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# Real-Time PCR analysis of *af4* and *dek* genes expression in acute promyelocytic leukemia t (15; 17) patients

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Abbreviations: APL, acute promyelocytic leukaemia

## Abstract

**Among several newly identified oncogenes, *dek* and *af4* are attractive targets for researchers interested with leukemia. In this study quantitative Real-Time RT-PCR technique was used to define alterations in expression of *dek* and *af4* genes associated with acute promyelocytic leukaemia (*APL*) t (15; 17). RNA samples obtained from bone marrow aspirates of fourteen *APL* patients, cDNA portions were labelled with Syber Green 1 dye and LightCycler analysis have been performed. Expression changes in patients were found not significant in comparison to healthy donors for *af4* (*P* = 0.192) and *dek* (*P* = 0.0895). We suggest that *af4* gene may have a role in leukomogenesis restricted to lymphoblastic lineage; also further studies must carry on with a larger series of patients in order to understand the relationship between the *dek* gene and *APL*. Our study was the first attempt for analysing *dek* and *af4* genes in *APL* t (15; 17) patients by quantitative Real-Time RT-PCR. This rapid and sensitive method could be used to screen these genes in different types of leukaemia.**

**Keywords:** *af4*; *APL*; *dek*; Gene expression; Real Time PCR

## Introduction

Translocation associated gene fusions are well known incidents in acute myeloid leukemia while the other genetic changes are less known. Acute promyelocytic leukaemia (*APL*) which is characterized by a reciprocal t (15; 17) translocation of fusing the *pml* gene to the retinoic acid receptor alpha (*rar-alpha*) gene, but probably there are more oncogenes responsible in *APL* pathogenesis. Among several newly identified oncogenes, *dek* and *af4* are attractive targets for researchers interested with leukemia. Single role of translocation partners *dek* and *af4* genes in leukemogenesis have been shown in previous studies (Domer *et al.,* 1993; Larramendy *et al.,* 2002). We also found that *dek* and *af4* genes were down regulated during vitamin D dependent differentiation of acute promyelocytic leukaemia cell line HL-60 cells, in our previous studies, using cDNA array technology (Savli *et al.,* 2002). These findings orientated us to further *dek* and *af4* gene expression studies in *APL* patient samples. In this study, we performed gene statement analysis in fourteen *APL* patients with t (15; 17), using quantitative real-time RT-PCR. Our study was the first attempt for the quantification of *dek* and *af4* genes in this specific translocation.

## Materials and Methods

Among the 14 *APL* patients; 10 were in adult and 4 in childhood age. Table 1 summarizes the patient data and some clinical features. Childhood patient's samples were obtained from Pediatric Hematology Division of SSK Bakirkoy Hospital and Our Children's Leukemia Foundation, Istanbul; adult patient's samples were collected from adult Hematology/Oncology Division of Istanbul Medical Faculty, Istanbul University, Turkey. Control group was consisted of bone marrow aspirates from 4 healthy volunteer donors.

Mononuclear cells from bone marrow aspirates were isolated by ficoll-hypaque centrifugation. Total RNA was extracted by using guanidium thiocyanate-phenol-chloroform extraction method as previously described (Chomznsky *et al.,* 1987). RNA samples were treated with DNase I and Quantitative Real-time

