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Genomic Aberrations and Their Evolution in Adult Acute Lymphoblastic Leukemia

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Suaugusiųjų ūminės limfoleukemijos genominių veiksnių ir jų evoliucijos tyrimas

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INTRODUCTION

Background

Acute lymphoblastic leukemia (ALL) is a malignant disease resulting from the accumulation of molecular genetic and cytogenetic alterations. The frequency of abnormal karyotypes in adult ALL is 64%–85%, and the outcome of the disease has been shown to be related to specific genetic and genomic abnormalities (Mrozek et al., 2004; Moorman et al., 2012). Single nucleotide polymorphism (SNP) array analysis provides a highly sensitive platform for detecting large and small genomic aberrations as well as copy number neutral loss of heterozygosity (CNN-LOH). Studies of childhood ALL are among the most advanced in this field. The pattern of aberrations at diagnosis is similar between adult and pediatric ALL, and thus leaving unexplained why the prognosis of adult ALL is poorer compared with childhood ALL. In contrast, SNP array profiling data in adult ALL is limited, and larger studies of this patient group are needed. SNP array genomic profiling of adult ALL at diagnosis has been reported in six studies and showed that aberrations of leukemia-associated genes, such as *CDKN2A*, *PAX5*, *IKZF1*, and *ETV6* occurred with high frequency (Paulsson et al., 2008, Okamoto et al., 2010; Safavi et al., 2014; Dirse et al., 2015; Forero-Castro et al., 2016; Mühlbacher et al., 2016). Furthermore, all these studies revealed recurrent genetic abnormalities in adult ALL, targeting genes not previously implicated in leukemogenesis. In addition to recurrent alterations, submicroscopic lesions have been detected in single cases, possibly indicating new candidate genes in the pathogenesis of ALL. *TP53* plays a crucial role in cell cycle regulation and apoptosis, and its role in tumorigenesis is well-recognized in hematological malignancies; still, *TP53* mutations have been poorly investigated in adult ALL (Chiaretti et al., 2013), and a detailed analysis is required.

In recent years, it has become apparent that childhood ALL harbors a substantial clonal genetic heterogeneity at diagnosis (Notta et al., 2011; Andersson et al., 2015). Some of the minor subclones present at diagnosis may eventually become the dominant clone at relapse. One way of investigating the clonal evolution of the disease is to perform genomic profiling of samples obtained both at diagnosis and relapse. Studies using single SNP array of childhood ALL have uncovered three distinct patterns of genomic evolution: (a) identical clones at diagnosis and relapse (i.e., the major clone at diagnosis is also the major clone at relapse), (b) clonal evolution, where the ALL has acquired additional aberrations at relapse, and (c) ancestral clones, where the relapse samples have lost some of the aberrations found in the diagnostic sample, indicative of an origin from a minor earlier clone (Mullighan et al., 2008, Davidsson et al., 2010, Olsson et al., 2014). In contrast, little is known about the clonal evolution of adult ALL. Only one study has previously investigated clonal evolution in relapsed adult ALL (Safavi et al., 2014).

The aim of the study – to identify the landscape of genomic aberrations and their prognostic significance of younger adult ALL, to investigate the clonal evolution of ALL at the genomic level.

The objectives of the study:

1. To determine the frequency, type, size, location in the chromosome of the genomic aberrations in adult ALL.
2. To detect new, recurrent aberrations that could have potential clinical significance in ALL and to identify new gene candidates in a population-based Lithuanian adult ALL cohort.

3. To evaluate the genetic risk factors and their prognostic significance of event free survival (EFS) and overall survival (OS) in adult ALL.
4. To detect clonal evolution of chromosomal aberrations and mutations in relapsed adult ALL and to suggest the pathogenicity of these aberrations in biological pathways in adult ALL.

Statments to be defended:

1. Lithuanian adult ALL patients have a lower frequency of genomic aberrations per case comparing with other adult ALL populations. In comparison with other adult ALL studies, we detected the same aberrant leukemia-related genes (*CDKN2AB*, *PAX5*, *IKZF1*, *ETV6*, *EBF1*, *TP53*), but their frequency was also lower.
2. New candidate genes related to leukemogenesis were detected in an adult ALL group.
3. Chromosomal regions and aberrant genes were determined as significant risk factors in EFS and OS in adult ALL.
4. Three distinct patterns of genomic evolution were detected: the identical clones at diagnosis and relapse, the clonal evolution from the diagnostic clone, and the ancestral clones, where the relapse samples have lost some of the aberrations found in the diagnostic sample, indicative of an origin from a minor earlier preleukemic clone. The most aberrant genes during the genomic evolution were *CDKN2AB*, *PAX5*, *IKZF1*, *ETV6*, *BTG1*, *NRAS*, *KRAS*. The relation between mutations of *NRAS/KRAS* genes and deletions of the *CDKN2AB* gene were identified. The association of these two genomic events could be important in leukemogenetic mechanisms and has an impact on clonal evolution in adult ALL.

