Novel Mutations in CEBPA in Korean Patients with Acute Myeloid Leukemia with a Normal Karyotype

Sollip Kim, M.D.1, Dong-Hwan (Dennis) Kim, M.D.2, Jun-Ho Jang, M.D.2, Chul-Won Jung, M.D.2, Mi-Ae Jang, M.D.3, Chang-Seok Ki, M.D.3, Jong-Won Kim, M.D.3, Sun-Hee Kim, M.D.3, and Hee-Jin Kim, M.D.3

Department of Laboratory Medicine1, Ilsan Paik Hospital, Inje University College of Medicine, Goyang; Departments of Medicine2, and Laboratory Medicine & Genetics3, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Mutations in the transcription factor CCAAT/enhancer binding protein α gene (CEBPA) are found in 5-14% of the patients with AML and have been associated with a favorable clinical outcome. In this study, we aimed to assess the frequencies and characteristics of mutations in CEBPA. Between 2006 and 2009, CEBPA mutations were assessed using archival DNA samples obtained from 30 consecutive adult patients diagnosed with AML with a normal karyotype at our institution. CEBPA mutations were detected using direct sequencing analyses. These mutations were detected and described with reference to GenBank Accession No. NM_004364.3. In our series, CEBPA mutations were detected in 4 patients (13.3%). These mutations occurred as double mutations in all 4 patients. Among the 8 mutant alleles, 5 were novel (c.179_180dupCG, c.50_53delGCCA, c.178_182delACGTinsTTT, c.243_244insGTCG, and c.923_924insCTC). The frequency of occurrence of CEBPA mutations in Korean patients with AML is comparable to that in previous reports. Long-term follow-up data from a larger series of patients with comprehensive molecular profiling are needed to delineate the prognostic implications.

Key Words: CEBPA, Mutation, Acute myeloid leukemia, Normal Karyotype, Korea

The karyotype at the time of diagnosis provides the most important prognostic information in AML patients, but 40-50% of patients do not have any clonal chromosomal aberrations [1-3]. All such cases of AML with normal karyotype (AML-NK) are currently categorized in the intermediate-risk group; however, this group is quite heterogeneous [4, 5]. In recent years, many studies have shown that acquired gene mutations in AML patients have prognostic relevance. In particular, the fms-related tyrosine kinase 3 gene-internal tandem duplications (FLT3-ITD) and myeloid-lymphoid or mixed-lineage leukemia gene-partial tandem duplications (MLL-PTD) have been associated with short relapse-free and overall survivals, whereas AML-NK with mutations in the nucleophosmin gene (NPM1), without concomitant FLT3-ITD, are associated with a more favorable outcome [6]. Other genes that are recurrently mutated in AML include KIT, DNMT3A, IDH1/IDH2, and TET2. Patients with core-binding factor AML commonly carry activating mutations of KIT (20-45%) and show inferior outcomes [7]. DNMT3A mutations were detected in 22.1% of the AML-NK patients and were associated with adverse outcomes [8]. Although IDH1 and IDH2 mutations were detected in 6% of AML patients and 11-12.1% of AML-NK patients, the prognostic impacts of these mutations have been controversial so far [9]. TET2 mutations were detected in 23% AML-NK patients and showed unfavorable outcomes in the favorable-risk group (AML-NK patients with mutated CEBPA and/or mutated NPM1, without FLT3-ITD) [10].

The CCAAT/enhancer binding protein α gene (CEBPA) is a member of the basic region leucine zipper family of transcription factors. It is an intronless gene located on chromosome 19q13.1. It is composed of 2 transactivation domains in the N-
terminal region—a basic leucine zipper region (bZIP) that mediates dimerization with other CEBP family members and a DNA-binding domain in the C-terminal region [11]. The importance of CEBPA in hematopoiesis can be attributed to its crucial role during the development of granulocytes and its deregulation associated with myeloid transformation [11]. Mutations in CEBPA are found in 5-14% of AML patients and have been associated with a favorable clinical outcome [12]. Most CEBPA mutant AML patients simultaneously exhibit 2 mutations (CEBPA-double-mut), which most frequently involve a combination of an N-terminal frame-shift mutation and a C-terminal insertion mutation, that are usually biallelic [12]. In a recent study involving a large cohort of AML-NK patients, only CEBPA-double-mut was associated with a unique gene expression profile and favorable overall and event-free survivals on multivariate analyses including factors such as age, white blood cell count, cytogenetic information, and FLT3-ITD and NPM1 mutation status. Therefore, CEBPA-double-mut was considered a separate disease entity in the classification of AML [13-15].

