Research Article

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Analysis of Chromosomal Aberrations and FLT3 gene Mutations in Childhood Acute Myelogenous Leukemia Patients

Çocukluk Çağı Akut Myeloid Lösemi Hastalarında Kromozomal Değişiklikler ve FLT3 Geni Mutasyonlarının Araştırılması

Ender Coşkunpınar[1], Sema Anak[2], Leyla Ağaoğlu[2], Ayşegül Ünüvar[2], Ömer Devecioğlu[2], Gönül Aydoğan[3], Çetin Timur[4], Ahmet Faik Öner[5], Yıldız Yıldırmak[6], Tiraje Celkan[7], İnci Yıldız[7], Nazan Sarper[8], Uğur Özbek[1]

[1]İstanbul University, Institute of Experimental Medical Research, Department of Genetics, İstanbul, Turkey
[2]İstanbul University, School of Medicine, Department of Pediatric Hematology-Oncology, İstanbul, Turkey
[3]Bakırköy Maternity and Children's Hospital, İstanbul, Turkey
[4]Göztepe Education and Research Hospital, Department of Pediatric Hematology, İstanbul, Turkey
[5]Yüzüncü Yıl University, School of Medicine, Department of Pediatrics, Van, Turkey
[6]Şişli Etfal Education and Research Hospital, Department of Pediatric Hematology, İstanbul, Turkey
[7]İstanbul University, Cerrahpaşa School of Medicine, Department of Pediatric Hematology-Oncology, İstanbul, Turkey
[8]Kocaeli University, School of Medicine, Department of Children's Health and Diseases, Kocaeli, Turkey

Abstract

Objective: To identify the well-known common translocations and FLT3 mutations in childhood acute myelogenous leukemia (AML) patients in Turkey.

Material and Methods: The study included 50 newly diagnosed patients in which t(15;17), t(8;21), and inv(16) chromosomal translocations were identified using real-time PCR and FLT3 gene mutations were identified via direct PCR amplification PCR-RE analysis.

Results: In all, t(15;17) chromosomal aberrations were observed in 4 patients (8.0%), t(8;21) chromosomal aberrations were observed in 12 patients (24.0%), inv(16) chromosomal aberrations were observed in 3 patients (6.0%), and FLT3-ITD mutations were observed in 2 patients (4.0%); FLT3-D835 point mutation heterozygosity was observed in only 1 patient (2.0%) patient.

Conclusion: Despite of the known literature, a patient with FLT3-ITD and FLT3-D835 double mutation shows a better survival and this might be due to the complementation effect of the t(15;17) translocation. The reportedmutation rate in this article (4%) of FLT3 gene seems to be one of the first results for Turkish population.

Key Words: Childhood AML, FLT3 gene mutations, ITD, D835 mutations, Chromosomal translocations

Address for Correspondence: Uğur ÖZBEK, M.D.,
İstanbul Üniversitesi DETAE Genetik AD, Vakıf Gureba Cad. Capa, Fatih 34093 İstanbul, Turkey
Phone: +90 212 414 20 00 (33312) E-mail: uozbek@istanbul.edu.tr

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Özet

Amaç: Bu çalışmada Türk çocukluk çağı AML hastalarında sık görülen kromozomal translokasyonların ve FLT3 mutasyonlarının belirlenmesi amaçlandı.

Gereç ve Yöntemler: Yeni tanı almış 50 hastada real time PCR yöntemi ile t(15;17), t(8;21), inv(16) kromozomal translokasyonlarının varlığı ve PCR yöntemi ile FLT3 geni ITD tipi mutasyonlar ve D835 nokta mutasyonları varlığı araştırıldı.

Bulgular: Olguların 4'ünde t(15;17) (8.0%), 12'sinde t(8;21) (24%), 3'ünde inv(16) (6.0%) kromozomal düzensizlikleri, 2 olguda FLT3-ITD mutasyonu (4.0%), 1 olguda FLT3-D835 nokta mutasyonu (2,0%) varlığı tespit edildi. t(15;17) pozitif AML M3'lü bir olguda hem FLT3-ITD hem de FLT3-D835 mutasyonları bakımından heterozigotluk tespit edildi.

