CLINICAL STUDY

Determination of CEBPA mutations by next generation sequencing in pediatric acute leukemia

Akin DF1, Oner DA2, Kurekci E2, Akar N3

Nigde Ömer Halisdemir University, Faculty of Medicine, Medical Biology, Nigde, Turkey.
dilarabali@ohu.edu.tr

ABSTRACT

OBJECTIVES: The CCAAT/enhancer-binding protein-alpha (CEBPA) is lineage-specific transcription factor in the hematopoietic system. In this study, we aimed on the clinical features and the prognostic significance associated with CEBPA mutations in 30 pediatric patients with acute leukemia.

METHODS: In addition, the association between found variants and mutations of Ten-Eleven-Translocation 2 (TET2), Kirsten rat sarcoma viral oncogene homolog (KRAS), and Casitas B-cell lymphoma (CBL), FLT3 (Fms-Related Tyrosine Kinase), JAK2 (Janus Kinase-2) and Nucleophosmin 1 (NPM1) were analyzed, which are important prognostic risk factors for pediatric acute leukemia patients. The entire CEBPA coding region was screened using the NGS method.

RESULTS: CEBPA mutations were detected in 16 (53.3 %) of 30 patients. In total, ten distinct of nucleotide changes were identified in 30 patients, including 6 novel and 4 known mutations by sequencing the entire CEBPA gene. We found 6 frame shift mutations, 1 missense mutation, 3 synonymous variants. The most common mutation was the c.487del G resulting p.Glu163Ser in 5 cases. Three patients carried CEBPA double mutations.

CONCLUSION: The detected variants in this article seemed to be the first screening results of genes studied by NGS in pediatric acute leukemia patients. Our results also showed some degree of association between FLT3-ITD, TET2, KRAS, CBL and CEBPA mutations (Tab. 4, Fig. 1, Ref. 24).

KEY WORDS: CEBPA, pediatric acute leukemia, next generation sequencing, molecular marker, mutation.

Introduction

Clinical and genetic prognostic markers are important in the classification of leukemia patients. Aberrant chromosomal translocations and gene mutations frequently occur in the transcriptional factor that lead to uncontrolled proliferation of lymphoid and myeloid progenitors (1–4). The CEBPA gene is a member of the leucine zipper family of the transcription factor family that is essential for the differentiation of myeloid cells (5). The CEBPA gene located on chromosome 19 q13.1 encodes the basic leucine zipper (bZIP) family of the transcription factors (6). It is expressed at high levels during myeloid cell differentiation and binds to the promoters of multiple specific genes at different levels of myeloid lineage maturation (2). In AML patients, it was reported that two types of mutations in CEBPA gene exist; N terminal and C terminal (2, 5, 7–11). The N terminal mutations are located between the major translational start site and the second ATG further downstream lead to premature stop of translation of the wild type p 42 CEBPA protein, while preserving translation of a short p30 isoform that suppresses the function of the full length protein. Mutations in the C terminal are usually in frame and deletions that affect homo-hetero-dimerization and DNA binding (6–8). CEBPA mutations are seen in approximately 4.5–6 % of pediatric Acute Myeloid Leukemia (AML) patients. In addition, CEBPA mutations are found in 5–14 % of adult patients with AML (5, 11–13). There is no study, which investigates CEBPA mutations in Turkish pediatric acute leukemia patients. Therefore, in our study, we investigated entire CEBPA coding region in 30 patients with Turkish pediatric acute leukemia patients. Our results also showed some degree of association between FLT3-ITD, TET2, KRAS, CBL and CEBPA mutations (Tab. 4, Fig. 1, Ref. 24).