**Table 1.** Clinical data of fourteen APL patients.

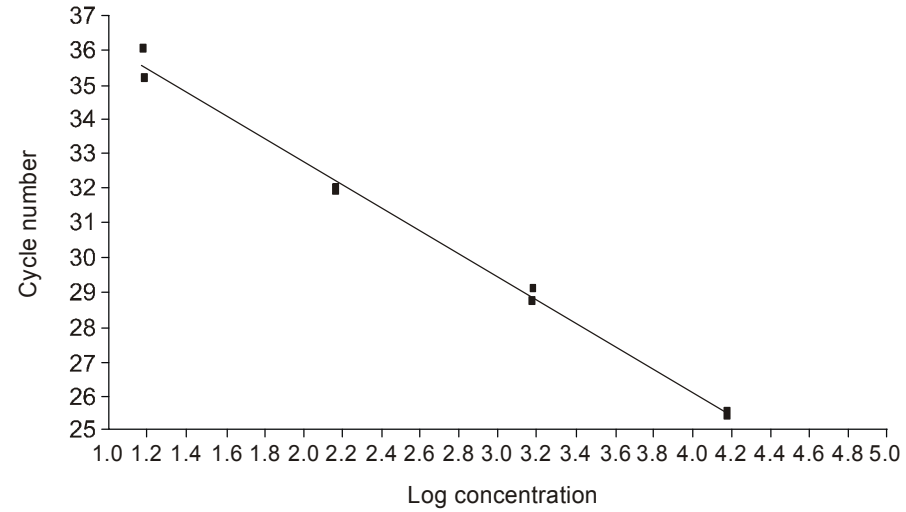
| Patients | Age/sex | BP | WBC ($10^9$/L) | Hb (g/dl) | P | S (m) |
|---|---|---|---|---|---|---|
| 1 | 44 (M) | bcr1/2 | 1,700 | 9.8 | 85% | 41 |
| 2 | 19 (F) | bcr3 | n.a | n.a | n.a | n.a |
| 3 | 31 (F) | bcr1/2 | 1,000 | 6.1 | 83% | 31+ |
| 4 | 15 (M) | bcr1/2 | 1,160 | 8.8 | n.a | n.a |
| 5 | 27 (F) | bcr1/2 | 2,500 | 7.8 | 85% | 19+ |
| 6 | 28 (F) | bcr1/2 | 4,300 | 12.0 | 65% | 29 |
| 7 | 48 (F) | bcr1/2 | 2,400 | 8.5 | n.a | 36+ |
| 8 | 26 (F) | bcr3 | 4,500 | 10.0 | 100% | 23+ |
| 9 | 49 (M) | bcr3 | 53,300 | 15.0 | 32% | n.a |
| 10 | 31 (F) | bcr1/2 | 4,600 | 5.7 | 100% | 24+ |
| 11 | 8 (M) | bcr1/2 | 4,700 | 11.5 | n.a | n.a |
| 12 | 12 (F) | bcr1/2 | n.a | n.a | n.a | n.a |
| 13 | 16 (F) | bcr3 | 400 | 5.7 | 100% | 14+ |
| 14 | 25 (F) | bcr3 | 86,000 | 3.8 | 96% | 15+ |

(Abbreviations: BP, Breakpoint; WBC, White Blood Cells; Hb, Hemoglobine; P, Promyelocyte amount in the bone marrow at diagnosis; S(m), Survival (months); n.a, Data is not available)

PCR (LightCycler, Roche Diagnostics GmbH, Germany) was performed as we described previously (Savli *et al.,* 2002; Savli *et al,* 2003). Standard curves were obtained by using serial dilutions of beta-globulin gene (DNA control kit, Roche, Mannheim, Germany). Gene-specific primers (Table 2) were obtained from TIB MOLBIOL (Berlin, Germany). Obtained gene expression values were normalized using a housekeeping gene *rps9* which belongs to a gene family accepted more reliable than either of the classical housekeeping genes, in human and mouse malignant cell lines (Bhatia *et al.,* 1994; Zhong *et al.,* 1999). A new software tool was used, named REST (relative expression software tool), and gene expression ratios compared in patient and control group. The mathematical model used is based on the PCR efficiencies and the mean crossing point deviations. Subsequently, the expression ratio results of the investigated transcripts are tested for significance by a randomization test. Randomization has been performed for 2000 times (Pfaffl *et al.,* 2002).

**Table 2.** Primer sequences of the studied genes.

| Genes | Chromosomal location | Accession number (GenBank) | Primer sequences |
|---|---|---|---|
| *rps9* (Housekeeping) | 19q13.4 | U14971 | CGTCTCGACCAAGAGCTGAGGTCCTTCTCATCAAGCGTC |
| *dek* | 6p23 | X64229 | GTGGGTCAGTTCAGTGGCAGGACATTTGGTTCGCTTAG |
| *af4* | 4q21 | L13773 | CCCATGGATGGTCAAGATCATGGGTCATTTCCTTCAGAATCTC |




Baseline adjustment: Anthmetic | Noise band cursor: 0.0777 | Analysis method: Fit points | Number of fit point: 2
Crossing line: 0.9305 | Slope: -3.344 | Intercept: 39.47 | Error: 0.0856
Color compensation: Dff | r: -1.00

**Figure 1.** Standart curve analysis of the serial dilutions of the external standard β globulin gene (Slope: -3.344, Intercept: 39.47, Error: 0.0856, R: -1.00).

