

# Does 8q24 region have a potential risk for childhood acute lymphoblastic leukemia? 8q24 bölgesi çocukluk çağı akut lenfoblastik lösemiler için potansiyel risk oluşturur mu?

## Runing title: 8q24 region in ALL

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### ÖZET

TCF transkripsiyon faktörünün bağlanma bölgesine denk gelen 8q24'de görülen polimorfizmler, bugün bir çok kanser türü için bir risk faktörü olarak belirlenmiştir. Bu çalışmada, akut lenfoblastik lösemilerde (ALL) TCF4'ün alele özgü regülasyonunu anlamak amacıyla 8q24 bölgesi genotiplendirilmesi ve TCF4'ün hedef genlerinin (MYC, LEF1, AXIN2) anlatımını incelemeyi hedeflenmiştir. rs6983267 akut lösemilerde anlamlı farklılık göstermemekle beraber alel frekanslarının kombinasyonları (GG ile GT+TT veya TT ile GT+GG) arasında da anlamı farklılık yoktur. Bunun yanında GG genotipi ve hedef genlerin mRNA anlatımı arasında korelasyon bulunmaktadır. Akut lösemi hücre serileri genotiplendirildiğinde de anlamlı bir farklılık bulunmamıştır. Bulgular, 8q24 bölgesinin ALL için direkt bir risk oluşturmakla beraber dokuya özgü farklılıkların TCF4 düzenlenmesini ve bu yol ile TCF4 hedef genlerinin düzenlenmesini etkilediğini açıkça göstermektedir.

**Anahtar kelimeler:** lösemi, kanser, TCF4, polimorfizm

### SUMMARY

8q24 region, localized in a TCF4 transcription factor consensus-binding sequence, harbors many independent risk polymorphisms and is to be associated to several cancers. In this study, we aimed to know if the allele specific regulation of TCF4 is applicable/admissible for childhood acute leukemia by genotyping 8q24 and investigating the expression of three main targets of TCF4 (c-MYC, LEF1, AXIN2). Allele frequencies of rs6983267 were not significantly different between the acute leukemia cases and the combined analysis of the frequencies (GG vs GT+TT or TT vs GT+GG) did not show a significant association but the GG genotype and mRNA expressions of target genes showed a correlation. Also in B-ALL patients, only c-MYC gene expression was increased. We also genotyped acute leukemia cell lines and cell line gene expressions did not show a consistent and significant correlation between the genotypes. To sum up our findings showed that, the frequency of the variation at 8q24 is not associated with ALL risk directly but it is clear that tissue specific differences and regulations may attend to TCF4 regulation and its targets.

**Key words:** leukemia, cancer, TCF4, polymorphism

### Introduction

Recent genome-wide association studies (GWAS) have revealed a substantial contribution of synonymous SNPs to human disease risk and other complex traits. The various molecular mechanisms that underlie these effects still need elucidation. A gene desert located in the chromosome 8q24 harbors many independent risk polymorphisms and is to be associated to several cancers (13).

Colorectal and prostate cancer studies have identified the rs6983267 risk variant, within the susceptibility 8q24 region (4, 1). The rs6983267 variant localized in a transcription factor consensus-binding sequence that is the strongest TCF4 binding site. Members of the TCF transcription family and  $\beta$ -CATENIN have been shown to regulate WNT signaling pathway and consequently regulate the expression levels of candidate genes, including c-MYC, AXIN2 and LEF1. Previously it has been shown that abnormal WNT signaling occurs in acute lymphoblastic leukemia (ALL) (9). The rs6983267 variant is in physical proximity with c-MYC promoter (335 Kb to telomeric region) and acts as a long-range regulator for c-MYC gene (6). Some studies showed that Tcf4 binds to G variant of the rs6983267 more strongly in colorectal carcinoma cells (14). On the other hand, some groups demonstrated the interaction of Tcf4 and T allele is more powerful depending on the cancer type (8). According to Sotelo et. al., in prostate cancer cell line (LnCAP) stronger interaction is reported between T allele and Tcf4 by an unorthodox explanation that is called "intrinsic tumor suppression". This interaction leads to higher expression of c-MYC, which in turn protects cells from producing tumors by inducing apoptosis (8).

Acute lymphoblastic lymphoma (ALL), which is characterized by the rapid proliferation of immature lymphoblast's, arises from B- or T-cell progenitors. Various signaling pathways play roles in leukemogenesis such as NOTCH and WNT pathways (2, 3). There are several genetic and epigenetic reasons for abnormal activation of WNT pathway. In prior work we showed abnormal activation of WNT pathway via increased  $\beta$ -Catenin mRNA and protein levels in a group of T-ALL patients (5). Herein, we wanted to know if the allele specific regulation of TCF4 is applicable/admissible for pediatric

acute leukemia by investigating the expression of three main targets of *TCF4* (*c-MYC*, *LEF1*, *AXIN2*).