In this study, we aimed to assess the frequencies and characteristics of CEBPA mutations in Korean patients with AML-NK. Between 2006 and 2009, CEBPA mutations were assessed in 30 consecutive adult patients diagnosed with AML-NK at our institution. Of these patients, 18 were men and 12 women, with a median age of 53 yr (range, 24-88 yr) at diagnosis. According to the WHO classification [16], 13 patients were diagnosed with AML with myelodysplasia-related changes, 8 with AML without maturation, and 1 each with AML with myelodysplasia-related changes, acute myelomonocytic leukemia, and acute erythroid leukemia. The patients were shown to have no recurrent molecular abnormalities on multiplex reverse-transcriptase PCR using the Hemavision-Full Kit (DNA technology A/S, Aarhus C, Denmark) and a panel of FISH assays (LSI 53 on 17p13.1, LSI D20S108 on 20q12, LSI D7S22 on 7q31/CEP 7, CEP 8, LSI EGR1 on 5q31/D5S23, D5S721 on 5p15.2, and LSI MLL dual color, LSI CBFB dual color, or LSI RUNX1/RUNX1T1 on 8q22/22q11q22 probes [Abbott Molecular/Vysis, Des Plaines, IL, USA]). All patients, except 3 who had refused treatment, received conventional induction chemotherapy with idarubicin and cytosine arabinoside. Data about complete blood counts and the proportion of blasts on peripheral blood (PB) and bone marrow (BM)-aspiration smear were obtained by reviewing electronic medical records. We also reviewed the data on immunophenotyping obtained by flow cytometry; FLT3/ITD, FLT3/TKD, and NPM1 mutations; and clinical outcomes after induction chemotherapy.

Genomic DNA extracted from the BM aspirates was analyzed using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer’s instructions. Written informed consent was obtained from the patients. The study protocol was approved by the Institutional Review Board of our institution. The coding sequences and flanking intronic regions of the CEBPA genes were amplified using the previously described primer sets [13, 17]. PCR was performed using a thermal cycler (model 9700; Applied Biosystems, Foster City, CA, USA). Cycle sequencing was performed using the ABI Prism 3100 Genetic Analyzer and the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems). When variations were observed in the sequences obtained using these methods and the reference sequences, we performed follow-up tests by using BM or PB specimens collected at complete remission; 2 PB specimens from healthy volunteers and 2 BM specimens showing no involvement of lymphoma during staging workup were used as controls. CEBPA mutations were described according to the guidelines of the Human Genome Variation Society by using the reference sequences NM_004364.3 and NP_004355.2. Only an insertion polymorphism or variation(s) not leading to amino acid changes was considered wild type [13, 18, 19].

In our study, CEBPA mutations were detected in 4 patients (13.3%), and all had double mutations (Fig. 1). CEBPA double mutations in these 4 patients were c.179_180dupCG at the N-terminal region [N] and c.929_930insSTCT at the C-terminal region [C]; c.50_53delGCCA [N] and c.912_913insTTG [C]; c.178_182delACGTinsTNT [N] and c.923_924insCTC [C]; and c.243_244insGTCG [N] and c.929_930insTCT [C]. Among these mutations, all but one (9/24) were observed in the sequences obtained using these methods. The only exception was c.243_244insGTCG [N] and c.923_924insCTC [C]. Among these mutations, all but one (9/24) were observed in the sequences obtained using these methods. The only exception was c.243_244insGTCG [N] and c.929_930insTCT [C]. Among these mutations, all but one (9/24) were observed in the sequences obtained using these methods. The only exception was c.243_244insGTCG [N] and c.929_930insTCT [C].

