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Original Investigation

Association of an Inherited Genetic Variant With Vincristine-Related Peripheral Neuropathy in Children With Acute Lymphoblastic Leukemia

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IMPORTANCE With cure rates of childhood acute lymphoblastic leukemia (ALL) exceeding 85%, there is a need to mitigate treatment toxicities that can compromise quality of life, including peripheral neuropathy from vincristine treatment.

OBJECTIVE To identify genetic germline variants associated with the occurrence or severity of vincristine-induced peripheral neuropathy in children with ALL.

DESIGN, SETTING, AND PARTICIPANTS Genome-wide association study of patients in 1 of 2 prospective clinical trials for childhood ALL that included treatment with 36 to 39 doses of vincristine. Genome-wide single-nucleotide polymorphism (SNP) analysis and vincristine-induced peripheral neuropathy were assessed in 321 patients from whom DNA was available: 222 patients (median age, 6.0 years; range, 0.1-18.8 years) enrolled in 1994-1998 in the St Jude Children's Research Hospital protocol Total XIII B with toxic effects follow-up through January 2001, and 99 patients (median age, 11.4 years; range, 3.0-23.8 years) enrolled in 2007-2010 in the Children's Oncology Group (COG) protocol AALL0433 with toxic effects follow-up through May 2011. Human leukemia cells and induced pluripotent stem cell neurons were used to assess the effects of lower *CEP72* expression on vincristine sensitivity.

EXPOSURE Treatment with vincristine at a dose of 1.5 or 2.0 mg/m².

MAIN OUTCOMES AND MEASURES Vincristine-induced peripheral neuropathy was assessed at clinic visits using National Cancer Institute criteria and prospectively graded as mild (grade 1), moderate (grade 2), serious/disabling (grade 3), or life threatening (grade 4).

RESULTS Grade 2 to 4 vincristine-induced neuropathy during continuation therapy occurred in 28.8% of patients (64/222) in the St Jude cohort and in 22.2% (22/99) in the COG cohort. A SNP in the promoter region of the *CEP72* gene, which encodes a centrosomal protein involved in microtubule formation, had a significant association with vincristine neuropathy (meta-analysis $P = 6.3 \times 10^{-9}$). This SNP had a minor allele frequency of 37% (235/642), with 50 of 321 patients (16%; 95% CI, 11.6%-19.5%) homozygous for the risk allele (TT at rs924607). Among patients with the high-risk *CEP72* genotype (TT at rs924607), 28 of 50 (56%; 95% CI, 41.2%-70.0%) developed at least 1 episode of grade 2 to 4 neuropathy, a higher rate than in patients with the *CEP72* CC or CT genotypes (58/271 patients [21.4%; 95% CI, 16.9%-26.7%]; $P = 2.4 \times 10^{-6}$). The severity of neuropathy was greater in patients homozygous for the TT genotype compared with patients with the CC or CT genotype (2.4-fold by Poisson regression [$P < .0001$] and 2.7-fold based on mean grade of neuropathy: 1.23 [95% CI, 0.74-1.72] vs 0.45 [95% CI, 0.3-0.6]; $P = .004$ by t test). Reducing *CEP72* expression in human neurons and leukemia cells increased their sensitivity to vincristine.

CONCLUSIONS AND RELEVANCE In this preliminary study of children with ALL, an inherited polymorphism in the promoter region of *CEP72* was associated with increased risk and severity of vincristine-related peripheral neuropathy. If replicated in additional populations, this finding may provide a basis for safer dosing of this widely prescribed anticancer agent.