The scientific novelty and clinical relevance of the study

It is the first time when a detailed analysis of genomic aberrations and their clonal evolution is analyzed in adult ALL in Lithuania. In parallel, there are five similar studies of which we have but the only one that had analyzed the influence of genomic factors of EFS and OS in adult ALL. In regard to the clonal evolution of genomic aberrations in adult ALL, there is only one analogical study in the world (Safavi et al., 2015). The prognosis of adult ALL is poorer compared with childhood ALL. To expand on the limited information available, we performed a genome-wide SNP array analysis of paired diagnostic and relapse samples from adult ALL to specify clonal evolution and its patterns. Due to the small population of Lithuania and centralized registration of ALL patients all around the country, we have an opportunity to perform a unique population-based study of genomic aberrations in adult ALL.

MATERIALS AND METHODS

Study population

In the first part of the research, we selected adult ALL patients in the 2007–2013 year period. DNA was extracted from bone marrow obtained at diagnosis. In this part, SNP array analysis was performed to detect the landscape of genomic aberrations and to evaluate the clinical significance of genetic factors in EFS and OS (Objectives 1–3).

In the second part of the research, we selected relapsed adult ALL patients in the 2007–2013 year period. Most of these relapsed patients were enrolled from the first part of the study (from the 2007–2013 period; published data). In contrast that little is known about the clonal evolution of adult ALL, additionally, several relapsed ALL patients were included from 2013–2018 year period (not published data). In this part we also used data of mutations detected by next generation (NGS) sequecing from Vilnius

University Hospital Santaros Klinikos Laboratory for Molecular Medicine database from the 2007–2018 year period. In both parts of the research, clinical and treatment outcome data were retrospectively retrieved from the Lithuanian population registry of hematological diseases, the Hematology Monitoring System (HESS), and the patients' medical records. The study was approved by the Vilnius Regional Research Ethics Committee.

SNP array and *TP53* mutation analysis

All cases were investigated using the Infinium HD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChip (Illumina Inc., San Diego, CA, USA), which covers the entire genome with an average spacing of 9.6 kb and allows an average resolution of 31 kb. Samples were processed and the assays were performed according to a routine protocol provided by the manufacturer. Genotypes were called using GenomeStudio GT module version 1.7 (Illumina Inc.) and further analyzed with QuantiSNP version 1.1 (Colella et al., 2007). Constitutional copy number polymorphisms were excluded based on a comparison with the Database of Genomic Variants (http://projects.tcag.ca/variation). We defined copy number-neutral LOH, which were >10 Mb in size as clinically relevant according to Simons et al. (2012).

Exons 3-11 of *TP53* gene were screened for mutations by High Resolution Melting (HRM) analysis as described in manufacture protocols (Krypuy et al., 2007). Mutations were confirmed by direct sequencing.

Analysis of database of mutations detected by NGS

In routine diagnostic practice, Vilnius University Hospital Santaros Klinikos Laboratory for Molecular Medicine is using RNA NGS sequencing for the detection of fusion translocations and genomic mutations in diagnostic and relapse ALL samples. RNA-seq

libraries are constructed using Illumina TruSight Pan-Cancer sequencing kit (Illumina, San Diego, CA, USA), following the manufacturer's protocol and recommendations. A total of 1385 cancer-related genes are analyzed. Mutation analysis from RNA-seq data is performed using the GATK pipeline and Isaac Variant Caller 2.3. For clonal evolution analysis, we compared the data of mutations detected in paired diagnostic and relapse adult ALL samples.

Statistical analysis

Descriptive statistics were used for the baseline patients' characteristics. The time from the ALL diagnosis to the event (resistant disease, relapse, secondary malignancy or death) or death were used to calculate EFS or OS, censoring patients not suffering an event or death, respectively. The risk factors for EFS and OS were assessed with a Cox regression analysis. Factors found to be significant in univariate analysis were entered into a multivariate Cox model with a backward selection process. A two-tailed p-value of less than 0.05 was considered significant. Statistical analysis was performed using the Statistical Analysis System (SAS) package version 9.2.

RESULTS

Patients

DNA extracted from bone marrow samples obtained at diagnosis was available from 66 (85%) ALL patients diagnosed in Lithuania between January 2007 and December 2013. The median (range) age at diagnosis was 34 (18–64) years. Forty-one (62%) cases were diagnosed with a B-cell precursor (BCP) ALL and 25 (38%) cases had T-ALL. The majority of the patients had good performance. All patients had genetic analysis performed for ALL risk assessment, and

31 (47%) of them had one or more genetic aberrations (Table 1). All patients were treated with a curative intent using multiagent chemotherapy protocols which included imatinib in *BCR-ABL1*-positive cases. Allogeneic stem cell transplantation was performed for 16 (24%) patients.

Sixteen patients between January 2007 and December 2013 experienced a relapse and these were included in the present study of analysis of clonal evolution. SNP array was performed for diagnostic and relapse samples respectively. Additionally, an analysis of mutations detected by NGS was performed in the database of diagnostic/relapsed adult ALL samples.

Table 1. Baseline characteristics of patients at acute lymphoblastic leukemia diagnosis according SNP-a test results in 2007–2013 year period in Lithuania.