All patients (4/4, 100%) with CEBPA mutations showed a CR after induction chemotherapy, whereas 83.6% (22/26) of patients without CEBPA mutations showed a CR after induction chemotherapy. The CEBPA mutations were not observed in the follow-up samples at complete remission. FLT3-ITD was detected in 10% (3/30) of the patients, and the NPM1 mutation in 37.5% (9/24). Among the 4 patients with CEBPA-double-mut, 1 also had the FLT3-ITD mutation, whereas none of them had the NPM1 mutation. Detailed clinical and molecular characteristics of these 4 patients are shown in Table 1. Six-nucleotide in-frame insertion polymorphisms of CEBPA were observed in 30% (9/30) of the patients. This polymorphism was also observed in the follow-up samples at complete remission and in control BM specimens.

Our results showed that the frequency of CEBPA mutations in Korean patients with AML-NK is 13.3% (4/30), which is compa-
Novel CEBPA mutations in AML-NK

CEBPA mutations occurred as double mutations in all 4 patients. CEBPA double mutations in these patients were c.179_180dupCG [N] and c.929_930insTCT [C], c.50_53delGCCA [N] and c.912_913insTTG [C], c.178_182delACGTinsTTT [N] and c.923_924insCTC [C], and c.243_244insGTCG [N] and c.912_913insTTG [C]. Among these mutations, c.179_180dupCG, c.50_53delGCCA, c.178_182delACGTinsTTT, c.243_244insGTCG, and c.923_924insCTC were novel mutations.

Figure 1. CEBPA mutations detected in 4 patients.

Table 1. Clinical and molecular characteristics of 4 Korean patients with AML-NK harboring CEBPA double mutations

| Patient No. | Age (yr) | Sex | BM diagnosis | Immunophenotype | CEBPA gene mutation | FLT3/ITD | NPM1 |
|------------|----------|-----|--------------|----------------|---------------------|----------|------|
|            |          |     |              |                | N-terminal          | C-terminal|      |
| 1          | 51/F     |     | AML with MRC | CD34+, HLA-DR+, CD13+, CD33+, CD117+, cMPO+, CD64+, ectopic CD7+ | c.179_180dupCG [p.S61Afs] | c.929_930insTCT [p.310_311insL] | Neg | Neg |
| 2          | 41/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w, ectopic CD7+ | c.50_53delGCCA [p.H18Qfs] | c.912_913insTTG [p.K304_Q305insL] | Pos | Neg |
| 3          | 48/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64+, ectopic CD7+ | c.178_182delACGTinsTTT [p.T60Ffs] | c.923_924insCTC [p.K304_Q305insL] | Neg | Neg |
| 4          | 42/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w, ectopic CD7+ | c.243_244insGTCG [p.F82Vfs] | c.912_913insTTG [p.K304_Q305insL] | Neg | Neg |

*Description at the cDNA level (reference sequence: NM_004364.3) and that at the protein level (NP_004355.2) in parentheses, according to the guidelines of the Human Genome Variation Society.

Novel mutations are in bold.

Abbreviations: AML, acute myeloid leukemia; NK, normal karyotype; BM, bone marrow; ITD, internal tandem duplication mutations; MRC, multilineage-related dysplasia; Neg, negative; Pos, positive.

Table 1. Clinical and molecular characteristics of 4 Korean patients with AML-NK harboring CEBPA double mutations

| Patient No. | Age (yr) | Sex | BM diagnosis | Immunophenotype | CEBPA gene mutation | FLT3/ITD | NPM1 |
|------------|----------|-----|--------------|----------------|---------------------|----------|------|
|            |          |     |              |                | N-terminal          | C-terminal|      |
| 1          | 51/F     |     | AML with MRC | CD34+, HLA-DR+, CD13+, CD33+, CD117+, cMPO+, CD64+, ectopic CD7+ | c.179_180dupCG [p.S61Afs] | c.929_930insTCT [p.310_311insL] | Neg | Neg |
| 2          | 41/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w, ectopic CD7+ | c.50_53delGCCA [p.H18Qfs] | c.912_913insTTG [p.K304_Q305insL] | Pos | Neg |
| 3          | 48/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64+, ectopic CD7+ | c.178_182delACGTinsTTT [p.T60Ffs] | c.923_924insCTC [p.K304_Q305insL] | Neg | Neg |
| 4          | 42/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w, ectopic CD7+ | c.243_244insGTCG [p.F82Vfs] | c.912_913insTTG [p.K304_Q305insL] | Neg | Neg |

