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REVIEW

Genomic and pharmacogenetic studies of childhood acute lymphoblastic leukemia

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Abstract **With the cure rate of childhood acute lymphoblastic leukemia (ALL) approaching 90%, further improvement in the treatment outcome and quality of life of patients will require better understanding of the mechanisms of drug resistance, identifying new leukemic cell genetic lesions that are amendable to available target therapy, and optimizing treatment based on host pharmacodynamics and pharmacogenomics. Deeper characterization of leukemic cell genetic abnormalities has discovered new subtypes of leukemia such as early T-cell precursor ALL and Philadelphia chromosome-like ALL, and identified many genomic alterations that have diagnostic, prognostic, or therapeutic implications. In this regard, several novel fusion transcripts are responsive to ABL tyrosine kinase inhibitors and potentially to JAK inhibitors. Genome-wide analyses have also unraveled the role of inherited cancer predisposing genes and small nucleotide polymorphisms of several genes in the development of childhood ALL. These advances promise to lead to more sophisticated personalized treatment strategies in the near future.**

Keywords acute lymphoblastic leukemia; genomics; pharmacogenetics; pharmacogenomics

Introduction

The 5-year event-free survival rates of childhood acute lymphoblastic leukemia (ALL) have improved to more than 80% in contemporary clinical trials [1], and the successful omission of carcinogenic treatment such as prophylactic cranial irradiation has pushed the cure rate to 90% in some studies by avoiding the late development of second neoplasm [2]. In parallel to the therapeutic gain, the advent of genome-wide analysis and the next generation sequencing to analyze the genome, transcriptome and epigenome of the leukemic cells has revised the genetic classification of ALL by identifying new ALL subtypes (Table 1), and similar studies of germline DNA from host normal cells have provided new insights into leukemogenesis, mechanisms of drug resistance, and genomic determinants of drug responsiveness and toxicities [3–5]. With the development of personalized targeted therapy based on these laboratory discoveries, we can expect further advances in the treatment outcome and quality of life of children with ALL in the coming decade. Some of the recent advances in leukemic cell genomic and pharmacogenetic studies that were identified by a search of Medline and PubMed of English literature published between 2009 and 2014, and can be readily translated into improved patient care are reviewed here.

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Table 1 Estimated frequency of specific subtypes of ALL by race in patients treated at St. Jude Children's Research Hospital between 2000 and 2007

	White/Non-black (%)	Black (%)
Hyperdiploidy>50	28.2	11.4
ETV6-RUNX1	18.6	21.5
BCR-ABL1-like	8.8	3.8
TCF3-PBX1	4.1	13.9
ERG	3.1	1.3
Hypodiploidy	2.6	–
BCR-ABL1	1.7	3.8
Rearranged *MLL*	1.4	3.8
CRLF2 (not *BCR-ABL1*-like)	1.7	1.3
Other B	16.0	20.3
T-cell	12.6	12.7
Early T-cell precursor	1.2	6.3

Genomic landscape of high-risk leukemia subtypes

Early T cell precursor ALL

ALL has traditionally been classified into T cell and B cell phenotypes. Recent studies have identified a high-risk immature T cell subtype, termed "early T-cell precursor" (ETP) ALL, which is characterized by a distinct immunophenotype (positive cytoplasmic CD3, weak or negative CD5, and negative CD1a and CD8 expression with aberrant expression of myeloid and/or stem cell markers), a profile reminiscent of the murine ETP at the earliest stage of thymic T cell maturation that retains lineage plasticity [6]. While having a distinct gene expression profile and immunophenotype, ETP cases lack a consistent chromosomal abnormality or genetic alterations common in T cell ALL (e.g., *NOTCH* mutations). Surprisingly, whole genome sequencing also failed to show a common structural rearrangement or sequencing mutation [7]. However, the majority of ETP cases harbored mutations in three pathways: loss-of-function mutations in genes encoding regulators of hematopoietic development (e.g., *GATA3, IKZF1, RUNX1, ETV6*), activating mutations in cytokine receptor and Ras signaling (e.g., *IL7R, NRAS, KRAS, FLT3, JAK1, JAK3*), and inactivating mutations targeting chromatin modification or epigenetic regulators (e.g., the polycomb repressor complex 2, *SETD2, EP300*). These findings suggest that the addition of myeloid-directed or pathway-directed therapies, such as JAK inhibitors or epigenetic modifiers might improve the clinical outcome of patients with this ALL subtype.

