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Polymorphisms of the vincristine pathway and response to treatment in children with childhood acute lymphoblastic leukemia

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Abstract

Background—Vincristine (VCR) is a standard component in the treatment of childhood acute lymphoblastic leukemia (ALL). VCR cytotoxicity is primarily due to its ability to disrupt the formation of microtubules of the mitotic spindle.

Patients & methods—A total of 17 polymorphisms in regulatory and coding regions of genes controlling VCR targets (*TUBB1*, *MAP4*, *ACTG1* and *CAPG*) or potentially influencing VCR levels (*ABCB1* and *CYP3A5*) were investigated for an association with peripheral neuropathy and outcome in childhood ALL patients.

Results—High-grade neurotoxicity was more frequent in carriers of the A allele of synonymous (Ala310) G to A (rs1135989) variation in the *ACTG1* gene. Substitution (rs4728709) in the promoter of the *ABCB1* gene had a protective effect against lower grade neurotoxicity and C to A variation (rs3770102) located 17 nucleotides upstream from the transcription start site had a

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

protective effect against high-grade neurotoxicity. Patients with the *ABCB1* 3435TT genotype had lower event-free survival; the association with event-free survival was not supported by the analysis in the replication patient set.

Conclusion—The polymorphisms in the *ACTG1, CAPG* and *ABCB1* genes may modulate VCR-related neurotoxicity, whereas the risk of relapse seems not to be affected by the genes of the VCR pathway.

Keywords

childhood leukemia; peripheral neurotoxicity; pharmacogenetics; polymorphisms; treatment outcome; vincristine

Background

Leukemia is the most common pediatric cancer, with acute lymphoblastic leukemia (ALL) comprising as many as 80% of all leukemia cases [1]. Survival rates for ALL have improved dramatically since the 1980s, with current 5-year overall survival rates estimated at greater than 85% [2,3]. Nevertheless, for a subgroup of patients, resistance to chemotherapy still remains an obstacle to successful treatment, whereas in others, drug intolerance may lead to a variety of related complications. Many factors contribute to the variability in treatment responses including patient genetic background that seems to play a substantial role [4]. A number of genetic variants have been identified to modulate treatment responses in ALL [5–8] and some of them, such as those in TPMT have become part of clinical management of ALL patients [9,10].

Vincristine (VCR) is a key component in the treatment regimen of ALL. VCR is a vinca alkaloid, whose cytotoxicity is primarily owing to its ability to disrupt the formation of microtubules of the mitotic spindle, thereby inducing metaphase arrest in dividing cells [11]. The dose-limiting toxicity of VCR consists of a peripheral neuropathy characterized by progressive motor, sensory and autonomic involvement in varying combinations [12]. No specific preventive or curative treatment is available to manage neurotoxicity, the only option is to discontinue or reduce VCR dose. The polymorphisms of the genes mediating VCR effects can underlie observed variability. VCR binds to the TUBB1 subunit of the α/β tubulin heterodimer, inhibiting microtubule polymerization [13]. MAP4, can slow this process down by stabilizing mitotic spindles [14]. Altered expression and/or mutations of genes encoding these VCR targets have been reported in ALL cells selected for resistance to vinca alkaloids [15]. Proteomic studies [14,16], subsequently confirmed through independent experiments [17,18], identified distinct changes in cytoskeleton protein expression associated with VCR resistance, revealing alterations in the actin- and tubulin-associated proteins, which besides TUBB, included changes in ACTG and in CapG, an actin-binding protein, involved in signal transduction to the actin cytoskeleton [18]. Variable VCR levels were observed in children with leukemia, with resistant cells accumulating lower amounts of the drug than responsive cells [19,20]. P-gp, which mediates VCR efflux was suggested to contribute to variable VCR levels and resistance to VCR [11,21]. P-gp is encoded by the multidrug-resistance (*MDR1* or *ABCB1*) gene known to harbor a number of functional polymorphisms [22–25]. The impact of *ABCB1* gene variations on VCR-related

neurotoxicity was assessed in one study with a limited sample size, without conclusive results [26]. VCR levels can also be affected by CYP3A5, which is involved in the metabolism of VCR [27]; the predicted intrinsic clearance was fivefold greater in CYP3A5 expressers (carriers of the *CYP3A5*1* allele) as compared to nonexpressers (*CYP3A5*3*3)*. [27,28]. One study suggested an association of low *CYP3A5* expression genotype with increased risk of VCR-associated neurotoxicity [29].

In this study we evaluated whether polymorphisms in genes coding for tubulin- and actin-associated proteins (*TUBB1*, *MAP4*, *ACTG*, *ACTG1* and *CAPG*), as well as known variations in the *CYP3A5* and *ABCB1* genes may affect VCR-related neurotoxicity in childhood ALL patients. The impact of these polymorphisms on ALL outcome was also evaluated.

