ACUTE LYMPHOBLASTIC LEUKEMIA

Inherited genetic variation in childhood acute lymphoblastic leukemia

Takaya Moriyama,[1,2] Mary V. Relling,[2] and Jun J. Yang[2]

[1]Department of Pediatrics, Mie University Graduate School of Medicine, Mie, Japan; and [2]Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN

Although somatically acquired genomic alterations have long been recognized as the hallmarks of acute lymphoblastic leukemia (ALL), the last decade has shown that inherited genetic variations (germline) are important determinants of interpatient variability in ALL susceptibility, drug response, and toxicities of ALL therapy. In particular, unbiased genome-wide association studies have identified germline variants strongly associated with the predisposition to ALL in children, providing novel insight into the mechanisms of leukemogenesis and evidence for complex interactions between inherited and acquired genetic variations in ALL. Similar genome-wide approaches have also discovered novel germline genetic risk factors that independently influence ALL prognosis and those that strongly modify host susceptibility to adverse effects of antileukemic agents (eg, vincristine, asparaginase, glucocorticoids). There are examples of germline genomic associations that warrant routine clinical use in the treatment of childhood ALL (eg, *TPMT* and mercaptopurine dosing), but most have not reached this level of actionability. Future studies are needed to integrate both somatic and germline variants to predict risk of relapse and host toxicities, with the eventual goal of implementing genetics-driven precision-medicine approaches in ALL treatment. (*Blood*. 2015;125(26):3988-3995)

Introduction

Acute lymphoblastic leukemia (ALL) is the most common cancer in children, accounting for 25% of all childhood malignancies.[1,2] Sentinel chromosomal abnormalities (translocations or aneuploidy) are characteristic of the majority of ALL cases, and recent genomic profiling of leukemic cells continues to broaden our appreciation of the complex genomic landscape of this disease.[3-6] These somatically acquired genomic aberrations are unique to ALL tumor cells; however, patients also carry inherited genetic variations (ie, germline variants) that are present in both normal and tumor cells. Although somatic genomic alterations have long been recognized as the hallmarks of ALL subtype classification, the last decade has shown that germline genetic variations are important determinants of interpatient variability in ALL susceptibility, drug response, and toxicities of ALL therapy (Table 1).

Common types of inherited genetic variations include single nucleotide polymorphisms (SNPs), insertions and deletions (gain or loss of short segments of sequence, indels), and structural variations (gain or loss of large segments of sequence; eg, copy number changes). Practically, nonmalignant cells from patients (eg, peripheral blood cells obtained during clinical remission) generally serve as the primary source of "germline" DNA. Recent advances in high-throughput genotyping technology enable agnostic screens of genetic variation across the entire human genome, with up to a few million genetic markers tested per patient. These "genome-wide" association studies, often referred to as GWASs do not rely on prior knowledge to focus on any subset of genes but, instead, systematically examine genetic variations in an unbiased fashion for their association with the phenotype of interest.[7]

Because of the large number of variants tested in GWASs, the required level of significance for association between a variant and a phenotype is generally set very high ($P < 5 \times 10^{-7}$) rather than the typical level of .05 for most power calculations.[8,9] Thus, it is not surprising that there is limited power to detect genotype-phenotype associations using genome-wide approaches, and only genomic variation with great impact (large effect sizes) can be expected in most ALL GWASs. For example, with a sample size of 1000 patients, at an α level of 5×10^{-7}, a frequency of the genomic variant in the population of 10%, and a phenotype that occurs in just 5% of patients (eg, such as central nervous system relapse of ALL or ALL therapy-related pancreatitis), in order to have 80% power to detect the genotype-phenotype association, the genomic variant would need to confer 4.5-fold higher risk of the trait than the wild-type or "normal" allele. For phenotypes or alleles that are less common, effect sizes would have to be even higher than 4.5-fold (or the sample size would need to be greater). Thus, in discovery studies, every effort must be made to minimize variation in nongenetic risk factors and to maximize sample size to improve the chance of observing associations between genomic variation and the phenotype of interest.

It should also be noted that commercial genotyping platforms that have been used in GWASs predominantly focus on relatively common genomic variants to achieve an even representation across all chromosomes, although with varying degrees of coverage and resolution.[10] Most of these variants are intronic and may not be directly functional; instead, they are in at least partial linkage with other variants that are likely biologically active.[11] As a result, findings from GWASs often require extensive follow-up studies to discover the true causal genetic variants underlying the GWAS signal. Although SNPs are the primary focus of GWASs, copy number variations can also be detected by most genome-wide SNP chip/arrays[12] (except for small indels; eg, promoter repeats in *TYMS*).

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Table 1. Examples of germline genetic variants associated with ALL susceptibility, treatment outcomes, and toxicities of ALL therapy

