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ORIGINAL ARTICLE

Analysis of Somatic Mutations in Epigenetic Genes in Major Leukemia Subtypes

Major Lösemi Alt Tiplerinde Epigenetik Genlerdeki Somatik Mutasyonların Analizi

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ABSTRACT

Aim: To investigate presence of somatic mutations in *DNMT3A, TET1, TET2, IDH1, IDH2, ASXL1, and SETBP1* genes playing a role in epigenetic regulation by NGS analysis of bone marrow samples of *13 ALL, 19 AML, 14 CLL, and 15 CML* patients.
Methods: DNA was isolated from bone marrow samples of 13 ALL, 19 AML, 14 CLL, and 15 CML patients, and Kapa NGS DNA extraction kit was used. Purity and concentration of DNA were measured by Qubit fluorometer, and NadPrep DNA Universal Library Preparation Kit was used for high-quality library preparation. Bioinformatic analyses were performed through the portal provided by Roche Diagnostics for NGS solutions. The pathogenicity status of the variants detected in the relevant genes was determined under the ACMG classification.
Results: Variations in at least one gene were found in all but one of the 13 patients diagnosed with ALL. Among seven genes, most distinct variant was detected in *TET3* gene, and all of them were VUS variants according to ACMG classification. In 19 patients diagnosed with AML, the gene with highest number of distinct variants was *TET3,* and all variants were in the VUS class, followed by *TET2* and *ASXL1* genes, respectively. Variants were detected in at least one gene in all but four AML patients. No variant was detected in four of 14 patients diagnosed with *CLL.* Variants were detected in at least one of the related genes in the remaining patients. The genes with the highest number of distinct variants in CML patients were *TET3, ASXL1, and SETBP1,* respectively.
Conclusions: At least one variant of related genes was detected in all leukaemia subtypes. The c.856-2A>T variant in the *DNMT3A* gene was detected in at least one patient with major leukaemia subtypes, suggesting this variant may play an important role in haematopoietic stem cell processes.

Keywords: Acute lymphoblastic leukaemia, acute myeloid leukaemia, chronic myeloid leukaemia, chronic lymphocytic leukaemia, epigenetics

ÖZ

Amaç: Bu çalışmada, 13 ALL, 19 AML, 14 KLL ve 15 KML hastasının kemik iliği örneklerinin NGS analizi ile epigenetik düzenlemede rol oynayan *DNMT3A, TET1, TET2, IDH1, IDH2, ASXL1 ve SETBP1* genlerinde somatik mutasyonların varlığının araştırılması amaçlanmıştır.
Gereç ve Yöntemler: 13 ALL, 19 AML, 14 KLL ve 15 KML hastasının kemik iliği örneklerinden DNA izolasyonu gerçekleştirilmiş ve dizi analizi için Kapa NGS DNA ekstraksiyon kiti kullanılmıştır. Elde edilen DNA'nın saflığı ve konsantrasyonu Qubit floremetre ile ölçülmüş, yüksek kalitede kütüphane hazırlanması için NadPrep DNA Universal Library Preparation Kit kullanılmıştır. Çalışmadan elde edilen verilerin biyoinformatik analizi, Roche Diagnostics tarafından NGS çözümleri için sağlanan portal aracılığıyla gerçekleştirilmiştir. İlgili genlerde tespit edilen varyantların patojenite durumu ACMG sınıflandırmasına göre yapılmıştır.
Bulgular: ALL tanısı alan 13 hastanın bir hasta hariç, kalan hastaların tamamında en az bir gende varyasyon saptandı. 7 gen arasından en fazla distinct varyant *TET3* geninde saptanmış olup tamamı ACMG klasifikasyonuna göre VUS varyantıydı. AML tanısı alan 19 hastada ilgili genler arasında en fazla distinct varyantın saptandığı gen *TET3* olup tüm varyantları VUS sınıfındaydı, sonrasında sırasıyla *TET2 ve ASXL1* genleri gelmekteydi. AML hastalarının 4'ü hariç diğer hastalarda en az bir gende varyant saptanmıştı. KLL tanısı alan 14 hastanın 4'ünde herhangi bir varyant saptanmadı. Kalan hastalarda ilgili genlerden en az birisinde varyant saptandı. KML tanısı alan 15 hastadan birisinde ilgili genlerde herhangi bir varyant saptanmadı. Kalan hastalarda ise ilgili genlerin en az birisinde varyant saptandı. KML hastalarında en fazla distinct varyant saptanan genler sırasıyla *TET3, ASXL1 ve SETBP1* genleriydi.
Sonuçlar: Çalışma örneklemini oluşturan tüm lösemi alt tiplerinde ilgili genlerden en az bir varyant saptanmıştır. Çalışmamızda başlıca lösemi alt tiplerinde olan hasta gruplarından en az bir hastada *DNMT3A* geninde c.856-2A>T varyantı saptanmıştır. Bu durum ilgili varyantın hematopoietik kök hücre süreçlerinde bu genin önemli bir rol oynayabileceğini düşündürmektedir.