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Cancer remains the leading cause of death by disease in US children despite major advances in the last 20 years. Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, and as cure rates have surpassed 85%,^{1,2} it becomes increasingly important to mitigate the acute and chronic toxicities of treatment that adversely affect quality of life and longevity. Vincristine is one of the most widely used and effective anticancer agents for treating leukemias, lymphomas, brain tumors, and solid tumors in both adults and children^{1,2} and is administered multiple times as part of treatment protocols for every child with ALL and for children with neuroblastoma, Wilms tumor, rhabdomyosarcoma, Ewing sarcoma, and retinoblastoma.³

Vincristine exerts its cytotoxic effects by interfering with microtubule formation and mitotic spindle dynamics, leading to mitotic arrest and cell death.⁴⁻⁶ The dose-limiting toxic effect of vincristine is peripheral neuropathy,^{7,8} characterized by neuropathic pain and sensory and motor dysfunction (eg, impaired manual dexterity, balance and

deep tendon reflexes, and altered gait). A substantial percentage of patients develop neuropathy that causes considerable morbidity and often disrupts curative treatment.⁹ Currently, there are no reliable

ALL acute lymphoblastic leukemia

CTCAE Common Terminology Criteria for Adverse Events

GWAS genome-wide association study

iPSC induced pluripotent stem cell

SNP single-nucleotide polymorphism

means of identifying patients at high risk of vincristine-induced neuropathy nor strategies to mitigate this drug toxicity. Although prior studies have documented racial differences in the incidence of vincristine neuropathy, candidate gene studies have failed to identify consistent genetic variants associated with an increased risk of vincristine-induced neuropathy.⁹⁻¹²

To determine whether there are genetic polymorphisms associated with vincristine-induced neuropathy, we performed a genome-wide association study (GWAS) in 2 independent cohorts of children receiving protocol-defined treatment for ALL.

Methods

Patients and Treatment Regimens

Two independent patient cohorts were treated either by St Jude Children's Research Hospital or the Children's Oncology Group (COG) according to contemporary institutional review board-approved ALL protocols that prospectively assessed and graded vincristine neuropathy according to National Cancer Institute (NCI) criteria. We analyzed all consenting patients who had germline DNA available for genome-wide single-nucleotide polymorphism (SNP) analyses in May 2011. The first cohort included children with newly diagnosed ALL enrolled and treated in the St Jude Total XIIIIB protocol¹³⁻¹⁵ (available from the authors). The second cohort included patients with relapsed ALL enrolled in the COG AALL0433 protocol (<https://clinicaltrials.gov/show/NCT00381680>). Written informed consent was ob-

tained from patients or their guardians and assent from patients, as appropriate.

The St Jude Total XIIIIB protocol was divided into 3 phases: 8 weeks of remission induction therapy, 2 weeks of consolidation therapy, and 120 weeks of continuation therapy (including a reinduction phase at weeks 16-21). Vincristine was administered as a component of combination chemotherapy during the induction and continuation phases of therapy at a dose of 1.5 mg/m² with a maximum dose of 2 mg. Altogether, St Jude patients were scheduled to receive a total of 36 vincristine doses. Consecutive patients were enrolled between August 1994 and July 1998 and were followed up for toxic effects through 2001.

The COG AALL0433 protocol was a cooperative group-wide phase 3 randomized study of intensive therapy for patients with recurrent childhood precursor B-cell ALL, including late bone marrow or combined relapse (≥ 36 months since diagnosis), or early (<18 months since diagnosis) isolated extramedullary relapse in the central nervous system or testes. The COG AALL0433 treatment was divided into induction, intensification (with a reinduction phase at weeks 28-32 or 29-33), and continuation phases. Vincristine was administered as a component of combination chemotherapy during the induction, intensification, reinduction, and continuation phases of therapy; a total of 39 doses of vincristine were scheduled to be given per this protocol. Patients were randomly assigned to receive a vincristine dose of 1.5 mg/m² with a maximum dose of 2 mg (group A) or 2 mg/m² with a maximum dose of 2.5 mg (group B). Group B treatment and the vincristine dose randomization were permanently suspended by COG in 2010 because of a high incidence of peripheral neuropathy, most prominently in patients aged 13 years or older. Patients were enrolled between March 2007 and September 2010 at the time of the suspension of group B treatment and were followed up for toxic effects through May 2011.