Patients & methods

Patients

The patient cohort (Québec ALL [Qc-ALL] cohort) was composed of 339 Caucasian children (98% of French–Canadian origin) who were diagnosed consecutively with ALL at the Sainte-Justine University Health Center (SJUHC; Québec, Canada) between January 1989 and July 2005 and consented to participate in the genetic ALL study. The patients underwent treatment with the Dana-Farber Cancer Institute (DFCI) ALL Consortium protocols DFCI 87-01, 91-01, 95-01 or 00-01 [30–33]. All patients were enrolled in event-free survival (EFS) and overall survival (OS) analysis. Children who had an induction failure, relapsed after achieving complete remission or died, were defined to have had an event.

During induction chemotherapy, each patient received a standard VCR dose of 1.5 mg/m^2 weekly for four doses and for protocol DFCI 91-01 a fifth dose at the same dosage, and for protocols DFCI 95-01 and 00-01, a fifth dose of 2 mg/m^2 for a maximum of 2 mg. For the consolidation and continuation phase (100 weeks of treatment), all four protocols included a VCR dose of 2 mg/m^2 (maximum of 2 mg), administered every 3 weeks.

Evaluation of neurotoxicity

VCR-related neurotoxicity was assessed by retrospective chart review. Severity of neurotoxicity (sensory, motor and autonomic) observed was graded (grades 1–4) using National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0 [34]. If lower and higher grades were assigned to the same patient (four such patients were identified), the highest grade obtained for any neurotoxicity item was retained. The data were collected for 320 patients. In the remaining 19 cases the data were either not available (n = 9) or patients did not complete at least 3 months of therapy (e.g., early event or bone marrow transplant) [29]. There was no difference in characteristics of patients enrolled in EFS and neurotoxicity analysis (Table 1) and none of the patients with early event/bone marrow transplant experienced neurotoxicity prior to that event. Occurrence of overall neurotoxicity (presence or absence) in relation to genotypes was assessed. Because patients with severe neurotoxicity are seriously affected with adverse events, defined as

limiting performance of basic activities of self-care (grade 3), or with life-threatening adverse events (grade 4), the analysis of lower and higher toxicity grades were performed separately.

The frequency of low- and high-grade neurotoxicity was 20 and 10.6%, respectively. Severity of neurotoxicity was defined as number of toxicity episodes of either lower or higher grades expressed per total number of treatment cycles. Tolerated VCR dose was defined as ratio of received and intended dose (change from the dose that would have been received if toxic episode did not occur).

A replication set for EFS (data on neurotoxicity are not available) is the Dana-Farber Cancer Institute (DFCI) group composed of a subset of nonconsecutively accrued patients who underwent treatment on DFCI 95-01 and 2000-01 protocol in nine remaining consortium institutions [6,32,35]. To minimize confounding due to the population stratification, similar to QcALL cohort and previous analysis [6,35], only Caucasians (self-reported) whose samples provided sufficient DNA to allow for genotyping (n = 275) were included in the study. The characteristics of patients for this cohort are provided in Table 1.

Genotyping

Forty six polymorphisms in *TUBB1* (n = 8), *MAP4* (n = 13), *ACTG1* (n = 18) and *CAPG* (n = 7) genes, located in regulatory and coding gene regions were selected from the NCBI SNP database [36]. Selected polymorphisms were analyzed in 60 controls to estimate allele frequency and linkage disequilibrium (LD; Supplementary Figure 1; see online at: www.futuremedicine.com/doi/suppl/10.2217/pgs.14.68). Twelve tagSNPs (sufficient to define common haplotypes) with a frequency ≥5% were retained for the analysis in patients (two SNPs in *TUBB1*, four in *MAP4*, three in *ACTG1* and three in *CAPG*; Supplementary Figure 1 & Supplementary Table 1). Additionally, known polymorphisms in the *ABCB1* and *CYP3A5* genes were investigated [5,37,38]. In the *ABCB1* gene these included synonymous C1236T and C3435T substitutions, a nonsynonymous triallelic polymorphism (G2677T/A) leading to Ser to Ala/Thr amino acid replacement at residue position 893 and rs4728709 variation reported to correlate with the risk of ALL relapse in the recent large genome-wide association study [5]. In the *CYP3A5* gene, the A6986G defining alleles **1* and **3* that create a cryptic splice site in intron 3 and altered mRNA splicing was studied. DNA was isolated from peripheral blood or bone marrow obtained at the end of induction, using a standard salting-out procedure [39]. The same QcALL cohort was used in our previous studies [6,35].