Anahtar Kelimeler: Akut lenfoblastik lösemi, akut miyeloid lösemi, epigenetik, kronik miyeloid lösemi, kronik lenfositik lösemi,

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INTRODUCTION

Cancer is defined as a genetic disease that occurs as a result of not only point mutations in DNA, but also translocations and gene amplifications affecting large genomic regions. However, many types of cancer involve not only genetic alterations but also disruptions at the epigenetic level. Indeed, comprehensive cancer genome projects such as TCGA have shown that mutations in essential chromatin-associated proteins are found in more than half of human cancers (1,2). Remarkably, the epigenetic signature can lead to more phenotypic variation than the genetic sequence. This is mainly because the error rate in the inheritance of DNA methylation is relatively high, approximately 4% for a given CpG site in each cell division. In contrast, genetic mutations that occur during DNA replication occur at much lower rates. Therefore, epigenetic variations can cause phenotypic selection- i.e., the onset of cancer- much sooner than genetic mutations, according to the conventional view. One of the major epigenetic changes seen in cancer is the disruption of CpG methylation patterns; in other words, the disorganization of the DNA methylome. Just as in the aging process, tumour formation is often associated with genome-wide DNA hypomethylation. This increases genomic instability, leading to reactivation of pluripotent transcription factors and retrotransposons located in repetitive DNA regions, which can exhibit oncogene-like behaviour. In contrast, hyper of CpG islands in the promoter regions of tumour suppressor genes leads to transcriptional silencing of these genes. For example, DNA hypermethylation in the promoter region of the tumour suppressor *MLH1* (MutL homolog 1) gene disrupts the DNA mismatch repair (MMR) mechanism, resulting in an increased risk of genome-wide mutation accumulation. This suggests that an epigenetic change- i.e., an epimutation- can indirectly trigger multiple genetic changes. Four main categories of epimutation have been identified that are effective in cancer formation: (1) DNA hypermethylation occurring in gene promoter regions, (2) DNA hypomethylation occurring genome-wide, (3) abnormal structural modification of histone proteins or defects in cellular recognition of these modifications, and (4) disordered chromatin organisation caused by malfunctioning chromatin remodelling complexes. These epigenetic defects may promote tumour development by having lasting effects on gene expression (3-6).

The results of genomic and epigenomic analyses in haematological malignancies have revealed that abnormal regulation of epigenetic processes is a prominent biological theme in this disease group. In particular, recurrent somatic alterations in DNA methylation, post-translational histone modifications, and basic proteins involved in chromatin remodelling have been frequently reported in myeloid malignancies. These findings strongly support that regulation of gene expression through epigenetic mechanisms plays a critical role in both the initiation and maintenance of various haematological malignancies. In addition, the effective and rational application of targeted epigenetic therapeutic approaches requires a comprehensive understanding of malignant transformation processes guided by abnormalities in epigenetic regulatory proteins at the molecular level (7). DNA methylation has long been recognised to maintain gene expression

patterns during mitotic cell division, as well as promoting transcriptional silencing and repressive chromatin structures (8,9). These epigenetic changes are highly disease-specific, with characteristic methylation patterns observed in haematological malignancies and even subtypes of these malignancies (10).

This study aimed to investigate the presence of somatic mutations in *DNMT3A, TET2, TET3, IDH1, IDH2, ASXL1,* and *SETBP1* genes involved in epigenetic regulation by NGS analysis of bone marrow samples of patients with 13 ALL, 19 AML, 14 CLL, and 15 CML.

MATERIALS and METHODS

The study was carried out with the permission of Selçuk University Faculty of Medicine Ethics Committee. (Date: 21.05.2025, Decision No: E-70632468-050.01-1009421). This study was designed retrospectively, and consent forms were also obtained from the patients.