As part of required clinical trial adverse event monitoring, children in both cohorts were prospectively assessed for the presence of peripheral neuropathy via physical examination by the treating oncologist at each clinic visit during treatment.^{16,17} Children in the St Jude cohort were assessed for toxicity weekly throughout all phases of therapy. Children in the COG cohort were assessed for toxicity weekly throughout the first year of therapy and monthly thereafter. For these analyses, children in the St Jude Total XIIIIB study were graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 1.0 and those in the COG cohort according to a modified Balis scale for neuropathy, which is a modification of the NCI CTCAE version 2.0 (eTables 1 and 2 in the Supplement). Both scales classify neuropathy events as mild (grade 1), moderate (grade 2), serious/disabling (grade 3), or life threatening (grade 4).¹⁸ Those with grades 2, 3, or 4 motor and/or sensory neuropathy were considered neuropathy cases in our analyses. There were no neuropathy-related deaths (grade 5). Follow-up for the St Jude cohort continued through January 2001; follow-up for the COG cohort continued through May 2011.

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Genotyping

Germline DNA (500 ng) was extracted from normal peripheral blood leukocytes, digested with restriction enzymes, amplified, labeled, and hybridized to the Affymetrix GeneChip Human Mapping 500K array (532 552 SNPs) or the SNP 6.0 array (906 600 SNPs) (Affymetrix). SNPs were excluded if genotyping call rates were less than 95% or if the minor allele frequency was less than 1% (eAppendix 1 in the Supplement). SNP imputation was carried out using MaCH version 1.0.15.¹⁹ The total number of SNPs that were imputed was 21 764 463 (eAppendix 1 in the Supplement).

Race was genetically determined following a method previously described.²⁰ Briefly, the genetic ancestry estimates were determined by applying STRUCTURE (version 2.2.3) using HapMap project data as references (eAppendix 1 in the Supplement). SNP genotype data for HapMap cells were downloaded from release 22 on the International HapMap Project website (<http://hapmap.ncbi.nlm.nih.gov/>).

Cellular Analyses

The human precursor B leukemia cell line Nalm6, the human T-lineage leukemia cell line CEM, and the neuroblastoma cell line SH-SY5Y were cultured in RPMI 1640 medium containing 2 mM of glutamine and 10% (vol/vol) fetal bovine serum at 37°C (98.6°F) with 5% CO₂. iCell neurons derived from human induced pluripotent stem cells were obtained from Cellular Dynamics International and maintained per manufacturer's protocol. Primary ALL cells were isolated by applying a Ficoll-Hypaque gradient to bone marrow aspirates obtained at diagnosis (with a median of 97% blast cells). *CEP72* expression (GenBank BC001750) was impaired by using short hairpin RNA. In vitro drug sensitivity of primary ALL cells and of Nalm6 and CEM cell lines was determined using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazoliumbromide) assay as previously reported.²¹ The drug concentration lethal to 50% of the leukemia cells (LC₅₀) was used as the measure of sensitivity to vincristine. Vincristine-induced neurotoxicity in iCell neurons was determined by individual cell measurements of total neurite outgrowth, number of processes, and number of branches in 1000 cells for each replicate experiment. Luciferase assays to measure *CEP72* expression were performed as described in eAppendix 1 in the Supplement. Gene expression of HapMap cells measured by the Affymetrix GeneChip Human Exon 1.0 ST Array was downloaded from <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE7761>.

Statistical Analysis

Analyses were conducted using R (<http://www.r-project.org/>) and SAS (SAS Institute Inc) software. Weighted logistic regression model fitting for recurrent events was used to test the associations between SNP genotypes and the phenotype of peripheral neuropathy (ie, occurrence of grade ≥ 2 vincristine-induced neuropathy during continuation therapy), including cumulative vincristine dose and genetically determined ancestry as covariates, as previously described (eAppendix 1 in the Supplement).¹⁵ After filtering out SNPs with a call rate of less than 95% (SNP call rate is the percentage of patients who

have a confident genotype call for each SNP according to Affymetrix quality control criteria) or minor allele frequency of less than 1%, the remaining SNPs (484 623 typed SNPs and 1 091 393 imputed SNPs) were assessed in a GWAS for their association with vincristine-induced neuropathy in the St Jude and COG cohorts separately using weighted logistics regression models, then combined by pooling the *P* values from the 2 cohorts via the Fisher combined probability test. All *P* values were computed using 2-sided tests; statistical significance was determined at the genome-wide significance level of $P < 5 \times 10^{-8}$.