Gene	SNP ID	Study design	Phenotype	Sample size (N)	Odds ratio (95% CI)	*P*	Reference
ALL susceptibility							
ARID5B	rs7089424	GWAS	ALL risk	907	1.65 (1.54-1.76)	6.7×10^{-19}	27
	rs10821936	GWAS		441	1.91 (1.6-2.2)	1.4×10^{-15}	28
IKZF1	rs4132601	GWAS	ALL risk	907	1.69 (1.58-1.81)	1.2×10^{-19}	27
	rs11978267	GWAS		441	1.69 (1.4-1.9)	8.8×10^{-11}	28
CEBPE	rs2239633	GWAS	ALL risk	907	1.34 (1.22-1.45)	2.9×10^{-7}	27
CDKN2A	rs17756311	GWAS	ALL risk	2450	1.36 (1.18-1.56)	1.4×10^{-5}	30
PIP4K2A	rs7088318	GWAS	ALL risk	2450	1.40 (1.28-1.53)	1.1×10^{-11}	30
GATA3	rs3824662	GWAS	ALL risk	3107	1.31 (1.21-1.41)	8.6×10^{-12}	31
		GWAS	Risk for Ph-like ALL	511	3.85 (2.7-5.4)	2.2×10^{-14}	32
TP63	rs17505102	GWAS	Risk for *ETV6-RUNX1* ALL	1370	0.65 (0.52-0.75)	8.9×10^{-9}	39
Treatment outcome							
TPMT	rs1800462 rs1800460 rs1142345	Candidate gene	Minimal residual disease	814	0.34 (0.13-0.86)	.02	44
	rs1800460 rs1142345	Candidate gene	Relapse	601	0.36 (0.15-0.88)	.03	45
IL15	rs17007695	GWAS	Minimal residual disease	487	2.67 (1.53-4.68)	8.9×10^{-7}	55
PYGL	rs7142143	GWAS	Relapse	2535	3.61 (2.34-5.57)	6.7×10^{-9}	58
PDE4B	rs6683977	GWAS	Relapse	2535	1.41 (1.22-1.64)	5.1×10^{-6}	58
GATA3	rs3824662	GWAS	Relapse	781	1.43 (1.10-1.86)	.007	32
			Minimal residual disease	710	1.38 (1.03-1.83)	.039	
		GWAS	Relapse	2258	2.0 (1.71-3.66)	2.3×10^{-6}	31
Toxicities							
TPMT	rs1800462 rs1800460 rs1142345	Candidate gene	Thiopurine-induced myelosuppression	180	9.3 (3.58-24.27)	.007	68
NUDT15	rs116855232	GWAS	Thiopurine intolerance	657	*	8.8×10^{-9}	70
ACP1	rs12714403	GWAS	Glucocorticoid-induced osteonecrosis	362	5.6 (2.7-11.3)	1.9×10^{-6}	80
GRIA1	rs4958351	GWAS	Asparaginase allergy	485	1.75 (1.41-2.17)	3.5×10^{-7}	84
HLA-DRB1	*HLA-DRB1**07:01	Candidate gene	Asparaginase allergy	1870	1.64 (1.28-2.09)	7.5×10^{-5}	86
			Anti-asparaginase antibody	502	2.92 (1.82-4.80)	1.4×10^{-5}	
ASNS	rs3832526	Candidate gene	Asparaginase allergy	533	14.6 (3.6-58.7)	<.0005	87
			Asparaginase pancreatitis		8.6 (2.0-37.3)	.008	
CBR3	rs1056892	Candidate gene	Anthracycline-induced cardiomyopathy	487	1.79 (1.08-2.96)	.02	88
HAS3	rs2232228	GWAS	Anthracycline-induced cardiomyopathy	362	3.7 (1.3-10.2)	.05	89
CEP72	rs924607	GWAS	Vincristine-induced neuropathy	321	*	4.7×10^{-8}	90
SLCO1B1	rs11045879	GWAS	Methotrexate clearance	640	*	8.2×10^{-11}	104, 105
			Methotrexate-induced GI toxicity	206	16.4 (8.7-26.7)	.004	
		Candidate gene	Methotrexate clearance	115	*	.008	106
	rs4149056	Candidate gene	Methotrexate clearance	415	*	3.5×10^{-4}	50

CI, confidence interval; GI, gastrointestinal; GWAS, genome-wide association study; ID, identification; Ph, Philadelphia chromosome; SNP, single-nucleotide polymorphism.

Inherited genetic basis of ALL susceptibility

The risk of developing ALL is highest between 2 and 5 years after birth, with initiating sentinel somatic genomic lesions (eg, translocations) detectable at the time of birth in many cases.[13,14] This early disease onset suggests a strong inherited genetic basis for ALL susceptibility. Inherited genetic risk factors for cancer can be divided into 2 main classes: rare penetrant variants associated with a high risk (may be observed in families with multiple members affected by ALL) and common less-penetrant variants associated with a modestly increased risk of ALL (such as those observed in population studies of ALL risk).

Rare germline mutations and familial ALL

A number of inherited genetic variants have been identified in excess in rare cases of familial ALL. For example, 50% of children with low-hypodiploid ALL have germline *TP53* mutations characteristic of Li-Fraumeni cancer syndrome,[15] an autosomal dominant familial

cancer syndrome characterized by a range of other solid and brain tumors. Germline mutations of *PAX5*, which encodes a transcriptional factor required for B-cell differentiation, were also found in 2 unrelated kindreds, each of which had 5 family members develop ALL.[16] However, the vast majority of childhood ALL is not familial, and *TP53* or *PAX5* mutations represent a very small population attributable risk (ie, proportion of ALL cases that can be explained by these risk factors).

Common variants and susceptibility to childhood ALL

Common genetic variants influencing leukemia susceptibility can be identified by association studies comparing the frequency of variations in unrelated ALL cases vs controls (individuals not affected by ALL); variants overrepresented in cases may contribute to the risk of developing this disease (examples given in Table 1). There is an extensive body of work that examines the contribution of a number of "candidate" pathways (eg, carcinogen metabolism, folate metabolism, DNA repair) to ALL risk, but with oftentimes conflicting results. A recent meta-analysis summarized 47 studies of 25 polymorphisms in 16 genes and observed statistically significant ($P < .05$), albeit modest, associations with ALL susceptibility for 8 variants (eg, *CYP1A1*2A* and *XRCC1* G28152A).[17] However, it should be noted that the false-positive probability in this study was estimated at 20%. Similar pooled analyses subsequently confirmed the association for multiple variants in *CYP1A1* and *XRCC1*,[18,19] although with some variability by ancestry and age. Several epidemiology studies noted significant associations between infection and risk of ALL in children, pointing to potential roles of host immune defense in ALL etiology.[20-22] In fact, germline SNPs at the *HLA-DP* and *HLA-DOA* loci were associated with ALL susceptibility in admixed populations in the United States.[23,24] However, a comprehensive analysis of the major histocompatibility complex region in 824 B-ALL cases and 4737 controls of European genetic ancestry did not find statistically significant association signals in this genomic region after correcting for multiple testing.[25] Caution needs to be exercised when examining *HLA* variants, especially in diverse populations, because of the complex linkage disequilibrium and excessive diversity at these loci in different races and ethnic groups. Variants in *IL15*, *IL12A*, and other genes related to adaptive immunity were also reported to potentially predispose children to ALL, although further validation is warranted.[23,26]

The first pair of GWASs of childhood ALL susceptibility were published in 2009, independently identifying *ARID5B*, *IKZF1*,[27,28] and *CEBPE*[27] as genome-wide significant risk loci in children of European descent. Subsequent GWASs with larger sample sizes and/or greater population diversity discovered additional susceptibility variants in *CDKN2A*, *BMI1-PIP4K2A*, and *GATA3*.[29-32] Unlike candidate-gene studies, these GWAS hits have been repeatedly validated by subsequent reports.[33-40] Interestingly, genomic loci implicated by ALL susceptibility GWASs are often also targeted by somatic genomic aberrations in ALL cells. For example, *IKZF1*, an important transcription factor in all lymphoid lineages, is frequently deleted in ALL blast cells (particularly in high-risk ALL), which confers a poor prognosis.[4] Loss of *CDKN2A*/*CDKN2B* tumor suppressor genes also occurs in up to 40% of B-precursor ALL and contributes to cell cycle deregulation in leukemia.[3] However, there does not appear to be any cosegregation of germline ALL risk variants and somatic abnormalities involving the same gene, suggesting that inherited and acquired variations occur and function independently.