Patients and study design

Subjects

The study population consisted of 30 patients aged between 1 and 15 years, who were admitted to Losante Children’s and Adult Hospital with the diagnosis of pediatric acute leukemia. Seventeen patients were diagnosed as AML, 4 as mixed leukemia, 9 as ALL. The study was carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The Ankara University, School of Medicine Ethics Committee approved the study protocol (project No.03-107-13/2013) and an informed consent was provided by the patients’ parents.
Cytogenetic techniques
Bone marrow samples were collected with Heparin-containing tubes, and chromosome analysis was performed using G-banding. After the slide preparation, G-banding using Giemsa staining was carried out according to the standard procedures. On each slide, 20 metaphases were analyzed by a light microscope (Nikon, Japan). Karyotypes were described according to International Standing Committee on Human Cytogenetic Nomenclature (ISCN) (14).

Fluorescence in situ hybridization (FISH) was performed on interphase nuclei and metaphase chromosomes of bone marrow cells using dual-color/dual-fusion probes for translocations inv (16;16), t(9;22), t(15;17), chromosome 19, and dual-color/deletion probe for del 7q, labeled in green and red spectra according to the manufacturer’s protocol provided by Cytocell, UK. Counterstaining was performed with 4,6-diamidino-2-phenylindole (DAPI).

At least 100 nuclei were analyzed under the Fluorescence microscope, and image capture was performed using Nikon Eclipse 80i equipped with a CCD-camera (CoolCube1), appropriate filters and Isis software (MetaSystems).

DNA isolation and Next Generation Sequencing
Blood samples were collected with EDTA-containing tubes and DNA was extracted from peripheral blood and bone marrow leukocytes with Mag-NA Pure automatic DNA isolation instrument (Roche Diagnostics, Mannheim, Germany).

We used NGS to study the entire CEBPA gene region that was presented by 4 overlapping amplicons. Primer sequences lists are shown in Table 1. NGS sample preparation and process stages were generated, previously described by Grossmann et al (8).

NGS was carried out using 454 GS Junior System Instrument Roche Applied Science. Data were analyzed using the GS Amplicon Variant Analyzer software version 2.3. (Roche Applied Science). We used it for determination of variances; filters were adjusted to show variances in more than 1% of bidirectional reads per amplicon in at least one patient. Figure 1 summarizes the location of the amplicons on the CEBPA protein.

Detection of FLT3, TET2, CBL, KRAS, JAK2 and NPM1 mutations
FLT3 mutation was analyzed by Real Time PCR on Light Cycler 480 II instrument (Roche Diagnostics, Gmbh, Mannheim, Germany). The results were analyzed with the High-Resolution Melting (HRM) method using genotype profiles. Different plots were created by selecting negative controls as the base-line. Therefore, fluorescence of all the other samples was diagramed relatively to this sample. Fluorescence signals were analyzed, and significant differences were used as indicators of the mutations (15–18).

| Tab. 1. Primers used for amplification of CEBPA gene. |
|-----------------------------------------------|
| Primer set | Forward Tm °C | Reverse Tm °C | Amplicon Length (bp) |
|------------|---------------|---------------|----------------------|
| CEBPA set 1 | 5’- GCCATGCCGGGAGAACT 3’ | 62 | 5’-CCCCGGTGATGTCAAAGTTCG-3’ | 59 | 357 |
| CEBPA set 2 | 5’- CTCCTCAAGCGAGGATCCTTG-3’ | 61 | 5’-CGGCTGTAAGGGAAGAGG-3’ | 62 | 335 |
| CEBPA set 3 | 5’- GAGGAGGGAGAAGCCGGAACG-3’ | 60 | 5’- CTCGTTTAGCTTTCTTGCCA-3’ | 60 | 357 |
| CEBPA set 4 | 5’- CGCCAGGCCGTCGCAAG-3’ | 65 | 5’- CCGAGGCGGCTCCCAA-3’ | 65 | 357 |