Philadelphia chromosome-like (BCR-ABL1-like) ALL

In 2009, using single-nucleotide-polymorphism (SNP) microarrays, transcriptional profiling and resequencing, two groups of investigators independently identified a novel high-risk subtype of B-ALL that exhibits a gene expression profile similar to that of Philadelphia chromosome (Ph)-positive ALL with a high frequency of deletions and less commonly sequence mutations of *IKZF1*, but lacks the BCR-ABL1 fusion protein [8,9]. This entity of "Ph-like" or "*BCR-ABL1*-like" cases occurred in approximately 10% of children with ALL, and was characterized by resistance to asparaginase and daunorubicin, high level of minimal residual disease at the end of induction and overall poor outcome [8,9]. While several studies confirmed the poor prognosis of this subtype of ALL [10,11], our recent study showed that minimal residual disease-based risk-directed therapy can yield a high rate of event-free survival and survival [12]. Overall, 92.5% of patients with Ph-like ALL as compared to 95.1% of other B-ALL patients in our study enjoyed 5-year overall survival. Importantly, 40% of Ph-like patients were treated with low-intensity chemotherapy because they had favorable clinical or biological features and no minimal residual disease at the end of 6-week remission induction. On the other hand, 15% of the Ph-like patients as opposed to 4.3% of the other B-ALL patients underwent allogeneic hematopoietic stem cell transplantation because of their high levels of minimal residual disease (1% or more) at the end of remission induction [12]. Retrospective studies of 25 patients with available cell samples demonstrated that six had fusion transcripts responsive to ABL tyrosine kinase inhibitors [12]. Together with the anecdotal reports of Ph-like ALL cases with ABL1-class rearrangements who had profound and lasting responsiveness to the addition of ABL tyrosine kinase inhibitors to the conventional chemotherapy [13,14], these findings suggest that a substantial proportion of Ph-like ALL cases could be spared from intensive chemotherapy or allogeneic transplantation with the additional tyrosine kinase inhibitor therapy.

Because the details on the frequency, the spectrum of underlying genetic alterations, and the potential for targeted therapy of Ph-like ALL have been lacking, we recently conducted a large study of 1725 children, adolescents and young adults with B-ALL, including 264 patients with Ph-like ALL [15]. We showed that Ph-like ALL was common, with increasing frequency with age to over 27% in young adults, and was associated with very poor outcome when treated with conventional therapy, the intensity of which was not based on the levels of minimal residual disease. Using genome-wide sequencing approaches in studying 154 Ph-like ALL cases, kinase activating alterations were identified in 91% of the cases. The genetic basis was complex, with 35 rearrangements targeting 13 cytokines, cytokine receptors and tyrosine kinases, but the majority converged on a limited number of signaling pathways, including "ABL-class" rearrangements targeting *ABL1*, *ABL2*, *CSF1R* and *PDGFRB* that were sensitive to imatinib and dasatinib; *EPOR* and *JAK2* rearrangements sensitive to JAK inhibitors (e.g., ruxolitinib); *CRLF2* rearrangements, frequently with concomitant activating *JAK* point mutations that could potentially be sensitive to JAK inhibitors; and other *JAK-STAT* activating mutations and deletions such as those involving *IL7R*, *FLT3* and *SH2B3*. In a separate cohort of 34 B-ALL patients with high risk features or poor response to therapy, 24 of 28 patients (86%) tested with a low-density gene expression array had Ph-like ALL and 22 of them had targetable kinase fusions involving *ABL1*, *ABL2*, *CRLF2*, *JAK2*, or *PDGFRB*. Of the 12 patients treated with tyrosine kinase inhibitors, 11 had rapid and durable responses.

Hypodiploid ALL

Hypodiploid ALL cases with less than 45 chromosomes have been associated with a poor prognosis [16–19] but