Sonuç: Literatürden farklı olarak, bir hastamızın daha uzun sağkalımının t(15;17) translokasyonunun mutasyonlu bireylerde iyi yönde düzeltici etkisinin neden olabileceği düşünülmektedir. FLT3 geninde görülen bu mutasyon oranı (4%) Türk toplumu için ilk sonuçlardan biridir.

Anahtar Sözcükler: Çocukluk çağı akut myeloid lösemi, FLT3 gen mutasyonları, İnternal tandem duplikasyon-ITD, D835 mutasyonları, Kromozomal translokasyonlar

Introduction

Acute myelogenous leukemia (AML) is a malignant disease of myeloid stem cells linked to oncogenic fusion proteins, which is due to chromosome translocations and inversions. Numerous translocations have been described in AML, of which the most common are t(8;21), t(15;17), and inv(16). These recurring translocations are currently used as the basis for classification of AML [1]. As such, AML-associated fusion proteins function as aberrant transcriptional regulators, with the potential to interfere with normal myeloid cell differentiation [1-3]. FMS-like tyrosine kinase 3 (FLT3)—a new member of the receptor tyrosine kinase (RTK) III subfamily—was originally identified in hematopoietic stem/progenitor cells and is important for normal lymphohematopoietic stem cell function [4]. FLT3 is aberrantly expressed in the most of AML patients. The *FLT3* gene is located on chromosome 13 (13q12) [5]. To date, 2 distinct types of *FLT3* gene mutations have been identified in AML cases: 1. Internal tandem duplication (ITD) mutations, which occur within the juxtamembrane region of the gene; 2. Point mutations that occur at codon 835 (D835) within the kinase domain. Both types of mutations constitutively activate FLT3 tyrosine kinase activity [6]. *FLT3* gene mutations are strongly associated with leukocytosis and poor prognosis in AML patients [5,7,8]. Patients with either of these mutations have a higher risk of recurrence and a lower survival rate [8]. It was recently reported that the *FLT3* gene mutant/normal ratio can be used as a marker for the selection of therapy [5-8]. The present study aimed to indentify the well-known common AML translocations and *FLT3* mutations in childhood AML patients in Turkey.

Materials and Methods

Patients

The study included 50 newly diagnosed childhood AML patients (28 male and 22 female) that presented for molecular diagnosis to Istanbul University, Institute of Experimental Medicine, Istanbul, Turkey, between October 2007 and July 2008. The Istanbul University, School of Medicine Ethics Committee approved the study protocol (project No. 1850/2007) and informed consent was provided by the patients' parents. Diagnostic bone marrow samples were divided into 2 parts; 2 x 10^7 cells were preserved in RTL buffer (cat. No. 79216, Qiagen, Germany) at –80 °C until RNA isolation and the remainder of the samples were used for DNA isolation, according to the manufacturer's instructions (cat. No. 11796828001 Roche Applied Sciences, Germany).

Determination of t(15:17), t(8:21) and inv(16) chromosome abnormalities

Total RNA was extracted from bone marrow specimens using a QIAamp RNA Blood Mini Kit (cat. No. 52304, Qiagen, GmbH, D-40724 Hilden, Germany), according to the manufacturer's instructions. cDNA was synthesized from 1 µg of total RNA, as previously described [7]. The quality of the obtained cDNA was evaluated via ß-globin PCR performed using the following primers: forward: 5' GAA GAG CCA AGG ACA GGT AC 3'; reverse: 5' CAA CTT CAT CCA CGT TCA CC 3'. Chromosomal abnormalities [t(15; 17), t(8; 21), and inv(16)] were identified via real-time PCR, using the LightMix primer/probe set (cat. No. 40-0135-16 cat. No. 40-0196-16 cat. No. 40-0229-16 TIB Molbiol GmbH, Berlin, Germany), and the Light Cycler FastStart

DNA Master Hyprobe Kit (cat. No. 03515575001, Roche Diagnostics, GmbH, Mannheim, Germany).