Genotyping was performed using the Sequenom genotyping platform at the Genome Québec and McGill Innovation Center (Montreal, Canada) and by allele specific oligonucleotide hybridization as previously described (primers used for amplification and allele specific oligonucleotide hybridization are provided in Supplementary Table 1) [40]. The subset of samples was genotyped in duplicate to ensure genotype reproducibility. Note that the amplification was not equally successful for all loci analyzed explaining the minor difference between SNPs in the total number of available genotypes.

Statistics

The estimates of LD and haplotype phase were obtained by PHASE software, version 2.0 [41]. The tagSNPs were selected based on LD information using Haploview software. Association of genotypes with overall neurotoxicity or with occurrence of at least one episode of either low- (1 and/or 2) or high- (3 and/or 4) grade neurotoxicity was assessed by χ^2 testing by comparing the frequency of genotypes to frequency in patients that did not have any of these toxicities. For significant associations, genotypes were grouped in two categories and the genotype-associated risk of neurotoxicity was expressed as odds ratio (OR) with 95% CI. For same SNPs, association with the frequency of neurotoxicity episodes and with tolerated VCR dose was assessed using the Mann–Whitney test. Significantly associated polymorphisms were also analyzed by multivariate logistic regression. Covariates that correlated independently with neurotoxicity (age, treatment protocol and risk classes) were included beside genotypes in the model. Body surface area (BSA) was also included since patients with BSA >1 m^2 get a fixed dose of VCR for a prescribed dose of 2 mg/m^2 (maximum of 2 mg).

ALL outcome was assessed by EFS and OS. Survival differences, estimated by Kaplan–Meier analysis for patients with different genotypes, were assessed using a log-rank test. Times to event, or death were measured as the time between diagnosis and the event of interest. For patients in continuous complete remission, survival times were censored at the date of last contact, for longer time durations all times were truncated at 5 years post-treatment. Patients with hematopoietic stem cell transplantation were censored at the time of transplantation. The hazard ratio (with a 95% CI) for genetic variants was estimated by Cox regression analysis. Cox regression was also used for multivariate analysis including genotype, common prognostic factors (sex, age, white blood cell count immunophenotype and ploidy) and treatment protocol in the model. As described previously [6,35], categories typically associated with lower risk of relapse were considered as references (age ≥1 and <10, white blood cell count lower than 50×10^9/l, B-cell type, female gender, hyperdiploidy [DNA index ≥1.16 or 50 chromosomes] and most recent treatment protocol).

The power estimates were performed with the Quanto (version 1.2) computer program for power and sample size calculation for genetic epidemiology studies [42]. Genotype-associated risk (OR) of overall neurotoxicity was estimated for the polymorphism with minor allele frequency ranging from 30 to 5%. For this frequency range, the study sample size has 80% power to detect a minimum OR of 1.7–2.5 and an OR of 2.0–2.6 assuming additive and dominant genetic models, respectively, and α set at 0.05.

Results

The relationship between each selected polymorphism in genes of the VCR pathway (*TUBB1*, *MAP4*, *ACTG1*, *CAPG*, *CYP3A5* and *ABCB1*) and neurotoxicity (low and high grade) in the QcALL cohort is given in Table 2. Significant association was seen with *ACTG1*, *CAPG* and *ABCB1* gene variations. Patients with the A allele of the synonymous (Ala310) G to A (rs1135989) variation in the *ACTG1* gene had higher risk of neurotoxicity grade 3/4 compared to patients with remaining genotypes (OR: 2.8; 95% CI: 1.3–6.3; p = 0.008). Individuals with the A allele also had more frequent episodes of high-grade

neurotoxicity (p = 0.008) and lower tolerated VCR dose compared to noncarriers (p = 0.02). Higher grade neurotoxicity was also associated with G>A rs2229668 SNP in the *CAPG* gene that leads to Val41Ile replacement. Association followed the additive model and the neurotoxicity risk increased with the number of A alleles (OR: 2.1; 95% CI: 1.1–3.7; p = 0.02). Toxicity episodes occurred more frequently in A allele carriers (p = 0.01) whereas no association with tolerated VCR dose was found. By contrast, C>A rs377010 SNP in the *CAPG* gene located 17 nucleotides upstream from transcription start site, had a protective effect against high-neurotoxicity grades. Individuals with the AA genotype had lower risk of this toxicity (OR: 0.1; 95% CI: 0.01–0.8; p = 0.009) and lower frequency of toxic episodes (p = 0.007). The C>T *ABCB1* substitution (rs4728709) also had a protective effect, but against low neurotoxicity grades. Patients with the T allele at *ABCB1* had lower risk of toxicity (OR: 0.3; 95% CI: 0.1–0.9; p = 0.02), less frequent toxicity episodes (p = 0.04) and tolerated higher VCR dose (p = 0.02). The results did not change when any neurotoxicity was considered as the phenotype of interest. There was difference between occurrence of neurotoxicity (both lower and higher grades) in relation to the treatment protocol with less toxicity in more recent (95-01 and 2000-01) than older (87-01 and 91-01) protocols (p ≤ 0.003). An association with the age and the risk classes was also found with a higher frequency of neurotoxicity in older children (≥10 years of age; p ≤ 0.008) and in patients assigned to the high-risk group (p = 0.01). There was no relationship between genotypes and these clinical covariates. The genotype associated risk did not change for *ACTG1*, *ABCB1* and *CAPG* rs3770102 variations in the multivariate model, which included risk/protective genotype, age, protocol, risk classes and BSA in the model (Table 3), whereas *CAPG* rs2229668 was not significant on multivariate analysis.