DNA isolation from bone marrow samples submitted to our laboratory from patients with haematological malignancies was performed using the Kapa NGS DNA extraction kit (Roche Molecular Systems, Inc., Germany). The purity and concentration of the DNA obtained were measured using a Qubit fluorometer (ThermoFisher Scientific, USA). To generate a high-quality library from double-stranded DNA (dsDNA), we used the NadPrep DNA Universal Library Preparation Kit (Nanodigmbio (Nanjing) Biotechnology Co., Ltd, China), which includes a Library Preparation Module and Adapter Primer Module. For libraries prepared using the NadPrep DNA Universal Library Preparation Kit (for MGI), NAD panels in 5′ biotinylated probes optimised for targeted capture applications in NGS were used. In this study, 500 ng of DNA from each library was used for hybrid capture. After this step, the Custom Myeloid Cancer Panel kit was used. In this study, a single-stranded circular DNA library was prepared using the MGIEasy Circularisation Kit (MGI Tech Co., Ltd, China). Single-stranded circular DNAs were converted into nanoballs (DNBs) by rolling circle amplification using the DNB SEQ-G50RS high-throughput sequencing kit (MGI Tech Co., Ltd, China). The sequencing cartridge was then prepared, and the DNBs were placed in the DNB tube and inserted into the instrument. The samples were passed through the flow cell in the device, and sequencing was performed on the DNBSEQ-G50RS device (MGI Tech Co., Ltd, China). Bioinformatic analysis of the data obtained from the study was performed through the portal provided by Roche Diagnostics for NGS solutions. ACMG (American College of Medical Genetics and Genomics) is the organisation that developed the most widely used standards and guidelines internationally to classify the clinical significance of genetic variants (mutations). The pathogenicity status of variants detected in *DNMT3A, TET2, TET3, IDH1, IDH2, ASXL1,* and *SETBP1* genes was determined under the ACMG classification. During variant analyses, coverage levels for each gene region were evaluated; variants with low read depth (<30x) and/or low quality scores were excluded from the study. This approach was applied to minimise false positive and false negative results, and only highly reliable variants were included in the analysis.

RESULTS

Variations in at least one gene were found in all but one of the 13 patients diagnosed with ALL. Among 7 genes, the most distinct

variant was found in the *TET3* gene, and all of them were VUS variants according to ACMG classification (Table 1). The genes with the highest number of distinct variants after the *TET3* gene were *TET2, ASXL1, SETBP1,* and *DNMT3A* genes, respectively (Figure 1). In 19 patients diagnosed with AML, the gene with the highest number of distinct variants was *TET3,* and all variants were in the VUS class, followed by *TET2* and *ASXL1* genes, respectively (Table 2). Variants were detected in at least one gene in all but 4 AML patients. No variant was detected in 4 of 14 patients diagnosed with CLL. Variants were detected in at least one of the related genes in the remaining patients. The genes with the highest number of variants were *TET3, TET2, and SETBP1* (Figure 1). All variants in these genes were in the VUS class (Table 3). One of the 15 patients diagnosed with CML did not have any variant in the related genes. In the remaining patients, variants were detected in at least one of the related genes. The genes with the highest number of distinct variants in CML patients were *TET3, ASXL1,* and *SETBP1,* respectively (Table 4). All of the variants detected in the *TET3* and *SETBP1* genes were in the VUS class in CML patients, as in ALL, AML, and CLL patients. Among all patients, the most common variants detected in *ASXL1* gene were p.S1248S, p.S1253S, p.E630V and p.R629S, while in *DNMT3A* gene; c.856-2 A>T, p.L422L and p. E294V, *IDH1* gene; p.G105G, p.R132H, *IDH2* gene; p.S332S, p.T350T, *TET2* gene; p. I1762V, p. L1721W, in the *TET3* gene; p.P505P, p.L846L, p.L907P, p.S1383T, p.R911Q, in the *SETBP1* gene; p.S1275S, p.T228fs, p.P1130T (Figure 2). VAF values and heterozygosity status of the variants detected in each gene are given in Supplementary File 1.

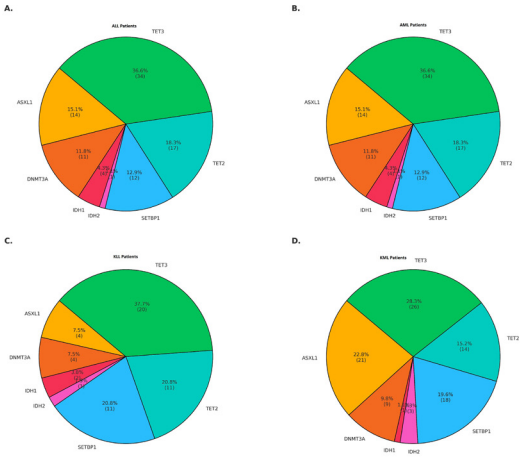

Figure 1. Number of distinct variants detected in related genes in leukemia subtypes