*Description at the cDNA level (reference sequence: NM_004364.3) and that at the protein level (NP_004355.2) in parentheses, according to the guidelines of the Human Genome Variation Society.

Novel mutations are in bold.

Abbreviations: AML, acute myeloid leukemia; NK, normal karyotype; BM, bone marrow; ITD, internal tandem duplication mutations; MRC, multilineage-related dysplasia; Neg, negative; Pos, positive.

Table 1. Clinical and molecular characteristics of 4 Korean patients with AML-NK harboring CEBPA double mutations

| Patient No. | Age (yr) | Sex | BM diagnosis | Immunophenotype | CEBPA gene mutation | FLT3/ITD | NPM1 |
|------------|----------|-----|--------------|----------------|---------------------|----------|------|
|            |          |     |              |                | N-terminal          | C-terminal|      |
| 1          | 51/F     |     | AML with MRC | CD34+, HLA-DR+, CD13+, CD33+, CD117+, cMPO+, CD64+, ectopic CD7+ | c.179_180dupCG [p.S61Afs] | c.929_930insTCT [p.310_311insL] | Neg | Neg |
| 2          | 41/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w, ectopic CD7+ | c.50_53delGCCA [p.H18Qfs] | c.912_913insTTG [p.K304_Q305insL] | Pos | Neg |
| 3          | 48/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64+, ectopic CD7+ | c.178_182delACGTinsTTT [p.T60Ffs] | c.923_924insCTC [p.K304_Q305insL] | Neg | Neg |
| 4          | 42/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w, ectopic CD7+ | c.243_244insGTCG [p.F82Vfs] | c.912_913insTTG [p.K304_Q305insL] | Neg | Neg |

*Description at the cDNA level (reference sequence: NM_004364.3) and that at the protein level (NP_004355.2) in parentheses, according to the guidelines of the Human Genome Variation Society.

Novel mutations are in bold.

Abbreviations: AML, acute myeloid leukemia; NK, normal karyotype; BM, bone marrow; ITD, internal tandem duplication mutations; MRC, multilineage-related dysplasia; Neg, negative; Pos, positive.

Table 1. Clinical and molecular characteristics of 4 Korean patients with AML-NK harboring CEBPA double mutations

| Patient No. | Age (yr) | Sex | BM diagnosis | Immunophenotype | CEBPA gene mutation | FLT3/ITD | NPM1 |
|------------|----------|-----|--------------|----------------|---------------------|----------|------|
|            |          |     |              |                | N-terminal          | C-terminal|      |
| 1          | 51/F     |     | AML with MRC | CD34+, HLA-DR+, CD13+, CD33+, CD117+, cMPO+, CD64+, ectopic CD7+ | c.179_180dupCG [p.S61Afs] | c.929_930insTCT [p.310_311insL] | Neg | Neg |
| 2          | 41/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w, ectopic CD7+ | c.50_53delGCCA [p.H18Qfs] | c.912_913insTTG [p.K304_Q305insL] | Pos | Neg |
| 3          | 48/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64+, ectopic CD7+ | c.178_182delACGTinsTTT [p.T60Ffs] | c.923_924insCTC [p.K304_Q305insL] | Neg | Neg |
| 4          | 42/F     |     | AML with maturation | CD34+, HLA-DR+, CD13+, CD33+, CD117+, MPO+, CD64w, ectopic CD7+ | c.243_244insGTCG [p.F82Vfs] | c.912_913insTTG [p.K304_Q305insL] | Neg | Neg |

*Description at the cDNA level (reference sequence: NM_004364.3) and that at the protein level (NP_004355.2) in parentheses, according to the guidelines of the Human Genome Variation Society.