We further analyzed whether selected polymorphisms of the VCR pathway affect ALL outcomes; the relationship with EFS in the QcALL cohort is given in Table 4. The significant association with EFS was seen for *ABCB1* C3435T and G2677T substitutions (p = 0.03; Table 3) with lowest EFS in TT individuals. A similar tendency, although not significant, was seen when haplotypes were analyzed with EFS; individuals homozygous for the TTT haplotype (at loci 1236, 2677 and 3435) had reduced EFS (p = 0.06). The results remain significant in the multivariate model for *ABCB1* 3435 variations (p = 0.02), but not for the 2677 polymorphism (p = 0.1). EFS did not differ across the treatment protocols. *ABCB1* 3435 was subsequently analyzed in the DFCI replication cohort and no positive association signal was detected (p = 0.2). There was no association between polymorphisms of the VCR pathway and OS in the QcALL cohort (data not shown).

Discussion

ALL patients who experience severe neurotoxicity are seriously affected, usually requiring a change in treatment plan with, including holding of dose(s) followed by gradual escalation to full dose as tolerated. Moderate neurotoxicity slightly affects the patient, requiring only minimal medical intervention. Given the importance of severe neurotoxicity from a clinical point of view, our analysis separately addressed the relationship between neurotoxicity of lower and higher grades and genetic variation in the VCR pathway. Given the apparent role that the *ABCB1* and *CYP3A5* genes may play in modulation of VCR pharmacokinetics, several studies of variable sample size addressed their role in VCR-related neurotoxicity in

childhood cancer patients. Aplenc *et al.* analyzed a large ALL patient cohort enrolled in the National Children's Cancer Group (CCG) pediatric ALL trial (CCG-1891) and found increased risk of high-grade neurotoxicity in *CYP3A5* expressers (**1* carriers) [43]. The result was statistically significant on univariate analysis, but not after controlling for multiple comparisons. The direction of association is not supported by a known role of CYP3A5 in VCR metabolism, which suggests higher hepatic VCR clearances in CYP3A5 expressers [28]. By contrast, Egbelakin *et al.* found a higher frequency of VCR neurotoxicity in CYP3A5 nonexpressers (*CYP3A5**3*3) [29]. The difference between genotypes was noted only when all grades of neurotoxicity were combined, and was not significant if higher grades were analyzed between genotype groups. The results nevertheless suggested different rates of VCR metabolism between *CYP3A5* genotype groups with higher primary metabolite plasma concentrations seen in the *CYP3A5* expressers. Our analysis does not support an association between *CYP3A5* genotype and neurotoxicity. Absence of association between *CYP3A5* polymorphism and motor performance in childhood ALL survivors [44], as well as with the incidence of high-grade neurotoxicity and VCR pharmacokinetics in children with solid tumors [26,45] was also reported, although in studies with a limited sample size.

There are only a few studies assessing the role of the *ABCB1* polymorphisms (position 1236, 2677 and 3435) in VCR-related neurotoxicity or VCR pharmacokinetics. In pediatric patients with solid tumors, these *ABCB1* polymorphisms did not significantly affect VCR pharmacokinetics [45], a trend towards higher intracellular:plasma ratio was found for patients that are heterozygous for minor alleles, with no association with neurotoxicity [26]. Absence of association between the same polymorphisms and motor performance in childhood ALL survivors [44] was also reported. Our results support the lack of association between these *ABCB1* polymorphisms and VCR-related neurotoxicity.

