCR) fragments were sequenced directly with the use of an ABI 377 DNA sequencer (Applied Biosystems). The sequences of the mutant genes were confirmed by cloning the PCR fragments with a TOPO TA Cloning kit (Invitrogen) and by sequence analysis. Long-range PCR of the biallelic mutation in Patient III-1 was carried out with the use of a TaKaRa LA Taq Kit (Takara Bio). An initial denaturation step at 95°C for 1 minute was followed by 35 cycles of denaturation at 98°C for 15 seconds, a combined annealing-and-extension step at 62°C for 12 minutes, and a final extension step at 72°C for 10 minutes. The primers for the first-round PCR step were designed from the *CEBPA* bacterial artificial chromosome sequence (GenBank accession number AC008738, clone CTD-2540B15); they were CHR19P1 (5'TTGCCAGATGAACTGCTTCTTTACTGCG3'), aligned from 173,185 to 173,156, and CHR19P4 (5'CTGGAATTAGCACTGAAGTCAGAGGGTTTG3'), aligned from nucleotide numbers 167,755 to 167,784, to generate a 5.4-kb fragment. The second, nested PCR step involved the use of the previously published primer pair³ — PP1F (5'TCGCCATGCCGGGAGAAGCTAAG3') and PP6R (5'CACGGTCTGGGCAAGCCTCGAGAT3') — to amplify a 1.2-kb fragment containing the entire *CEBPA* coding region. Position numbering is based on the GenBank DNA sequence number Y11525 and Swiss-Prot protein sequence number P49715.

RESULTS

Patient III-1 was found to have a mutation involving the deletion of a cytosine residue at nucleotide 212 of *CEBPA* (212delC) in DNA extracted from a peripheral-blood sample obtained at the time of diagnosis (Fig. 3). The corresponding protein is predicted to terminate prematurely, at codon 158. His sister, Patient III-5, had an identical *CEBPA* mutation in DNA extracted from a diagnostic peripheral-blood sample. DNA extracted from peripheral-blood samples obtained during remission and germ-line DNA from buccal-swab specimens from both these patients and from their father (Patient II-3) also had the 212delC *CEBPA* mutation.

CEBPA in the diagnostic blood sample from Patient III-1 also had a 36-bp duplication, which comprised nucleotides 1050 to 1085 and resulted in an internal tandem duplication of amino acids 302 to 313 (KAKQRNVETQQK [where amino acids are denoted by their single-letter codes]) (Fig. 1C). Long-range PCR and cloning confirmed that the





two mutations were on separate alleles. The duplication was not found in DNA from blood cells taken from this patient during a remission or in DNA from his buccal-swab specimen (germ-line DNA); it also was not found in any of the samples from Patients II-3 and III-5. No other mutations were identified in screened exons or motifs of *KRAS*, *NRAS*, *KIT*, *PTPN11*, *FES*, *FLT3*, or *RUNX1* from DNA samples taken at the time of diagnosis from Patient III-1 or III-5 (data not shown).

After these results were obtained, screening for the 212delC mutation was offered to all members of this kindred. After pretest counseling and with the involvement of the Genetics Department of St. Bartholomew's Hospital, written informed consent for mutation analysis was obtained from five healthy family members: II-1, II-2, III-2, III-3, and III-4. Direct sequencing of *CEBPA* from DNA extracted from peripheral-blood mononuclear cells confirmed the presence of the wild-type sequence in these five family members. All the other family members declined testing.

DISCUSSION

CEBPA mutations occur in sporadic AML at a frequency of 5 to 10 percent, primarily in patients with normal cytogenetic characteristics and the FAB subtypes M1 and M2. It is therefore notable that the two siblings with AML in the family we studied had a normal karyotype and that they both had an uncommon form of the disease — namely, subtype M2Eo. This subtype of AML is occasionally seen in the 10 to 15 percent of patients with a t(8;21)(q22;q22) translocation, which generates an *AML1/ETO* fusion product. This chimeric protein is known to suppress C/EBP α expression by inhibiting autoregulation of the *CEBPA* promoter.⁹ Hence, the M2Eo subtype may arise from the deregulation of a molecular pathway that includes *CEBPA*.

The 212delC mutation found in this family has also been reported in two cases of sporadic AML.¹⁰ The *CEBPA* mutation was not found in 32 normal subjects or in 330 samples from patients with other cancers.^{3,11} Frame-shift mutations, such as the 212delC mutation, cause truncation of the 42-kD C/EBP α protein and overproduction of a 30-kD isoform that lacks the first transactivating domain but retains the bZIP region required for dimerization (Fig. 1B). Such transactivating domains are necessary for the activity of C/EBP α in the regulation of its target genes by way of specific promoter sequences. In the absence of the functional 42-kD protein, this shorter isoform predominates and, after dimerization, functions in a dominant negative fashion, causing loss of function of C/EBP α (Fig. 4).^{3,11}

The forced expression of a dominant negative mutant *CEBPA* in cultures of cord-blood-derived hematopoietic progenitor cells by means of a retroviral vector dramatically inhibits differentiation of both myeloid and erythroid lineages.¹² In *CEBPA*^{-/-} (knockout) mice, a myeloproliferative disease with a block in neutrophilic differentiation develops,¹³ whereas patients with sporadic AML and a *CEBPA* mutation tend to have a high white-cell count at presentation.^{8,14} Hence, after chemotherapy, marrow regeneration by stem cells bearing a dominant negative mutation might result in a proliferative phenotype. Such a scenario could explain the “regeneration leukocytosis” observed in the two siblings we studied, Patients III-1 and III-5, after they completed therapy.

The findings in this kindred are reminiscent of pediatric acute lymphoblastic leukemia in twins,¹⁵ in whom there may be a long latent period between

birth and the development of disease. In the family we studied, the *CEBPA* mutation appeared to be fully penetrant: AML developed in all carriers of the 212delC mutation, albeit after a long latency period. This latency period (10 years in Patient II-3, 18 years in Patient III-5, and 30 years in Patient III-1) suggests that one or more additional mutations is necessary for the development of overt acute leukemia. Mutation analysis was extended to include several other genes implicated in the development of AML (*KRAS*, *NRAS*, *KIT*, *PTPN11*, *FES*, *FLT3*, and *RUNX1*), but no mutations were identified in these genes. However, a mutation in the other *CEBPA* allele was identified in Patient III-1. This mutation, a duplication, was undetectable during remission and was absent from his germ-line DNA. Such a mutation is predicted to prevent dimerization of C/EBP α

because it disrupts the leucine zipper and therefore results in loss of function of the normal allele (Fig. 1C).

Three large studies of uniformly treated patients have found that mutation of *CEBPA* is an independent favorable prognostic factor.^{10,14,16} Furthermore, *CEBPA* mutations such as 212delC appear to carry an even better prognosis than bZIP mutations. These data may partly explain why Patient II-3 remains in complete remission after receiving inadequate therapy by today's standards.

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