Detection of *FLT3*-ITD mutations

FLT3-ITD mutations were indentified via PCR. The forward primer was in exon 14 (14F 5'-GCAATTTAG-GTATGAAAGCCAGC-3') and the reverse primer was in exon 15 (15R 5'-CTTTCAGCATTTTGACGGCAACC-'3), as described by Wang et al. [4]. Amplification was performed in a reaction volume of 50 µL with 100 ng μL^{-1} of DNA, 10 pmol of each primer, 10 mmol dNTP, and 2.5 U of Ex-Taq DNA polymerase (cat. No. RR001A Takara, Japan) in the buffer (10 mmol L^{-1} of TrispHCl [pH 8.3], 50 mmol L^{-1} of KCl, and 1.5 mmol L^{-1} of $MgCl_2$). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for 30 cycles, and elongation for 10 min at 72 °C. Amplification products were analyzed on 3% agarose gel stained with ethidium bromide and samples with the specific PCR products (329bp) were considered as positive for *FLT3*-ITD mutations. The specific amplicons were purified using the QIAEX II Gel Extraction Kit (cat. No. 20021, Qiagen, Hilden, Germany), according to the manufacturer's instructions, and directly sequenced for confirmation of PCR.

Detection of *FLT3*-D835 mutations

FLY3-D835 mutations were identified using the PCR-RFLP method. The primers employed were 20F 5'-CGC-CAGGAACGTGCTTG-3' and 20R 5'-GCAGCCTCACATT-GCCCC-3', as described by Wang et al. [4]. At codon 835 an aspartate amino acid is encoded, providing a recognition site for restriction enzyme *Eco*RV; as such, mutants can be detected via the loss of this enzyme restriction site. The PCR setup was as described above. The specific products were detected on agarose gel, followed by EcoRV (cat. No. 50-720-3590 Takara, Japan) digestion at 37 °C for 4 h. Restriction products were detected on a 3.5% agarose gels and undigested PCR product indicated the presence of the mutation (Figure). The results were confirmed via direct sequencing.

Statistical analysis

Clinical and laboratory characteristics at diagnosis were statistically correlated (age, sex, WBC count, hemoglobin level, PLT count, blast cells rate) with t(15;17), t(8;21), inv(16) chromosomal aberrations, and *FLT3* mutations (Table 1). Fisher's exact test and Pearson's chi-square test were performed using SPSS v.12.0. P values less than 0.05 were considered statistically significant.

Results

Diagnoses—based on French-American-British (FAB) classification—were as follows: M0 (n = 3); M1 (n = 9); M2 (n = 15); M3 (n = 12); M4 (n = 3); M5 (n = 5); M7 (n = 1). Additionally, 1 patient was lacking clinical data and could not be classified, and another patient that died following BMT and couldn't be classified was thought to