Of 6 genome-wide significant ALL risk loci, lead variants in *ARID5B*, *IKZF1*, *GATA3*, and *PIP4K2A* are significant regardless of genetic ancestry, whereas the effects of *CEBPE* and *CDKN2A* variants were more restricted to Europeans.[30,32,38] Also, frequencies of ALL risk alleles at *ARID5B*, *PIP4K2A*, and *GATA3* differ significantly by ancestry in a pattern that is consistent with racial differences in ALL incidence (Africans < Europeans < Hispanics), and are therefore likely to contribute to ancestry-related differences in ALL susceptibility.

ALL consists of subgroups with different genomic abnormalities, each of which may have distinct genetic susceptibility. Initial GWASs have already noted considerable differences in the effects of susceptibility variants by ALL molecular subtype. For example, an *ARID5B* variant is significantly overrepresented in ALL cases with hyperdiploid karyotypes and less so in children with T-cell ALL.[28,38] A *PIP4K2A* variant was also enriched in hyperdiploid ALL among B-cell ALL.[30,31,37] In populations of European descent, variants in the *TP63* gene were genome-wide significantly associated with the acquisition of the t(12;21) translocation in ALL.[39] Similarly, intronic variants in *GATA3* strongly influence the risk of developing Ph-like ALL and were also associated with the risk of relapse.[32] A contemporaneous GWAS also identified these *GATA3* SNPs overrepresented in childhood ALL cases with high-risk clinical features (older age and higher leukocyte count at diagnosis), although the Ph-like phenotype was not explicitly ascertained in this study.[31] These data collectively illustrate the complex interactions between genetic variations in the host (inherited) and those in ALL cells (acquired) and their unique contributions to disease pathogenesis and treatment outcomes.

It is fair to argue that these GWASs have produced unequivocal evidence for an inherited genetic basis of ALL susceptibility. However, the molecular mechanisms by which these variants are linked to ALL risk are largely unknown. For example, the vast majority of susceptibility variants identified are intronic, and their effects on gene functions are not clearly understood. In some instances (eg, rs3824662 in the *GATA3* gene),[32] the risk variant is located in a genomic region rife with enhancer elements active in hematopoietic tissues and is directly linked to *GATA3* transcription. Therefore, we posit that ALL risk loci identified by GWASs are likely to overlap with regulatory DNA elements in the genome, possibly influencing gene function by modulating transcription. Future functional studies are needed to describe the details of these molecular processes.

Germline genetic variation and ALL treatment outcome

Although the survival rates of childhood ALL increased significantly in the past few decades due to risk-directed therapy, there is still substantial variation in treatment response, with 15% of children with ALL experiencing relapse.[41] In fact, ALL relapse is the fifth most common cancer in children and a leading cause of death in this cancer. The interindividual variation in relapse risk can arise from both tumor- and host-related factors. Gene expression profiling and, more recently, whole-genome sequencing studies discovered tumor genetic features associated with outcome and drug resistance.[6,15,42,43] In parallel, there is increasing evidence that inherited genetic variations play important roles in determining patients' risk of relapse (Table 1).

Candidate genes related to response to ALL therapy

Inherited genetic variation can contribute to ALL treatment response by influencing host disposition of antileukemic agents, interactions between ALL and tumor, and tumor biology itself. In particular, it was widely hypothesized that variation in genes involved in antileukemic

drug metabolism would be associated with treatment outcome of ALL therapy. For example, patients with loss-of-function variants in the *TPMT* gene had significantly lower levels of minimal residual disease (MRD) compared with those with wild-type *TPMT* after 2 weeks of therapy including mercaptopurine.[44] In a subsequent study of 601 children treated on the Nordic Society of Paediatric Haematology and Oncology ALL-92 protocol, TPMT deficiency was associated with a lower risk of relapse, plausibly due to higher levels of active mercaptopurine metabolites in patients with defective TPMT.[45] In contrast, *TPMT* genotype was not predictive of hematologic relapse risk in the St Jude Children's Research Hospital (St Jude) Total Therapy XIIIB protocol, most likely because mercaptopurine dose was already individualized on the basis of *TPMT* status to achieve comparable exposure to active metabolites.[46] More recently, a 2.9-kb intronic germline deletion in the *BIM* gene was shown to alter the splicing pattern and consequently result in the loss of proapoptotic isoforms of BIM, required for glucocorticoid cytotoxicity in ALL.[47,48] This intronic deletion of *BIM* in ALL cells also conferred significant resistance to dexamethasone,[49] although the exact impact of this polymorphism on ALL relapse risk in patients remained unclear. Other candidate-gene studies have identified relapse risk variants in *MTHFR*, *TYMS*, *GSTM1*, and *ABCC4*, but the degree of association at these loci varied significantly among studies, plausibly due to differences in ALL treatment regimens.[50-54]

GWASs of ALL treatment outcome

In 2009, Yang et al reported one of the first GWASs of ALL treatment response in which the authors identified 102 SNPs associated with end-of-induction MRD in 487 children with newly diagnosed ALL on St Jude and Children's Oncology Group (COG) frontline clinical trials.[55] Twenty percent of the MRD-related SNPs were also associated with pharmacokinetics and pharmacodynamics of antileukemic agents, generally linking the same allele to MRD eradication and greater drug exposure. In particular, germline intronic variants in *IL15* were consistently associated with MRD in both cohorts; these SNPs positively regulate *IL15* expression, and higher *IL15* levels protect hematologic cancer cells from cytotoxic agents.[56] A recent independent report confirmed that autocrine and paracrine *IL15* signaling led to significant growth advantage of primary B-precursor ALL cells in vitro through induction of *STAT5*, *ERK1/2*, and to a lesser extent *PI3K* and *NF-κB* signaling.[57] A subsequent GWAS focused on relapse risk in 2535 children with ALL and discovered 134 relapse-related SNPs, of which 133 (99%) remained prognostic after adjusting for known relapse risk factors (ALL subtypes defined by tumor cytogenetics, age, and leukocyte count at diagnosis, and MRD).[58] The top-ranked hit in this study was an intronic variant in the *PYGL* gene, which was associated with a 3.6-fold higher risk of relapse ($P = 6.7 \times 10^{-9}$). Glycogen phosphorylase (PYGL) is a target of adenosine monophosphate, which plays a critical role in response to antileukemic agents such as mercaptopurine and methotrexate. Also notable was the highly significant association with relapse observed for *PDE4B* variants. Prior studies have already shown that inhibition of PDE4B induces apoptosis in chronic lymphoblastic leukemia and diffuse large cell lymphoma[59,60] and sensitizes cells to glucocorticoid-induced cell death.[61,62] In ALL, pharmacologic inhibition of *PDE4* results in growth suppression and dexamethasone sensitivity,[63] suggesting glucocorticoid response as a plausible mechanism by which *PDE4B* is linked to ALL relapse. In a more recent study of 34 000 preselected potentially clinically relevant SNPs in 778 European children with newly diagnosed ALL, the authors discovered 11 cross-validated SNPs associated with relapse risk.[64] Combined analyses of host genomic profiles, clinical presenting features, and MRD status further identified 3 distinct risk groups with highly divergent prognoses.