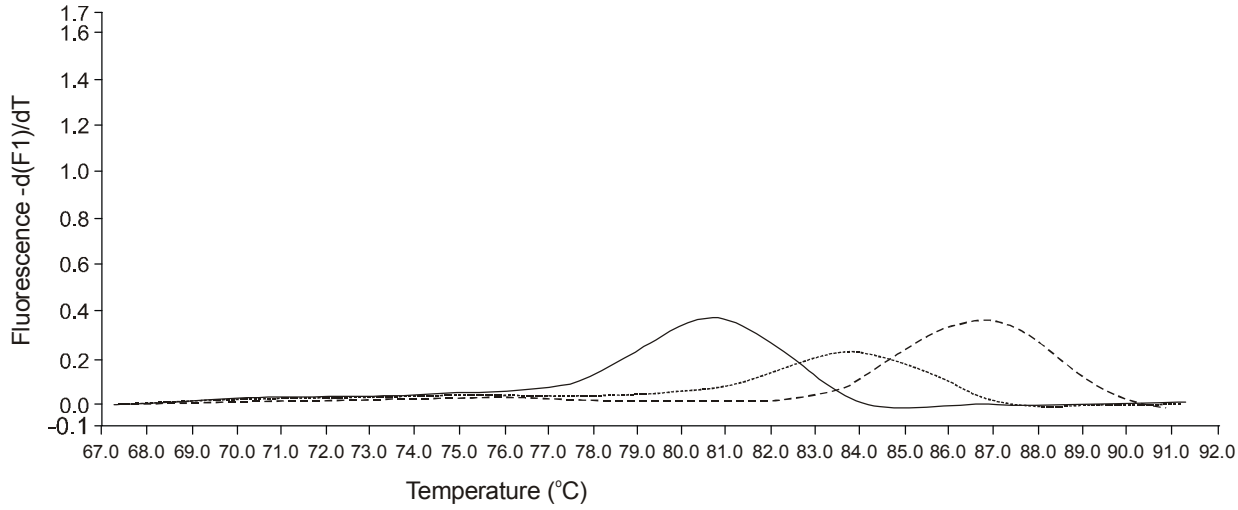



**Figure 2.** Melting curve analysis demonstrating the gradual reduction in fluoresence as temperature increases. The melting peaks at 81°C for *dek*, 84°C for *af4*, 87°C for *rps9* indicate the specific products that melt at this temperature.

## Results and Discussion

*dek* and *af4* gene expression were investigated in fourteen t (15; 17) *APL* patients. *af4* was found up-regulated in patient sample group (in comparison to control group) by the factor 13.825 and absolute gene regulation value was calculated as 7.85167. This expression value was not significant ($P$ = 0.192). *dek* was found down-regulated in patient sample group (in comparison to control group) by the factor 4.629 and absolute gene regulation value was calculated as 0.29689. This expression value also was not significant ($P$ = 0.0895). Exact means, ratios and $P$ values are shown in Table 3. These findings indicate that *dek* and *af4* genes might not be involved in t (15; 17) *APL* pathogenesis. Standart curve analysis of the serial dilutions of the external standart β globulin gene shown in Figure 1. Gene specific amplifications were demonstrated with melting curve peaks in Figure 2.

There are evidences that *dek* and *af4* are working as transcription regulators in the cell physiology. *dek* on 6p23 is known as a translocation partner that forms a fusion gene with the one on 9q34 in a subset of patients with acute myeloid leukemia who carry a t (6; 9) (p23; q34) fusion. It is thought to be a member of a new family of site-specific DNA-binding factors involved in signal transduction and transcriptional regulation might play a role in chromatin architecture (Fu *et al.,* 1997; Kappes *et al.,* 2001). Larramendy *et al.* found up-regulated *dek* levels in 15 AML patients compared to five bone marrow samples of healthy donors, using cDNA array technology. Our results were not in concordance with their findings (Larramendy *et al.,* 2002). Nevertheless, it should be taken into consideration that patient and control populations in both of these studies are not very wide.