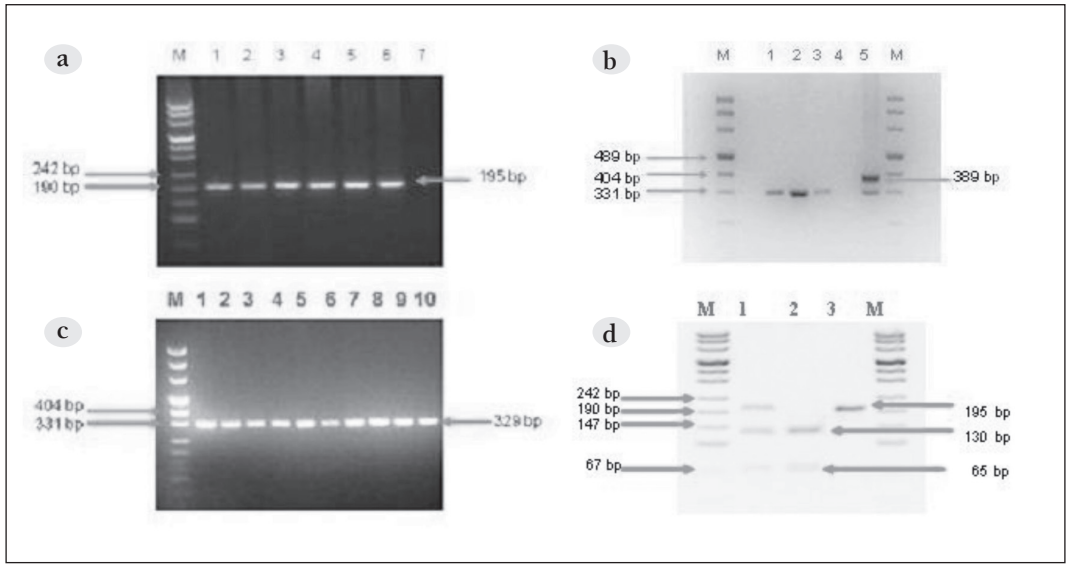

Figure. a. PCR amplification of the *FLT3*-ITD region (lane M: size marker; lanes 1-10: normal samples). **b.** PCR amplification of the *FLT3*-ITD region (lane M: size marker; lanes 1-3: normal samples; lane 4: negative control; lane 5: *FLT3*/ITD-positive case). **c.** PCR amplification of *FLT3*-D835 (lane M: size marker; lanes 1-6: normal samples; lane 7: negative control). **d.** D835 mutation detection (lane M: size marker; lane 1; *FLT3*-D835-positive case; lane 2: wild type; lane 3: EcoRV undigested sample).

Table 1: Characteristics of childhood AML patients carrying *FLT3* gene mutations and/or chromosomal aberrations.

Case No.	SEX	AGE (months)	WBC count (x10^9 L^{-1})	Hb (g dL^{-1})	PLT count (x10^9 L^{-1})	BM Blast (%)	T(15;17)	T(8;21)	Inv(16)	ITD	D835	CD34 (%)	FAB Classification
1	M	20	15.920	8.90	15.000	44	Negative	Negative	Positive	Negative	WT	47.4	M0
2	M	1	132.000	NA	NA	NA	Negative	Negative	Negative	Positive	WT	NA	NA
3	M	132	1.200	5.90	32.000	60	Positive	Negative	Negative	Negative	WT	NA	M3
4	F	120	4.100	7.00	99.000	40	Negative	Negative	Positive	Negative	WT	1.38	M1
5	F	120	1.100	7.90	31.000	100	Positive	Negative	Negative	Negative	WT	4.0	M3
6	F	180	3.600	11.20	104.000	22	Negative	Positive	Negative	Negative	WT	45.2	M2
7	F	132	5.200	6.90	43.000	45	Negative	Positive	Negative	Negative	WT	33.8	M2
8	F	60	8.500	11.00	71.000	53	Negative	Positive	Negative	Negative	WT	15.9	M2
9	M	96	12.100	7.40	22.000	48	Negative	Positive	Negative	Negative	WT	44.2	M2
10	M	144	10.710	10.20	29.000	47	Negative	Positive	Negative	Negative	WT	69.5	M2
11	M	84	23.700	10.70	86.000	72	Positive	Negative	Negative	Positive	HET	8.81	M3
12	M	120	9.800	7.40	28.000	61	Negative	Positive	Negative	Negative	WT	86.5	M2
13	M	24	5.450	7.70	61.000	37	Negative	Positive	Negative	Negative	WT	1.76	M1
14	M	180	1.300	8.90	291.000	81	Negative	Positive	Negative	Negative	WT	NA	M1
15	M	204	1.400	12.80	25.000	51	Negative	Positive	Negative	Negative	WT	70.7	M2
16	F	168	5.500	3.60	6.000	48	Negative	Positive	Negative	Negative	WT	58.6	M2
17	M	14	74.600	8.10	79.000	80	Negative	Negative	Positive	Negative	WT	31.7	M5
18	F	84	11.900	7.20	45.000	49	Negative	Positive	Negative	Negative	WT	NA	M2
19	F	54	1.800	8.20	17.000	94	Positive	Negative	Negative	Negative	WT	1.73	M3
20	F	72	27.000	8.30	38.000	48	Negative	Positive	Negative	Negative	WT	48.8	M2
21	M	144	3.730	11.50	24.000	73	Negative	Positive	Negative	Negative	WT	NA	M2

WBC: White blood cell, Hb: hemoglobin, PLT: platelet, BM: bone marrow, FAB: French-American-British;
WT: wild type; HET: heterozygous; NA: not available.

Table 2: Patient mutation status and clinical features.