Germline genetic variants characteristic of Native American ancestry have been associated with increased risk of ALL relapse, explaining the inferior treatment outcome in children with ALL of self-declared Hispanic ethnicity.[65] Ancestry-related poor prognosis was abrogated by the addition of a single extra phase of chemotherapy (delayed intensification), pointing to the potential utility of treatment individualization based on germline genetic variants. In fact, the aforementioned susceptibility variants in *GATA3* for Ph-like ALL are significantly overrepresented in individuals with higher Native American genetic ancestry (characteristic of self-reported Hispanics), potentially contributing to ancestry-related differences in ALL relapse.[32] These *GATA3* variants were associated with MRD and relapse in 2 cohorts of children treated on COG frontline protocols, which was also true in >2000 children enrolled on the Berlin-Frankfurt-Munster clinical trials for newly diagnosed ALL.[31]

Taken together, both candidate-gene and genome-wide studies have identified inherited genetic variations related to interpatient variability in ALL treatment outcomes. However, the extent to which the effects of inherited germline variants on MRD and relapse are confounded by (or independent of) ALL tumor genetic factors is unclear, and integrated analyses including both germline and somatic genetic variations will hopefully provide comprehensive characterization of genetic risk factors for ALL relapse.

Pharmacogenomics of adverse effects of ALL therapy

Discovering the genomic basis for adverse effect phenotypes in ALL is complicated by the fact that all drug-induced phenotypes will be at least partly dependent on drug therapy; thus, it is critical to control for variability in drug exposure when conducting studies to elucidate the genomic basis of the adverse effect. Because relatively subtle differences among ALL regimens can have substantial impacts on the frequency and severity of adverse effects and because most ALL regimens differ from each other (eg, drugs used, doses used, combinations, and schedules), the power to detect genomic influences on adverse-effect phenotypes is diminished as each treatment group is added as a stratification variable, in that effective sample size decreases with each new grouping. Other covariates that must be included in analyses of how genotype variation may influence adverse-effect phenotypes include genomic ancestry and, often, age. Because collection of germline DNA has not been a routine component of many ALL trials, and not all ALL trials routinely capture adverse effects of therapy, the field is still in its infancy in terms of discovering genetic variants that are associated with ALL adverse effects.

Although some adverse effects (eg, myelosuppression) due to ALL therapy can be linked to a number of antileukemic agents, some can largely be linked to specific drugs. These include glucocorticoid-induced osteonecrosis, vincristine neuropathy, anthracycline cardiomyopathy, asparaginase-induced allergy and pancreatitis, and methotrexate-induced mucositis and neurotoxicity. There have been candidate-gene and genome-wide approaches to identify inherited variants that can explain some of the risk of these drug-specific adverse effects in ALL (Table 1). Interestingly, although myelosuppression can be caused by many agents, a substantial portion of myelosuppression during continuation therapy is due to a monogenic defect in *TPMT*,[66-68] which has led to the use of *TPMT* genetic testing

to modify starting dosages of thiopurines.[69] More recently, a coding variant in *NUDT15* has been reported to account for thiopurine intolerance, particularly in those with East Asian ancestry and of Hispanic ethnicity,[70,71] arguing that contemporary ALL treatment regimens (eg, drug dose) developed in populations of European descent may require modifications to be appropriate for non-European populations due to differences in genetic variations.

Glucocorticoids

Osteonecrosis is associated with glucocorticoid use. It has been hypothesized that several mechanisms can lead to the loss of blood supply to bone, which causes the ultimate phenotype, including in some cases thrombosis and hyperlipidemia. It is likely that additional treatment-related factors (eg, asparaginase)[72,73] play a role not only in the incidence but also in the mechanism of glucocorticoid-related osteonecrosis, and given the strong association with adolescent age, it is possible that some genetic risk factors may be more penetrant in some age groups than in others. Candidate-gene studies have implicated inherited variation in *PAI-I*, *TYMS*, *VDR*, and factor V Leiden in the risk of osteonecrosis among patients with ALL.[74-79] A GWAS has implicated *ACP1* and genes related not only to osteonecrosis but also to hypoalbuminemia and hypercholesterolemia (supportive of a role for drug-induced lipidemias) as contributors to osteonecrosis risk.[80] Additional genome-wide studies for osteonecrosis risk in the setting of differing age groups and differing ALL therapeutic protocols are needed to define genetics of this disorder.

Asparaginase

Asparaginase use has increased in several recent ALL regimens, bolstered by data indicating that relapses are prevented by increased asparaginase exposure.[81-83] Although its frequency has decreased with the more common use of pegylated formulations, up to 40% of patients develop allergy to asparaginase. Asparaginase allergy is detrimental not only because of morbidity associated with allergy, but because allergy is associated with lower serum asparaginase concentrations and because asparaginase doses may be missed and thus therapy can be compromised. In a frontline St Jude trial,[84] the top-ranked SNP associated with allergy was in *GRIA1* on chromosome 5q33. SNPs in this locus have previously been associated with asthma and atopy in non-ALL settings.[85] In a larger study of St Jude and COG patients, *HLA* variants were imputed using genome-wide SNP data and external reference sets; the *HLA-B-07:01* variant was associated with asparaginase allergy and the presence of antibodies against asparaginase, and the variants were predicted to alter binding between HLA proteins and asparaginase epitopes.[86] Using a candidate-gene approach and pooling together the reactions of allergies, pancreatitis, and thrombotic events, it has been reported that variants in *ASNS* were associated with these asparaginase-related adverse effects.[87]

Anthracyclines

The risk of cardiomyopathy from anthracyclines has been assessed in long-term survivors including those treated for ALL. Candidate-gene studies implicated *CBR3* in the risk of cardiomyopathy, particularly at lower doses of anthracyclines[88]; patients exposed to higher doses were at high risk of cardiomyopathy, regardless of genotype. Broader genomic studies, using a platform directed at cardiovascular variants, identified that *HAS3* predisposed to cardiomyopathy, most strongly in those exposed to higher anthracycline doses.[89] These findings illustrate the principle that pharmacogenetic risk factors may be highly dependent on the exact therapeutic regimen, with some genetic risk factors most evident at lower drug doses and others most evident at higher drug doses.

