

Significance of Primary Immunodeficiency Disorder (PID)–associated Germline Mutations in Korean patients with ALL

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Abstract

Background In addition to somatic mutations, germline genetic predisposition to hematologic malignancies is an emerging research issue. In this study, we investigated genetic alterations (both somatic and germline) in Korean acute lymphoblastic leukemia/lymphoma (ALL) patients using targeted gene panel sequencing. **Method** We selected ALL patients diagnosed at Samsung Medical Center. Eighty-two well known genes associated with 23 predisposition syndromes were included in the gene panel. In addition to sequence variants, gene-level copy number variants (CNVs) were investigated. **Results** Ninety-three ALL patients (65 children, 28 adults) were enrolled in this study. We identified 197 somatic sequence variants (excluding synonymous variants) and 223 somatic CNVs. Overall survival (OS) and relapse-free survival (RFS) differed significantly by cytogenetic group in childhood B-ALL. The *IKZF1* alteration had an adverse effect on OS and RFS in childhood ALL. Among the 82 genes associated with 23 predisposition syndromes, known mutations (*TP53*, *LIG4*) and novel mutations (*TINF2*, *CTC1*) were identified. Nine patients (9.7%) had pathogenic or likely pathogenic variants associated with primary immunodeficiency disorder (PID) in a heterozygous state. **Conclusions** We found recurrent mutations and clinical effects similar to those reported in previous studies. We also found an unexpectedly high prevalence of PID-associated germline mutations. Our results suggest several important points: 1) paired leukemic and normal control samples are recommended to discriminate somatic and germline mutations; 2) gene panels must include genes associated with predisposition; 3) gene panels must include genes associated with PID.

Background

B-cell precursor and T-cell precursor acute lymphoblastic leukemia/lymphoma (B-ALL, T-ALL) are two of the most common malignancies in children. ALL can be classified by genetic alterations, which are various and heterogeneous. Chromosome aneuploidy, structural alterations and rearrangements, copy number variations (CNVs), and sequence mutations all contribute to leukemogenesis. In 2016, the fourth edition of the World Health Organization (WHO) classification of lymphoid and myeloid neoplasms and acute leukemia included new provision entities of ALL: *BCR-ABL 1*-like (or Ph-like) ALL, *iAMP21* (intrachromosomal amplification of chromosome 21), and early T-cell precursor ALL (ETP-ALL) [1].

BCR-ABL 1-like ALL is a high-risk form of ALL with peak incidence in young adults. *IKZF1* deletions, mutations of JAK-STAT and RAS signaling genes (*NRAS*, *KRAS*, *PTPN11*, *NF1*, et al.), and structural rearrangements (*CRLF2*, *ABL*-class tyrosine kinase genes, *JAK2*, *EPOR*, et al.) have been identified in this group [2]. *iAMP21* accounts for about 2% of ALL in older children and is associated with adverse outcomes [3]. In *iAMP21*, three or more additional copies of *RUNX1* (*AML 1*) are observed on chromosome 21 in metaphase fluorescence *in situ* hybridization (FISH). ETP-ALL is defined as CD1a⁻, CD8⁻, CD5⁻ (dim), and positive for one or more stem-cell or myeloid antigens. Genetic alterations in ETP are somewhat different than in non-ETP, with *FLT3*, *DNMT3A*, and *WT1* mutations found more frequently in ETP than in non-ETP [4, 5].

In addition to somatic mutations, germline genetic predisposition to hematologic malignancies is an emerging issue in current research. Genes found to be associated with predisposition to myeloid malignancy have been included in the WHO classification, "Myeloid neoplasms with germ line predisposition" [1]. *CEBPA*, *DDX41*, *RUNX1*, *ANKRD26*, *ETV6*, *GATA2*, et al. are included in that category. Several syndromes, such as bone marrow failure syndrome and telomere biology disorders, also are included in that category. One early study estimated that childhood leukemia with hereditary genetic causes accounted for 2.6% of all cancers [6]. Down syndrome (DS) is the most common underlying genetic predisposition for ALL [6, 7]. Various syndromes, such as Li Fraumeni (*TP53*), Bloom syndrome (*BLM*), Wiskott Aldrich syndrome (*WAS*), ataxia telangiectasia (*ATM*), and Nijmegen breakage syndrome (*NBN*), also increase susceptibility to ALL [8]. A germline mutation of *PAX5* is highly associated with B-ALL [9].

Some genes associated with ALL are found to have both germline and somatic mutations. For example, *PAX5*, *ETV6*, *TP53*, and *IKZF1* are known to have important somatic alterations, and germline mutations of those genes also cause susceptibility to ALL. Furthermore, somatic and germline mutations of those genes can be found at the same time in leukemic samples [9, 10]. Therefore at initial ALL diagnosis, discrimination of somatic and germline mutations are crucial to accurately classify ALL patient genetic subtypes/risk groups and detect predisposition genes.

In this study, we used extensive gene panel sequencing to investigate genetic alterations (both somatic and germline) in Korean ALL patients. We also evaluated the clinical significance of recurrent somatic mutations and germline predisposition mutations in Korean ALL patients.

Patients And Methods

Study population and samples

We selected paired initial-diagnosis and complete remission (CR) bone marrow samples from patients diagnosed with ALL at Samsung Medical Center from 2008 to 2012. The Institutional Review Board at Samsung Medical Center approved this study (IRB No. 2015-11-053).

To detect germline mutations, bone marrow slides obtained when the patients were in CR were used. Morphology, chromosome, FISH, and immunophenotyping results were reviewed, and bone marrow slides with no apparent residual leukemic cells were selected as control samples.

Conventional study

A chromosome study was performed using a standard method, and the karyotypes are described according to the International System for Human Cytogenetic Nomenclature. Multiplex reverse transcription polymerase chain reaction (RT-PCR) was performed to detect recurrent translocation (HemaVision kit, DNA Technology, Aarhus, Denmark). *FLT1*-ITD (internal tandem duplication) mutation analyses (by fragment length polymorphism) and FISH for *CDKN2A/B* were performed.