**Table 3.** Means, ratios and $P$ values of investigated gene expression levels.

| Genes | *rps9* | *af4* | *dek* |
|---|---|---|---|
| Control means | 17.81 | 29.263 | 19.073 |
| Sample means | 21.688 | 29.532 | 25.162 |
| Absolute gene regulation | | 7.8516 | 0.2968 |
| Expression Ratios | | 13.825 | 0.216 |
| $P$ values | | 0.192 | 0.895 |

Randomisations have been performed for 2000 times

*af4* is the 4q21 gene involved in the acute lymphoblastic leukemia (Domer *et al.,* 1993). This gene remains poorly understood but there may be a potential role in regulating transcription. It has been suggested that *af4* plays an important role in the development of the hematopoietic, cardiovascular, and skeletal and central nervous systems and the disruption of normal *af4* function by the translocation may contribute to leukemogenesis. (Baskaran *et al.,* 1997). Yamamoto *et al.* suggest that the instability of the *af4* gene may be associated with the leukemogenesis of infant leukemia (Yamamoto *et al.,* 1998). Taken together, we believe that *af4* gene may have a role in the transcription regulation of various cell types but its role in leukemogenesis restricted to lymphoblastic lineage.

*dek* and *af4* may not be involved in (15; 17) *APL* pathogenesis and their expression differences may be related to other types of leukemia. Another possibility is that these genes are working as transcriptional

modulators of drug induced differentiation only. Our study was first attempt for the quantification of *dek* and *af4* genes in *APL* t (15; 17) patients. Further analyses are required to demonstrate the exact role of these genes and our approach will enable to screen them among different types of leukemia. Quantitative Real-Time RT-PCR is a sensitive-rapid method and could be used for this aim.

## References

Baskaran K, Erfurth F, Taborn G, Copeland NG, Gilbert DJ, Jenkins NA, Iannaccone PM, Domer PH. Cloning and developmental expression of the murine homolog of the acute leukemia proto-oncogene *af4*. Oncogene 1997;15: 1967-78

Bhatia P, Taylor WR, Greenberg AH, Wright JA. Comparison of glyceraldehyde-3-phosphate dehydrogenase and 28S-ribosomal RNA gene expression as RNA loading controls for northern blot analysis of cell lines of varying malignant potential. Anal Biochem 1994;216:223-6

Chomczynsky P, Sacchi N. Single step metod of RNA isolation by acide guanidium thiocyonate-phenol chloroform extraction. Anal Biochem 1987;152

Domer PH, Fakharzadeh SS, Chen CS, Jockel J, Johansen L, Silverman GA, Kersey JH, Korsmeyer SJ. Acute mixed-lineage leukemia t(4;11)(q21;q23) generates an MLL-*af4* fusion product. Proc Natl Acad Sci USA 1993;90:7884-8

Fu GK, G Grosveld, Markovitz DM. *dek*, an autoantigen involved in a chromosomal translocation in acute myelogenous leukemia, binds to the HIV-2 enhancer. Proc Natl Acad Sci 1997;94:1811-5

Kappes F, Burger K, Baack M, Fackelmayer FO, Gruss C. Subcellular Localization Of The Human Proto-Oncogene Protein *Dek*. J Biol Chem 2001;13:26317-23

Larramendy ML , Niini T, Elonen E, Nagy B, Ollila J, Vihinen M, Knuutila S. Overexpression of translocation-associated fusion genes of FGFRI, MYC, NPMI, and DEK, but absence of the translocations in acute myeloid leukemia. A microarray analysis. Haematologica 2002;87:569-77

Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 2002;1:e36

Savli H, Aalto Y, Nagy B, Knuutila S, Pakkala S. Gene expression analysis of 1,25(OH)2D3 dependent differentiation of HL-60 cells-a cDNA array study. Br J Haematol 2002;118: 1065-70

Savli H, Karadenizli A, Kolayli F, Gundes S, Ozbek U, Vahaboglu H.Expression stability of six housekeeping genes: a proposal for resistance gene quantification studies of Pseudomonas aeruginosa by real-time quantitative RT-PCR. J Med Microbiol 2003;52:403-8

Yamamoto S, Zaitsu M, Ishii E, Yatsuki H, Mizutani S, Eguchi M, Ihara K, Okamura T, Hara T, Miyazaki S. High frequency of fusion transcripts of exon 11 and exon 4/5 in AF-4 gene is observed in cord blood, as well as leukemic cells from infant leukemia patients with t(4;11)(q21;q23). Leukemia 1998;12:1398-403

Zhong H, Simons JW. Direct comparison of GAPDH, beta-actin, cyclophilin, and 28S rRNA as internal standards for quantifying RNA levels under hypoxia. Biochem Bioph Res 1999;259:523-6