In addition to VCR, dexamethasone and anthracyclines are also substrates for the ABCB1 transporter. Thus the polymorphisms in this gene may affect both intrinsic blast resistance and pharmacokinetics of several drugs used in ALL protocols, affecting the efficacy of treatment and EFS. Better EFS and a lower rate of CNS relapse associated with the T3435 allele has been reported in childhood ALL [46,47], whereas others [48,49] reported no association of this polymorphism with prognosis. We observed lower EFS in individuals with the T3435 allele in the discovery cohort in univariate and multivariate analysis; however, this finding was not subsequently replicated in a validation patient set arguing against the role of this polymorphism in modulation of ALL outcome. A recent large genome-wide association study [5] reported that two *ABCB1* variations (rs10264856 and rs4728709) are associated with increased risk of relapse in childhood ALL patients. Since these two SNPs are in LD (but not in LD with SNPs at 3435, 2677 or 1236 positions) we analyzed one of these two SNPs, rs4728709. No association with EFS or OS was found. By contrast, we noted its protective effect against lower grades of neurotoxicity, which may suggest that this polymorphism (or other SNPs in LD) indeed has an impact on ALL treatment outcomes.

The effect of other *ABCB1* substitutions beyond those studies mentioned about cannot be ruled out. For example, *in vitro* experiments have shown that G1199A (Ser400Asn) [50]

affects VCR efflux, and that G554T confers amino acid Gly185Val substitution and changed drug specificity of vinblastine and colchicine [51,52]. Both SNPs have very low minor allele frequencies (as reported in the NCBI SNP database) and are therefore not assessed here.

Our study is the first to examine the role of polymorphisms in VCR target genes in response to leukemia treatment. Only one study assessed *MAPT* polymorphisms in relation to motor performance in childhood ALL survivors [44] and reported absence of association. We did not see an association between EFS and polymorphisms investigated in VCR target genes, but a relationship was found with high-grade neurotoxicity. Two polymorphisms were retained on multivariate analysis, G>A (rs1135989) variations in the *ACTG1* gene that increased risk of higher neurotoxicity grades, and C>A variation (rs3770102) upstream from the transcription start site of the *CAPG* gene that had a protective effect against this neurotoxicity. ACTG encoded by the *ACTG1* gene is a major cytoskeletal protein, whereas CapG mediates a crosstalk between actin and the microtubule cytoskeleton [16,53]. The expression of both proteins has been reduced in ALL xenografts that are intrinsically resistant to VCR [16]. Reduction of *ACTG1* mRNA levels was found in relapsed ALL cases and *ACTG1* mutations were detected in leukemia cell lines selected for VCR resistance [17]. The functional role of associated polymorphisms in the *ACTG1* and *CAPG* genes is not yet known. The *CAPG* sequence prior to the transcription start site might affect mRNA regulation whereas synonymous *ACTG1* variation is predicted to affect exonic splicing regulatory sequences [54,55]; the mechanism behind the associations as well as their confirmation in other childhood ALL cohorts remain to be assessed.

In conclusion, our study suggests no major role of polymorphisms in the VCR pathway in the risk of relapse, whereas particular variations in *ABCB1*, *ACTG1* and *CAPG* genes might influence VCR-related neurotoxicity.

Conclusion & future perspective

A number of polymorphisms that influence efficacy of ALL treatment or drug-related complications have been identified through ALL pharmacogenetic studies, which include analysis of single candidate genes or several genes in the same pathway, and more recently, genome-wide association studies. However, there is yet no clear conclusion (with the exception of 6-mercaptopurine and TPMT) as to which treatment in the context of patient genetic background will be the most suitable or the least likely to improve the efficacy of the treatment and predict drug-related side effects. With the advent of next-generation sequencing technologies, the way we think about scientific approaches in basic, applied and clinical research is changing. It will be possible to obtain a more in depth view of genomic variations involved in human health and diseases and to identify variations (common and rare, inherited and somatic) leading to variability in treatment responses. This information coupled with clinical and treatment information will likely lead to personally tailored treatment in ALL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Executive summary

Background

- Vincristine (VCR) is a standard component in the treatment of childhood acute lymphoblastic leukemia (ALL).
- VCR cytotoxicity is primarily due to its ability to disrupt the formation of microtubules of the mitotic spindle.
- Altered expression of the actin- and tubulin-associated proteins have been reported in ALL cells resistant to VCR and in mouse xenograft models. VCR is metabolized by CYP3A5 and drug efflux is mediated by P-gp.

Patients & methods

- Polymorphisms in genes coding for VCR targets (*TUBB1*, *MAP4*, *ACTG1* and *CAPG*) or potentially influencing VCR levels (*ABCB1* and *CYP3A5*) were investigated for an association with peripheral neuropathy and outcome in childhood ALL patients.
- VCR-related neurotoxicity was assessed by retrospective patients chart review. Because patients with severe neurotoxicity are seriously affected, an association with presence of low- and high-grade toxicities was separately assessed.