### Material and methods

Total 87 pediatric acute leukemia patients (n=41 T-ALL and n=46 B-ALL), healthy controls (n=106) and cell lines (T-ALL cell lines: Molt-4, Jurkat, CEM and RPMI 8402; B-ALL cell lines: REH, Nalm 6 and SUPB27) were included in this study. Genomic DNA was isolated by Qiagen DNA Mini Kit (Qiagen, GmbH, Germany). Total RNA was isolated by Qiagen RNeasy Plus Mini Kit (Qiagen, GmbH, Germany) and cDNA was synthesized by random hexamers and *MMLV* reverse transcriptase, from 1µg of total RNA according to manufacturer procedures (MBI Fermentas Life Sciences, Lithuania). First, the patient samples were genotyped for rs6983267 variant [G/T] by using the Light Cycler 480 Instrument (Roche Applied Sciences, Mannheim, Germany) and the results were confirmed by direct sequencing. The expression levels of *TCF4* and its downstream targets *c-MYC*, *LEF1* and *AXIN2* genes were studied by real time quantitative PCR and analyzed according to the genotyping results. Most stable  $\beta$ -*ACTIN* and *CYCLOPHILIN* genes were used for normalization. Primers, probes and conditions are available upon request.

Relative expressions were calculated according to the delta-delta Ct method, based on the mathematical model described by Livak *et al.*. Expression differences were examined by two-sided t test. Hardy Weinberg Equilibrium (HWE) and the difference between allelic and genotypic frequencies in cases and controls were examined by  $\chi^2$  test. Risk was estimated by odds ratio (ORs) with a confidence interval of 95%. A *p* value of <0.05 was considered statistically significant in all analysis.

### Results

The observed genotype frequencies were in accordance with HWE. Allele frequencies of rs6983267 were not significantly different between the acute leukemia cases (for G allele, 51% and for T allele, 49%) and the controls (for G allele 50% and for T allele 50%) (*p*=0,76 and OR=1,06 and CI 95% 0,71-1,60, respectively). No significant difference was found between the cases and the controls in genotype frequencies (Table 1). Combined analysis of the frequencies (GG vs GT+TT or TT vs GT+GG) did not show a significant association (*p*=0,61 OR=1,22, CI% 0,64-2,37 and *p*=1,00 OR=0,97, CI% 0,50-1,90, respectively). But the GG genotype and mRNA expressions of target genes showed a correlation. Although it is not statistically significant, T-ALL patients had elevated *TCF4* and its downstream genes' (*c-MYC*, *LEF* and *AXIN2*) expression in the GG genotype compared to GT+TT genotypes (Figure 1A). Where as in B-ALL patients, only *c-MYC* gene expression was increased (Figure 1B). Here we also genotyped acute leukemia cell lines. The distribution of genotypes in cell lines were as followed: GG genotype (Molt-4), GT genotype (Jurkat, RPMI 8402, REH and Nalm 6) and TT genotype (SUPB27 and CEM). Cell line gene expressions did not show a consistent and significant correlation between the genotypes (data not shown).

### Discussion

GWAS studies are identifying an increasing number of SNPs associated with disease susceptibility. The challenge is to unravel the causative mechanisms related to these variants. The only study with leukemia patients, which was examining the association between the chronic lymphocytic leukemia (CLL) patients and rs6983267 variant distribution, was not found to be significant (7). In another study, linkage disequilibrium was obtained between the rs6983267 allele and multiple myeloma patients, but G allele frequency was not different than in controls (12).

In previous studies, lymphoblastic cell lines and colorectal carcinoma patients did not show significant *c-MYC* expression between the GG and TT genotypes (10, 11), however they have not examined the *TCF4* and its target genes' expressions in this concept. Our findings support the allele specific regulation of the expressions of *TCF4* transcription factor and its target genes *c-MYC*, *LEF1* and *AXIN2* in T-ALL subgroup. In B-ALL patients, *c-MYC* oncogene, which is the nearest gene to the rs6983267 region, mRNA expression was increased. Although rs6983267 variant allele frequency and the risk of ALL did not show a significant association, the up regulation of *c-MYC* may depend on the physical proximity with 8q24 region, independent from *TCF4* regulation. Previous studies support a model in which the *c-MYC* chromatin loop itself is not responsive to active WNT signaling (14). Moreover *c-MYC*, itself, is a downstream target of WNT pathway, the biological and tissue-specific responses to *MYC* levels are highly dose-dependent, is an independent oncogene and regulated by different pathways (15).

Although the frequency of the variation at 8q24 is not associated with ALL risk directly, it is clear that tissue specific differences and regulations exist in the *TCF4* driven genes which gives a result that colon cells, prostate cells or T-cell or B-cell blasts may act in different ways. Having a GG genotype might be an indirect, independent and significant effector in the *TCF4* regulated WNT pathway regulation in pediatric ALL. Molecular consequences of non-protein coding risk factors have not come to light completely and non-protein coding risk factors like 8q24 region can be secret targets of additional somatic changes in cancer.