| Tab. 2. Patient characteristics according to CEBPA mutation status. |
|-----------------------------|
| Characteristic | No CEBPA mutation | CEBPA mutation | p value |
|-----------------------------|
| Gender | Male (n = 21) | 11 | 10 | 0,33 |
| | Female (n = 9) | 3 | 6 | |
| Diagnosis | AML (n = 17) | 7 | 10 | 0,94 |
| | ALL (n = 9) | 4 | 5 | |
| | Biphenotypic Acute Leukemia (n=4) | 2 | 2 | |
| Risk Groups | High Risk (n = 19) | 11 | 8 | 0,20 |
| | Median Risk (n = 2) | 1 | 1 | |
| | Standard Risk (n = 9) | 2 | 7 | |
| Status | Remission (n = 17) | 8 | 9 | 0,07 |
| | Relapse (n = 6) | 0 | 5 | |
| | Exitus (n = 7) | 3 | 4 | |
| Genetic Syndrome | Down Syndrome (n = 4) | – | 4 | 0,52 |
| | Fragile X Syndrome (n = 1) | – | 1 | |
| Cytogenetic/Genetics | Normal Karyotype (n = 19) | 10 | 9 | 1 |
| | t(15;17) (n = 1) | 1 | | |
| | t(9;22) (n = 1) | – | 1 | |
| | t(4;11) (n = 1) | 1 | | |
| | t(8;21) (n = 1) | 1 | | |
| | t(16;16) (n = 1) | – | 1 | |
| | FLT3 mutations (n = 8) | 5 | 3 | |
| | JAK2 mutations (n = 2) | – | 2 | |
| | TET2 mutations (n = 5) | – | 5 | |
| | CBL mutations (n = 1) | – | 1 | |
| | KRAS mutations (n = 4) | – | 4 | |
| | NPM1 mutations (n = 1) | – | 1 | |

Male – Acute Myeloid Leukemia, ALL – Acute Lymphoblastic Leukemia, Ex – Exitus
Statistics

Statistical analyses were performed with SPSS 15.0 (SPSS IBM, USA). Chi-square test, independent sample t-test, or Mann–Whitney U-test, as appropriate for the type of data being analyzed, were used to assess the statistical significance of the differences between the two groups. p-values less than 0.05 were considered statistically significant.

Results

Incidence of CEBPA mutations

Among 30 pediatric acute leukemia patients, CEBPA mutations were found in 16 cases (53.3 %). Patient characteristics of the 30 pediatric acute leukemia cases are shown in Table 2. Ten distinct nucleotide changes were identified in 30 patients, including 6 novel and 4 known mutations by sequencing the entire gene as shown in Table 3. The frame shift mutation was the most common mutation subtype that was found in 6 types. The remaining mutation subtypes were 3 synonymous variants and 1 missense mutation.

Tab. 3. Characterization of CEBPA mutations in pediatric acute leukemia.

| Mutation No | Type of mutations | No of patients | Nucleotide change | Amino-acid change | Localization | Mutation Type | Rs number | Clinical Significance |
|-------------|-------------------|----------------|-------------------|-------------------|-------------|--------------|-----------|----------------------|
| 1           | Nucleotide change | 3              | g.5800G>T         | c.690G>T          | p.Thr230Thr | Between TAD2-DNA binding domain | Synonymous variant | 34529039 | Benign               |
| 2           | Duplication       | 3              | g.5316_5319dupTCAC | c.198_201dupTCAC  | p.Ile68LeufsTer41 | TAD-1 domain | Frameshift mutation in regulatory region | 137852731 | Pathogenic for AML   |
| 3           | Insertion         | 1              | g.5327_5328insC   | c.217_218insC     | p.Phe73SerfsTer35 | N-terminal domain | Frameshift mutation | 137852733 | Pathogenic for AML   |
| 4           | Nucleotide change | 1              | g.5683C>T         | c.573C>T          | p.His191His | Between TAD2-DNA binding domain | Synonymous variant | 192240793 | Benign               |
| 5           | Nucleotide change | 3              | g.5444C>T         | c.336C>T          | p.Pro112Pro | Between TAD1-TAD2 domain | Synonymous variant | Novel | NA                   |
| 6           | Deletion          | 1              | g.5492_5493delC   | c.382delC         | p.Pro128Pro | Between TAD1-TAD2 domain | Frameshift mutation | Novel | NA                   |
| 7           | Deletion          | 5              | g.5597            | c.487delG         | p.Glu163Ser | Between TAD1-TAD2 domain | Frameshift mutation | Novel | NA                   |
| 8           | Deletion          | 1              | g.5410-5411delC   | c.300delC         | p.Gly100GlysTer59 | TAD-1 domain | Frameshift mutation | Novel | NA                   |
| 9           | Insertion         | 1              | g.1107-1113ins TTGACC | c.955-961ins TTGACC | p.Ser319TrpsTer13 | C-terminal domain | Frameshift mutation | Novel | NA                   |
| 10          | Nucleotide change | 1              | g.5599C>A         | c.489C>A          | p.Glu163Asp | Between TAD1-TAD2 domain | Missense variant | Novel | NA                   |