Results

- An association was found between G to A (rs1135989) variation in the *ACTG1* gene and the risk of high-grade neurotoxicity (odds ratio [OR]: 2.8; 95% CI: 1.3–6.3; $p = 0.008$).
- Protective effects against lower grade of neurotoxicity was noted for the promoter polymorphism of the *ABCB1* gene (rs4728709; OR: 0.3; 95% CI: 0.1–0.9; $p = 0.02$) and protective effects against higher neurotoxicity grade was seen for the rs3770102 polymorphism of the *CAPG* gene (OR: 0.1; 95% CI: 0.01–0.8; $p = 0.009$).
- In univariate and multivariate analyses, lower event-free survival was associated with *ABCB1* 3435 TT genotype in the discovery cohort ($p \leq 0.03$), but not in the replication patient set.

Conclusion

- Polymorphisms in genes of the VCR pathway might play a role in VCR-related neurotoxicity.

Table 1

Characteristics of acute lymphoblastic leukemia patients in the test and validation cohorts.

Characteristic	Subjects, n (%)		
	QcALL		DFCI (EFS data)
	Neurotoxicity data	EFS data	
Sex			
Male	177 (55.3)	186 (54.9)	125 (45.5)
Female	143 (44.7)	153 (45.1)	150 (54.5)
Age			
<10 years	256 (80)	269 (79.4)	225 (81.8)
Older than 10 years	64 (20)	70 (20.6)	50 (18.2)
WBC			
$<50 \times 10^9$/l	267 (83.4)	277 (81.7)	222 (80.7)
$>50 \times 10^9$/l	53 (16.6)	60 (17.7)	53 (19.3)
Lineage			
B-cell	289 (90.3)	305 (90)	251 (91.3)
T-cell	26 (8.1)	29 (8.6)	22 (8)
Unknown	5 (1.6)	5 (1.5)	2 (0.7)
Risk group			
Standard	156 (48.8)	161 (47.5)	168 (61.1)
High and very high	164 (51.3)	178 (52.5)	107 (38.9)
DNA index			
>1.16	56 (17.5)	58 (17.1)	
<1.16	237 (74.1)	247 (72.9)	
Unknown	27 (8.4)	34 (10)	
DFCI protocol			
87-01	25 (7.8)	31 (9.1)	
91-01	60 (18.8)	63 (18.6)	
95-01	118 (36.9)	124 (36.6)	94 (34.2)
00-01	117 (36.6)	121 (35.7)	181 (65.8)

Number of patients evaluated for neurotoxicity and EFS in the QcALL cohort is 320 and 339, respectively.

Patients in the validation cohort whose DNA index was not available are shown by empty table cells.

DFCI: Dana-Farber Cancer Institute; EFS: Event-free survival; QcALL: Quebec acute lymphoblastic leukemia; WBC: White blood cell.

Table 2

Relationship of the polymorphisms studied with low and high neurotoxicity grade.