Vincristine

Vincristine neuropathy can be a major dose-limiting adverse effect in ALL. In a genome-wide study, a higher frequency of neuropathy has been associated with a promoter variant in *CEP72* (rs924607).[90] The frequency of the risk allele was lower in individuals with African ancestry compared with the other ancestral groups, consistent with a lower incidence of vincristine neuropathy in African American patients.[91] A candidate-gene study found that variants in *ABCB1*, *ACTG1*, and *CAPG* were associated with vincristine neurotoxicity during ALL therapy,[92] although other candidate-gene studies found no associations with *ABCB1* variants, despite its likely role in vincristine transport.[93,94] Although *CYP3A5* affects vincristine metabolism, candidate-gene studies indicate that there are conflicting data on its association with neuropathy.[92,93,95,96]

Methotrexate

There have been extensive pharmacogenetic studies of methotrexate in ALL.[97,98] Candidate-gene studies have focused on common variants in genes clearly involved in the folate pathway, such as *MTHFR*, *SLC19A1*, *TYMS*, and *DHFR*.[50,99] Despite multiple candidate-gene studies for toxicity, results have been conflicting (or based on single, nonreplicated small studies), and thus it is currently not possible to recommend changes to methotrexate dosing based on inherited variants in these candidate genes.[97,98] Genome-wide studies identified variants associated with leukoencephalopathy,[100] but these findings have not yet been replicated. Methotrexate effects are influenced by interindividual variation in its plasma clearance, leading some to implement an approach that targets systemic exposure based on clearance.[101-103] Genome-wide analyses identified multiple common genomic variants in *SLCO1B1* that were associated with methotrexate clearance,[104] a finding that has been replicated in several studies[50,99,105,106] and confirmed in preclinical models.[107,108] The high degree of replication for *SLCO1B1* variants as a determinant of methotrexate clearance stands in contrast to the lack of replicated findings using a candidate-gene approach.[97,98]

Perspectives

Studies of germline genomic determinants in ALL have multiple objectives, one of which is to gain new biological insights into the mechanisms of leukemogenesis or ALL response (desired antileukemic effects or host toxicities) that could eventually yield improvements in diagnosis or therapy. Another, more elusive objective, is to discover genetic variation that can itself be used as a diagnostic or therapeutic test. For example, it is possible that tests of germline *TP53* status can be used in families of patients with hypodiploid ALL to provide risk estimates for individuals in the family. Likewise, germline tests of *TPMT* status can be used for individualizing the dose of thiopurines to minimize host toxicity without adversely affecting outcomes.[69] Currently, there are relatively few germline genomic associations that have the required level of evidence on clinical utility to permit routine use as a clinical test. However, the field is likely to change as new data emerge over the next few years, especially with the rapid advances in next-generation sequencing

that raises the exciting possibility of exhaustively interrogating all variants in the genome (eg, rare variants with large effects).

Ultimately, one can foresee that somatically acquired ALL-specific genetic alterations as well as inherited genomic variants will be used to predict each patient's risk of relapse and host toxicities with differing treatment regimens, and the choice of treatment protocol can be informed by balancing the probability of cure vs the probability of adverse effects based on genetic and other patient characteristics. For example, patients carrying highly penetrant germline variants related to life-threatening toxicities (pancreatitis) may be considered for treatment regimens that are not highly dependent on asparaginase, especially if his/her germline and/or tumor genetic profiles indicate sensitivity to other chemotherapeutics. Conversely, optimizing antileukemic effect is weighted more in patients with high-risk ALL, particularly if they are predicted to experience modest toxicities based on germline genetic variations. The delicate balance between toxicity and efficacy in this context is challenging,[109] and large collaborations are needed to comprehensively evaluate outcome- or toxicity-related genetic variants in diverse treatment regimens and to develop genetics-based decision support systems. Childhood ALL is uniquely positioned for this type of translational research, given the impressive progress already made in genomics and pharmacogenomics of this disease and the exceptionally organized clinical trials for children with ALL.

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Correspondence: Jun J. Yang, Pharmaceutical Sciences MS313, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678; e-mail: jun.yang@stjude.org.