Characteristics	*FLT3* Negative Patients (n)	*FLT3* Positive Patients (n)
	48	2
Age (years)		
0-5	15	-
5-10	11	2
10-18	22	-
Sex		
Male	26	2
Female	22	-
WBC (x10^9 L^{-1})		
≤50	38	1
>50	8	1
Unknown	2	-
Hemoglobin (g dL^{-1})		
≤10	31	-
>10	16	1
Unknown	1	1
Platelet (x10^9 L^{-1})		
≤50	21	-
>50	25	1
Unknown	2	1
Blast cells (%)		
≤80	30	1
>80	13	-
Unknown	5	1

be M0 or M7. Median age of the patients was 8.42 ± 5.24 years (range: 0-18 years). The median white blood cell (WBC) count was 30,394.17 ± 57,255.86 mL^{-1} (range: 890-260,000 mL^{-1}), the median platelet (PLT) count was 83,851.06 ± 76,349.87 L^{-1} (range: 2 x 10^9-291 x 10^9 L^{-1}), the median hemoglobin level was 3.4 ± 2.67 g dL^{-1} (range: 3.4-14.3 g dL^{-1}), and the median bone marrow blast rate was 60.86% ± 22.9% (range: 12%-100%).

FAB classification and clinical features of the 50 childhood AML patients are summarized in Table 2. In all, 4 patients were positive for t(15;17), 12 were positive for t(8; 21), and 3 were positive for inv(16) (Table 3). The 4 t(15;17)-positive patients were classified as M3, and 9 of the 12 t(8;21)-positive patients were M2, 2 were M1, and 1 was M4. Among the inv(16)-positive patients, 1 was M0, 1 was M1, and 1 was M5. None of the patients were classified as M6; therefore, statistical evaluation of the FAB M6 patients was excluded. In total, 2 patients had *FLT3* gene mutations, 1 of which was classified as AML-M3 and interestingly the *FLT3*-D835 mutation was not a deletion, but a point mutation (g.IVS20 +49 A>G) that also changed the EcoRV restriction site. Both of these changes were previously described and are known to increase expression of FLT3 [1,5,6].

Table 3: The frequency of translocations in the childhood AML patients.

Chromosome translocation breakpoints	Positive (n = 50)	Rate (%)
t(8;21)(q22;q22)	13	26
inv(16)(p13;q22)	3	6
t(15;17)(q22;q21)	4	8

The hemoglobin level in the patients with *FLT3*-ITD mutations was significantly lower than in the patients without the mutation. In the present study there was a correlation between FAB M2 classification and t(8;21) positivity (P = 0.005), and between FAB M3 classification and t(15;17) positivity (P = 0.009), which is in agreement with previous reports. In addition inv(16) was positive in the FAB M0, M1, and M5 patients with P values of P = 0.001, P = 0.003, P = 0.002 respectively.None of the FAB M4 patients were positive. Bone marrow blast rates below and higher than 80% were compared with t(8; 21) positivity and found that patients with t(8;21) had higher blast rates than non translocated ones (P = 0.049).

Discussion

In addition to observation of the standard clinical features and laboratory analysis, the diagnosis of AML requires additional procedures, including pathological examination, immunophenotyping, cytogenetics examination, and molecular diagnostics. Identification of the specific cytogenetic abnormality is important for selection of appropriate therapy and prognostic analysis [1,9]. Numerous translocations have been described in AML, of which the most frequent are t(15;17), t(8;21), and inv(16), accounting for 20%-30% of all chromosomal aberrations [1,10-12].These aberrations, depending on their structure, lead to expression of a chimeric protein with new functions [8,13].Prognosis is considered goodin cases oft(15;17)

associated with AML-M3, t(8;21), inv(16) associated with AML-M2, and inv(16) associated with AML-M4 [3,14,15]. It is known that the blast level in AML-M2 patients is 30%-90% [16-18].