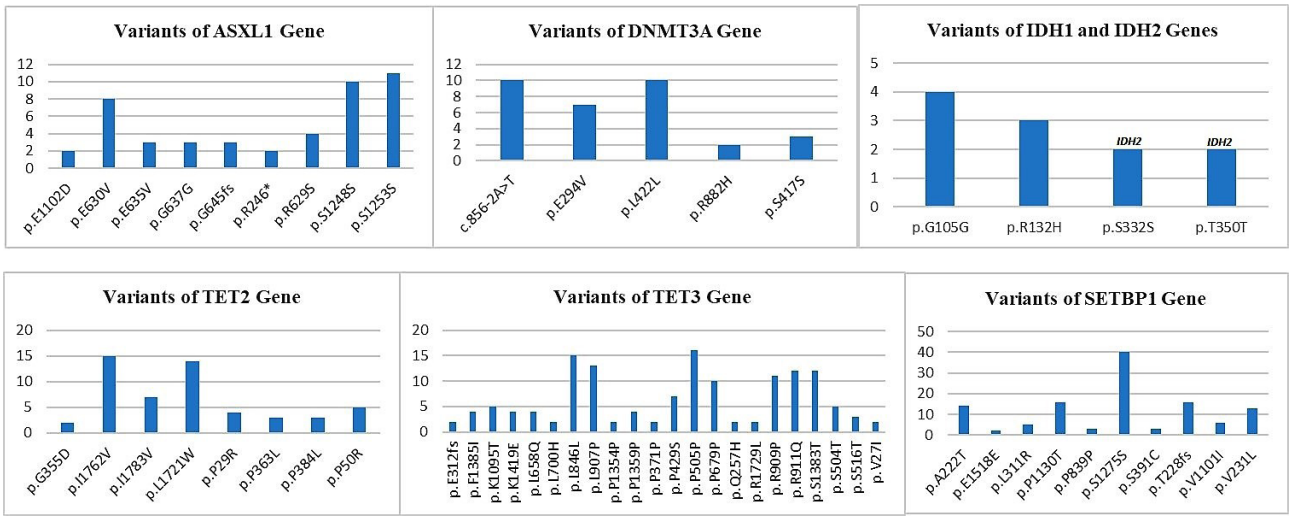

Figure 2. The most frequently detected variants in related genes in all leukemia groups

Table 1: Distribution of variant-detected cases according to genes in ALL patients

CASES	ASXL1	DNMT3A	IDH1	IDH2	SETBP1[x]	TET2[x]	TET3[x]
Case 1	p.R629S [b]	c.8562A>T [b]			p.A222T	p.V239M	p.L846L
	p.E630V [d]	p.I292F [d]			p.V231L	p.I1783V	p.V27I
	p.S1248S [d]				p.P1130T	p.S1060S	p.S516T
	c.1869+6A>T [d]				p.T228fs	p.H1401H	p.K1095T
					p.S1275S		p.A1613T
					p.E1518E		p.P505P
							p.P679P
							p.P702P
							p.C1684C
							p.P371P
Case 2	p.P1525S [d]	p.S417S [d]			p.A222T	p.P50R	p.P429S
	p.G637G [d]				p.P1130T	p.T1621T	p.L846L
					p.T228fs	p.Q918L	p.R911Q
					p.A1082T	p.A1886T	p.P561S
					p.P256P		p.L907P
					p.S1275S		p.R909P
					p.C897C		p.G1125D
					p.L311R		p.L1374Q
							p.A1477T
							p.P505P
							p.P1359P
Cse 3	p.S1253S [d]	p.L422L [d]			p.P1130T	p.L1721W	p.L846L
					p.S1275S		p.R911Q
							p.L907P
							p.R909P
Case 4	p.R629S [b]	c.856-2A>T [b]		p.T350T [d]	p.A222T	p.I1783V	p.V567A
	p.E630V [d]	p.S881S [d]			p.T228fs		p.S504T
	p.E1102D [a]	p.E294V [d]			p.S1275S		p.L846L
							p.T431S
							p.S1383T
Case 5				p.R140Q [c]			
Case 6	p.G645fs [b]	c.856-2A>T [b]			p.S1275S	p.P363L	p.F1385I
	p.G652S [a]				p.T1387R	p.L1721W	p.S1389T
	p.P237S [d]				p.P839P		p.S1383T
							p.P505P
							p.P679P
							p.L700H
							p.P745P
							p.P1359P
Case 7	p.S1248S [d]				p.V1101I	p.F540fs	
	p.S301R [d]				p.A222T	p.I1783V	
	p.P932P [d]				p.T228fs	p.S275R	
					p.S391C		
					p.S1275S		
					p.S333N		