References

1. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. *Lancet.* 2013; 381(9881):1943-1955.
2. Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. *J Clin Oncol.* 2011;29(5):551-565.
3. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature.* 2007; 446(7137):758-764.
4. Mullighan CG, Su X, Zhang J, et al; Children's Oncology Group. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med.* 2009;360(5):470-480.
5. Moorman AV, Enshaei A, Schwab C, et al. A novel integrated cytogenetic and genomic classification refines risk stratification in pediatric acute lymphoblastic leukemia. *Blood.* 2014; 124(9):1434-1444.
6. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med.* 2014;371(11):1005-1015.
7. McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet.* 2008;9(5):356-369.
8. Sham PC, Purcell SM. Statistical power and significance testing in large-scale genetic studies. *Nat Rev Genet.* 2014;15(5):335-346.
9. Pe'er I, Yelensky R, Altshuler D, Daly MJ. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. *Genet Epidemiol.* 2008;32(4): 381-385.
10. Ha NT, Freytag S, Bickeboeller H. Coverage and efficiency in current SNP chips. *Eur J Hum Genet.* 2014;22(9):1124-1130.
11. Porcu E, Sanna S, Fuchsberger C, Fritsche LG. Genotype imputation in genome-wide association studies. *Curr Protoc Hum Genet.* 2013;1.1.25.
12. Craddock N, Hurles ME, Cardin N, et al; Wellcome Trust Case Control Consortium. Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. *Nature.* 2010;464(7289): 713-720.
13. Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. *Nat Rev Cancer.* 2003;3(9):639-649.
14. Greaves MF, Maia AT, Wiemels JL, Ford AM. Leukemia in twins: lessons in natural history. *Blood.* 2003;102(7):2321-2333.
15. Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. *Nat Genet.* 2013;45(3): 242-252.
16. Shah S, Schrader KA, Waanders E, et al. A recurrent germline PAX5 mutation confers susceptibility to pre-B cell acute lymphoblastic leukemia. *Nat Genet.* 2013;45(10):1226-1231.
17. Vijayakrishnan J, Houlston RS. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. *Haematologica.* 2010;95(8): 1405-1414.
18. Zhang H, Liu H, Jiang G. Genetic polymorphisms of XRCC1 and leukemia risk: a meta-analysis of 19 case-control studies. *PLoS ONE.* 2013;8(11): e80687.
19. Han F, Tan Y, Cui W, Dong L, Li W. Novel insights into etiologies of leukemia: a HuGE review and meta-analysis of CYP1A1 polymorphisms and leukemia risk. *Am J Epidemiol.* 2013;178(4):493-507.
20. Kinlen L. Evidence for an infective cause of childhood leukaemia: comparison of a Scottish new town with nuclear reprocessing sites in Britain. *Lancet.* 1988;332(8624):1323-1327.
21. Kinlen LJ, Balkwill A. Infective cause of childhood leukaemia and wartime population mixing in Orkney and Shetland, UK. *Lancet.* 2001;357(9259):858.
22. Greaves MF. Aetiology of acute leukaemia. *Lancet.* 1997;349(9048):344-349.
23. Chang JS, Wiemels JL, Chokkalingam AP, et al. Genetic polymorphisms in adaptive immunity genes and childhood acute lymphoblastic leukemia. *Cancer Epidemiol Biomarkers Prev.* 2010;19(9):2152-2163.
24. Urayama KY, Chokkalingam AP, Metayer C, et al. HLA-DP genetic variation, proxies for early life immune modulation and childhood acute lymphoblastic leukemia risk. *Blood.* 2012; 120(15):3039-3047.
25. Hosking FJ, Leslie S, Dilthey A, et al. MHC variation and risk of childhood B-cell precursor acute lymphoblastic leukemia. *Blood.* 2011; 117(5):1633-1640.
26. Lin D, Liu C, Xue M, et al. The role of interleukin-15 polymorphisms in adult acute lymphoblastic leukemia. *PLoS ONE.* 2010;5(10):e13626.
27. Papaemmanuil E, Hosking FJ, Vijayakrishnan J, et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. *Nat Genet.* 2009;41(9): 1006-1010.
28. Treviño LR, Yang W, French D, et al. Germline genomic variants associated with childhood acute lymphoblastic leukemia. *Nat Genet.* 2009; 41(9):1001-1005.
29. Sherborne AL, Hosking FJ, Prasad RB, et al. Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. *Nat Genet.* 2010;42(6):492-494.
30. Xu H, Yang W, Perez-Andreu V, et al. Novel susceptibility variants at 10p12.31-12.2 for childhood acute lymphoblastic leukemia in ethnically diverse populations. *J Natl Cancer Inst.* 2013;105(10):733-742.
31. Migliorini G, Fiege B, Hosking FJ, et al. Variation at 10p12.2 and 10p14 influences risk of childhood B-cell acute lymphoblastic leukemia and phenotype. *Blood.* 2013;122(19):3298-3307.
32. Perez-Andreu V, Roberts KG, Harvey RC, et al. Inherited GATA3 variants are associated with Ph-like childhood acute lymphoblastic leukemia and risk of relapse. *Nat Genet.* 2013;45(12): 1494-1498.