their clinical characteristics and genetic basis have not been well defined. Cytogenetically, hypodiploid cases can be subdivided into 4 groups: near-haploid with 23 to 29 chromosomes, low-hypodiploid with 33 to 39 chromosomes, high-hypodiploid with 40 to 43 chromosomes, and hypodiploid with 44 chromosomes [19]. Among patients with hypodiploid ALL, those with near-haploidy or low-hypodiploidy have particularly poor outcome [18,19]. In a study of genomic profiling of 126 hypodiploid ALL cases with whole genome and exome sequencing of a subset of 40 cases, distinct submicroscopic genetic alterations were observed in each subtype [20]. Near-haploid cases had a high frequency of activating *Ras* signaling pathway mutations (particularly novel deletions in *NF1*) and alterations of lymphoid transcription factor gene *IKZF3* (encoding AIOLOS), and low-hypodiploid cases *IKZF2* (encoding HELIOS) and *RB1* mutations. Remarkably, there was a near universal presence of *TP53* mutations in low-hypodiploid ALL, which were in non-tumor cells in over half of cases and shown to be inherited in a kindred with other non-ALL tumor (e.g., glioblastoma multiforme). Both near-haploid and low-hypodiploid ALL leukemic cells had activation of the phosphoinositide 3-kinase (PI3K) and were sensitive to PI3K/mTOR inhibitors, suggesting that these inhibitors should be explored in patients with hypodiploid ALL. Of interest, in a recent study of 29 adults with low-hypodiploid ALL, *TP53* mutations were found in 27 cases (93%), of whom 26 lost the second *TP53* allele due to monosomy 7 [21]. Hence, *TP53* mutation is a hallmark of low-hypodiploid ALL.

Pharmacogenetic studies of inherited genetic determinants of leukemogenesis

Inherited predispositions to childhood ALL via highly penetrant mutations are uncommon, including children with constitutive trisomy 21 (Down's syndrome), inherited germline *TP53* (in low-hypodiploid ALL) [20], and inherited mutations of *PAX5* (in familial ALL) [22]. Previous candidate gene approach has failed to yield reproducible results. Recent genome-wide association studies comparing single nucleotide polymorphisms between patients with ALL (usually DNA derived from remission sample) and ethnically matched controls have greatly improved our understanding of inherited genetic susceptibility of childhood ALL. These studies requires hundreds or thousands of patients and controls to obtain robust results (i.e., a P value $< 10^{-7}$) which should be validated in a second, independent cohorts of patients, and preferably confirmed by another group of investigators. Multiple statistical testing should be addressed by Bonferroni correction, and the results of association analyses should not only be reported as P values but also as estimates of an association measure (odds ratio or relative risk).

In 2009, two genome-wide association studies independently described the first two genes, *ARID5B* and *IKZF1*, with the strongest association with the risk of developing ALL in Caucasians [23,24]. Thus far, seven common allelic variants have been unequivocally associated with the development of childhood ALL (Table 2) [23–30], including the *PIP4K2A-BMI1* locus identified for the first time in multi-ethnic populations [26]. The inclusion of non-European subjects in this study not only improved the statistical power of detecting association signal at the *PIP4K2A* variants, but also revealed striking differences in the prevalence of these genetic risk variants among groups of different ancestries and their contribution to racial and ethnic differences in the incidence of ALL.

Notably, several germline susceptibility loci are also targeted by somatic genetic alterations in leukemia blasts (*IKZF1*, *CDKN2A/CDKN2B*) [27], suggesting that inherited and acquired genetic variations may act synergistically in the leukemogenesis of childhood ALL. To this end, some germline susceptibility loci were associated with specific genetic subtypes of ALL: the *ARID5B* gene in hyperdiploid ALL [23,24], the *TP63* and *PTPRJ* genes in the t(12;21)/*ETV6-RUNX1* ALL [28], and the *GATA3* gene in Ph-like ALL [29]. Of interest, the same *GATA3* variants were associated with not only ALL susceptibility but also poor outcome [29,30], illustrating interactions between genetic variations in the host and those in the cancer cells.

Genomic determinants of drug resistance and toxicities

Advances in technologies to interrogate inherited and acquired genome variations have accelerated the discovery of pharmacogenomic determinants of inter-patient differences in treatment response and outcome. Early microarray studies have linked the expression of a relatively small number of genes in leukemia cells with *de novo* sensitivity to glucocorticoids, vincristine, asparaginase and daunorubicin [31], chemotherapy cross-resistance [32], and treatment response to up-front high-dose methotrexate [33]. More recent genome-wide association studies have related inherited genome variants to drug disposition, response to remission induction, or relapse [34], and identified germline *SCLO1B1* variants affecting methotrexate clearance [35,36]. Ongoing genome-wide interrogation of germline and somatic DNA variations (genetic and epigenetic) should lead to novel treatment approaches to enhance the effectiveness of treatment.