Characteristics	No.	%
Total N	66	100
Age, years		
Median	34	
Range	18-64	
Gender		
Male	34	52
Female	32	48
Performance status		
0-1	58	88
≥2	8	12
Immunophenotype and genetics		
B	41	62
BCR-ABL1	11	27
ETV6-RUNX1	0	0
MLL-AF4	6	15
TCF3-PBX1	2	5
iAMP21*	0	0
Other	6	15
T	25	38
STIL-TAL1*	3	12

iAMP21*	0	0
Other	2	8
Splenomegaly or Hepatomegaly		
Present	18	27
Absent	48	73
CNS involvement		
Present	10	18
Absent	46	82
Nd	10	-
WBC, $\times 10^9$/l		
Median	14.34	
Range	0.9-481.9	
NEU, $\times 10^9$/l		
Median	2.00	
Range	0.14-33.71	
Hgb, g/l		
Median	109.3	
Range	52.4-169	
Plt, $\times 10^9$/l		
Median	58	
Range	6.62-443	
Blasts, %		
Median	41.6	
Range	0-95	

SNP array analysis of 66 adult ALL samples

The SNP array analysis detected a total of 192 genetic abnormalities not corresponding to known copy number polymorphisms in 54 patients (82%). The aberrations comprised 86 (45%) hemizygous deletions, 21 (11%) homozygous deletions and 85 (44%) copy-number gains (Fig 1). The average, median and mode number of aberrations per patient was 3.6, 2 and 1, respectively. The range of the aberrations was 1–12. CNN-LOH (>10 Mb) was detected in 27 genomic regions. Recurrent regions of CNN-LOH

(>10 Mb) included chromosome 9p (6 cases), chromosome arm 5q (3 cases) and chromosome arm 6p (3 cases).

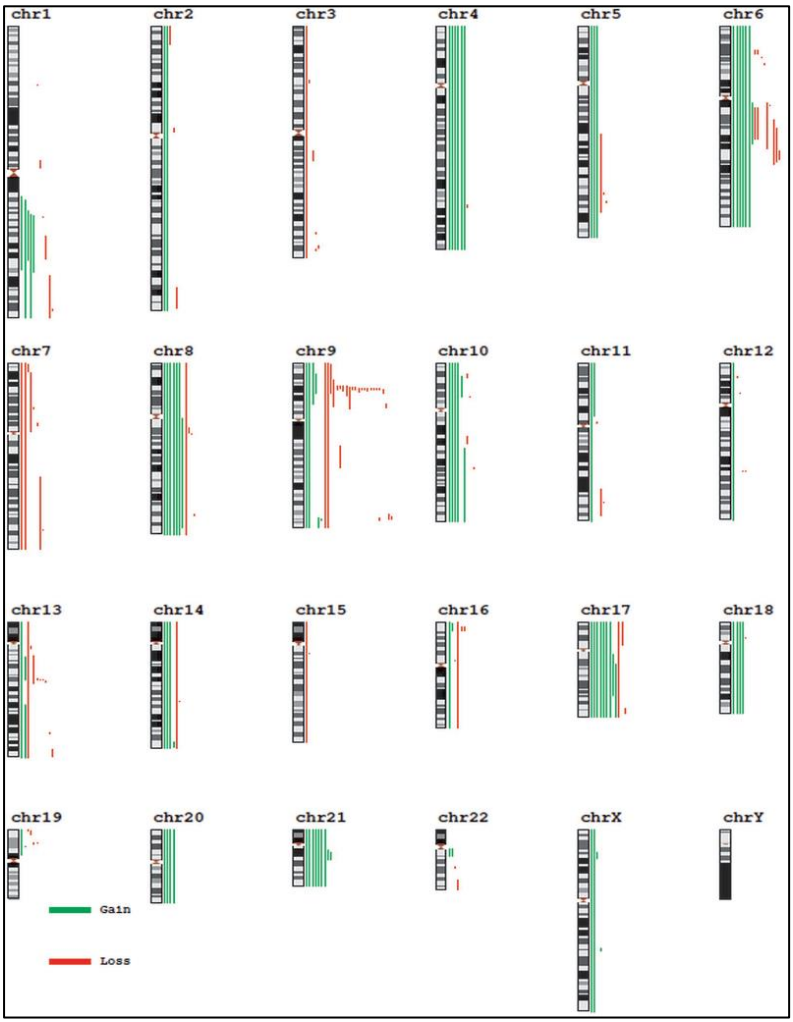

Figure 1. Overview of all genetic aberrations found with SNP array in 54 adult ALL cases. For each type of aberration, each line represents a different case. Red lines are deletions and green lines are copy-number gains.

Whole genome genotyping analysis of the patients revealed 14 hyperdiploid and 2 hypodiploid karyotypes. In the hyperdiploid group, 8 cases had low hyperdiploidy (chromosome number 47–50) and 6 cases had high hyperdiploidy (chromosome number 51–60). The most common trisomies were +4 (6 cases), +6 (6 cases), +10 (4 cases), +17 (5 cases), +18 (4 cases) and +21 (6 cases). In the hypodiploid group, both patients had high hypodiploidy (41 and 45 chromosomes, respectively).

The distribution of the aberrations according to chromosomes showed that most aberrations were detected in chromosome arm 9p (n = 34; 18%), chromosome arm 6q (n = 18; 9%), chromosome 17 (n = 12; 6%) and chromosome arm 13q (n = 12; 6%). According to different types of aberrations, there was difference between T and BCL.