Targeted gene sequencing

Gene panel

From a literature review, we selected 500 genes found to be significantly mutated in ALL (Additional file 1: Table S1). Our gene panel included [11]: cell cycle and p53 signaling pathway (*ATM*, *CDKN1B*, *CDKN2A*, *CDKN2B*, *RB*, *TP53*, et al.), chromatin structure modifiers and epigenetic regulators (*ARID1A*, *BMI1*, *CHD1*, *CHD4*, *CHD9*, *CREBBP*, *CTCF*, *DNMT3A*, *EED*, *EP300*, *EZH2*, *KDM5C*, *KDM6A*, et al.), JAK-STAT signaling pathway (*CRLF2*, *IL2RB*, *IL7R*, *JAK1*, *JAK2*, *JAK3*, *PTPN2*, *SH2B3*, *STAT3*, *TYK2*, et al.), DNA repair (*MSH2*, *MSH6*, *ZFX4*, et al.), the NOTCH pathway (*FBXW7*, *NOTCH1*, et al.), PI3K-AKT-mTOR signaling pathway (*AKT2*, *PIK3CD*, *PIK3R1*, *PTEN*, et al.), RAS pathway (*BRAF*, *CBL*, *FLT3*, *KRAS*, *NF1*, *NRAS*, *PTPN11*, et al.), and transcriptional processes (*BCL11B*, *DNM2*, *ERG*, *GATA3*, *LMO2*, et al.).

Syndromes known to cause predisposition to hematologic malignancies

Twenty-three well known predisposition syndromes (82 genes) were included in our next generation sequencing (NGS) panel: Ataxia pancytopenia syndrome, Ataxia telangiectasia, Bloom syndrome, Constitutional mismatch repair deficiency syndrome, Diamond-Blackfan anemia, Dyskeratosis congenita, Familial acute myeloid leukemia, Familial platelet disorder with propensity to myeloid malignancy, Fanconi anemia, GATA2-spectrum disorders, Li Fraumeni, Ligase IV syndrome, Neurofibromatosis, Nijmegen breakage syndrome, Noonan syndrome, Noonan-like syndrome, Severe congenital neutropenia 3, Severe congenital neutropenia, Shwachman-Diamond syndrome, susceptibility to ALL 3, Thrombocytopenia 2, Thrombocytopenia 5, and Wiskott Aldrich Syndrome.

Data analysis

Data analysis was conducted using previously described methods (Additional file 1: Fig. S1) [12]. After sequencing, we aligned the reads to human genomic reference sequences (GRCh37) using the Burrows-Wheeler alignment tool. The Genome Analysis Tool Kit (Broad Institute) was used for variant calling. Pindel was used to crosscheck insertion and deletion mutations. All mutations were annotated using ANNOVAR and VEP software. Variants were further examined by visual inspection using the Integrative Genomic Viewer. Annotated variants were classified by automated algorithm software, DxSeq Analyzer (Dxome, Seoul, Korea), by applying the standards and guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [13]. An R package, ExomeDepth (1.1.10), was used to detect exon- and gene-level CNVs in target regions, followed by visualization using a base-level read depth normalization algorithm implemented in DxSeq Analyzer (Dxome, Seoul, Korea) [12].

Results

Patients

Characteristics of the 93 enrolled Korean ALL patients are summarized in Table 1. We enrolled 65 pediatric (<20 years old) ALL patients and 28 adult ALL patients, including 12 T-ALL patients (7 children, 5 adults).

B-ALL with *BCR-ABL1* was the most common form of adult B-ALL (11/23). B-ALL, hyperdiploidy and B-ALL, Not Otherwise Specified (NOS) were the most common forms of childhood B-ALL (41/58). B-ALL with t(12;21)(p13.2;q22.1);*ETV6-RUNX1* and B-ALL with t(v;11q23.3);*KMT2A* rearranged occurred in 9 patients and 4 patients, respectively. According to the immunophenotyping results, two adults with T-ALL were diagnosed with early T-cell precursor acute leukemia.

B-ALL was classified into three risk groups following a previous study [14]: (1) good risk (Gcyto), *ETV6-RUNX1*, and high hyperdiploidy (51–65 chromosomes); (2) intermediate risk (Icyto), *TCF3-PBX1*, *IGH* translocations, and B-other (none of the established abnormalities); and (3) high risk (Hcyto), *BCR-ABL1*, *KMT2A* translocations, near haploidy (30–39 chromosomes), low hypodiploidy (fewer than 30 chromosomes), *iAMP21*, and *TCF3-HLF*.

Germline sequence variants among 23 syndrome-associated genes

Pathogenic or likely pathogenic variants

Four variants were identified in four patients (Table 2). Two variants were previously known, and two variants were novel. The *TP53* rs28934575 variant was identified in a B-ALL, NOS patient. The rs28934575 variant was previously reported in Li-Fraumeni syndrome patients (multiple cancers, including breast cancer, liver cancer, and lung cancer) [15]. The *LIG4* rs772226399 variant was identified in a patient with B-ALL with hyperdiploidy

that had previously been reported in patients with clinical feature of dyskeratosis congenita (DC) [16]. The *TINF2* c.1130-1G>C variant is novel and associated with DC and Revesz syndrome. It is found in about 10% of all DC cases in an autosomal dominant (AD) pattern [17]. The mutation hot spot of *TINF2* is exon 6 [17], but the *TINF2* c.1130-1G>C variant is located in an intron between exon 7 and exon 8. The *CTCF* c.2249dup variant was identified in a patient with B-ALL with hyperdiploidy. *CTCF* is a causative gene for DC in an autosomal recessive (AR) pattern [18].

Germline sequence variants in genes other than those associated with the 23 syndromes

Pathogenic or likely pathogenic variants

We identified 30 variants in this study (Table 3 and Additional file 1: Table S2), of which four were novel and 26 had been previously reported. Most variants have AR pattern or AD/AR pattern inheritance. The *PTCH2*, *THBD*, *PDGFRB*, and *CSF1R* genes are associated with AD pattern disorders.