[a]Benign variant according to ACMG Classification,[b]Likely Pathogenic variant according to ACMG classification, [c]Pathogenic variant according to ACMG classification, [d]Variant of uncertain significance (VUS) according to ACMG classification, xAll variants detected in this gene were VUS, *stop gained mutation, fs: frame shift mutation

Table 1 Cont.: Distribution of variant-detected cases according to genes in ALL patients

CASES	ASXL1	DNMT3A	IDH1	IDH2	SETBP1[x]	TET2[x]	TET3[x]
Case 8	p.E630V [d] p.S1248S [d] p.G637G [d]				p.A222T p.V1101I p.T228fs p.S1275S p.L311R	p.I1783V	p.P505P p.P679P p.LL1374QH
Case 9	p.S1253S [d]			p.A346A [d]	p.V231L p.S1275S		p.L846L p.R911Q p.L907P p.R909P
Case 10	p.A666V [d]		p.F32V [d]		p.V231L p.P1130T p.S1275S p.L311R		p.S504T p.R911Q p.G1733D p.R303K p.I352N p.Q529L p.K1095T p.F1485I
Case 11	p.R629S [b] p.E630V [d] p.S1248S [d]	p.R882C [c] c.856-2A>T [b] p.E294V [d]	p.R132H [c]		p.A222T p.V231L p.T228fs p.S1275S	p.I1783V	p.S504T p.L907P p.S1383T p.P505P
Case 12	p.S1248S [d] p.P566L [d] p.S151T [d] p.Q289L [d] p.P139P [d]	p.T671T [d]		p.L438L [d]	p.A222T p.P1130T p.T228fs p.S1275S p.S381T p.P569P	p.L1742W p.H1799R p.R555S p.I1202N p.M1750L	p.P411L p.T170fs

[a]Benign variant according to ACMG Classification,[b]Likely Pathogenic variant according to ACMG classification, [c]Pathogenic variant according to ACMG classification, [d]Variant of uncertain significance (VUS) according to ACMG classification, [x]All variants detected in this gene were VUS, *stop gained mutation, fs: frame shift mutation

Table 2: Distribution of variant-detected cases according to genes in AML patients

CASES	ASXL1	DNMT3A	IDH1	IDH2	SETBP1[x]	TET2[x]	TET3[x]
Case 1	p.G1420R [d] p.S1253S [d]	p.R882H [c]	p.R132H [c]		p.V1101I p.S1275S		p.R909P p.R1729L p.R911Q
Case 2	p.P599S [d] p.S1253S [d]				p.E1466D p.S1275S p.R1458W p.P906P	p.G355D [d] p.P363L [d] p.I1762V [d] p.L1721W [d] p.A1241V [d] p.G1946D [d]	p.P429S p.L846L p.R303R p.G314D p.A343V p.P1011S p.E312fs p.G314G p.G1008G p.S1478S p.G1540G
Case 3	p.G637G [d]				p.A222T p.P1130T p.T228fs p.S1275S p.P235S	p.P50R [d] p.P384L [d] p.L1742W [d]	p.P50R [d] p.P384L [d] p.L1742W [d]

[a]Benign variant according to ACMG Classification, [b]Likely Pathogenic variant according to ACMG classification, [c]Pathogenic variant according to ACMG classification, [x]All variants detected in this gene were VUS, *stop gained mutation, fs: frame shift mutation,

Table 2 Cont.: Distribution of variant-detected cases according to genes in AML patients