ALL (Table 2). The fewest number of aberrations were found in chromosome 15 (n = 2; 1%), 20 and 22 (n = 4; 2%). As regards the number of the different types of the aberrations, chromosomes 17 and 21 had the most copy-number gains (9 copy number gains in each of the chromosome); chromosome 9 had the most hemizygous deletions (16 hemizygous deletions) and homozygous deletions (12 homozygous deletions). Chromosomes 20 and 21 only harbored copy-number gains, whereas chromosomes 3, 7 and 15 only harbored deletions (hemizygous and homozygous). Of note, chromosome X had only 2 trisomies and 2 duplications and chromosome Y was without aberrations. We did not identify any correlations among the primary cytogenetic subgroups and copy number aberrations (data not shown).

The overall length distribution of observed aberrations varied. The median size of all aberrations was 2.755 Mb (whole chromosome aberrations were excluded). The median size of hemizygous deletions, homozygous deletions, and copy-number gains was 2.710 Mb, 273 Kb and 19.548 Mb, respectively.

The recurrent aberrations and genes related to leukemogenesis of adult ALL

The analysis revealed 98 regions recurrently targeted by copy-number changes. These copy number abnormalities encompassed several leukemia-related genes – *CDKN2A* (19 cases, 29%), *CDKN2B* (18 cases, 27%), *MLL* (2 cases, 3%), *IKZF1* (4 cases, 6%), *PAX5* (4 cases, 6%), *RB1* (4 cases, 6%), *TP53* (5 cases, 7%), and *ETV6* (2 cases, 3%). We identified several novel deleted recurrent regions which, based on their biological functions, included possible target genes: *SMARCA4* in 19p13.2 (450 Kb-12Mb in size) (3 cases, 4%), *RNASEL* in 1q25.3 (1.2-5 Mb in size) (3 cases, 4%), *ARHGEF12* in 11q23.3 (100 Kb-10 Mb in size) (3 cases, 4%) and *LYL1* in 19p13.2 (500 Kb-1 Mb in size) (2 cases, 3%) (Fig 2). T and B ALL showed the difference in recurrent copy-number changes (Table 2).

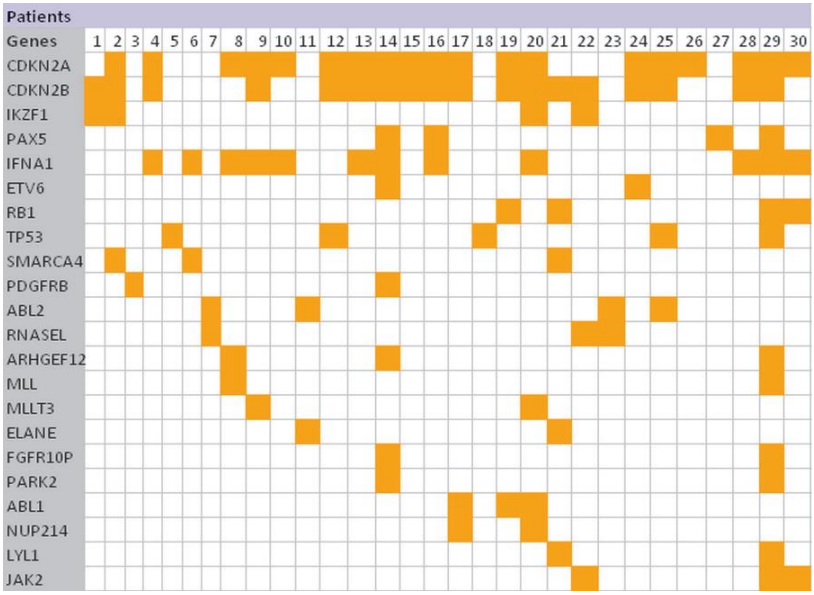

Figure 3. Recurrent aberrations and genes found with SNP array in adult ALL.

Table 2. The types of aberrations by T and B ALL.

Aberration type	T-ALL, n (%)	B-ALL, n (%)
Hemizygous deletion	15 (60)	24 (59)
Homozygous deletion	5 (20)	10 (24)
Copy number gains	12 (48)	18 (44)
The most frequent aberrations by chromosome per case		
Chromosome arm 9p	9 (36)	18 (44)
Chromosome arm 6q	5 (20)	11 (27)
Chromosome arm 13q	2 (8)	8 (20)
Chromosome 17	3 (12)	9 (22)
The recurrent aberrations/genes per case		
CDKN2A/B deletions	7 (28)	12 (29)
IKZF1 deletions	0 (0)	4 (10)
PAX5 deletions	1 (4)	3 (7)
TP53 deletions	1 (4)	4(10)
MLL deletions	1 (4)	1 (2)
RB1 deletions	1 (4)	3 (7)
ETV6 deletions	0(0)	2(4)

TP53 mutation analysis of 66 adult ALL samples

TP53 mutations were detected in 3 of 66 patients (4.5%). Of these three, one was a point mutation, one was a complex mutation including point mutation and deletion, and one was a complex mutation including duplication and point mutation. Exons 5, 6, and 7 were affected. Of the cases with mutations in *TP53*, one had a complex karyotype, one a complex karyotype including the deletion of chromosome arm 17p, and one had hypodiploidy including the monosomy of chromosome 17.