NA = Not Available, Rs = Reference SNP
| Patient No | Age/Gender | Diagnosis | Risk Group | Cytogenetics-Molecular abnormalities | FLT3 | CEBPA Mutations | KRAS | JAK2 | FLT3 ITD | FLT3 TKD | TET2 | CBL | \(164\_179\) 183delITCTTA-intronic |
|------------|------------|-----------|------------|-----------------------------------|------|-----------------|------|------|-----------|----------|------|------|-----------------------------------|
| 1          | 5.5/M      | Biphenotypic (Mixed Phenotypic) Acute Leukemia | HR | Ex-46, XY | c.690 G>T p.Thr230Thr | - | c.690 G>T p.Thr230Thr | - | - | - | - | - | - |
| 2          | 2/M        | Biphenotypic (Mixed Phenotypic) Acute Leukemia | HR | Ex-46, XY | c.690 G>T p.Thr230Thr | - | c.487 del G p.Glu163Ser | - | - | - | - | - | - |
| 3          | 12/M       | AML-M2    | SR         | 46, XY | c.690 G>T | - | c.487 del G p.Glu163Ser | - | - | - | - | - | - |
| 4          | 11/M       | AML-M1    | HR         | Ex-46, XY | c.198_201dupCTAC p.Ile68LeufsTer41 | + | p.1c1762Val | - | - | - | - | - | - |
| 5          | 4/M        | Pre-B-ALL | SR         | 46, XY | c.336 C>T p.Pro112Pro | - | p.Pro363Leu p.Gly614Gly p.Val178Met c.*128-7564G>A p.Lys1678Glu | - | - | - | - | - | - |
| 6          | 7/M        | AML-M4    | HR         | 47, XY; 22[12] Inv (16;16), Fragile X syndrome | c.198_201dupCTAC p.Ile68LeufsTer41 | - | p.1c1762Val | - | - | - | - | - | - |
| 7          | 6/F        | AML-NA    | SR         | 46, XY (15;17) | c.487 del G p.Glu163Ser | - | p.Pro363Leu p.Gly614Gly | - | - | - | - | - | - |
| 8          | 13/M       | AML-M4    | HR         | 46, XY, Ms/Ts 14, | c.336 C>T p.Pro112Pro | - | p.1c1762Val | - | - | - | - | - | - |
| 9          | 4/M        | AML-M4    | HR         | 46, XY | c.382 del C | - | p.Gly614Gly | - | - | - | - | - | - |
| 10         | 5,5/F      | AML-M2    | SR         | 46, XX | c.300 del C | p.376delG p.Glu163Ser | - | p.Gly614Gly p.Glu614Gly p.Ile20Thr | - | - | - | - | - | - |
| 11         | 8/F        | AML-M5    | SR         | 46, XX | c.198_201dupCTAC p.Ile68LeufsTer41 | - | p.Gly614Gly p.Glu163Ser | p.Ile20Thr | - | - | - | - | - | - |
| 20         | 6,5/F      | AML-M5    | HR         | 46, XX | c.487 del G p.Glu163Ser | - | p.336 C>T | - | - | - | - | - | - |
| 22         | 3/F        | AML-NA    | HR         | Ex, 47, XX (+21) c.21 der(14) (14q1.2→q3.2i 1q21→q43;der(19) (19gdel) →p13.3: 11q13;11q1del [9]; t(9;22)) + | p.217_218insC | + | p.Pro363Leu p.Pro761Leu p.Ser217Ser | p.1c1762Val C714-72237T>C | p.Ile20Thr | - | - | - | - | - | - |
| 28         | 3/M        | Pre-B-ALL | MR         | 47, XY (+21) | c.573C>T | - | - | - | - | - | - | - | - |
| 29         | 2.5/M      | Pre-B-ALL | SR         | 47, XY (+21) | c.555-561 ins TT-GACC | - | - | - | - | - | - | - | - |
| 30         | 3/F        | Pre-B-ALL | SR         | 47, XY (+21) | c.489 C>A | - | - | - | - | - | - | - | - |