CASES	ASXL1	DNMT3A	IDH1	IDH2	SETBP1[x]	TET2[x]	TET3[x]
Case 4	p.E630V [d]	c.856-2A>T [b] p.L422L [d] p.P385P [d]			p.A222T p.T228fs p.S391C p.S1275S		p.T546I p.M689K p.S1383T p.F1385I p.K1419E p.P679P p.P505P
Case 5	p.E635V [d]	c.856-2A>T [b] p.L422L [d]				p.I1762V [d]	p.S504T p.L846L p.P371P p.S1383T p.P505P p.V27I p.P1359P
Case 6	p.E635V [d] p.G642G [d] p.R625R [d]	c.856-2A>T [b] p.L639fs [b] p.D286V [d] p.L422L [d]	p.R132H [c]		p.V231L p.S1275S	p.L1721W [d] p.I1762V [d]	p.L846L p.L658Q p.S1377T p.S1383T p.S516T p.P505P p.P679P p.T697T p.L700H p.P1354P p.P1359P p.LL1374QH
Case 7	p.S1248S [d] p.S301R [d] p.P932P [d]				p.V1101I p.A222T p.T228fs p.S391C p.S1275S p.S333N	p.F540fs p.I1783V p.S275R	
Case 8	p.E630V [d] p.S1248S [d] p.G637G [d]				p.A222T p.V1101I p.T228fs p.S1275S p.L311R	p.I1783V	p.P505P p.P679P p.LL1374QH
Case 9	p.S1253S [d]			p.A346A [d]	p.V231L p.S1275S		p.L846L p.R911Q p.L907P p.R909P
Case 10	p.A666V [d]		p.F32V [d]		p.V231L p.P1130T p.S1275S p.L311R		p.S504T p.R911Q p.G1733D p.R303K p.I352N p.Q529L p.K1095T p.F1485I
Case 11	p.R629S [b] p.E630V [d] p.S1248S [d]	p.R882C [c] c.856-2A>T [b] p.E294V [d]	p.R132H [c]		p.A222T p.V231L p.T228fs p.S1275S	p.I1783V	p.S504T p.L907P p.S1383T p.P505P p.P679P
Case 12	p.S1248S [d]	p.T671T [d]		p.L438L [d]	p.A222T	p.L1742W	p.P411L
Case 13	p.G645fs [b]				p.S1275S		p.P429S p.L846L
Case 14	p.D690fs [b]	p.S417S [d]			p.S1275S	p.P29R [d] p.I1762V [d]	p.R911Q p.R909P p.L907P
Case 15		p.E294V [d]	p.G105G [d]				

[a]Benign variant according to ACMG Classification, [b]Likely Pathogenic variant according to ACMG classification, [c]Pathogenic variant according to ACMG classification, [x]All variants detected in this gene were VUS, *stop gained mutation, fs: frame shift mutation,

Table 3. Distribution of variant-detected cases according to genes in KLL patients

CASES	ASXL1	DNMT3A	IDH1	IDH2	SETBP1[x]	TET2[x]	TET3[x]
Case 1		p.L422L [d]	p.G105G [d]	p.S332S [d]	p.A222T p.P1130T p.T228fs p.V1377L p.S1275S	p.Q502* [b] p.L1742W [d]	p.A317V p.K1095T
Case 2	p.I636M [d] p.S1248S [d]		p.G105G [d]			p.P50R [d] p.P384L [d] p.L1742W [d]	p.A197V
Case 3					p.A222T p.V231L p.P1130T p.T228fs p.S391C p.S1275S	p.P50R [d]	p.K1419E
Case 4					p.V231L p.S1275S c.4171+2T>A	p.S1497T [d]	p.S1588T p.C382C
Case 5	p.E630V [d]	c.856-2A>T [b] p.L422L [d]			p.A222T p.V231L p.T228fs p.S1275S	p.P50R [d]	p.P505P p.S516T p.L907P p.S1383T p.P679P
Case 6		p.E294V [d]					
Case 7	p.S1248S [d]				p.V1101I p.P1130T p.T228fs p.S1275S p.L311R	p.Q1720K [d] p.T820T [d]	p.L846L p.R911Q p.R909P p.V1028D p.K1095T p.P505P p.L66L
Case 8		p.R882H [c]					
Case 9	p.R629S [b] p.E630V [d] p.S1248S [d]				p.A222T p.V231L p.T228fs p.S1275S	p.L1742W [d]	p.L658Q p.S1383T p.P505P p.P679P
Case 10					p.P1130T p.S1275S p.P1563S	p.G355D [d] p.P363L [d] p.L1721W [d] p.I1762V [d]	p.P429S p.L846L p.P1616S p.R1729L

[a]Benign variant according to ACMG Classification, [b]Likely Pathogenic variant according to ACMG classification, [c]Pathogenic variant according to ACMG classification, [x]All variants detected in this gene were VUS, *stop gained mutation, fs: frame shift mutation,

Table 4. Distribution of variant-detected cases according to genes in KML patients