In the present study the bone marrow blast rate in 11 of the 12 FAB M2 patients with t(8;21) translocation was over 80%, which shows that the bone marrow blast level in childhood AML patients classified as FAB M2 is high. Additionally, inv(16) was strongly correlated with FAB classification (P = 0.043). The 3 inv(16)-positive AML patients were classified as follows: M0 (n = 1); M1 (n = 1); M5 (n = 1). According to the literature, inv(16) occurs more frequently in patients classified as FAB M4 [19] and AML-M4 occurs more frequently in patients classified as FAB M4, primarily in patients aged ≥50 years [17,18]. The presence of inv(16) is indicative of a good prognosis in FAB M4 patients, but data concerning the relationship between inv(16) and other AML FAB classifications are lacking. In the present study inv(16) was not observed in any of the FAB M4 patients. The present findings are in agreement with those reported by Dash et al. and the American National Cancer Institute AML guideline [14,20].The *FLT3* gene is expressed primarily in hematopoietic stem cells [5,21,22]. Moreover, human leukemia and lymphoma cell lines also express FLT3 protein [22]. The 2 most common mutations of the *FLT3* gene are *FLT3*-ITD and *FLT3*-D835. Among the 50 childhood AML patents in the present study, only 2 had *FLT3*-ITD mutations, of which 1 also had *FLT3*-D835 point mutation. The incidence of *FTL3*-ITD mutation in childhood AML patients (5-16%) is lower than that in adult AML patients (20-25%) [22,25]. Meshinchi et al. reported that the incidence of *FLT3*-TKD (tyrosine kinase domain) mutations in childhood AML patients was 6.7% [27], versus 7% reported by Karabacak et al. [23]. The known *FLT3* gene mutation rate in adult AML patients is 20%-25% [22,25]; however, Liang et al. [24] reported a mutation rate in childhood AML patients of 11.3%, and Kondo et al. [22], Iwai et al. [25], and Krstovski et al. (26) reported rates of 5.3%-16.5%.

Our data is one of the first results *FLT3*-ITD mutation evaluation in Turkish pediatric AML patients and the mutation rate is 4.0%, which is much lower than the other study groups [23,27]. In the present study there wasn't a correlation between *FLT3* gene mutations, and age, gender, the WBCcount, blast cell rate, or FAB classification. Although it was reported that the *FLT3*-ITD mutation rate increases with age Meshinchi et al. [27], Kondo et al. [22] and Wang et al. [4] and was not observed a significant difference between the mutation rate and the age in our results.

In the present study there wasn't a significant difference between *FLT3*-ITD mutations and the WBC count. The WBC count in 95% of the present study's patients was less than 50 x 10^9 L^{-1}, which is in agreement with the findings reported by Moreno et al. [28]. The most common feature of AML is chronic, severe anemia, which damages bone marrow. The present results show that there was a relationship between a low hemoglobin level and *FLT3*-ITD mutations, but not between a low hemoglobin level and *FLT3*-D835 mutation, which might have been due to the small number of patients with*FLT3*-D835 point mutation as well as the small study population. Pre-clinical studies highlighted the potential use of inhibitors against FLT3 kinase activity. Most of patients carrying *FLT3* gene mutation have a 50% decrease in the peripheral blast count, along with inhibition of receptor autophosphorylation.

Herein we described the development of anemia in patients carrying *FLT3* gene mutation. It is also known that *FLT3* mutations are poor prognostic markers of AML. A 7-year-old male patient in the present study classified as AML-M3 had double *FLT3* mutations and t(15;17) translocation. He was in remission for 1 year, and died after 19 months of diagnosis. While *FLT3* mutation causes continuous tyrosine kinase activity, t(15;17) translocation deregulates RARα transcription factor and cell differentiation stops. Thus, AML occurs due to the combined effects of *FLT3* gene mutations and t(15;17) translocation. To the best of our knowledge our 7-year-old male patient is the first reported case to have been in remission for approximately 1 year with double *FLT3* mutation and translocation. This is also the first case with double *FLT3* mutation and t(15;17) translocation that is reported inTurkish population. Most *FLT3* gene mutations are reported as individual mutations and only a few cases of double mutation have been reported [5,22,29,30]; Moreno et al. reported 4 cases in 2003 [28] and Wang et al. reported 2 cases in 2005 [4]. All the reported double mutation patients died after induction therapy or relapsed in first months after diagnosis [28]. These results indicate that *FLT3*-ITD and *FLT3*-D835 mutations are markers of poor prognosis. In contrast to previous reports our 7-year-old patient survived longer, which might have been due to the combined effect of t(15;17) translocation (31,32); however, the quality of clinical response to FLT3 inhibitors has been minor, with many patients transiently responding with a decreased blast count(31,32). This finding needs to be confirmed by in vitro studies in which patients are followed-up for longer periods of time. It is not clear if *FLT3* double mutation causes upregulation in tyrosine kinase activity or increases cell survival. AML occurs as a result of excessive prolifera-

tion and differentiation of myelogenous blasts. The present study is the first to perform detailed molecular analysis of Turkish childhood AML patients. The low *FLT3* gene mutation rate (4%) seems to be unique to this study's population. This result and the effects of double mutations need to be evaluated in larger patient groups.