M = Male, F = Female, HR = High Risk, MR = Medium Risk, SR = Standard Risk, AML = Acute Myeloid Leukemia, ALL = Acute Lymphoblastic Leukemia, Pre B-ALL = Precursor B- Acute Lymphoblastic Leukemia, NA = Not Available, Ex = Exitus, Del = Deletion, Ins = Insertion, Dup = Duplication

Tab. 4. CEBPA mutations in sixteen children with acute leukemia.
All relapse patients (Patient No 1–4, 9, 20) had CEBPA mutations. The one patient with t(15;17)/PML-RARα, 1 patient with t(9;22)/BCR-ABL, and 1 with inv(16)/CBFß-MYH11 had CEBPA mutations.

Four patients including CEBPA mutations did not have cytogenetic and molecular aberrations. The presence of CEBPA mutations with no correlation with prognostic classification were divided into “high”, “moderate” and “standard” risk groups of acute leukemia. There was no difference in FAB classification between CEBPA wild type and CEBPA mutant type. Four patients (Patients No 22, 28–30), who had Down syndrome had CEBPA mutations.

Two mutations (198_201dupCTAC and 217_218insC) reported in the previous study were described as pathogenic for AML. Ten (33.3 %) patients died during the treatment or relapse. The clinical and laboratory features of the study group were summarized in Table 4. No statistical significant differences were detected in the two groups.

Discussion

Acute leukemia is a heterogeneous disorder of hematopoietic stem cells, characterized by multiple genetic events, which have an impact on proliferation and differentiation. Some of the genetic and epigenetic alterations play a major role in leukemogenesis; gene mutations, deletions, translocations, and DNA methylation. Compared with adult leukemia, there were fewer studies of gene mutations in pediatric leukemia patients. We previously reported the frequencies of TET2, CBL, and KRAS mutations by using NGS in pediatric AML patients and the frequencies of FLT3-ITD and FLT3-TKD mutations in acute leukemia patients (18).

Although CEBPA mutations have been studied for many years in AML, there were no data about its prevalence and prognostic significance in Turkish patients with AML or ALL. In this study, we investigated CEBPA aberrations in pediatric acute leukemia patients to determine their frequency and prognostic impact. In previous studies, several common patterns of CEBPA mutations have been reported in AML. In the N-terminus, small out-of-frame insertions or deletions occurred resulting in a premature stop codon, which inhibits transcription of the p42 product (4, 5, 6, 9–13, 20).