CASES	ASXL1	DNMT3A	IDH1	IDH2	SETBP1[x]	TET2[x]	TET3[x]
Case 1	p.Q623L [d]	p.L422L [d] p.D11N [d] p.E294V [d]			p.P1130T p.S1275S p.P1527A p.A1561A	p.I1762V [d] p.G1647V [d]	p.R911Q p.L907P p.R909P p.L384H p.L1375H p.L658Q p.S1383T p.P699P p.P1354P p.P505P p.P679P
Case 2	p.R246* [d] p.E635V [d] p.A1312V [a] p.S1253S [d] p.P937P [d]	p.E294V [d]		p.T350T [d]	p.V231L p.P1130T p.S1275S p.M1123L	p.Q701* [b] p.I1762V [d]	p.S504T p.W113* p.V1692D p.K1419E p.S515T
Case 3	p.S1253S [d]	p.L422L [d]			p.P1130T p.S1275S p.A692T	p.I1762V [d]	
Case 4	p.G646fs [c] p.S1253S [d]		p.G105G [d]	p.S332S [d]	p.V231L p.V1101I p.S1275S	p.R550* [c] p.P29R [d] p.I1762V [d] p.Y1902H [d]	
Case 5	p.P937T [d] p.K163N [d] p.P255L [d]	p.P307S [d] p.A269V [d]			p.P325S p.S1275S p.S295N	p.I1762V [d] p.V1417V [d]	p.L620H p.Q257H
Case 6	p.S1253S [d]	p.L422L [d]			p.P1130T p.S1275S	p.N1489fs [b]	p.R911Q p.L907P p.R909P
Case 7	p.S1248S [d]		p.G105G [d]		p.A222T p.P1130T p.T228fs p.S1275S	p.L1742W [d] p.C1379Y [d]	p.L1742W d p.C1379Y d
Case 8	p.K1303 [b] p.K1302M [d] p.H995H [d]	p.D747V [d]			p.L311R p.V1495E	p.L1721W d	p.Q257H p.A853A
Case 9	p.G1420R [d]				p.S1275S p.P1540P p.L1530L	p.P29R [d]	p.R911Q p.V119A p.L907P p.R909P
Case 10	p.T1001fs [b] p.G1397S [a] p.S1253S [d]	c.856-2A>T [b]			p.S1275S p.P839P	p.I1762V [d]	p.P505P
Case 11		p.R899C [d]			p.V1101I p.S1275S p.H1206H	p.P29R [d]	p.L907P p.R909P
Case 12	p.G972D [d] p.Q1491R [d] p.A746A [d]	p.M864K [d]			p.P839P	p.S441R [d] p.P1568S [d]	p.P429S p.P735S p.P597P
Case 13	p.S1253S [d] p.C605* [b]			p.R172K [b]	p.V231L p.S1275S	p.I1762V [d]	p.L846L p.S1383T p.P505P p.P1359P
Case 14		p.L422L [d]				p.L567* [b] p.L668fs [b] p.I1762V [d]	p.S1383T p.F1385I p.P505P

[a]Benign variant according to ACMG Classification, [b]Likely Pathogenic variant according to ACMG classification, [c]Pathogenic variant according to ACMG classification, [x]All variants detected in this gene were VUS, *stop gained mutation, fs: frame shift mutation,

DISCUSSION

Compared to normal cells of the same individual, the epigenome of tumour cells shows a large overall loss of DNA methylation, while for some genes, hypermethylation at CpGs is also observed. Distortions in the DNA methylome are seen in most cases of cancer, with the DNMT3A gene being mutated in approximately 25% of adult acute myeloid leukaemia cases, which disrupts the methylome and makes the regulatory landscape of pre-leukaemia blood stem cells more vulnerable to additional mutations (11).

ASXL1 functions as a scaffold protein with several proteins, including the histone deubiquitinase BAP1, to epigenetically regulate gene expression, playing a role in both transcriptional activation and repression (12). Mutations in ASXL1 are found in cancers, including haematological, colorectal, breast, prostate, and skin cancer. Truncation mutations that protect the BAP1 interaction promote malignancy by causing increased ASXL1/BAP1 activity and dysregulation of genes involved in cell division and differentiation (13, 14). In a clinical study on patients with myelofibrosis, ASXL1 mutations were associated with worse survival compared to patients with wild-type ASXL1. No significant difference was observed in terms of survival between patients carrying ASXL1 frameshift, nonsense, and missense mutations (15). In our study, the most frequently detected mutations in the *ASXL1* gene were synonymous (p.S1248S, p.S1253S), followed by missense, nonsense, and frameshift mutations. Among the missense mutations, p.R629S, p.E630V, and p.E1102D are the most frequently observed exon 12 mutations. Various studies refer to *ASXL1* exon 12 mutations, but only nonsense and frameshift mutations have been shown to independently affect prognosis (16). In addition, in a clinical study, it was reported that *ASXL1* mutations were associated with a worse prognosis in patients with myelofibrosis. In the study, a patient carrying *ASXL1* E1102D was treated with a JAK1/2 inhibitor, progressed to leukaemia 11 months later, and died 23 months later (17). In our study, *ASXL1* frameshift variation (p. G645fs) was detected in some of our patients with ALL and AML, and it has been shown that these mutations cause a truncated ASXL1 protein lacking the PH domain and lead to an increase in interactions with BAP1 and BRD4, a decrease in H2AK119 ubiquitination, an increase in H3K122 acetylation, and changes in gene regulation (13, 18).