Survival outcomes

The median (range) follow-up was 18 (0–74) months. There were 2 resistant disease cases, 14 relapses, and 18 deaths as primary events. Thirty-four patients remain alive. The median EFS and OS were 21 (95% CI: 5–37) and 33 (95% CI: 19–47) months,

respectively. In univariate analysis, older age, abnormalities in chromosome 13 and undeleted *CDKN2A/B* gene region were associated with shorter EFS. Abnormalities in chromosome 13 and undeleted *CDKN2A/B* gene region remained significant risk factors for shorter EFS in multivariate analysis. The only predictor of shorter OS was older age at diagnosis.

A multivariate analysis of the BCPALL group showed that older age, abnormalities in chromosomes 13 and 14 were indicative of shorter EFS, while older age, abnormalities in chromosomes 3 and 14, as well as the deletion of the *RB1* gene were predictive of poor OS. No factors of prognostic significance were identified in T-ALL group (Table 3).

Table 3. Cox regression analysis for EFS and OS of ALL patients.

Factor	Univariate			Multivariate		
	Hazard ratio		p value	Hazard ratio		p value
	Estimate	95% CI		Estimate	95% CI	
All ALL patients						
Overall survival						
Older age, years	1.024	1.001-1.047	0.040	1.024	1.001-1.047	0.040
Abnormal chromosome 13	2.041	0.915-4.553	0.082	-		
Undeleted CDKN2AB	2.434	0.997-5.944	0.051	-		
Event free survival						
Older age, years	1.026	1.004-1.049	0.022	n.s.		
Abnormal chromosome 13	2.498	1.163-5.363	0.019	3.017	1.388-6.556	0.005
Undeleted CDKN2AB	2.607	1.075-6.324	0.034	3.034	1.236-7.451	0.015
B-ALL patients						
Overall survival						
Older age, years	1.048	1.019-1.078	0.001	1.078	1.041-1.116	<.001
Abnormal chromosome 3	3.760	1.218-11.602	0.021	5.349	1.248-22.926	0.024
Abnormal chromosome 5	3.290	1.178-9.195	0.023	n.s.		

Abnormal chromosome 13	3.415	1.332-8.751	0.011	n.s.		
Abnormal chromosome 14	3.103	1.107-8.702	0.031	4.659	1.227-17.687	0.024
Abnormal RB1 gene	3.491	1.004-12.141	0.049	10.699	2.332-49.087	0.002
Event free survival						
Older age, years	1.050	1.021-1.079	0.001	1.073	1.038-1.110	<.001
Abnormal chromosome 3	3.582	1.180-10.873	0.024	n.s.		
Abnormal chromosome 5	3.220	1.173-8.838	0.023	n.s.		
Abnormal chromosome 13	4.697	1.906-11.572	0.001	8.912	2.991-26.559	<.001
Abnormal chromosome 14	2.920	1.053-8.098	0.040	3.781	1.242-11.509	0.019
Abnormal RB1 gene	3.215	0.941-10.984	0.062	-		

Genomic clonal evolution

For this part of research we selected all relapsed adult ALL patients from the first part of this study (study population from 2007–2013, published data). Addionally, we added all relapsed patients from the 2013–2018 year period (not published data). In total, sixteen patients experienced a relapse and these were included in the present study. SNP array analysis was performed for all 16 patients with paired diagnostic and relapse samples in the same conditions. Two relapse SNP array results (patients No. 4, 8). showed no chromosomal aberrations or loss of heterozygosity and were deemed to contain a high proportion of normal cells (blast counts 0,5–1,5%); these were excluded from the analysis. One patient was excluded from analysis because of relapse being in the lymph node but not in bone marrow (the blast count was normal in bone marrow).

Table. Clonal evolution of adult ALL in chromosomal and mutation level (recurrent aberrations are marked in bold).

Patient No.	Chromosomal aberrations at diagnosis	Chromosomal aberrations at relapse	Evolution type of chromosomal aberrations	Gene variants at diagnosis	Gene variants at relapse	Evolution type of gene variants
1	**5 trisomy**	**5 trisomy** 9p del (*CDKN2AB*, *PAX5*) 3q del 12p del 17p del (*TP53*)	Clonal evolution	*NRAS* G12D *TAL1* Y174H	undetected	unclassified
2	9p del (*CDKN2AB*, *PAX5*)	9p del (*CDKN2AB*, *PAX5*)	Identical clones	*PAX5* D193Ter *NRAS* G12D	undetected	unclassified
3	**5 trisomy** **13q del**	**5 trisomy** **13q del** 9p del (*CDKN2AB*, *PAX5*) 7p del (*IKZF1*)	Clonal evolution	***WT1* Q155Ter**	***WT1*** **Q155Ter** *NRAS* G12D	Clonal evolution
4	normal karyotype	normal karyotype	unclassified	*KRAS* G12R *KMT2A* R420Q	undetected	unclassified
5	hyperdiploidy	not done (relapse in lymph node)	unclassified	***JAK2* R1063H** *CBL* L272FX	***JAK2*** **R1063H** *KRAS* L52P	Evolution from ancestral clone
6	**1p del** **8q del** 2q del 12p del (*ETV6*)	**1p del** **8q del** 9p HP (*CDKN2A*, *PAX5*) 8q dup	Evolution from ancestral clone	***NRAS* G12D** ***JAK2* R122H** ***FLT3* V94M**	***NRAS* G12D** ***JAK2*** **R122H** ***FLT3* V94M** *TP53* R282P	Clonal evolution
7	**7p del (*IKZF1*)**	**7p del (*IKZF1*)** 9p del (*CDKN2AB*, *PAX5*) 8q del 16q del	Clonal evolution	***SETD2*** **I1960M** ***NUP98*** **R1052L** *ETV6* R291S	***SETD2*** **I1960Met** ***NUP98*** **R1052L** *ZNF384* D220dup	Evolution from ancestral clone
8	normal karyotype	normal karyotype	unclassified	***NRAS* G13R** ***FLT3* D835Y** ***IL7R* I258F**	***NRAS* G13R** ***FLT3*** **D835Y** ***IL7R* I258F** *WT1* H465N	Clonal evolution
9	**9p LOH (*CDKN2AB, PAX5*)** **8q del** **12q del (*BTG1, PTPN11*)**	**9p LOH (*CDKN2AB, PAX5*)** **8q del** **12q del (*BTG1, PTPN11*)** +21, +X	Clonal evolution	*KRAS* G12D ***ABL1* G1079C** *JAK2* R683G *PAX5* Q71Ter	*NRAS* Q61K ***ABL1*** **G1079C** *KRAS* G12V *KRAS* G12C *EBF1* S238Y	Evolution from ancestral clone