Genome-wide approaches have disclosed novel mechanisms and new risk factors of drug toxicities, such as *HLA-DRB1*07:01* in association with the development of anti-asparaginase antibody and asparaginase hypersensitivity [37], *SLCO1B1* with delayed methotrexate clearance [38,39], *PACSIN2* with mercaptopurine-related

Table 2 Genome-wide association of studies of susceptibility of childhood acute lymphoblastic leukemia

Study group	Ethnic study population	Sample size (*n*)	Susceptibility loci	Comment	References
COG, SJCRH	Caucasian	441	*ARID5B* *IKZF1*	*ARID5B* associated with hyperdiploid ALL	Treviño *et al*. [23]
UKCCS, MRS	Caucasian	907	*ARID5B* *IKZF1* *CEBPE*	*ARID5B a*ssociated with hyperdiploid ALL	Papaemmanuil *et al.* [24]
UK, BFM, Spain, Hungary,Canada	Caucasian	3293	*ARID5B* *IKZF1* *CEBPE* *CDKN2A*	*CDKN2A* associated with both B- and T-ALL	Sherborne *et al*. [25]
COG, SJCRH	Caucasian, Black, Hispanic	2450	*ARID5B* *IKZF1* *CEBPE* *CDKN2A* *BMI1-PIP4K2A*	The number of risk alleles associated positively with the risk of ALL	Xu *et al*. [26]
AIEOP, BFM, COALL	Caucasian	1370	*TP63* *PTPRJ*	*TP63* and *PTPRJ* associated with *ETV6-RUNX1*-rear ranged ALL	Ellinghaus *et al.* [28]
COG, SJCRH	Caucasian, Black, Hispanic	682	*GATA3*	*GATA3* associated with Phila delphia chromosome-like ALL and poor prognosis	Perez-Andreu *et al.* [29]
UKCCS, MRC UKALL, BFM	Caucasian	3107	*ARID5B* *IKZF1* *CEBPE* *CDKN2A* *BMI1-PIP4K2A* *GATA3*	*GATA3* associated with increased risk of ALL risk and poor prognosis	Migliorini *et al*. [30]

Abbreviations: AIEOP, Associazione Italiana di Ematologia Pediatrica Group; ALL, acute lymphoblastic leukemia; BFM, Berlin-Frankfurt-Münster; COG, Children's Oncology Group; COALL, Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia; MRC UKALL, Medical Research Council UK Acute Lymphoblastic Leukemia; UK, United Kingdom; SJCRH, St. Jude Children's Research Hospital; UKCCS, UK Childhood Cancer Study.

gastrointestinal toxicity [40], *CRHR1* with corticosteroid-associated osteopenia [41], *PAI-1* with dexamethasone-related osteonecrosis [42], *HACS3* with anthracycline-related cardiomyopathy [43], and *CEP72* with vincristine-induced neuropathy [44] (Table 3). Validation of these associations is important to determine their clinical relevance. The underlying genomic risk factors for some toxicities are likely to differ by treatment regimens. For example, asparaginase may potentiate osteonecrosis risk of glucocorticoids, partly because of its effects on lipid homeostasis [45], and partly because of its effects on glucocorticoid disposition [46]. Dosage may also play an important role. For example, inherited variants in *HAS3* is associated with anthracycline-induced cardiomyopathy only in patients who received higher cumulative doses of anthracycline [43], and *CEP72* polymorphism is associated with vincristine neuropathy only at lower doses because virtually all patients would develop the adverse effect at high doses [44].

Pharmacogenomics-guided therapy

Patients with an inherited deficiency of thiopurine methyltransferase, an enzyme which catalyzes S-methylation of thiopurines to inactive methylated metabolites, are at increased risk of mercaptopurine-induced toxicities because more parent drug is converted to active metabolites. Prospective identification of patients with the enzyme deficiency to allow preemptive dosage reduction of mercaptopurine may reduce the likelihood of acute myelosuppression without compromising disease control [47]. In theory, this approach could also decrease the risk of secondary myeloid malignancy induced by mercaptopurine [48]. Guidelines for thiopurine therapy (updates at http://www.pharmgkb.org), based on the association between clinical effects and genotype/phenotype of the enzyme, has been developed by the Clinical Pharmacogenetics Implementation Consortium [49]. A recent study of Korean patients with inflammatory bowel diseases showed that a common missense variant in *NUDT15* (encoding p. Arg139Cys) was strongly associated with thiopurine-induced leukopenia, an association also replicated in patients of European descent albeit rare [50]. This finding partly explains the higher frequency of thiopurine-induced leukopenia observed in Asians than in individuals of European descent.