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Conflict of Interest Statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

References

1. Lee S, Chen J, Zhou G, Shi RZ, Bouffard GG, Kocherginsky M, Ge X, Sun M, Jayathilaka N, Kim YC, Emmanuel N, Bohlander SK, Minden M, Kline J, Ozer O, Larson RA, LeBeau MM, Green ED, Trent J, Karrison T, Liu PP, Wang SM, Rowley JD. Gene expression profiles in acute myeloid leukemia with common translocations using SAGE. Proc Natl Acad Sci USA 2006; 103 (4): 1030-1035
2. Smith M, Barnett M, Bassan R, Gatta G, Tondini C, Kern W. Adult acute myeloid leukaemia. Crit Rev Oncol Hematol 2004; 50 (3): 197-222
3. Karen S. Acute myelogenous leukemia. June 22, 2010 Available at: www.emedicine.com/med/topic34.htm
4. Wang L, Lin D, Zhang X, Chen S, Wang M, Wang J. Analysis of FLT3 internal tandem duplication and D835 mutations in Chinese acute leukemia patients. Leuk Res 2005; 29 (12): 1393-1398
5. Liang DC, Shih LY, Hung IJ, Yang CP, Chen SH, Jaing TH, Liu HC, Wang LY, Chang WH. FLT3-TKD mutation in childhood acute myeloid leukemia. Leukemia 2003; 17 (5): 883-886
6. Levis M, Allebach J, Tse KF, Zheng R, Baldwin BR, Smith BD, Jones-Bolin S, Ruggeri B, Dionne C, Small D. A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells in vitro and in vivo. Blood 2002; 99 (11): 3885-3891
7. Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S, Asou N, Kuriyama K, Yagasaki F, Shimazaki C, Akiyama H, Saito K, Nishimura M, Motoji T, Shinagawa K, Takeshita A, Saito H, Ueda R, Ohno R, Naoe T. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood 2001; 97 (8): 2434-2439
8. Sayitoğlu MA, Ozbek U. Molecular genetics of acute leukemias. Türkiye klinikleri J Int Med Sci 2007; 3(2)
9. Jaffe ES, Harris NL, Diebold J, Muller-Hermelink HK. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. A progress report. Am J Clin Pathol 1999; 111: 8-12
10. Valk PJ, Delwel R, Löwenberg B. Gene expression profiling in acute myeloid leukemia. Curr Opin Hematol 2005; 12 (1): 76-81
11. Alcalay M, Tiacci E, Bergomas R, Bigerna B, Venturini E, Minardi SP, Meani N, Diverio D, Bernard L, Tizzoni L, Volorio S, Luzi L, Colombo E, Lo Coco F, Mecucci C, Falini B, Pelicci PG. Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. Blood 2005; 106 (3): 899-902
12. Ali R. Classification and differential diagnosis of the acute leukemias. Türkiye Klinikleri J Int Med Sci 2007; 3(2)
13. Diverio D, Rossi V, Avvisati G, De Santis S, Pistilli A, Pane F, Saglio G, Martinelli G, Petti MC, Santoro A, Pelicci PG, Mandelli F, Biondi A, Lo Coco F. Early detection of relapse by prospective reverse transcriptase-polymerase chain reaction analysis of the PML/RARalpha fusion gene in patients with acute promyelocytic leukemia enrolled in the GIMEMA-AIEOP multicenter "AIDA" trial. GIMEMA-AIEOP Multicenter "AIDA" Trial. Blood 1998; 92 (3): 784-789
14. Adult Acute Myeloid Leukemia-PDQ [database online]. Nationale Cancer Institute; 2007. Updated August 07, 2010
15. O'Brien MM, Lacayo NJ. Acute leukemia in children. Dis Mon 2008; 54 (4): 202-225
16. Brunning RD, Matutes E, Haris NL, et al; Acute myeloid leukaemia: introduction. In: Jaffe ES, Harris NL, Stein H, et al., eds.: Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC press, 2001. World Health Organization Classification of Tumours, 3: p. 77-80
17. Litchman MA & Liesveld JL. The chronic myelogenous leukemias [Chapter 88]. In: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal J (eds).Williams Hematology New York: McGraw Hill Book Co 2006
18. Turgeon ML. Clinical Hematology, Theory and procedures. Philadelphia: JB Lippincott, 3rd ed. 1999
19. Colovic M, Jurisic V, Pavlovic S, Terzic T, Colovic N. FLT3/D835 mutation and inversion of chromosome 16 in leukemic transformation of myelofibrosis. Eur J Intern Med 2006; 17 (6): 434-435
20. Dash A, Gilliland DG. Molecular genetics of acute myeloid leukaemia. Best Pract Res Clin Haematol 2001; 14 (1): 49-64
21. Drexler HG. Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. Leukemia 1996; 10 (4): 588-599