10	3q del **6q del** 9p del homozygous (*CDKN2AB*) **13q del** **14q dup**	**6q del** **13q del** **14q dup**	Evolution from ancestral clone	not done	not done	unclassified
11	6q del 17p del **+21 trisomy**	7q dup **+21 trisomy**	Evolution from ancestral clone	NGS not done, ***TP53*** **P152dup** ***TP53*** **P153L fs180X**	NGS not done, ***TP53*** **P152dup** ***TP53*** **P153L fs180X**	Identical clones
12	hyperdiploidy	hyperdiploidy	Identical clones	not done	not done	unclassified
13	+10	+10	Identical clones	not done	not done	unclassified
14	13q del	13q del	Identical clones	not done	not done	unclassified
15	**9p del (*CDKN2AB*)**	3q dup **9p del (*CDKN2AB*)**	Clonal evolution	not done	not done	unclassified
16	**7p del**	**7p del** 9p dup	Clonal evolution	not done	not done	unclassified

Four patients (31%) displayed identical genetic changes at diagnosis and relapse, six (46%) had clonal evolution with additional imbalances at relapse, and three (23%) showed the evolution from the ancestral clones, with some chromosomal aberrations present at diagnosis lacking at relapse. We identified a mean of 3.1 (range 1–8) aberrations/sample at diagnosis and 3.3 (range 1–8) at relapse.

At diagnosis, 54% of the cases had deletions/duplications/LOH in genes known to be associated with leukemogenesis, including *CDKN2AB*, *PAX5*, *IKZF1*, *ETV6*, *BTG1*, *PTPN11*, whereas at relapse the same frequency of the cases had deletions/duplications/LOH including *TP53*, *CDKN2AB*, *PAX5*, *IKZF1*, *BTG1*, *PTPN11*. The genes most frequently targeted at relapse were *CDKN2A/B* and *PAX5*.

In regard to the analysis of mutations detected ny NGS, the patterns of clonal evolution were the same, but the ratio was diffrent in comparison with chromosomal aberrations. NGS data were available for 10 relapsed adult ALL patients in the 2007–2018 year period. Of these, three patients were lacking of mutations at relapse, so the genomic evolution was not possible to evaluate. Further analysis of seven patients detected three types of evolution patterns: one patient (14%) displayed identical genetic changes at diagnosis and relapse, three (43%) had clonal evolution with additional imbalances at relapse, and three (43%) showed the evolution from the ancestral clone. At diagnosis 85% of ALL patients had mutations in leukemia-related genes: *TP53*, *KRAS*, *NRAS*, *ABL1*, *JAK2*, *FLT3*, *SETD2*, *NUP98*, *ETV6*, *IL7R*, *CBL*. At relapse, mutations were detected in 100% of cases in these genes: *TP53*, *KRAS*, *NRAS*, *ABL1*, *JAK2*, *FLT3*, *SETD2*, *NUP98*, *ETV6*, *IL7R*, *CBL*. At the time of relapse, the most of mutations appeared in RAS biological pathway genes – *NRAS* and *KRAS*.

DISCUSSION

In the present population-based study, we have investigated 66 cases of adult ALL using genomewide SNP array analysis. Our analysis of adult ALL showed that 82% of samples had one or more genomic abnormalities, with the median number of 3.56 abnormalities per case. This may be compared with five previous adult ALL studies in which 5,4 to 8 abnormalities were detected per case. Comparing the types of aberrations, we detected nearly equal numbers of other studies detected significantly higher number of hemizygous deletions than duplications/amplifications (Paulsson et al., 2008; Okamoto et al., 2010; Safavi et al., 2014; Mühlbacher et al., 2016; Forero-Castro et al., 2016). We found most aberrations at chromosome arm 9p and 6q, in line with previous four reports, (Paulsson et al., 2008; Okamoto et al., 2010; Safavi et al., 2014;

Mühlbacher et al., 2016) whereas one adult ALL study detected the 7p aberration to be more frequent than 6q (Forero-Castro et al., 2016).