CEBPα mutations were detected in 30 pediatric acute leukemia cases (53.3 %), with 9 cases combination of mutations and 7 cases single mutations. As the frequency of CEBPA mutations in this study was quite high, the number of patients was not enough to assess the clinical association and mutations. We found no statistical differences in the clinical parameters. The combined mutations (CEBPα, FLT3, NPM1) have been detected in adult AML, their frequencies varied considerably, ranging between 25 and 35 % (21). Mutations in CEBPA have been described in approximately 5–14 % of adult patients with AML (4, 5, 22–24). The frequency of CEBPA mutations have been reported to be lower in pediatric AML when compared to the adult AML (5, 19, 20). The study by Hollink et al showed CEBPA mutations in 20 out of the 252 (7.9 %), including 14 double mutant and 6 single mutant cases in the pediatric AML patients (5). Fröhling et al showed that frequency of CEBPA was 15 % in young adults with AML. They reported that mutations of CEBPA predicted a favorable prognosis and improved risk stratification in AML patients with normal cytogenesis (10).

We identified 10 mutations in CEBPA by NGS. Six novel mutations were detected in the present study. The remaining 4 types of CEBPA mutations have been reported in the previous studies. We used an amplicon-based sequencing method to find possible new genetic markers for leukemia diagnosis. The c.690G>T, c.198_201dupCTAC, C.217_218insC and c.573C>T variants had been reported in previous studies, whereas we detected 6 novel variants in CEBPA gene in this present study. We detected three types of variants in CEBPA; frame-shift, missense and synonymous (Tab. 3). Totally ten variants were identified involving N-terminal, TADs and C-terminal domains. The most frequent type of variants...
in CEBPA in pediatric acute leukemia patients was 487 deletion of G, which led to a glutamin-serin deletion between the TAD1 and TAD2 domain in CEBPA protein.

In our study, we detected that frame-shift mutations (68.75 %) resulted in a premature terminal of the full length 42–kd protein of CEBPA, which is shown in Table 3. The 198_201 duplication of CTAC and 217_218 insertion of C was previously reported as pathogenic mutations for AML. The four of sixteen patients had the pathogenic mutations and one patient had benign mutations, which were previously reported. Two types of mutations including c.198_201dupCTAC and c.300delC occurred in TAD1 domain. These two types of mutations could be predicted to loss the transactivation activity of CEBPA protein. The one type mutation (c.955_961delITTGAC) in the C terminal domain was caused by truncated the b-ZIP domain of CEBPA protein. In our study as well as in previous studies, the patients with frame shift mutations in encoding regions were associated with favorable clinical outcome (11, 23).

The only patient (patient no 22) in our study group had combination mutations of CEBPA, FLT3, TET2, CBL and KRAS genes (Tab. 4). This patient was a three year old girl diagnosed with AML and classified as a high risk group and 18 months later she was diagnosed with a relapse and died during the treatment. The combination of mutations cases were very few. The patients with combination of mutations died in the reported studies either during treatment or after relapse (11, 13). We screened 6 patients at relapse: All the relapse-screened patients carried CEBPA mutations (Tab. 4). Fröhling et al. detected both types of FLT3 mutations and found no correlation with prognostic influence among AML patients with CEBPA mutations (10). In this study, we found 3 patients, who carried both CEBPA and FLT3 mutations and two of them died during treatment.

The 5-base deletion in the intronic region (1641+179_1641+183delTCTTA-intronic) of JAK2 gene was first reported in a Down syndrome patient associated with B-cell precursor ALL was detected with CEBPA synonymous variant in pediatric biphenotypic acute leukemia and AML patients in this present study. In our study, we found no significant correlation between CEBPA mutations and other gene mutations and clinical parameters. In addition, four patients with CEBPA mutations did not have cytogenetic and molecular aberrations. This study is the first report of the frequency of CEBPA mutations and its correlation with other gene mutations in Turkey. We think that c.487delG and c.198_201dupCTAC could be important prognostic markers for pediatric acute leukemia patients at relapse. There may be biologic differences between adults and children, which may require another study on a larger group of patients to validate the prognostic significance of CEBPA mutations in pediatric acute leukemia.