Germline copy number variants

Only one patient (male, 18 years old, B-ALL-NOS) had a known CNV, *CASP10* (deletion of exon 6-exon 9, Additional file 1: Fig. S2). This same CNV was previously found in a patient with systemic juvenile idiopathic arthritis [19]. *CASP10* is a causative gene for autoimmune lymphoproliferative syndrome (ALPS) type IIa, and its mutation hot spot is the protease domain (CASC) with an almost missense mutation.

Somatic sequence variants

We identified 197 variants, excluding synonymous variants (Additional file 1: Table S3). The RAS pathway and chromatin structure modifiers and epigenetic regulators were the most common variants in ALL (Additional file 1: Table S4).

Sixty variants were previously known (registered in the COSMIC database), and 33 variants were in genes associated with the 23 predisposition syndromes.

T-ALL

NOTCH1, *IL7R*, *FBXW7*, and *NRAS* were the most common variants overall in T-ALL (Additional file 1: Fig. S3). In childhood T-ALL, *NOTCH1* (4/7, 57.1%), *FBXW7* (2/7, 28.6%), *GATA3* (2/7, 28.6%), *WT1* (2/7, 28.6%), and *BCL11B* (2/7, 28.6%) were the most common variants. In adult T-ALL, *NRAS* (3/5, 60.0%), *NOTCH1* (2/5, 40.0%), *IL7R* (2/5, 40.0%), *PHF6* (2/5, 40.0%), *DNMT3A* (2/5, 40.0%), *PTEN* (2/5, 40.0%), and *CREBBP* (2/5, 40.0%) were most common.

One ETP patient (ALL0043) had a *WT1* mutation (c.822_823insTCCCT), and *FLT3*-ITD was identified in it by fragment length polymorphism. The other ETP patient (ALL0047) had several sequence variants, including *NOTCH1* and *IL7R* (Additional file 1: Table S3).

B-ALL

NRAS, *FLT3*, *SETD2*, and *KRAS* were the most common variants overall in B-ALL (Additional file 1: Fig. S3).

In childhood Gcyto B-ALL, *NRAS* (8/26, 30.8%), *FLT3* (6/26, 23.1%), *KRAS* (6/26, 23.1%), *SETD2* (3/26, 11.5%), and *PTPN11* (2/26, 7.7%) variants were the most common (Additional file 1: Fig. S4). In adult Gcyto B-ALL (n=2), *FLT3*, *PTPN11*, *GNB1*, *CREBBP*, *HUWE1*, and *IL3RA* variants were identified.

NRAS, *SETD2*, *PAX5*, *FLT3*, *CREBBP*, and *NF1* variants were the most common variants in the Icyto B-ALL. *NRAS* was found in 19.2% (5/26) of child patients and 11.1% (1/9) of adult Icyto B-ALL patients.

The *ASXL1* variant was the most common variant in adult Hcyto B-ALL (2/12, 16.7%).

Somatic copy number variants

We identified 223 somatic CNVs in this study (Additional file 1: Table S5). Lymphoid development and differentiation and the cell cycle and p53 signaling pathway were the most common CNVs overall.

CDKN2A/B by NGS and FISH

FISH for *CDKN2A/B* was performed at the initial diagnosis of ALL. We compared the *CDKN2A/B* results between the FISH and NGS CNV analysis. The overall agreement rate for *CDKN2A/B* was 83.7% (Table 4). Nine cases were positive for *CDKN2A/B* deletion by NGS but negative by FISH. Six cases were normal by NGS analysis, but deletion/duplication was confirmed by FISH.

T-ALL

CDKN2A/B was the most common CNV in T-ALL (Additional file 1: Figure S5). Five of seven childhood T-ALL patients had a *CDKN2A/B* CNV. One ETP patient (ALL0047) had a *CDKN2A/B* deletion.

B-ALL

CDKN2A/B, *IKZF1*, *ERG*, *ETV6*, *RB1*, and *PAX5* were the most common CNVs in B-ALL (Additional file 1: Figure S5). In the adult Hcyto B-ALL group, *IKZF1*, *RB1*, and *PAX5* CNVs were the most common (Additional file 1: Figure S6).

Fifteen patients had an *IKZF1* alteration (Table 5). Seven patients had B-ALL with t(9;22)(q34;q11.2);*BCR-ABL1*. Six B-ALL, NOS patients had an *IKZF1* gene deletion. Seven of thirteen B-ALL with t(9;22)(q34;q11.2);*BCR-ABL1* patients had an *IKZF1* deletion (53.8%). Three of thirteen B-ALL with t(9;22)(q34;q11.2);*BCR-ABL1* patients had a *PAX5* deletion (23.1%).

Clinical effects of genetic alteration

Overall survival and relapse-free survival

Overall survival (OS) and relapse-free survival (RFS) are shown by cytogenetic groups in Figure 1. Differences in OS and RFS were statistically significant in childhood ALL but not adult ALL.

Clinical effect of *IKZF1*

IKZF1 alteration had an adverse effect on OS and RFS only in childhood ALL (Figure 2). No other genes had a consistent clinical effect in childhood or adult ALL (data not shown).

Discussion

NGS technology has been applied to various hematologic diseases. Many gene panels and several methods have been used to detect not only sequence variants but also large gene deletions and duplications or fusion genes [12, 20, 21]. In this study, we found a significant agreement rate between NGS and FISH for *CDKN2A/B* CNV detection. We also found significant *IKZF1* deletions using an NGS CNV analysis. A presumed diagnosis of *BCR-ABL1*-like ALL can be enabled by using an NGS CNV analysis to test for a high prevalence of genetic alterations in *IKZF1* and *JAK2* because *IKZF1* alterations (~80%) and *JAK2* alterations (~25%) are more prevalent in *BCR-ABL1*-like ALL than in other B-ALL sub-types [2]. Although we did not perform gene express profiling and FISH or RT-PCR for alterations commonly found in *BCR-ABL1*-like ALL, we found seven cases with *IKZF1* alterations in non-ALL with *BCR-ABL1*, and they had adverse clinical effects. We assume that the seven patients with non-ALL with *BCR-ABL1* and an *IKZF1* alteration probably had *BCR-ABL1*-like ALL.