All adult ALL studies found *CDKN2A/B* gene aberrations to be the most frequent. Conversely, we have identified very few *PAX5* gene aberrations (the frequency of *PAX5* gene aberrations in other adult ALL studies – 14%–33%) (Paulsson et al., 2008; Okamoto et al., 2010; Safavi et al., 2014; Mühlbacher et al., 2016; Forero-Castro et al., 2016). Notably, the frequency of *PAX5* gene aberrations in childhood ALL is also high (31.7%) (Inaba et al., 2013). We also detected a few *IKZF1* gene deletions. The lower aberration rate per case and few *PAX5* and *IKZF1* aberrations could be related to the population specificity of Lithuanian adult ALL. The low frequency of these aberrations compared to other studies could be because our study was designed to be population-based to avoid patient selection bias.

We detected three *TP53* mutations (4.5%) in our cohort of adult ALL patients, which is fewer than in a recent report that found *TP53* mutations in 8% of the cases (Chiaretti et al., 2013). One patient had both a *TP53* mutation and the loss of one copy of chromosome arm 17p, effectively knocking-out both copies of *TP53*. Notably, another patient with a *TP53* mutation had a high hypodiploid karyotype including the monosomy of chromosome 17, indicating the loss of a second copy of *TP53*. A study of 124 hypodiploid childhood ALL cases showed that 91.2% low-hipodipoid cases had *TP53* alterations (Holmfeldt et al., 2013). Adult low-hypodiploid ALL also had a high frequency of *TP53* mutations (90.9%), indicating that the mutations of *TP53* are a hallmark of low-hypodiploid ALL (Holmfeldt et al., 2013). In our SNP array analysis, we detected one high hypodipoid case which also had a *TP53* mutation. Thus, alterations in *TP53* may be an important event in the pathogenesis of both low and high hypodiploid ALL.

The present SNP array analysis identified several target genes possibly related to adult ALL leukemogenesis, although it should be noted that the minimally deleted regions contained additional genes. Three cases had the deletion of *SMARCA4*, encoding a protein which is part of the large ATP-dependent chromatin remodeling complex SNF/SWI required for transcriptional activation of genes normally repressed by chromatin. The *SMARCA4* deletion could disrupt the function of the SNF/SWI complex and play a leukemogenic role in adult ALL (Pottier et al., 2008). This complex is also required for glucocorticoid transcriptional activity. Resistance to glucocorticoids is an important adverse prognostic factor in newly diagnosed ALL patients (Holmfeldt et al., 2013).

Three patients had aberrations in the *ARHGEF12* gene. The encoded protein may form a complex with G proteins and stimulate Rho-dependent signals. This protein has been observed to form a myeloid/lymphoid fusion partner in acute myeloid leukemia (Pottier et al., 2008). The *ARHGEF12* gene as a partner was involved in a translocation t(7;11) in one pediatric acute lymphoblastic case (Poppe et al., 2005). Two cases had deletions of the *LYL1* gene, which may play role in blood vessel maturation and hematopoeisis (Poppe et al., 2005). A translocation between this locus and the T-cell receptor beta locus on chromosome 7 has been associated with T-ALL. Although it may participate in T-cell leukemogenesis, an oncogenic potential has thus far not been demonstrated (De Keersmaecker et al., 2005). In our SNP array study, both cases with *LYL1* aberrations were T-ALL. Finally, three cases had deletions of *RNASEL*, which plays an important role in the inflammatory response pathway and was first identified as a candidate gene for prostate cancer (Dong et al., 2008).

A multivariate Cox regression analysis showed that older age, *CDKN2AB* gene status, aberrations in chromosomes 3, 13, 14 and deletions in the *RB1* gene were associated with EFS and/or OS.

In our overall group, the presence of an aberrant chromosome 13 was associated with shorter EFS. A multivariate analysis of BCP ALL indicated that aberrations in chromosome 13 were associated with shorter EFS, whereas the deletion of *RB1* predicted poor OS. Deletions of the *RB1* were detected in a number of previous studies of adult ALL (Paulsson et al., 2008; Okamoto et al., 2010; Yasar et al., 2010). Several studies detected deletions of the *RB1* gene in both BCP and T-ALL; however, other authors suggested that the alterations in genes which control the G1/S phase, such as the *RB1*, play an important role only in the differentiation of the B-lineage but not of the T-lineage leukemias (Studniak et al., 2013). We detected *RB1* gene deletions in three patients with BCP ALL and in one patient with T-ALL. Although a large study of 3239 children's BCP ALL cases indicated *RB1* gene deletion to be associated with poor prognosis, *RB1* gene deletions as a predictive factor of poorer outcome in adult ALL schould to be confirmed in larger studies (Hamadeh et al., 2019).

We found that an undeleted *CDKN2AB* gene was related to shorter EFS. A previous study of 61 children and 30 adult ALL showed that *CDKN2AB* deletion occurs in adults more frequently than in children and adversely affects OS only in adult BCP ALL (Kim et al., 2009). The analysis of 227 and 109 children with BCP ALL showed that *CDKN2AB* deletion did not influence the patient outcome (van Zutven et al., 2005; Mirebeau et al., 2006). Another study of 54 patients aged 10–25 years was unable to demonstrate the prognostic value of the *CDKN2AB* deletion (Usvasalo et al., 2008). On the other hand, two other studies confirmed that the deletion of the *CDKN2AB* gene is a poor prognoctic factor for EFS and OS in ALL (Moorman et al., 2014; Hamadeh et al., 2019). These discordant results may show a random association of the *CDKN2AB* deletion status with the outcome or may be related to very different treatment strategies used by clinicians.