Ongoing pharmacogenomics studies hold great promise to yield additional genetic polymorphisms that can be used to further individualize the dosages of other drugs. To this

Table 3 Selected examples of genomic determinants of drug toxicities

Drug	Genes	Comments	References
Asparaginase	*HLA-DRB1*07:01*	*HLA-DRB1*07:01*-encoded protein is associated with higher incidence of the development of anti-asparaginase antibody and a higher frequency of asparaginase hypersensitivity, probably through its high-affinity binding to asparaginase epitopes.	Christian *et al.* [37]
Methotrexate	*SLCO1B1*	Methotrexate clearance is associated with polymorphisms of *SLCO1B1* which encode a hepatic solute carrier organic anion transporter that mediates disposition of many medications including methotrexate.	Ramsey *et al.* [38,39]
Mercaptopurine	*TPMT*, *PACSIN2*	Genetic polymorphisms in *TPMT* (thiopurine S-methyltransferase) are known to have a marked effect on mercaptopurine metabolism and toxicity. By modulating TPMT activity, polymorphism in *PACSIN2* (protein kinase C and casein kinase substrate in neurons protein 2) increases the severity of gastrointestinal toxicity associated with mercaptopurine therapy.	Stocco *et al.* [40]
Corticosteroids	*CRHR1*	Polymorphisms of *CRHR1* that encodes corticotropin-releasing hormone receptor-1 are associated with low bone density in male patients treated with corticosteroids, probably due to its effect on the release of corticotropin from the anterior pituitary, altering levels of circulating endogenous glucocorticoids.	Jones *et al*. [41]
Corticosteroids	*PAI-1*	A polymorphism of *PAI-1* that encodes plasminogen activator inhibitor-1 is associated with increased risk of dexamethasone-related osteonecrosis. It was speculated that high levels of PAI-1, by inhibiting fibrinolysis and resulting increased intraosseous venous pressure blocking blood flow to the bone, cause osteonecrosis.	French *et al*. [42]
Anthracycline	*HAS3*	A variant of hyaluronan synthase 3 gene is associated with increased risk of anthracycline-related cardiomyopathy, which could be due to inadequate remodeling or inadequate protection of the heart from reactive oxygen species after anthracycline treatment.	Wang *et al*. [43]
Vincristine	*CEP72*	A polymorphism of *CEP72* reduces expression of its encoded centrosomal protein 72kDa that functions as the major microtu bule-organizing center and regulates proper bipolar spindle formation and is associated with an increased risk of vincristine-induced neuropathy.	Diouf *et al.* [44]

end, we have already released the testing results for four genes into the electronic medical record for pre-emptive clinical implementation: *TPMT* (mercaptopurine, thioguanine, azathioprine use), *CYP2D6* (codeine, tramadol, oxycodone, amitriptyline, ondansetron, fluoxetine, paroxetine), *SLCO1B1* (simvastatin, potentially methotrexate) and *CYP2C19* (clopidogrel) [51]. Each result is coupled with an interpretive consult, and patients and the guardians are informed not only of the genotyping results but also the clinical relevance to the medication use.

Conclusions

Recent advances in the next-generation genome sequencing of leukemic cells have improved our understanding of the molecular basis of ALL by discovering new genes and pathways, refining classification schema with prognostic and therapeutic implications, and identifying new targets for therapeutic intervention. However, these studies are only works in progress and many more cases are needed to be examined to define the full landscape of genetic alterations. Because subclonal and *de novo* mutations may be important in disease progression and treatment failure [52], single-cell DNA sequencing should allow a more detailed understanding of mutational heterogeneity and the development of drug resistance, leading to more rational approach to treatment [53].

Genome-wide association studies have disclosed a number of genes whose polymorphisms were associated with treatment response, toxicity or prognosis. The inherited susceptibility genes associated with the familial cancer syndromes or familial leukemia [20,22,54] should be thoroughly identified for the counseling purposes, and the role between common inherited variants and susceptibility of ALL [23–26,28,29] should be investigated in details for the potential development of preventive measures. Finally, the role of genetic alterations in the noncoding genome and epigenetic profiles in the development of ALL and treatment outcome remain to be explored. To this end, recent studies showed that different genetic subtypes of ALL have distinct DNA methylation signatures that correlate with gene expression profiles [55], and that T cell ALL has a high frequency of somatic mutations in epigenetic regulators [56]. Because epigenetics can influence chemo-resistance in ALL as manifested by increased global promoter methylation at relapse [57] and demethylating agents could restore chemo-

sensitivity in experimental models [58], incorporation of epigenetic therapies such as histone deacetylase inhibitors or demethylating agents to standard chemotherapy backbone should be tested.

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Compliance with ethics guidelines

Ching-Hon Pui declares that he has no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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