In B-ALL, RAS signaling (*NRAS*, *KRAS*, *PTPN11*, *FLT3*, *NF1*, et al.) and B-cell differentiation and development (*PAX5*, *IKZF1*, *EBF1*, et al.) are the most common pathogenic pathways [11, 22], and we found a similar distribution of genetic changes in this study. In the adult high-risk cytogenetic group, *IKZF1* deletion was the most common alteration (6/12, 50%). This high prevalence might have occurred because most cases (11/12) were ALL with *BCR-ABL1*.

In T-ALL, we found recurrent somatic sequence variants and CNVs similar to those reported in previous studies [4, 5]. Sequence variants of *NOTCH1*, *IL7R*, and *FBXW7* and *CDKN2A/B* deletion are common mutations in T-ALL [5, 23]. Although only two cases of ETP were enrolled in this study, one of those patients had an *FLT3*-ITD and *WT1* mutation [4]. More ETP cases are needed to reveal genetic alterations with T-ALL among Koreans.

Detection of gene mutations that predispose individuals to cancer is important for several reasons, including modification of cancer management, early detection of secondary malignancies or non-malignant complications, and genetic counseling. The characteristic clinical and physical manifestations of multiple cancers and familial cancer histories can be suggestive of predisposing gene mutations. Some researchers have published a guideline for referral indications for cancer predisposition assessment [8]. However, cancer predisposition assessments can be delayed or ignored for several reasons. Heterogeneous clinical and physical manifestations can be missed by physicians. Sometimes, patients have no familial history because they have a *de novo* mutation [24]. Therefore, the germline mutation detection rate from guideline-based referrals for cancer predisposition assessment (using clinical, physical, and familial history criteria) is lower than from universal, targeted panel sequencing of cancer genes using tumor-normal matched samples [25]. In this study, we analyzed unselected sporadic ALL cases to investigate the overall germline mutation rate in Korea.

In this study, we used CR-state bone marrow slides for germline mutation detection. Skin fibroblasts are the only recommended control sample for germline mutations. Peripheral blood (PB) and bone marrow can be contaminated with leukemic cells. Other samples, such as saliva or buccal swab, can be also contaminated with PB. Furthermore, Age-related clonal hematopoiesis can be observed in ~10% of the healthy population, which increases with age [26]. However, a skin biopsy is an invasive procedure, so such samples are not easily available. Therefore, we used CR-state bone marrow slides (acquired to test for residual leukemic cells) as the control for germline mutations. No apparent leukemic samples were

obtained in our review of bone marrow morphology or the FISH, chromosome, flow-cytometry, and RT-PCR results. The variant allele fraction (VAF) and public population databases were used as filtering tools [27]. Germline variants can have a VAF of >33% even in tumor samples [27]. Variants within that range have a high possibility of germline origin. Variants registered in public databases, such as the Single Nucleotide Polymorphism database (dbSNP), the 1000 Genomes Project, the Exome Aggregation Consortium (ExAC) database, and the Human Gene Mutation Database (HGMD), are probably also of germline origin. We double checked the germline variants both in CR and leukemic samples. True germline variants (identified in CR samples) were also identified in paired leukemic samples with similar VAF. On the contrary, true somatic variants (identified in leukemic samples) were not found or were found with very low VAF in paired CR samples.

At initial diagnosis, only leukemic samples without a normal control might be available to analyze genetic alterations. In that case, discrimination of somatic and germline genetic alterations is difficult. Some genes could have both somatic and germline alterations. For example, *PAX5* is a predisposition gene for ALL, and a *PAX5* alteration can also be found in B-ALL as a somatic alteration. Moreover, if the VAF of a *PAX5* alteration is around 50%, it is difficult to determine whether its origin is somatic or germline. Many clinically important genes in ALL, such as *IKZF1*, *ETV6*, and *RUNX1*, can have both somatic and germline alterations.

To overcome the difficulty of tumor-only analysis, some researchers have sought computational or automatic algorithms to discriminate somatic and germline alterations using VAF and population databases. The accuracy of those methods depends on tumor purity, CNVs, and population allele frequency cut-off values. Sensitivity and specificity of more than 90% were reported [28, 29], but no consensus guidelines or cut-off values for tumor-only filtering strategies have been established. Therefore, further studies are still needed to accurately discriminate somatic and germline mutations in tumor-only samples.

Various syndromes increase the risk of ALL, with variable penetrance and preference. DS is the most common genetic cause of childhood leukemia. In an analysis of the National Registry of childhood tumors in the United Kingdom, 131 of 142 leukemia patients with underlying genetic causes were DS patients [6], and an analysis of approximately 18,000 European childhood ALL cases found that 2.4% of ALL patients also had DS [7]. Other genetic diseases with connections to ALL are ataxia telangiectasia, Nijmegen breakage syndrome, neurofibromatosis type 1, familial ALL, and Noonan syndrome [7]. Germline *PAX5* and *ETV6* mutations carry a high risk (high penetrance) of cancer, mainly ALL [7, 9, 30]. Bloom syndrome and constitutional mismatch repair deficiency syndrome also carry a moderate risk of ALL. Constitutional mismatch repair deficiency syndrome is more associated with T-cell lineage leukemia and lymphoma than B-cell lineage [31]. However, in this study, we did not find pathogenic or likely pathogenic variants among those gene mutations (*PAX5*, *ETV6*, *NF1*, *BLM*, *ATM*, et al.), which have high penetrance for ALL [7], possibly because we enrolled a relatively small number of unselected sporadic cases. Moriyama et al. reported that only 0.79% (35/4,405) of sporadic childhood ALL cases have a potentially pathogenic *ETV6* variant [30].