Conclusion

CEBPA may be potential genetic markers for pediatric acute leukemia diagnosis. However, these results need to be confirmed by further studies on a larger number of patients.

References

1. Pabst T, Mueller BU. Transcriptional dysregulation during myeloid transformation in AML. Oncogene 2007; 15: 6829–6837.
2. Kassem N, Fahmy EA, Desoky M, Medhat N, Zawam H. CCAAT/enhancer binding protein α gene expression in Egyptian patients with acute myeloid leukemia. J Egypt Nat Cancer Inst 2013; 25: 115–120.
3. Roe JS, Vakoc CR. C/EBPα: critical at the origin of leukaemic transformation. J Exp Med 2014; 13: 1–4.
4. Preudhomme C, Sagot C, Boissel N, Cayuela JM, Tigaud I, de Botton T, Thomas X, Raffoux E, Lamandin C, Castaigne S, Fenaux P, Donnem H. ALFA Group. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood 2002; 15: 17–23.
5. Holliink I, van den Heuvel-Eibrink MM, Arentsen-Peters ST, Zimmermann M, Peeters JK, Vaik P, Balobidad BV, Sonneveld E, Kaspers GJ, de Bont ES, Trka J, Barcheluc A, Creutzig U, Pieters R, Reinhardt D, Zwaan CM. Characterization of CEBPA mutations and promoter hypermethylation in pediatric acute myeloid leukemia. Haematologica 2011; 96: 384–392.
6. Fasan A, Haferlach C, Alpermann T, Jeromin S, Grossmann V, Eder C, Weissmann S, Dicker F, Kohlmann A, Schindela S, Kern W, Haferlach T, Schnittger S. The role of different genetic subtypes of CEBPA mutated AML. Leukemia. 2014; 28: 794–803.
7. Liss A, Ooi CH, Zjablovskaja P, Benoukraf T, Radomska HS, Ju C, Wu M, Balastik M, Delwel R, Brdicka T, Tan P, Tenen DG, Alberich-Jorda M. The gene signature in CCAAT-enhancer-binding protein α dysfunctional acute myeloid leukemia predicts responsiveness to histone deacetylase inhibitors. Haematologica 2014; 99: 697–705.
8. Grossmann V, Schnittger S, Schindela S, Klein HU, Eder C, Dugas M, Kern W, Haferlach T, Haferlach C, Kohlmann A. Strategy for robust detection of insertions, deletions, and point mutations in CEBPA, a GC-rich content gene, using 454 next-generation deep-sequencing technology. J Mol Diagn 2011; 13: 129–136.
9. Fuchs O, Kostecka A, Provazniková D, Kránsá B, Kotlin R, Stanková M, Kobylka P, Dostálová G, Zeman M, Chochola M. CCAAT/enhancer-binding protein alpha (CEBPA) polymorphisms and mutations in healthy individuals and in patients with peripheral artery disease, ischaemic heart disease and hyperlipidaemia. Folia Biol (Praha) 2010; 56: 51–57.
10. Fröhling S, Schlenk RF, Stolze I, Bihlmayer J, Benner A, Kreitmeier S, Tobis K, Döhner H, Döhner K. CEBPA mutations in younger adults with acute myeloid leukaemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. J Clin Oncol 2004; 15: 624–633.
11. Pabst T, Eyholzer M, Haefliger S, Scharf J, Mueller BU. Somatic CEBPA mutations are a frequent second event in families with germline CEBPA mutations and familial acute myeloid leukemia. J Clin Oncol 2008; 1: 5088–5093.
12. Taskesen E, Bullinger L, Corbachoglu A, Sanders MA, Erpelinck CA, Wouters BJ, van der Poel-van de Luytgaarde SC, Damm F, Krauter J, Ganser A, Schlenk RF, Löwenberg B, Delwel R, Döhner H, Vaik P, Döhner K. Prognostic impact, concurrentgenetic 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; 24: 2469–2475.
13. Leroy H, Roumier C, Huysge P, Biggio V, Fenaux P, Preudhomme C, CEBPA point mutations in hematological malignancies. Leukemia 2005;19: 329–334.
14. Basel S. ISCN An International System for Human Cytogenetic Nomenclature. Karger and Cytogenetic and Genome Research 2013.