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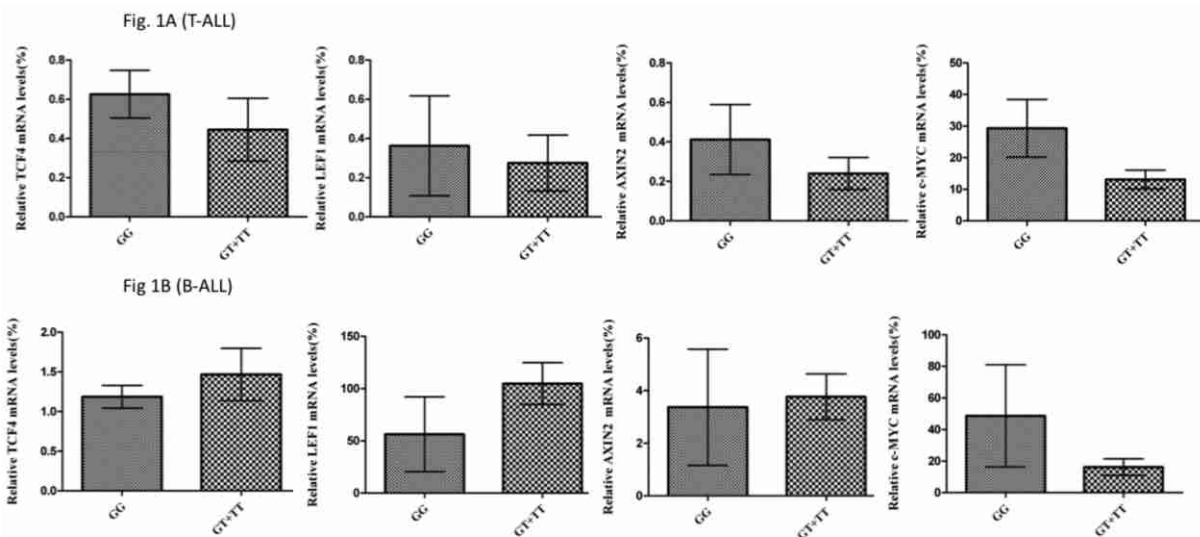
The authors declare no conflict of interest

Table 1: Allele and genotype frequencies of rs6983267 in acute leukemia patients

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| Allele          | ALL (n=87) |         | TALL (n=41) |         | BALL (n=46) |         | HC (n=106) |
|-----------------|------------|---------|-------------|---------|-------------|---------|------------|
|                 | n (%)      | P value | n (%)       | P value | n (%)       | P value | n (%)      |
| G               | 89(51)     | 0,76    | 40 (49)     | 1,00    | 49 (53)     | 0,61    | 105(50)    |
| T               | 85 (49)    |         | 42 (51)     |         | 43 (47)     |         | 107(50)    |
| <b>Genotype</b> |            |         |             |         |             |         |            |
| GG              | 23 (26)    | 0,61    | 10 (24)     | 0,82    | 13 (28)     | 0,53    | 24 (23)    |
| TT+GT           | 64 (74)    |         | 31 (76)     |         | 33 (72)     |         | 82 (77)    |
| TT              | 21 (24)    | 1,00    | 11 (27)     | 0,25    | 10 (22)     | 1,00    | 25 (24)    |
| GG+GT           | 66 (76)    |         | 30 (73)     |         | 36 (78)     |         | 81 (76)    |
| GG              | 23 (26)    | 0,83    | 10 (24)     | 1,00    | 13 (28)     | 0,61    | 24 (23)    |
| TT              | 21 (24)    |         | 11 (27)     |         | 10 (22)     |         | 25 (24)    |
| GG              | 23 (26)    | 0,79    | 10 (24)     | 0,85    | 13 (28)     | 0,75    | 24 (23)    |
| GT              | 43 (50)    |         | 20 (49)     |         | 23 (50)     |         | 57 (53)    |
| TT              | 21 (24)    |         | 11 (27)     |         | 10 (22)     |         | 25 (24)    |

ALL; acute lymphoblastic leukemia, TALL; T-cell acute lymphoblastic leukemia, BALL; B-cell acute lymphoblastic leukemia, HC; healthy controls, p value<0.05 represents significance.



**Figure 1A:** Relative expression levels of *TCF4*, *AXIN2*, *LEF1* and *c-MYC* genes in T-ALL and **1B:** Relative expression levels of *TCF4*, *AXIN2*, *LEF1* and *c-MYC* genes in B-cell acute leukemia patients compared to GG vs GT+TT genotypes. Relative expression levels normalized to *CYP4* were given as percentage. Mann Whitney test was used to detect the expression differences .p value<0.05 represents significance.

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