In this study, we did identify four pathogenic or likely pathogenic variants (*TP53*, *CTC1*, *TINF2*, and *LIG4*) among 82 genes associated with 23 syndromes well known for their connection to hematologic malignancy. All four of the variants were heterozygous. Li-Fraumeni syndrome (*TP53*) is a well-known rare cancer syndrome. The most common cancers in patients with Li-Fraumeni syndrome are solid cancers (such as breast cancer, lung cancer, and bladder cancer) [15]. However, a somatic *TP53* alteration is strongly associated with low hypodiploidy ALL (~90%), disease relapse, and germline origin (~40%) [32]. Although hypodiploid ALL accounts for only 5% of childhood ALL cases, hypodiploid ALL patients should be tested for Li-Fraumeni syndrome because of its poor prognosis and the possibility of a germline *TP53* mutation [33].

CTC1, *TINF2*, and *LIG4* are genes associated with inherited bone marrow failure syndrome, whose clinical features overlap with DC [16]. Fanconi anemia, DC, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome are also associated predominantly with solid cancers, whereas myelodysplastic syndrome and acute myeloid leukemia are predominant among hematologic malignancies rather than ALL [34]. Therefore, it is unclear whether these variants are causative genetic factors of ALL in our patients. A functional study or familial study, along with a physical and clinical investigation, is needed to confirm the correlations between ALL and these variants.

We also found 31 germline pathogenic or likely pathogenic variants in genes other than those associated with the 23 syndromes, including a CNV (*CASP10*). Among these 31 germline variants, *ADA*, *CASP10*, *IL12RB1*, *JAK3*, *LPIN2*, *MEFV*, and *TYK2* are genes associated with PID [35]. Given that *CTC1*, *TINF2*, and *LIG4* are also associated with PID, nine of our patients (9.7%) had pathogenic or likely pathogenic heterozygous variants associated with PID. All these genes are inherited in the AR pattern except *MEFV* (AR or AD, familial Mediterranean fever) and *TINF2* (AD, DC).

More than 300 distinct disorders and genes of PID have been classified by the International Union of Immunological Societies PID expert committee [35]. Familial Mediterranean fever (*MEFV*) is an auto-inflammatory disorder causing polyserositis, abdominal pain, arthritis, and other symptoms. *ADA*, *JAK3*, and *LIG4* are causative genes for severe combined immunodeficiencies (SCID), which affect cellular and humoral immunity. ALPS (*CASP10*) is a disease of immune dysregulation. *CTC1* is a causative gene of COATS plus syndrome, which overlaps with DC. Chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia (Majeed syndrome, *LPIN2*) are auto-inflammatory disorders. IL-12 and IL-23 receptor b1 chain deficiency (*IL12RB1*) and Tyk2 deficiency (*TYK2*) cause mendelian susceptibility to a mycobacterial disease associated with defects in intrinsic and innate immunity.

An increase in leukemia/lymphoma with PID is well known. A large analysis of PID patients in the United States Immune Deficiency Network database found significant increases in lymphoma (>8 fold-changes in PID cancer incidence over what was expected) [36]. Leukemia also

increased, with 1.43 fold-changes in men and a 1.0 fold-change in women. The mechanism of leukemogenesis in PID is unclear, but intrinsic (cancer predisposition parallel to the immunological defect) and extrinsic (following chronic infections, inflammation, or loss of immunosurveillance) mechanisms were proposed by Hauck et al [37].

We were unable to determine whether our patients with variants associated with PID were primary immunodeficiency patients, carriers, or carrying non-pathogenic variants because all of them were heterozygous, and appropriate clinical/laboratory findings or functional studies for the identified variants were unavailable. Nevertheless, the high prevalence of PID-associated gene variants in our ALL patients suggests that PID-associated germline mutations are important in hematologic malignancies in Koreans, especially ALL. However no comprehensive study of the prevalence of cancer or lymphoma/leukemia among Korean PID patients has been done. There is not even a national registry for PID in Korea. One large study of PID in Korea observed 152 PID patients (<19 years) from 2001 to 2005 [38]. The most common diseases were antibody deficiencies (such as congenital agammaglobulinemia or IgA deficiency) and chronic granulomatous disease, though one patient died from lymphoma [38]. Further study of PID and the risk of malignancies is needed because of the relatively high prevalence of genetic alterations association with PID that we found in Korean ALL patients.

In addition to gene variants associated with hematologic/immunologic disorders, we also identified various pathogenic or likely pathogenic variants in this study. Genes associated with bilirubin metabolism (*UGT1A1*), thrombophilia (*THBD*), thalassemia (*HBD*), platelet glycoprotein IV deficiency (*CD36*), and medulloblastoma (*PTCH2*), among others, were identified in this study. As reported in previous studies, variants not directly associated with hematologic malignancies are frequently identified incidentally [7, 24]. The genes/diseases identified are representative of common genetic diseases in this ethnic group. The clinical significance of these variants is the diagnosis of unexpected co-morbid genetic diseases and supporting the management of specific complications [7].

Our study has several limitations. First, we enrolled a relatively small number of cases. We enrolled few or no patients with ETP, hypodiploid ALL, and adult ALL. Second, skin fibroblasts were not used in our search for germline mutations. Third, we did not perform a familial study or a clinical or physical investigation of the identified germline variants.

Conclusions

In conclusion, using targeted NGS, we found recurrent somatic alterations (sequence variants and CNVs) in Korean ALL patients. The somatic alteration results enabled us to perform a comprehensive genetic analysis. The presumed diagnosis of clinically important sub-types of ALL, such as *BCR-ABL 1*-like ALL and ETP, was also enabled by targeted NGS. We identified significant germline mutations in Korean ALL patients. In addition to well-known predisposition genes, we identified variants associated with PID in 9.7% of our Korean ALL patients. Taken together, the results of our study suggest several important points: 1) paired tumor–normal matched sample analyses are recommended to discriminate between somatic and germline mutations; 2) gene panels must include genes associated with predisposition, in addition to recurrent somatic genes; 3) genes associated with PID are recommended for the gene panel, in addition to genes known to predispose patients for hematologic malignancies.