Gene/polymorphisms	Genotype	Neurotoxicity grade 1/2, n (%)			Neurotoxicity grade 3/4, n (%)		
		Grade 1/2	Grade 0	p-value	Grade 3/4	Grade 0	p-value
ABCB1, rs1045642 (3435)	CC	14 (21.9)	65 (30.5)	0.2	15 (39.5)	65 (30.5)	0.08
	CT	30 (46.9)	103 (48.4)		11 (28.9)	103 (48.4)	
	TT	20 (31.2)	45 (21.1)		12 (31.6)	45 (21.1)	
ABCB1, rs2032582 (2677)	GG	22 (34.4)	90 (41.3)	0.6	19 (50)	90 (41.3)	0.6
	GT	28 (43.7)	88 (40.4)		14 (36.8)	88 (40.4)	
	TT	14 (21.9)	40 (18.3)		5 (13.2)	40 (18.3)	
ABCB1, rs1128503 (1236)	TT	23 (36.5)	85 (39)	0.7	18 (47.4)	85 (39)	0.6
	TC	26 (41.3)	95 (43.6)		15 (39.5)	95 (43.6)	
	CC	14 (22.2)	38 (17.4)		5 (13.1)	38 (17.4)	
ABCB1, rs4728709	TT/TC	5 (7.9)	44 (20.6)	0.01†	5 (13.2)	44 (20.6)	0.2
	CC	58 (92.1)	170 (79.4)		33 (86.8)	170 (79.4)	
CYP3A5, rs776746 (**1/*3*)	AA/AG	6 (9.5)	29 (13.4)	0.3	8 (21.1)	29 (13.4)	0.2
	GG	57 (90.5)	188 (86.6)		30 (78.9)	188 (86.6)	
ACTG1, rs7406609	CC	11 (17.5)	49 (23)	0.6	5 (13.1)	49 (23)	0.3
	CT	29 (46)	97 (45.5)		21 (55.3)	97 (45.5)	
	TT	23 (36.5)	67 (31.5)		12 (31.6)	67 (31.5)	
ACTG1, rs1135989	GG	26 (41.3)	100 (46.7)	0.7	9 (23.7)	100 (46.7)	0.02‡
	GA	25 (39.7)	82 (38.3)		23 (60.5)	82 (38.3)	
	AA	12 (19)	32 (15)		6 (15.8)	32 (15)	
ACTG1, rs1139405	TT	42 (64.6)	134 (61.5)	0.9	25 (65.8)	134 (61.5)	0.7
	TC	20 (30.8)	72 (33)		12 (31.6)	72 (33)	
	CC	3 (4.6)	12 (5.5)		1 (2.6)	12 (5.5)	
CAPG, rs6886	GG	29 (45.3)	84 (38.9)	0.2	13 (34.2)	84 (38.9)	0.8
	GA	29 (45.3)	90 (41.7)		16 (42.1)	90 (41.7)	
	AA	6 (9.4)	42 (19.4)		9 (23.7)	42 (19.4)	
CAPG, rs2229668	GG	52 (81.3)	170 (79.4)	0.3	25 (64.1)	170 (79.4)	0.04§
	GA	9 (14.1)	40 (18.7)		11 (28.2)	40 (18.7)	
	AA	3 (4.7)	4 (1.9)		3 (7.7)	4 (1.9)	
CAPG, rs3770102	CC	18 (29)	78 (36.4)	0.4	16 (41)	78 (36.4)	0.03¶
	AC	28 (45.2)	95 (44.4)		22 (56.4)	95 (44.4)	
	AA	16 (25.8)	41 (19.2)		1 (2.6)	41 (19.2)	

Gene/polymorphisms	Genotype	Neurotoxicity grade 1/2, n (%)			Neurotoxicity grade 3/4, n (%)		
		Grade 1/2	Grade 0	p-value	Grade 3/4	Grade 0	p-value
MAP4, rs11268924	Del	5 (8.1)	16 (7.6)	0.8	4 (10.5)	16 (7.6)	0.8
	Hetero	30 (48.4)	92 (43.8)		15 (39.5)	92 (43.8)	
	Ins	27 (43.5)	102 (48.6)		19 (50)	102 (48.6)	
MAP4, rs1137524	GG	27 (41.5)	104 (47.7)	0.1	20 (52.6)	104 (47.7)	0.4
	GA	31 (47.7)	105 (48.2)		15 (39.5)	105 (48.2)	
	AA	7 (10.8)	9 (4.1)		3 (7.9)	9 (4.1)	
MAP4, rs1875103	GG	42 (22.1)	148 (77.9)	0.6	27 (15.4)	148 (84.6)	0.8
	GA/AA	22 (24.7)	67 (75.3)		11 (14.1)	67 (85.9)	
MAP4, rs11711953	GG	53 (21.1)	198 (78.9)	0.07	35 (15)	198 (85)	0.8
	GA/AA	11 (35.5)	20 (64.5)		4 (16.7)	20 (83.3)	
TUBB, rs6070697	CC	44 (68.8)	153 (71.2)	0.8	23 (59)	153 (71.2)	0.1
	CT/TT	20 (31.3)	62 (28.8)		16 (41)	62 (28.8)	
TUBB, rs10485828	CC	0	6 (3)	0.3	2 (5.3)	6 (3)	0.7
	CG	15 (23.8)	58 (28.7)		12 (31.6)	58 (28.7)	
	GG	48 (76.2)	138 (68.3)		24 (63.2)	138 (68.3)	

Carriers of the minor allele (homozygous and heterozygous individuals) are grouped together for SNPs with low minor allele frequencies. The risk values for significantly associated genotype are as follows:

† Odds ratio [OR]: 0.3; 95% CI: 0.1–0.9; p = 0.02;

‡ OR: 2.8; 95% CI: 1.3–6.3; p = 0.008;

§ OR: 2.1; 95% CI: 1.1–3.7 (p = 0.02);

¶ OR: 0.1; 95% CI: 0.01–0.8; p = 0.009.

Table 3

Risk of neurotoxicity associated with the genetic variants of the vincristine pathway in the presence of clinical covariates.