Novel mutations are in bold.

Abbreviations: AML, acute myeloid leukemia; NK, normal karyotype; BM, bone marrow; ITD, internal tandem duplication mutations; MRC, multilineage-related dysplasia; Neg, negative; Pos, positive.
we also confirmed the disappearance of CEBPA double-mut in the follow-up samples at complete remission (leukemia-specific genetic changes). N-terminal mutations are located between the major translational start codon and the second ATG in the same open reading frame [13]. These mutations introduce a premature termination of translation of the p42 CEBPA protein, while preserving the translation of a p30 isoform that has been reported to inhibit the function of the full-length protein. In contrast, mutations in the C-terminal bZIP region are in-frame mutations, and they may impair DNA binding and/or homodimerization and heterodimerization [13].

We found that c.584_589dupACCGC (9/30, 30%) and c.690G>T (2/30, 6.7%) were the most common types of CEBPA polymorphisms. Although c.584_589dupACCGC was first reported as a mutation [25], this 6-nucleotide in-frame duplication has recently been shown to represent a germline polymorphism (P194_H195dup) [18, 26, 27]. We detected this variation in the follow-up samples of patients at CR and in control samples.

Of the 4 patients with CEBPA double-mut, 1 also had the FLT3-ITD mutation, whereas none of them had the NPM1 mutation. Acquired gene mutations in AML fall into either of the 2 broadly defined complementation groups (Class I and II) [1]. Class I comprises mutations that activate signal-transduction pathways and thereby increase the proliferation or survival, or both, of hematopoietic progenitor cells [1]. Mutations that activate members of the receptor tyrosine kinase FLT3 or RAS families are considered Class I mutations. Class II comprises mutations that affect transcription factors or components of the transcriptional coactivator complex and cause impaired differentiation. On the basis of their known physiological functions, mutations in CEBPA, MLL, and possibly NPM fall into this group [6].

A recent study that comprehensively analyzed gene mutations in AML showed that 103 of 165 patients had multiple gene mutations, which most frequently occurred as a combination of Class I and Class II mutations [20]. In addition to gene mutations, hypermethylation of the distal promoter region of CEBPA has been reported to have a prognostic implication in a significant proportion of AML patients [28, 29]. Notably, hypermethylation and gene mutation were mutually exclusive in AML [28]. These evidences suggest that methylation testing may be needed along with gene mutation studies to detect CEBPA mutations in AML.

**Authors’ Disclosures of Potential Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

**Acknowledgement**

This study was supported by the Samsung Medical Center Clinical Research Development Program grant, #CRS-108-62-3.

**REFERENCES**

1. Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002;100:4325-36.

2. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children’s Leukaemia Working Parties. Blood 1998;92:2322-33.

3. Mrozek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004;18:115-36.

4. Estey E and Dohner H. Acute myeloid leukaemia. Lancet 2006;368:1894-907.

5. Lowenberg B, Griffin JD, Tallman MS. Acute myeloid leukemia and acute promyelocytic leukemia. Hematology Am Soc Hematol Educ Program 2003:82-101.

6. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Builinger L, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. New Engl J Med 2008;358:1909-18.

7. Scholl S, Fricke HJ, Sayer HG, Hoffken K. Clinical implications of molecular genetic aberrations in acute myeloid leukemia. J Cancer Res Clin Oncol 2009;135:491-505.

8. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med 2010;363:2424-33.

9. Abbas S, Lugthart S, Kavelaars FG, Schelen A, Koenders JE, Zeilmaker A, et al. Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value. Blood 2010;116:2122-6.

10. Metzeler KH, Maharry K, Radmacher MD, Mrozek K, Margeson D, Becker H, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol 2011;29:1373-81.

11. Pabst T and Mueller BU. Complexity of CEBPA dysregulation in human acute myeloid leukemia. Clin Cancer Res 2009;15:5303-7.