Abbreviations

AD: autosomal dominant; ALPS: Autoimmune lymphoproliferative syndrome; AR: autosomal recessive; B-ALL: B-cell precursor acute lymphoblastic leukemia/lymphoma; CNVs: copy number variations; CR: complete remission; dbSNP: Single Nucleotide Polymorphism database; DC: dyskeratosis congenital; DS: Down syndrome; ETP-ALL: early T-cell precursor ALL; ExAC: Exome Aggregation Consortium; F: female; FISH: fluorescence *in situ* hybridization; HGMD: Human Gene Mutation Database; iAMP21: intrachromosomal amplification of chromosome 21; M: male; NGS: next generation sequencing; NOS: Not Otherwise Specified; OS: overall survival; RFS: relapse-free survival; T-ALL: T-cell precursor acute lymphoblastic leukemia/lymphoma; WHO: World Health Organization.

Declarations

Ethics approval and consent to participate

This study was performed in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The Institutional Review Board at Samsung Medical Center approved this study (IRB No. 2015-11-053).

Consent for publication

All patients provided written informed consent for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors have declared no conflicts of interest.

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Authors' contributions

SHK and STL came up with the idea for, and designed and planned the study. SYS and HL acquired the data and analyzed the data. SYS and LST interpreted the results. JRC, CWJ, HHK and SHK advised about overall study. SYS drafted the report. All authors read and approved the final manuscript.

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Tables

Table 1. Clinical characteristics of 93 ALL patients

		Children	Adults
Sex	Male	35	10
	Female	30	18
Diagnosis	B-ALL, NOS	25	8
	B-ALL with t(9;22)(q34.1;q11.2); <i>BCR-ABL1</i>	2	11
	B-ALL with t(v;11q23.3); <i>KMT2A</i> rearranged	4	1
	B-ALL with t(12;21)(p13.2;q22.1); <i>ETV6-RUNX1</i>	9	
	B-ALL with hyperdiploidy	16	1
	B-ALL with t(1;19)(q23;p13.3); <i>TCF3-PBX1</i>	2	2
	T-ALL	7	3
Cytogenetic Risk Group (B-ALL)	Early T-cell precursor acute leukemia		2
	Good	26	2
	Intermediate	26	9
	High	6	12
Total		65	28

(1) low risk—*ETV6-RUNX1* and high hyperdiploidy (51–65 chromosomes); (2) intermediate risk—*TCF3-PBX1*, IGH translocations, B-other (none of these established abnormalities); (3) high risk—*BCR-ABL1*, *KMT2A* translocations, near haploidy (30–39 chromosomes), low hypodiploidy (fewer than 30 chromosomes), *iAMP21*, *TCF3-HLF*

Table 2. Germline pathogenic or likely pathogenic variants identified in Korean ALL patients

ID	Sex/ Age	Diagnosis	Gene	Accession	Nucleotide	Amino acid	%Variant	Syndrome	Inheritance
ALL0009	Female/51	B-ALL, NOS	<i>TP53</i>	NM_000546.5	c.733G>A	p.Gly245Ser	40.3	Li-Fraumeni	AD
ALL0069	Male/16	B-ALL with BCR-ABL	<i>TINF2</i>	NM_001099274.1	c.1130-1G>C		45.1	Dyskeratosis congenita	AD
ALL0071	Female/2	B-ALL with hyperdiploidy	<i>CTC1</i>	NM_025099.5	c.2249dup	p.Gly751ArgfsTer40	47.0	Dyskeratosis congenita	AR
ALL0021	Male/2	B-ALL with hyperdiploidy	<i>LIG4</i>	NM_002312.3	c.1271_1275del	p.Lys424ArgfsTer20	42.6	Ligase IV	AR

AD autosomal dominant, AR autosomal recessive.

Table 3. Thirty germline pathogenic or likely pathogenic variants (excluding genes associated with 23 syndromes)