Post-GWAS analyses focusing on the *CEP72* SNP rs924607 were performed as follows: the relationship between the time to first episode and genotype was analyzed using cumulative incidence function estimates²² and the Gray test,²³ Cox regression, and accelerated failure time regression²² with cumulative dose and genetic ancestry as covariates. The relationship between the cumulative vincristine dose at the first toxic episode and genotype was analyzed by Cox regression with genetically determined ancestry as a covariate. The relationship between severity (grade) of toxicity at the first episode and genotype was analyzed by Poisson regression model with cumulative dose and genetic ancestry as covariates. The *t* test was used to examine the differences in luciferase reporter activity. A linear regression model was used to test the association between vincristine sensitivity (LC₅₀) and the SNP genotype in primary ALL cells. In post-GWAS analyses and laboratory experiments, all tests were 2-sided and significance was determined at the $P < .05$ level.

Results

Patient Population

Genome-wide SNP analysis and vincristine-induced neuropathy were assessed in 321 patients enrolled in 2 ALL treatment protocols. Of 247 consecutive patients enrolled in the St Jude Total XIIIIB protocol, 222 patients with newly diagnosed ALL (median age at diagnosis, 6.0 years; range, 0.1-18.8 years) had germline DNA available for analysis and were included in this study (eFigure 1 in the Supplement). Of the 137 patients who were enrolled in the COG AALL0433 protocol, 99 patients with relapsed ALL (median age at diagnosis, 11.4 years; range, 3.0-23.8 years) had germline DNA available for analysis and were included in this study (eFigure 2 in the Supplement).

The demographic and clinical characteristics of these 321 patients are summarized in **Table 1** and for each cohort in eTables 3 and 4 in the Supplement.

Vincristine-Associated Neurotoxicity

Grade 2 to 4 vincristine-induced peripheral neuropathy during continuation therapy occurred in 28.8% (64/222) and 22.2% (22/99) of patients in the St Jude and COG protocols, respectively. Genetically determined ancestry and vincristine cumulative dose were the demographic or clinical characteristics significantly related to the occurrence of vincristine-induced neuropathy in each of the cohorts, as summarized in eTables 3 and 4 in the Supplement. Within the COG trial, the

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Table 1. Characteristics of Patients in the Combined Cohort of St Jude and COG Patients Assessed for Vincristine Neuropathy

Characteristics	Overall No. (%) of Patients (N = 321) ^a	Grade of Vincristine Neuropathy, No. of Patients			No. of Episodes of Grade 2-4 Vincristine Neuropathy ^b	P Value ^c
		None to Grade 1	1 Episode of Grade 2-4	≥2 Episodes of Grade 2-4		
Age at diagnosis, y						
<1	8 (2.5)	7	1	0	1	
1-10	192 (59.8)	137	34	21	103	.03
>10	121 (37.7)	91	16	14	84	
Sex, No. (%)						
Male	187 (58.3)	142	29	16	88	
Female	134 (41.7)	93	22	19	100	.02
Genetically determined ancestry						
European	209 (65.1)	156	29	24	123	
African	43 (13.4)	33	8	2	15	
Asian	2 (0.6)	1	1	0	1	.002
Hispanic	44 (13.7)	30	7	7	36	
Other	23 (7.2)	15	6	2	13	
Cumulative vincristine dose, median (range), mg/m ²	47 (2-120)	46 (2-120)	51 (8-92)	49 (10-120)	52 (22-120)	.28

^a Cohort includes patients treated in the St Jude Total XIII B (n = 222) and Children's Oncology Group AALL0433 (n = 99) protocols.

^c P values are derived from univariate weighted logistic regression using each episode as an observation.

^b Number of events.