DNMT3A, DNA methyltransferase 3A, is a member of the DNA methyltransferase family that catalyses cytosine methylation during embryonic development and haematopoietic stem cell differentiation to regulate gene expression (19). Alterations in *DNMT3A* have been detected in various cancers, including haematological malignancies. Biallelic loss of function or dominant-negative mutations in the R882 codon in *DNMT3A* result in uncontrolled stem cell expansion, abnormal DNA methylation, and have been shown to lead to haematological malignancies, although this mechanism is not well understood. It has also been reported that AML patients harbouring *DNMT3A* mutations respond well to decitabine, an inhibitor of DNA methyltransferase (19). Although DNMT3A mutations are frequently associated with poor prognosis in acute myeloid leukaemia (AML), some studies have reported that they do not affect prognosis (20). Complete response rates with decitabine are higher in

AML patients harbouring *DNMT3A* mutations compared to wild-type patients (21). In a multivariate analysis performed concerning mutations in *DNMT3A* and/or *TET2,* it was reported that patients responded better to treatment with 5-azacitidine and/or decitabine, and the absence of *DNMT3A* or *TET2* mutation constituted a poor risk factor for DNMT inhibitor treatment (22). The c.856-2A>T variant in the *DNMT3A* gene, which was detected in all leukaemia groups in our study, is a *DNMT3A* inactivating variant, and it has been shown by experimental studies that variants in this group lead to loss of function of the DNMT3A protein (23, 24). In our study, two different variants were detected in the R882 codon of the *DNMT3A* gene in leukaemia groups except CML (p.R882C, p.R882H), and these variants inactivate the *DNMT3A* gene. *DNMT3A* R882H is located in the dimer interface region and SAM-dependent MTase C5 type domain of DNMT3A protein (24). This mutation has been shown by experimental studies to disrupt the tetramerisation ability of the DNMT3A protein, leading to decreased DNA binding affinity, decreased methylation activity, and increased proliferation compared to wild-type protein (24-26).

IDH1, a cytoplasmic isocitrate dehydrogenase, catalyses the conversion of isocitrate to α-ketoglutarate (α-KG) in metabolic pathways (27). In our study, the p.R132H variant in the *IDH1* gene was found in some patients diagnosed with AML and ALL. IDH1 R132H variant is located in the active site of *IDH1* protein and causes a decrease in the conversion of isocitrate to alpha-ketoglutarate by IDH1, but it also provides a new function to IDH1 as it has been shown that the conversion of alpha-ketoglutarate to the onco-metabolite 2HG (R(-)-2-hydroxyglutarate) increases in cell culture and increased 2HG levels are associated with tumour formation and progression (28, 29). In addition, it has been reported in the literature that *IDH1* mutant AML patients respond better to ivosidenib and azacitidine treatment (30). TET2 plays a critical role in DNA methylation, transcriptional regulation, and myeloproliferation. In addition, TET proteins have been reported to have a function in epigenetic regulation during embryogenesis (31, 32). In our study, pathogenic/likely pathogenic variants (nonsense, frameshift) inactivating the *TET2* gene were detected in all leukaemia groups. Mutations that cause inactivation of *TET2* have been shown to lead to loss of transcriptional regulation and increased proliferation of haematopoietic stem cells, and *TET2* is being investigated as a potential prognostic marker in many haematological cancer types and various solid tumour types in which *TET2* gene and protein expression is reduced (33, 34). It has been reported that the most frequently observed frameshift mutations in pathogenic and likely pathogenic variants in our study typically lead to loss or disruption of DNA-binding and substrate-binding sites, resulting in loss of function (35).

CONCLUSION

In our study, many variants were found in the *SETBP1* and *TET3* genes in all leukaemia groups, and all of these variants were in the VUS class under the ACMG classification. The classification of these variants may change with future large cohort studies and experimental studies. Therefore, the clinical significance of many missense variants detected in these two genes in our study may be important in the coming years.

Conflict of interest

The author has no conflicts of interest to declare.

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