ID	Age/Sex	Gene	Accession	Nucleotide	Amino acid	%Variant	Global (ExAC)	Korean (KRGDB)
ALL0007	56/F	<i>THBD</i>	NM_000361.2	c.-151G>T		52.2	0.0004	0.0064309
ALL0009	51/F	<i>HOXA10</i>	NM_018951.3	c.81C>G	p.Asn27Lys	49.6	0.00009896	0.0008039
ALL0009	51/F	<i>JAK3</i>	NM_000215.3	c.1503G>T	p.Gln501His	51.3	0.00002825	0.0032154
ALL0011	63/M	<i>PTCH2</i>	NM_001166292.1	c.1172_1173del	p.Ser391Ter	49	0.0003	
ALL0011	63/M	<i>ADA</i>	NM_000022.2	c.715G>A	p.Gly239Ser	50.8	0.00003766	
ALL0011	63/M	<i>MEFV</i>	NM_000243.2	c.1508C>G	p.Ser503Cys	50.5	0.0001	0.0008039
ALL0030	8/F	<i>ANK1</i>	NM_001142446.1	c.3022G>A	p.Glu1008Lys	50.6	0.00007644	0.0032154
ALL0035	58/F	<i>CD36</i>	NM_001001547.2	c.332_333delCA	p.Thr111SerfsTer22	48.5	0.0011	
ALL0047	13/M	<i>VWF</i>	NM_000552.3	c.5026C>T	p.Gln1676Ter	49.8		
ALL0063	59/F	<i>THBD</i>	NM_000361.2	c.-151G>T		47.4	0.0004	0.0064309
ALL0070	19/M	<i>HBD</i>	NM_000519.3	c.-127T>C		48.2		0.0040193
ALL0074	10/M	<i>EPB42</i>	NM_000119.2	c.424G>A	p.Ala142Thr	45.2	0.00008475	0.0024116
ALL0090	3/F	<i>JAK3</i>	NM_000215.3	c.1503G>T	p.Gln501His	52.3	0.00002825	0.0032154
ALL0077	10/M	<i>TYK2</i>	NM_003331.4	c.209_212del	p.Cys70SerfsTer21	40.2	0.00006591	
ALL0006	65/M	<i>CD36</i>	NM_001001547.2	c.332_333del	p.Thr111SerfsTer22	39.6	0.0011	
ALL0044	2/F	<i>USH2A</i>	NM_206933.2	c.14835del	p.Val4946TrpfsTer4	48.2		
ALL0003	72/F	<i>UGT1A1</i>	NM_000463.2	c.1091C>T	p.Pro364Leu	48.6	0.0014	0.016881
ALL0053	2/M	<i>IL12RB1</i>	NM_005535.1	c.1897G>T	p.Glu633Ter	50.7	0.00001967	
ALL0089	42/M	<i>RELN</i>	NM_005045.3	c.8352_8355dup	p.Trp2786GlufsTer13	47.5		
ALL0088	11/F	<i>EPB42</i>	NM_000119.2	c.1772del	p.Phe591SerfsTer54	49.4	0.000009416	
ALL0088	11/F	<i>PTCH2</i>	NM_001166292.1	c.1172_1173del	p.Ser391Ter	51.7	0.0003	
ALL0032	2/F	<i>FAT1</i>	NM_005245.3	c.10331A>G	p.Asn3444Ser	52.1	0.00006638	0.0040193
ALL0001	8/M	<i>CD36</i>	NM_001001547.2	c.332_333delCA	p.Thr111SerfsTer22	49.4	0.0011	
ALL0004	55/F	<i>UGT1A1</i>	NM_000463.2	c.1091C>T	p.Pro364Leu	51.7	0.0014	0.016881
ALL0020	0/M	<i>ITGA2B</i>	NM_000419.3	c.2602-3C>G		60.2		
ALL0022	12/F	<i>PDGFRB</i>	NM_002609.3	c.2053C>T	p.Arg685Cys	46.6	0.0002	0.0136656
ALL0023	3/M	<i>CSF1R</i>	NM_005211.3	c.2906_2909dupATCA	p.Phe971SerfsTer7	34.5	0.00005759	
ALL0024	18/F	<i>HBD</i>	NM_000519.3	c.-127T>C		46.2		0.0040193
ALL0057	3/F	<i>SLC4A1</i>	NM_000342.3	c.388G>A	p.Gly130Arg	50.7	0.0002	0.0032154
ALL0060	40/M	<i>LPIN2</i>	NM_014646.2	c.480_483del	p.Lys160AsnfsTer23	44		

ExAC Exome Aggregation Consortium database, F female, KRGDB Korean Reference Genome Database, M male.

Table 4. Comparison of FISH and NGS results for *CDKN2A/B* deletion/duplication

		Next generation sequencing			
Fluorescence <i>in situ</i> hybridization		deletion	duplication	normal	Total
	deletion	20		1	21
	duplication		1	5	6
	normal	9		56	65
	not tested	1			1
Total	30	1	62	93	

Agreement rate: 83.7%

Table 5. *IKZF1* alteration cases

ID	Sex	Age group	Diagnosis	Bone marrow transplant	<i>IKZF1</i>	<i>PAX5</i>	Relapse	Clinical outcome
LL0003	F	adult	B-ALL with <i>BCR-ABL1</i>		deletion	deletion		
LL0004	F	adult	B-ALL with <i>BCR-ABL1</i>		deletion	deletion		expired
LL0010	F	adult	B-ALL with <i>BCR-ABL1</i>	Allogenic	deletion			
LL0011	M	adult	B-ALL, NOS		deletion		Yes	expired
LL0024	F	child	B-ALL, NOS		deletion		Yes	
LL0026	M	child	B-ALL, NOS	Allogenic	deletion		Yes	expired
LL0028	F	adult	B-ALL, NOS	Allogenic	deletion		Yes	expired
LL0047	M	child	T-ALL (ETP)	Allogenic	missense mutation		Yes	expired
LL0049	F	adult	B-ALL, NOS	Allogenic	deletion	deletion		
LL0051	M	child	B-ALL, NOS		deletion	deletion		
LL0062	M	adult	B-ALL with <i>BCR-ABL1</i>		deletion			
LL0064	F	adult	B-ALL with <i>BCR-ABL1</i>	Allogenic	deletion			
LL0065	F	adult	B-ALL, NOS	Allogenic	frameshift mutation			
LL0069	M	child	B-ALL with <i>BCR-ABL1</i>	Allogenic	deletion			
LL0083	F	adult	B-ALL with <i>BCR-ABL1</i>		deletion	deletion		

female, M male.

Additional File Legends

Additional file 1

Supplementary Figures

Figure S1. Workflow for the NGS data analysis.

Figure S2. *CASP10* gene deletion detected by the NGS CNV analysis in a patient with B-ALL.

Figure S3. Somatic sequence variants in ALL patients.

Figure S4. Somatic sequence variants by cytogenetic group.

Figure S5. Somatic gene CNVs in ALL patients.

Figure S6. Somatic gene CNVs by cytogenetic group.

Supplementary Tables

Table S1. Genes included in the hematologic cancer panel.

Table S2. Thirty germline pathogenic or likely pathogenic variants (excluding genes associated with 23 syndromes).

Table S3. Somatic sequence variants identified in Korean ALL patients.

Table S4. Somatic sequence variants by pathway.

Table S5. Somatic CNVs by pathway.