33. Prasad RB, Hosking FJ, Vijayakrishnan J, et al. Verification of the susceptibility loci on 7p12.2, 10q21.2, and 14q11.2 in precursor B-cell acute lymphoblastic leukemia of childhood. *Blood.* 2010;115(9):1765-1767.
34. Yang W, Treviño LR, Yang JJ, et al. ARID5B SNP rs10821936 is associated with risk of childhood acute lymphoblastic leukemia in blacks and contributes to racial differences in leukemia incidence. *Leukemia.* 2010;24(4): 894-896.
35. Healy J, Richer C, Bourgey M, Kritikou EA, Sinnett D. Replication analysis confirms the association of ARID5B with childhood B-cell acute lymphoblastic leukemia. *Haematologica.* 2010;95(9):1608-1611.
36. Pastorczak A, Górniak P, Sherborne A, et al. Role of 657del5 NBN mutation and 7p12.2 (IKZF1), 9p21 (CDKN2A), 10q21.2 (ARID5B) and 14q11.2 (CEBPE) variation and risk of childhood ALL in the Polish population. *Leuk Res.* 2011;35(11):1534-1536.
37. Walsh KM, de Smith AJ, Chokkalingam AP, et al. Novel childhood ALL susceptibility locus BMI1-PIP4K2A is specifically associated with the hyperdiploid subtype. *Blood.* 2013;121(23): 4808-4809.
38. Xu H, Cheng C, Devidas M, et al. ARID5B genetic polymorphisms contribute to racial disparities in the incidence and treatment outcome of childhood acute lymphoblastic leukemia. *J Clin Oncol.* 2012;30(7):751-757.
39. Ellinghaus E, Stanulla M, Richter G, et al. Identification of germline susceptibility loci in ETV6-RUNX1-rearranged childhood acute lymphoblastic leukemia. *Leukemia.* 2012;26(5): 902-909.
40. Orsi L, Rudant J, Bonaventure A, et al. Genetic polymorphisms and childhood acute lymphoblastic leukemia: GWAS of the ESCALE study (SFCE). *Leukemia.* 2012;26(12): 2561-2564.
41. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med.* 2006; 354(2):166-178.
42. Roberts KG, Morin RD, Zhang J, et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. *Cancer Cell.* 2012; 22(2):153-166.
43. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature.* 2012; 481(7380):157-163.
44. Stanulla M, Schaeffeler E, Flohr T, et al. Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia. *JAMA.* 2005;293(12):1485-1489.
45. Schmiegelow K, Forestier E, Kristinsson J, et al; Nordic Society of Paediatric Haematology and Oncology. Thiopurine methyltransferase activity is related to the risk of relapse of childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. *Leukemia.* 2009;23(3): 557-564.
46. Relling MV, Pui CH, Cheng C, Evans WE. Thiopurine methyltransferase in acute lymphoblastic leukemia. *Blood.* 2006;107(2): 843-844.
47. Abrams MT, Robertson NM, Yoon K, Wickstrom E. Inhibition of glucocorticoid-induced apoptosis by targeting the major splice variants of BIM mRNA with small interfering RNA and short hairpin RNA. *J Biol Chem.* 2004;279(53): 55809-55817.
48. Ng KP, Hillmer AM, Chuah CT, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nat Med.* 2012;18(4): 521-528.
49. Soh SX, Lim JY, Huang JW, Jiang N, Yeoh AE, Ong ST. Multi-agent chemotherapy overcomes glucocorticoid resistance conferred by a BIM deletion polymorphism in pediatric acute lymphoblastic leukemia. *PLoS ONE.* 2014;9(8): e103435.
50. Radtke S, Zolk O, Renner B, et al. Germline genetic variations in methotrexate candidate genes are associated with pharmacokinetics, toxicity, and outcome in childhood acute lymphoblastic leukemia. *Blood.* 2013;121(26): 5145-5153.
51. Rocha JC, Cheng C, Liu W, et al. Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. *Blood.* 2005; 105(12):4752-4758.
52. Takanashi M, Morimoto A, Yagi T, et al. Impact of glutathione S-transferase gene deletion on early relapse in childhood B-precursor acute lymphoblastic leukemia. *Haematologica.* 2003; 88(11):1238-1244.
53. Brüggemann M, Trautmann H, Hoelzer D, Kneba M, Gökbuget N, Raff T. Multidrug resistance-associated protein 4 (MRP4) gene polymorphisms and treatment response in adult acute lymphoblastic leukemia. *Blood.* 2009; 114(26):5400-5401, author reply 5401-5402.
54. Ansari M, Sauty G, Labuda M, et al. Polymorphisms in multidrug resistance-associated protein gene 4 is associated with outcome in childhood acute lymphoblastic leukemia. *Blood.* 2009;114(7):1383-1386.
55. Yang JJ, Cheng C, Yang W, et al. Genome-wide interrogation of germline genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. *JAMA.* 2009; 301(4):393-403.
56. Tinhofer I, Marschitz I, Henn T, Egle A, Greil R. Expression of functional interleukin-15 receptor and autocrine production of interleukin-15 as mechanisms of tumor propagation in multiple myeloma. *Blood.* 2000;95(2):610-618.
57. Williams MT, Yousafzai Y, Cox C, et al. Interleukin-15 enhances cellular proliferation and upregulates CNS homing molecules in pre-B acute lymphoblastic leukemia. *Blood.* 2014; 123(20):3116-3127.
58. Yang JJ, Cheng C, Devidas M, et al. Genome-wide association study identifies germline polymorphisms associated with relapse of childhood acute lymphoblastic leukemia. *Blood.* 2012;120(20):4197-4204.
59. Meyers JA, Su DW, Lerner A. Chronic lymphocytic leukemia and B and T cells differ in their response to cyclic nucleotide phosphodiesterase inhibitors. *J Immunol.* 2009; 182(9):5400-5411.
60. Smith PG, Wang F, Wilkinson KN, et al. The phosphodiesterase PDE4B limits cAMP-associated PI3K/AKT-dependent apoptosis in diffuse large B-cell lymphoma. *Blood.* 2005; 105(1):308-316.
61. Kim SW, Rai D, Aguiar RC. Gene set enrichment analysis unveils the mechanism for the phosphodiesterase 4B control of glucocorticoid response in B-cell lymphoma. *Clin Cancer Res.* 2011;17(21):6723-6732.
62. Meyers JA, Taverna J, Chaves J, Makkinje A, Lerner A. Phosphodiesterase 4 inhibitors augment levels of glucocorticoid receptor in B cell chronic lymphocytic leukemia but not in normal circulating hematopoietic cells. *Clin Cancer Res.* 2007;13(16):4920-4927.
63. Ogawa R, Streiff MB, Bugayenko A, Kato GJ. Inhibition of PDE4 phosphodiesterase activity induces growth suppression, apoptosis, glucocorticoid sensitivity, p53, and p21(WAF1/CIP1) proteins in human acute lymphoblastic leukemia cells. *Blood.* 2002;99(9):3390-3397.
64. Wesołowska-Andersen A, Borst L, Dalgaard MD, et al. Genomic profiling of thousands of candidate polymorphisms predicts risk of relapse in 778 Danish and German childhood acute lymphoblastic leukemia patients. *Leukemia.* 2015;29(2):297-303.
65. Yang JJ, Cheng C, Devidas M, et al. Ancestry and pharmacogenomics of relapse in acute lymphoblastic leukemia. *Nat Genet.* 2011;43(3): 237-241.
66. Evans WE, Hon YY, Bomgaars L, et al. Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. *J Clin Oncol.* 2001;19(8): 2293-2301.
67. Lennard L, Gibson BE, Nicole T, Lilleyman JS. Congenital thiopurine methyltransferase deficiency and 6-mercaptopurine toxicity during treatment for acute lymphoblastic leukaemia. *Arch Dis Child.* 1993;69(5):577-579.
68. Relling MV, Hancock ML, Rivera GK, et al. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *J Natl Cancer Inst.* 1999;91(23):2001-2008.
69. Relling MV, Gardner EE, Sandborn WJ, et al. Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. *Clin Pharmacol Ther.* 2013;93(4):324-325.
70. Yang JJ, Landier W, Yang W, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. *J Clin Oncol.* 2015;33(11):1235-1242.
71. Yang SK, Hong M, Baek J, et al. A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia. *Nat Genet.* 2014;46(9):1017-1020.
72. Liu C, Kawedia JD, Cheng C, et al. Clinical utility and implications of asparaginase antibodies in acute lymphoblastic leukemia. *Leukemia.* 2012; 26(11):2303-2309.
73. Yang L, Boyd K, Kaste SC, Kamdem Kamdem L, Rahija RJ, Relling MV. A mouse model for glucocorticoid-induced osteonecrosis: effect of a steroid holiday. *J Orthop Res.* 2009;27(2):169-175.
74. Powell C, Chang C, Gershwin ME. Current concepts on the pathogenesis and natural history of steroid-induced osteonecrosis. *Clin Rev Allergy Immunol.* 2011;41(1):102-113.
75. Gong LL, Fang LH, Wang HY, et al. Genetic risk factors for glucocorticoid-induced osteonecrosis: a meta-analysis. *Steroids.* 2013;78(4):401-408.
76. Vora A. Management of osteonecrosis in children and young adults with acute lymphoblastic leukaemia. *Br J Haematol.* 2011; 155(5):549-560.
77. Bond J, Adams S, Richards S, Vora A, Mitchell C, Goulden N. Polymorphism in the PAI-1 (SERPINE1) gene and the risk of osteonecrosis in children with acute lymphoblastic leukemia. *Blood.* 2011;118(9):2632-2633.
78. French D, Hamilton LH, Mattano LA Jr, et al; Children's Oncology Group. A PAI-1 (SERPINE1) polymorphism predicts osteonecrosis in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Blood.* 2008; 111(9):4496-4499.
79. Relling MV, Yang W, Das S, et al. Pharmacogenetic risk factors for osteonecrosis of the hip among children with leukemia. *J Clin Oncol.* 2004;22(19):3930-3936.