In the first study of genomic evolution of adult ALL in Lithuania, we detected an equal ratio of three patterns of genomic evolution (33% of each) (Dirse et al., 2016). Still, when we extended the number of relapsed patients (from 2013–2018; data not published), the ratio of genomic evolution changed and became the following: four patients (31%) displayed identical genetic changes at diagnosis and relapse, six (46%) had clonal evolution with additional imbalances at relapse, and three (23%) showed the evolution from the ancestral clones, with some aberrations present at diagnosis lacking at relapse. In comparison with another adult ALL study, we detected a quite equal frequency of identical genetic changes at diagnosis and relapse (31% vs. 27%), more of clonal evolution (46% vs. 22%) and less of ancestral clones (23% vs. 50%) (Safavi et al., 2015). Though given the small number of cases included in most series and the different composition of the cohorts regarding age, immunophenotypes, and genetic subtypes, the exact proportions of different evolutionary pathways may vary, but it can be concluded that all three patterns exist also in adult ALL.

At both adult ALL studies, the genes most frequently targeted at relapse were *CDKN2A/B* and *PAX5*. The transcription factor PAX5 represses lineage-inappropriate genes and activates B cell-specific genes in B lymphocytes. By identifying 110 PAX5-repressed genes, it is demonstrated that PAX5 downregulates diverse biological activities including receptor signaling, cell adhesion, migration, transcriptional control, and cellular metabolism at B-cell commitment. Knowing that we hypothesized that PAX5 has a higher frequency of aberrations of this gene at relapse, this could be related with the progression of the disease (Delogu et al., 2006). The good example could be the genetic situation in patients No. 2 and No. 9 (Table), where at diagnosis both patients have hemizygous *PAX5* deletions, and at relapse, besides these two deletions, we detected *PAX5* mutations, resulting in the knocking out of both copies of *PAX5*.

By analyzing the connections between chromosomal aberrations and mutations in diagnosis-relapse samples, we noticed the association between deletions of gene *CDKN2AB* and mutations in the RAS pathway. Here we could state that the first genetic event are RAS pathway mutations, followed by a second genetic event – *CDKN2AB* gene deletion. For example, patient No. 6 has a *NRAS* gene mutation at diagnosis and then a *CDKN2AB* gene deletion at relapse. The same genetic situation is in patient No. 1 (Table) as well. Our hypothesis is supported by a mouse model study, where it was showed that RAS pathway activation cooperates with *CDKN2AB* locus deletion in B-cells to induce a fully penetrant lymphoma/leukemia phenotype in mice. These tumors resemble high-risk subtypes of human B-ALL, providing a convenient and highly reproducible model of refractory B-ALL (Sewastianik et al., 2017).

CONCLUSION

1. The SNP array analysis of adult ALL detected chromosomal aberrations in 82% of the patients (3.6 aberrations per case). The most aberrations were detected in chromosome arms 9p, 6q, 13q. We identified an equal ratio of hemizygous deletions (45%) and duplications/amplifications (44%). The most aberrant leukemia-related genes were*: CDKN2A* (29% of the patients), *CDKN2B* (18.27%), *MLL* (2.3%), *IKZF1* (4.6%), *PAX5* (4.6%), *RB1* (4.6%), *TP53* (5.7%) and *ETV6* (2.3%). We detected a lower frequency of aberrations per case and lower frequency of aberrant leukemia-related *CDKN2AB*, *PAX5*, *ETV6*, *IKZF1*, *EBF1*, *TP53* genes than other adult ALL studies.
2. The SNP array analysis identified new target genes possibly related to adult ALL leukemogenesis – *ARHGEF12*, *LYL1*, *RNASEL*, *SMARCA4*. Although the *ARHGEF* gene has been observed to form a myeloid/lymphoid fusion partner in acute

myeloid/lymphoid leukemia, the deletion of this gene could be important in adult ALL. *SMARCA4* encodes a protein that is part of the large ATP-dependent chromatin remodeling complex SNF/SWI. The *SMARCA4* deletion could disrupt the function of this complex and play a leukemogenic role in adult ALL. Deletions of the *LYL1* gene could be important in blood vessel maturation and hematopoeisis and may participate in T-cell leukemogenesis. The *RNASEL* gene, which encodes endoribonulease, acts as a tumor suppressor in an interferon-dependent process and could inhibit the proliferation of cancer cells.

3. Abnormalities in chromosome 13 and the *CDKN2A/B* gene status remained significant risk factors for shorter EFS in the multivariate analysis of adult ALL. A multivariate analysis of the BCP ALL group showed that abnormalities in chromosomes 13 and 14 were indicative of shorter EFS, while abnormalities in chromosomes 3 and 14, as well as the deletion of the *RB1* gene, were predictive of poor OS.
4. Three distinct patterns of genomic evolution were detected: the identical clones at diagnosis and relapse, the clonal evolution from the diagnostic clone, and the evolution from the ancestral clones. The association between the deletion of the *CDKN2AB* gene and mutations of the *NRAS/KRAS* genes were observed. These two genomic events and their dynamics could be important in leukemogenetic mechanisms and have an impact of clonal evolution in adult ALL.

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