Figures

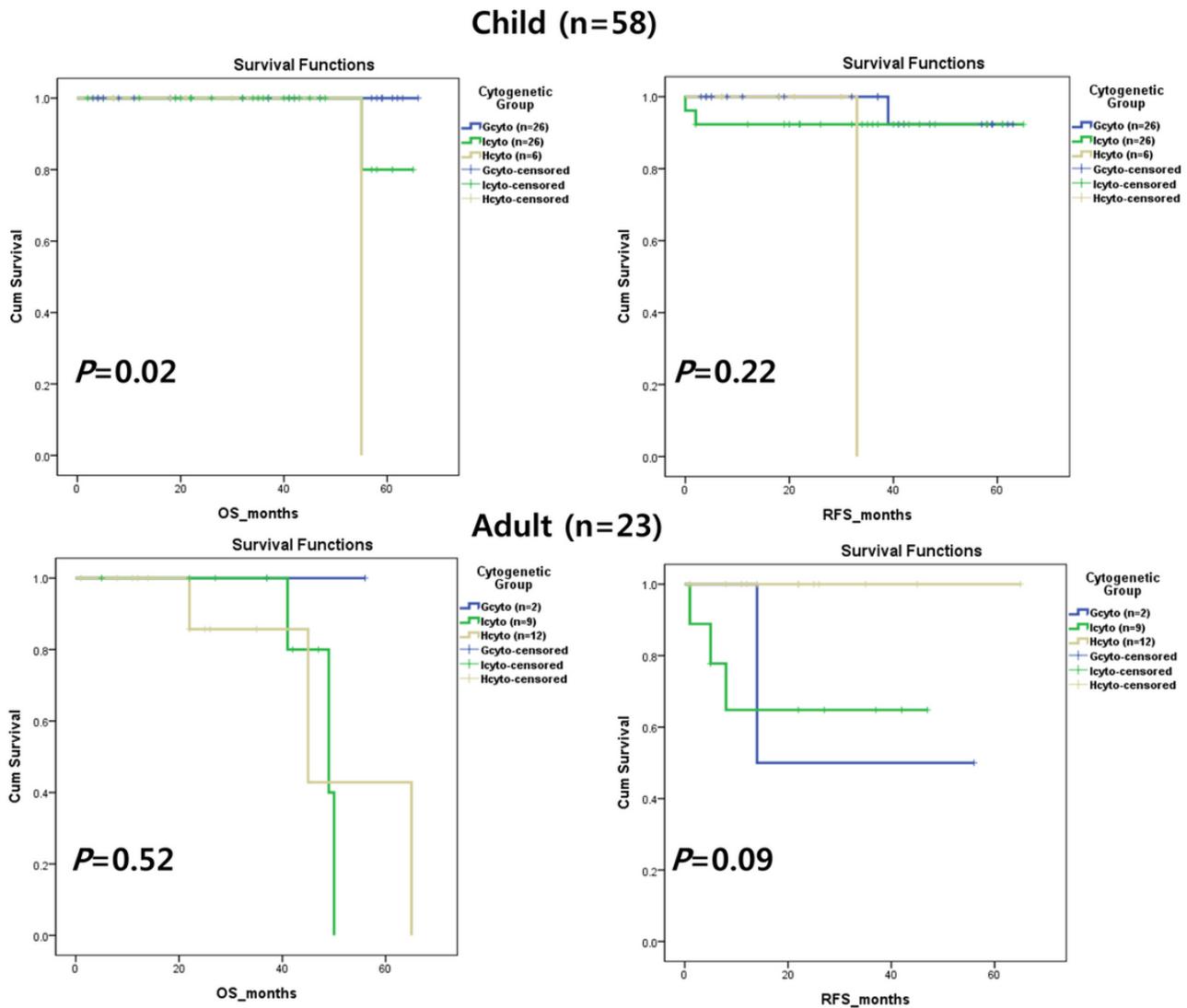


Figure 1

Clinical outcomes by cytogenetic group.

Child (n=52, Gcyto=26 and Icyto=26)

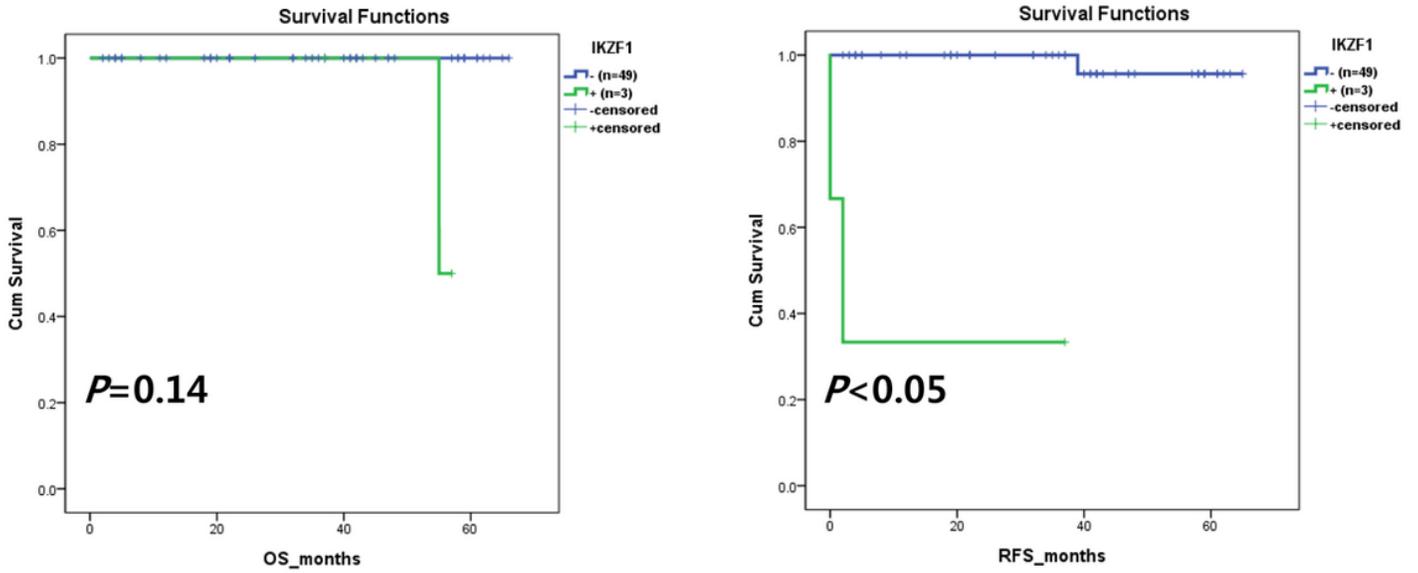


Figure 2

Clinical outcomes by IKZF1 alteration.

Supplementary Files

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