12. Henneville A, Boissel N, Gachard N, Naguib D, Bastard C, de Botton S, et al. The favorable impact of CEBPA mutations in patients with acute myeloid leukemia is only observed in the absence of associated cytogenetic abnormalities and FLT3 internal duplication. Blood 2009;113:5090-3.

13. Wouters BJ, Lowenberg B, Erpelinck-Verschueren CA, van Putten WL, Valk PJ, Delwel R. Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. Blood 2009;113:3088-91.

14. Dufour A, Schneider F, Metzeler KH, Hoste E, Schneider S, Zellmeier E, et al. Acute myeloid leukemia with biallelic CEBPA gene mutations and normal karyotype represents a distinct genetic entity associated with a favorable clinical outcome. J Clin Oncol 2010;28:570-7.
15. Taskesen E, Bullinger L, Corbacioglu A, Sanders MA, Erpelinck CA, Wouters BJ, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. Blood 2011;117:2469-75.

16. Swardlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., eds. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer, 2008.

17. Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with CEBPA mutations; only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis. Br J Cancer 2009;100:1343-6.

18. Lin LI, Chen CY, Lin DT, Tsay W, Tang JL, Yeh YC, et al. 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. Clin Cancer Res 2005;11:1372-9.

19. Fuchs O, Provaznikova D, Kocova M, Kostecak A, Cvekova P, Neuwirthova R, et al. CEBPA polymorphisms and mutations in patients with acute myeloid leukemia, myelodysplastic syndrome, multiple myeloma and non-Hodgkin’s lymphoma. Blood Cells Mol Dis 2008;40:401-5.

20. Ishikawa Y, Kiyoi H, Tsujimura A, Miyawaki S, Miyazaki Y, Kuriyama K, et al. Comprehensive analysis of cooperative gene mutations between class I and class II in de novo acute myeloid leukemia. Eur J Haematol 2009;83:90-8.

21. Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (CEBPalpha), in acute myeloid leukemia. Nat Genet 2001;27:263-70.

22. Snaddon J, Smith ML, Neat M, Cambal-Parrales M, Dixon-McIver A, Arch R, et al. Mutations of CEBPA in acute myeloid leukemia FAB types M1 and M2. Genes Chromosomes Cancer 2003;37:72-8.

23. Barjesteh van Waalwijk van Doorn-Khosrovani S, Erpelinck C, Meeijer J, van Oosterhoud S, van Putten WL, Valk PJ, et al. Biallelic mutations in the CEBPA gene and low CEBPA expression levels as prognostic markers in intermediate-risk AML. Hematol J 2003;4:31-40.

24. Gombart AF, Hofmann WK, Kawano S, Takeuchi S, Krug U, Kwok SH, et al. Mutations in the gene encoding the transcription factor CCAAT/enhancer binding protein alpha in myelodysplastic syndromes and acute myeloid leukemias. Blood 2002;99:1332-40.

25. Frohling S, Schlenke RF, Stolze I, Bihlmayer J, Benner A, Kreitmeier S, et al. CEBPA mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. J Clin Oncol 2004;22:624-33.

26. Resende C, Regalo G, Duraes C, Carneiro F, Machado JC. Genetic changes of CEBPA in cancer: mutations or polymorphisms? J Clin Oncol 2007;25:2493-4; author reply 4-5.

27. Wouters BJ, Louwers I, Valk PJ, Lowenberg B, Delwel R. A recurrent in-frame insertion in a CEBPA transactivation domain is a polymorphism rather than a mutation that does not affect gene expression profiling-based clustering of AML. Blood 2007;109:389-90.

28. Szankasi P, Ho AK, Bahler DW, Efimova O, Kelley TW. Combined testing for CCAAT/enhancer-binding protein alpha (CEBPA) mutations and promoter methylation in acute myeloid leukemia demonstrates shared phenotypic features. Leuk Res 2011;35:200-7.

29. Lin TC, Hou HA, Chou WC, Ou DL, Yu SL, Tien HF, et al. CEBPA methylation as a prognostic biomarker in patients with de novo acute myeloid leukemia. Leukemia 2011;25:32-40.