22. Kondo M, Horibe K, Takahashi Y, Matsumoto K, Fukuda M, Inaba J, Kato K, Kojima S, Matsuyama T. Prognostic value of internal tandem duplication of the FLT3 gene in childhood acute myelogenous leukemia. Med Pediatr Oncol 1999; 33 (6): 525-529
23. Karabacak BH, Erbey F, Bayram I, Yilmaz S, Acipayam C, Kilinç Y, Tanyeli A. Fms-like tyrosine kinase 3 mutations in childhood acute leukemias and their association with prognosis. Asian Pac J Cancer Prev 2010; 11 (4): 923–927
24. Liang DC, Shih LY, Hung IJ, Yang CP, Chen SH, Jaing TH, Liu HC, Chang WH. Clinical relevance of internal tandem duplication of the FLT3 gene in childhood acute myeloid leukemia. Cancer 2002; 94 (12): 3292-3298
25. Iwai T, Yokota S, Nakao M, Okamoto T, Taniwaki M, Onodera N, Watanabe A, Kikuta A, Tanaka A, Asami K, Sekine I, Mugishima H, Nishimura Y, Koizumi S, Horikoshi Y, Mimaya J, Ohta S, Nishikawa K, Iwai A, Shimokawa T, Nakayama M, Kawakami K, Gushiken T, Hyakuna N, Fujimoto T. Internal tandem duplication of the FLT3 gene and clinical evaluation in childhood acute myeloid leukemia. The Children's Cancer and Leukemia Study Group, Japan. Leukemia 1999; 13 (1): 38-43
26. Krstovski N, Tosic N, Janic D, Dokmanovic L, Kuzmanovic M, Spasovski V, Pavlovic S. Incidence of FLT3 and nucleophosmin gene mutations in childhood acute myeloid leukemia: Serbian experience and the review of the literature. Med Oncol 2010; 27 (3): 640-645
27. Meshinchi S, Alonzo TA, Stirewalt DL, Zwaan M, Zimmerman M, Reinhardt D, Kaspers GJ, Heerema NA, Gerbing R, Lange BJ, Radich JP. Clinical implications of FLT3 mutations in pediatric AML. Blood 2006; 108 (12): 3654-3661
28. Moreno I, Martín G, Bolufer P, Barragán E, Rueda E, Román J, Fernández P, León P, Mena A, Cervera J, Torres A, Sanz MA. Incidence and prognostic value of FLT3 internal tandem duplication and D835 mutations in acute myeloid leukemia. Haematologica 2003; 88 (1):19-24
29. Abu-Duhier FM, Goodeve AC, Wilson GA, Care RS, Peake IR, Reilly JT. Identification of novel FLT-3 Asp835 mutations in adult acute myeloid leukaemia. Br J Haematol 2001; 113 (4): 983-988
30. Colovic N, Tosic N, Aveic S, Djuric M, Milic N, Bumbasirevic V, Colovic M, Pavlovic S. Importance of early detection and follow-up of FLT3 mutations in patients with acute myeloid leukemia. Ann Hematol 2007; 86 (10): 741-747
31. Alvares CL, Schenk T, Hulkki S, Min T, Vijayaraghavan G, Yeung J, Gonzalez D, So CW, Greaves M, Titley I, Bartolovic K, Morgan G. Tyrosine kinase inhibitor insensitivity of non-cycling CD34+ human acute myeloid leukaemia cells with FMS-like tyrosine kinase 3 mutations. Br J Haematol 2011; 154 (4): 457-465
32. Kindler T, Lipka DB, Fischer T. FLT3 as a therapeutic target in AML: Still challenging after all these years. Blood 2010; 116 (24): 5089-5102