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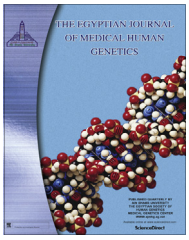

ORIGINAL ARTICLE

Detection of *TET2*, *KRAS* and *CBL* variants by Next Generation Sequencing and analysis of their correlation with *JAK2* and *FLT3* in childhood AML

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KEYWORDS

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Abstract *Background:* Acute myeloid leukemia (AML) is a heterogeneous clonal disorder in terms of cytogenetic and molecular aberrations. Ten-Eleven-Translocation 2 (*TET2*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), and Casitas B-cell lymphoma (*CBL*) have an important role pathogenesis of acute myeloid leukemia (AML) and their activated mutations confer proliferative and survival signals.

Aim: In this study, we aimed to find possible genetic markers for molecular analysis in childhood AML by screening hot-spot exons of *TET2, KRAS*, and *CBL* using Next Generation Sequencing (NGS) analysis. In addition, association between found variants and mutations of Januse Kinase-2 (*JAK2*) and Fms-Related Tyrosine Kinase (*FLT3*) were analyzed which are important prognostic risk factors for AML.

Methods: Eight patients who were diagnosed with pediatric AML at Losante Pediatric Hematology–Oncology Hospital were included to the study. Hot-spot exons of *TET2, KRAS* and *CBL* genes were screened using the NGS method. Furthermore, *FLT3*-Internal Tandem Duplicate (*FLT3-ITD*) and *JAK2*-V617F were analyzed by Real Time Polymerase chain Reaction (Real Time-PCR).

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Results: In total, we identified 20 variants in studied genes by NGS. In our patient group, 16 variants in the *TET2* (seven novel, seven missense and two silent), two variants in the *KRAS* (one missense and one intronic) and two variants in the *CBL* (two novel) were found. All of AML patients were found negative for JAK V617 F. Three of the eight patients (37.5%) showed mutations of both FLT3-ITD and *TET2, KRAS, CBL*.

Conclusion: We found novel mutations for *TET2, KRAS*, and *CBL*. The detected variants in this article seem to be the first screening results of genes studied by NGS in childhood AML patients. Our results also showed some degree of association between FLT3-ITD and *TET2, KRAS, CBL* mutations.

1. Introduction

AML is a heterogeneous clonal neoplasm characterized by accumulated genetic aberrations, which causes enhanced proliferation, maturation arrest, increased survival of the leukemic blast cells and variable response to therapy [1–5].

During the past decades, a number of recurrent cytogenetic and molecular genetic abnormalities have been identified in AML such as *t*(8;21), inv(16), *FLT3, NPM1, CEBPA, TET2, KRAS*, and *CBL*.

TET family gene members (*TET1, TET2*, and *TET3*) have functions mostly in hematopoietic differentiation. The TET oncogene family member 2 (*TET2*) gene located at chromosome band 4q24 catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine [1,6,7]. *TET2* mutations are frequently detected during progression of MPN (myeloproliferative neoplasms) or MDS (myelodysplastic syndromes) to AML [1]. *TET2* mutations may contribute to leukemogenesis by altering epigenetic regulation of transcription via DNA methylation. The incidence of *TET2* mutations is approximately 10–20% in AML [2].

RAS (Rat Sarcoma Virus) proteins including *HRAS* (Harvey Rat Sarcoma Viral Oncogene Homolog), *KRAS* and *NRAS* (Neuroblastoma RAS Viral V-Ras Oncogene Homolog) are members of the small GTPases superfamily [8]. Abnormal RAS function is related to hyperproliferative developmental diseases and cancers [2]. *RAS* mutations, especially *KRAS*, represent about 90% of cancer-associated mutations. RAS proteins play a major role in cell signaling pathway of cell proliferation, differentiation, and survival [9]. The RAS mutations are the most common mutations in AML which are seen in approximately 25–44% of patients. Among RAS mutations, *KRAS* mutations are the most frequently seen and found in 10–15% of these patients [10].

CBL, CBL-B, and CBL C/3 are the members of the *CBL* which is localized on human chromosome 11 q23 containing several functional domains [11]. *CBL* is a mammalian gene encoding the protein CBL which is an E3 ubiquitin-protein ligase involved in cell signaling and protein ubiquitination. Mutations of this gene have been implicated in a number of human cancers, particularly in AML. These mutations have also been observed in 1% of AML. *CBL* mutations have been reported in myeloid malignancies and uniformly affect either the linker region or the RING finger domain. Loss of ubiquitination of activated receptor tyrosine kinases is thought to contribute to the transforming potential of leukemia-associated mutant CBL proteins [12].

JAK2 (located in chromosome 9p24) encodes a cytoplasmic tyrosine kinase. JAK2V617F presents a somatic point mutation (including exon 12 of the *JAK2*) resulting in the substitution of valine by phenylalanine amino acid at codon 617. This mutation, which causes JAK homology 2 (JH2) negative regulatory domain, derails JAK2 kinase regulatory activity which effects cytokine independent proliferation of hematopoietic cells [13]. The JAK2 V617F mutation is found in 1.8–28% of patients with AML [14].

FMS-like tyrosine kinase 3 (*FLT3*) is a member of the receptor tyrosine kinase (RTK) III subfamily. The *FLT3* receptor gene (located in chromosome 13q12) encodes a 993 amino acid protein. This protein is expressed in bone marrow, thymus and lymph nodes [15]. *FLT3* plays a major role in cell survival, proliferation, and differentiation of hematopoietic stem cells [16]. *FLT3-ITD* is found in about one quarter of newly diagnosed AML patients. This mutation causes the main activation of the receptor tyrosine kinase activity in the absence of ligand [17].

In our study, we aimed to screen whole *TET2, KRAS*, and *CBL* by NGS analysis, evaluating the association of mutations of JAK2 and FLT3 which is known as prognostic risk factors and finding possible genetic markers for molecular leukemia analysis. The NGS method is a powerful tool to discover novel disease mutations and candidate biomarkers. Therefore we chose NGS as a screening method for our study.

2. Subjects and methods

2.1. Subjects

The study population consisted of eight patients aged between one and 15 years who were admitted to Losante Hospital for Children with Leukemia with the diagnosis of AML. An informed written consent was obtained from all the patients' parents. Patient characteristics of the eight pediatric AML cases are shown in Table 1.

2.2. 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 ISCN [24].