15. Kohlmann A, Grossmann V, Klein HU, Schindela S, Weiss T, Kazak B, Dicker F, Schnittger S, Dugas M, Kern W, Haferlach C, Haferlach T. Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8% of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1. J Clin Oncol 2010; 28: 3858–3865.

16. Tan AY, Westerman DA, Carney DA, Seymour JF, Juneja S, Dobrovic A. Detection of NPM1 exon 12 mutations and FLT3- internal tandem duplications by high resolution melting analysis in normal karyotype acute myeloid leukemia. J Hematol Oncol 2008; 1: 1–10.

17. Murugesan G, Aboudola S, Szpurka H, Verbie MA, Maciejewski JP, Tubbs RR, Hsi ED. Identification of the JAK2 V617F mutation in chronic myeloproliferative disorders using FRET probes and melting curve analysis. Am J Clin Pathol 2006; 125: 625–633.

18. Akin DF, Öner D, Mumcuoğlu M, Baççe M, Kurecki E, Ezer Ü, Akar N. Detection of TET2, KRAS and CBL variants by Next Generation Sequencing and analysis of their correlation with JAK2 and FLT3 in childhood AML. The Egyptian Journal of Medical Human Genetics 2016; 17: 209–215.

19. Ho PA, Alonzo TA, Gerbing RB, Pollard J, Stirewalt DL, Hurwitcz C, Heerema NA, Hirsch B, Raimondi SC, Lange B, Franklin JL, Radich JP, Meshinchi S. Prevalence and prognostic implications of CEBPA mutations in pediatric acute myeloid leukemia (AML): a report from the Children’s Oncology Group. Blood 2009; 25: 6558–6566.

20. Liang DC, Shih LY, Huang CF, Hung IJ, Yang CP, Liu HC, Jaing TH, Wang LY, Chang WH. CEBPalpha mutations in childhood acute myeloid leukemia. Leukemia 2005; 19: 410–414.

21. Rubio P, Campos B, Digiorge JA, Gallego MS, Medina A, Rossi JG, Felice MS, Alonso CN. NPM1, FLT3 and CEBPA mutations in pediatric patients with AML from Argentina: incidence and prognostic value. Int J Hematol 2016; 104: 582–590.

22. Barjesteh van Vaalwijk van Doorn-Khosrovanis S, Erpelinck C, Meijer J, van Oosterhoud S, van Putten WL, Valk PJ, Berna Beverloo H, Tenen DG, Löwenberg B, Delwel R. Biallelic mutations in the CEBPA gene and low CEBPA expression levels as prognostic markers in intermediate-risk AML. Hematol J 2003; 4: 31–40.

23. Gombart AF, Hofmann WK, Kawano S, Takeuchi S, Krug U, Kwok SH, Larsen RJ, Asou H, Miller CW, Hoelzer D, Koeffler HP. Mutations in the gene encoding the transcription factor CCAAT/enhancer binding protein alpha in myelodysplastic syndromes and acute myeloid leukemias. Blood 2002; 15: 1332–1340.

24. Snaddon J, Smith ML, Neat M, Cambal-Parrales M, Dixon-McIver A, Arch R, Amess JA, Rohatiner AZ, Lister TA, Fitzgibbon J. Mutations of CEBPA in acute myeloid leukemia FAB types M1 and M2. Genes Chromosomes Cancer 2003; 37: 72–78.

Received February 19, 2018.
Accepted March 14, 2018.