Variable	Neurotoxicity 1/2			Neurotoxicity 3/4		
	Sig. (p)	OR	95% CI	Sig. (p)	OR	95% CI
Age	0.06	3.9	0.9–16.1	0.2	2.9	0.6–15.1
Risk group	0.6	1.2	0.6–2.5	0.3	0.6	0.2–1.6
BSA	0.4	0.5	0.1–2.4	0.4	2.3	0.4–13.7
Treatment protocol	0.002	1.7	1.2–2.4	0.002	1.9	1.3–2.9
ABCB1 rs4728709	0.02	0.3	0.1–0.9			
ACTG1 rs1135989				0.03	2.6	1.1–6.0
CAPG rs3770102				0.02	0.07	0.01–0.6

The risk (OR and 95% CI), of neurotoxicity grades 1/2 and 3/4 associated with the minor allele of indicated *ABCB1*, *ACTG1* and *CAPG* polymorphisms in the presence of clinical covariates. The referent categories for these covariates were age ≥1 and <10, standard risk group and the most recent treatment protocol (00-01); BSA was a continuous variable.

Blank cells show that the p-value was not significant for the association between neurotoxicity and polymorphism.

BSA: Body surface area; OR: Odds ratio; Sig.: Significance.

Table 4

The relationship between polymorphisms of the vincristine pathway and event-free survival.

Gene/polymorphisms	Genotype	Frequency, n (%)		
		Event	Nonevent	p-value
ABCB1, rs1045642 (3435)	CC	11 (17.2)	88 (32.4)	0.03†
	CT	33 (51.5)	120 (44.1)	
	TT	20 (31.3)	64 (23.5)	
ABCB1, rs2032582 (2677)	GG	20 (31.7)	117 (42.4)	0.03‡
	GT	25 (39.7)	111 (40.2)	
	TT	18 (28.6)	48 (17.4)	
ABCB1, rs1128503 (1236)	TT	22 (34.9)	111 (40.3)	0.1
	TC	16 (38.1)	82 (42.7)	
	CC	14 (27)	73 (17)	
ABCB1, rs4728709	TT/TC	12 (19.4)	47 (17.3)	0.7
	CC	50 (80.6)	225 (82.7)	
CYP3A5, rs776746 (**1/*3*)	AA/AG	4 (6.5)	41 (14.9)	0.08
	GG	58 (93.5)	235 (85.1)	
ACTG1, rs7406609	CC	15 (24.2)	57 (21)	0.6
	CT	28 (45.2)	128 (47.2)	
	TT	19 (30.6)	86 (31.8)	
ACTG1, rs1135989	GG	24 (38.7)	120 (44)	0.6
	GA	33 (53.2)	108 (39.6)	
	AA	5 (8.1)	45 (16.4)	
ACTG1, rs1139405	TT	35 (55.6)	179 (64.6)	0.3
	TC	25 (39.7)	85 (30.7)	
	CC	3 (4.7)	13 (4.7)	
CAPG, rs6886	GG	22 (35.5)	110 (40.1)	0.7
	GA	30 (48.4)	115 (42)	
	AA	10 (16.1)	49 (17.9)	
CAPG, rs2229668	GG	50 (79.4)	211 (77.6)	0.9
	GA	10 (15.9)	54 (19.8)	
	AA	3 (4.7)	7 (2.6)	
CAPG, rs3770102	CC	26 (42)	95 (35.1)	0.3
	AC	27 (43.5)	125 (46.1)	
	AA	9 (14.5)	51 (18.8)	
MAP4, rs11268924	Del	2 (3.2)	23 (8.6)	0.2

Gene/polymorphisms	Genotype	Frequency, n (%)		
		Event	Nonevent	p-value
	Hetero	28 (45.2)	121 (45.1)	
	Ins	32 (51.6)	124 (46.3)	
MAP4, rs1137524	GG	32 (50.8)	127 (45.8)	0.4
	GA	29 (46)	133 (48)	
	AA	2 (3.2)	17 (6.2)	
MAP4, rs1875103	GG	40 (64.5)	190 (69.5)	0.5
	GA/AA	22 (35.5)	83 (30.5)	
MAP4, rs11711953	GG	55 (87.3)	248 (89.5)	0.4
	GA/AA	8 (12.7)	29 (10.5)	
TUBB, rs6070697	CC	42 (67.7)	193 (70.4)	0.8
	CT/TT	20 (32.3)	81 (29.6)	
TUBB, rs10485828	CC	2 (3.3)	7 (2.7)	0.9
	CG	16 (26.2)	75 (28.8)	
	GG	43 (70.5)	178 (68.5)	

†Hazard ratio *ABCB1*$_{3435T}$: 2.2; 95% CI: 1.1–4.2; p = 0.02.

‡Hazard ratio *ABCB1*$_{2677TT}$: 1.7; 95% CI: 1.0–3.0; p = 0.05.