80. Kawedia JD, Kaste SC, Pei D, et al. Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. *Blood.* 2011;117(8):2340-2347, quiz 2556.

81. Kawedia JD, Liu C, Pei D, et al. Dexamethasone exposure and asparaginase antibodies affect relapse risk in acute lymphoblastic leukemia. *Blood.* 2012;119(7):1658-1664.

82. Duval M, Suciu S, Ferster A, et al. Comparison of Escherichia coli-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia Group phase 3 trial. *Blood.* 2002;99(8):2734-2739.

83. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood.* 2001;97(5): 1211-1218.

84. Chen SH, Pei D, Yang W, et al. Genetic variations in GRIA1 on chromosome 5q33 related to asparaginase hypersensitivity. *Clin Pharmacol Ther.* 2010;88(2):191-196.

85. Mathias RA, Grant AV, Rafaels N, et al. A genome-wide association study on African-ancestry populations for asthma. *J Allergy Clin Immunol.* 2010;125(2):336-346.e4.

86. Fernandez CA, Smith C, Yang W, et al. HLA-DRB1*07:01 is associated with a higher risk of asparaginase allergies. *Blood.* 2014;124(8): 1266-1276.

87. Ben Tanfous M, Sharif-Askari B, Ceppi F, et al. Polymorphisms of asparaginase pathway and asparaginase-related complications in children with acute lymphoblastic leukemia. *Clin Cancer Res.* 2015;21(2):329-334.

88. Blanco JG, Sun CL, Landier W, et al. Anthracycline-related cardiomyopathy after childhood cancer: role of polymorphisms in carbonyl reductase genes—a report from the Children's Oncology Group. *J Clin Oncol.* 2012; 30(13):1415-1421.

89. Wang X, Liu W, Sun CL, et al. Hyaluronan synthase 3 variant and anthracycline-related cardiomyopathy: a report from the children's oncology group. *J Clin Oncol.* 2014;32(7): 647-653.

90. Diouf B, Crews K, Lew G, et al. Genome-wide association analyses identify susceptibility loci for vincristine-induced peripheral neuropathy in children with acute lymphoblastic leukemia. *JAMA.* 2015;313(8):815-823.

91. Renbarger JL, McCammack KC, Rouse CE, Hall SD. Effect of race on vincristine-associated neurotoxicity in pediatric acute lymphoblastic leukemia patients. *Pediatr Blood Cancer.* 2008; 50(4):769-771.

92. Ceppi F, Langlois-Pelletier C, Gagné V, et al. Polymorphisms of the vincristine pathway and response to treatment in children with childhood acute lymphoblastic leukemia. *Pharmacogenomics.* 2014;15(8):1105-1116.

93. Kishi S, Cheng C, French D, et al. Ancestry and pharmacogenetics of antileukemic drug toxicity. *Blood.* 2007;109(10):4151-4157.

94. Guilhaumou R, Solas C, Bourgarel-Rey V, et al. Impact of plasma and intracellular exposure and CYP3A4, CYP3A5, and ABCB1 genetic polymorphisms on vincristine-induced neurotoxicity. *Cancer Chemother Pharmacol.* 2011;68(6):1633-1638.

95. Egbelakin A, Ferguson MJ, MacGill EA, et al. Increased risk of vincristine neurotoxicity associated with low CYP3A5 expression genotype in children with acute lymphoblastic leukemia. *Pediatr Blood Cancer.* 2011;56(3): 361-367.

96. Hartman A, van Schaik RH, van der Heiden IP, et al. Polymorphisms in genes involved in vincristine pharmacokinetics or pharmacodynamics are not related to impaired motor performance in children with leukemia. *Leuk Res.* 2010;34(2):154-159.

97. Kodidela S, Suresh Chandra P, Dubashi B. Pharmacogenetics of methotrexate in acute lymphoblastic leukaemia: why still at the bench level? *Eur J Clin Pharmacol.* 2014;70(3): 253-260.

98. Schmiegelow K. Advances in individual prediction of methotrexate toxicity: a review. *Br J Haematol.* 2009;146(5):489-503.

99. Lopez-Lopez E, Ballesteros J, Piñan MA, et al. Polymorphisms in the methotrexate transport pathway: a new tool for MTX plasma level prediction in pediatric acute lymphoblastic leukemia. *Pharmacogenet Genomics.* 2013; 23(2):53-61.

100. Bhojwani D, Sabin ND, Pei D, et al. Methotrexate-induced neurotoxicity and leukoencephalopathy in childhood acute lymphoblastic leukemia. *J Clin Oncol.* 2014; 32(9):949-959.

101. Pauley JL, Panetta JC, Crews KR, et al. Between-course targeting of methotrexate exposure using pharmacokinetically guided dosage adjustments. *Cancer Chemother Pharmacol.* 2013;72(2):369-378.

102. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med.* 2009;360(26): 2730-2741.

103. Evans WE, Relling MV, Rodman JH, Crom WR, Boyett JM, Pui CH. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *N Engl J Med.* 1998; 338(8):499-505.

104. Treviño LR, Shimasaki N, Yang W, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. *J Clin Oncol.* 2009;27(35):5972-5978.

105. Ramsey LB, Panetta JC, Smith C, et al. Genome-wide study of methotrexate clearance replicates SLCO1B1. *Blood.* 2013;121(6): 898-904.

106. Lopez-Lopez E, Martin-Guerrero I, Ballesteros J, et al. Polymorphisms of the SLCO1B1 gene predict methotrexate-related toxicity in childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer.* 2011;57(4):612-619.

107. Ramsey LB, Bruun GH, Yang W, et al. Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. *Genome Res.* 2012;22(1):1-8.

108. van de Steeg E, van der Kruijssen CM, Wagenaar E, et al. Methotrexate pharmacokinetics in transgenic mice with liver-specific expression of human organic anion-transporting polypeptide 1B1 (SLCO1B1). *Drug Metab Dispos.* 2009;37(2):277-281.

109. Levinsen M, Rotevatn EO, Rosthøj S, et al; Nordic Society of Paediatric Haematology, Oncology. Pharmacogenetically based dosing of thiopurines in childhood acute lymphoblastic leukemia: influence on cure rates and risk of second cancer. *Pediatr Blood Cancer.* 2014; 61(5):797-802.