Table 2. SNPs Associated With the Incidence of Vincristine-Induced Neuropathy (grades 2-4)^a

SNP	Chromosome	Position	Nearest Gene	Allele, Major/Minor	St Jude Total XIII B Protocol			COG AALL0433 Protocol			P Value for Meta-analysis	
					MAF, %	OR (95% CI)	P Value	MAF, %	OR (95% CI)	P Value	Univariate	Multivariate ^b
rs924607 ^c	5	663093	CEP72	C/T	36.7	2.43 (1.70-3.49)	1.25×10 ⁻⁶	36.4	4.1 (1.86-9.01)	.0004	6.33 × 10 ⁻⁹	4.68 × 10 ⁻⁸
rs17032980	2	67156247	ETAA1	A/G	26.6	3.17 (1.95-5.17)	3.67×10 ⁻⁶	19.2	10.4 (2.97-36.15)	.0002	9.77 × 10 ⁻⁹	9.01 × 10 ⁻⁷
rs12786200	11	91618666	MTNR1B	C/T	22.7	0.23 (0.13-0.40)	1.58×10 ⁻⁷	20.7	0.24 (0.08-0.76)	.02	2.56 × 10 ⁻⁸	6.30 × 10 ⁻⁷
rs4463516 ^c	9	32857481	TMEM215	C/G	33.6	2.89 (1.90-4.39)	6.83×10 ⁻⁷	24.2	4.94 (1.65-14.79)	.004	3.02 × 10 ⁻⁸	1.19 × 10 ⁻⁷
rs7818688 ^c	8	96093258	NDUFA6	C/A	12.6	4.26 (2.45-7.42)	3.05×10 ⁻⁷	14.1	4.59 (1.35-15.59)	.01	4.46 × 10 ⁻⁸	5.03 × 10 ⁻⁷

Abbreviations: COG, Children's Oncology Group; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

When there were multiple SNPs in linkage disequilibrium, only the one with the lowest P value is represented.

^a SNPs were selected based on their univariate meta-analysis P values, and the SNPs with the lowest P values are shown. These SNPs had the lowest P values among the 5051 typed SNPs and 10 195 imputed SNPs with a P < .05 in each cohort separately and with concordant direction (effect) in the 2 cohorts.

^b Including genetically defined ancestry and cumulative vincristine dose as covariates.

^c Imputed SNP.

rate of vincristine-induced neuropathy was higher in the cohort of patients randomized to the higher dose of vincristine (2.0 mg/m² with a maximum dose of 2.5 mg) compared with those randomized to the conventional dose (1.5 mg/m² with a maximum dose of 2 mg) (19 of 48 patients [39.6%] vs 3 of 51 patients [5.9%]; P < .0001), leading to early closure of the higher-dose treatment group of the COG protocol.

Meta-analysis of Genome-wide Association Results

Assessing SNPs that had a P < .05 in both cohorts and concordant effects in both populations yielded 5051 typed SNPs and 10 195 imputed SNPs. The Manhattan plot (eFigure 3 in the Supplement) depicts P values of SNPs from the 2 patient cohorts assessed via a meta-analysis. The SNPs with the lowest P values for their association with vincristine-induced neu-

ropathy in the 2 cohorts are summarized in Table 2 and in eTable 5 in the Supplement. In a multivariable analysis that included genetically defined ancestry and cumulative dose of vincristine, only rs924607 remained genome-wide significantly (P = 4.7×10⁻⁸) related to the development of vincristine-induced neuropathy (Table 2 and eTable 5). rs924607 is in the promoter region of the CEP72 gene in chromosome 5 (Figure 1).

Twenty (62%) of 32 patients with the TT genotype in the St Jude cohort and 8 (44%) of 18 patients with the TT genotype in the COG cohort developed at least 1 episode of vincristine-induced neuropathy. In total, 28 of 50 patients (56%) with the TT genotype developed at least 1 episode of grade 2 to 4 vincristine-induced neuropathy (Table 3) compared with 58 (21.4%) of 271 patients with the CC or CT genotype (P = 2.4×10⁻⁶). The allelic odds ratios for the development of

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neuropathy for rs924607 were 2.43 (95% CI, 1.70-3.49) and 4.1 (95% CI, 1.86-9.01) in the St Jude and COG cohorts, respectively. As summarized in eTable 6 in the Supplement, the frequency of the *CEP72* risk allele (T) differed by ancestry, with a lower frequency in patients with African ancestry and in the HapMap cells from individuals of African ancestry (Yoruba in

Ibadan, Nigeria; African ancestry in southwestern United States; Luhva in Webuye, Kenya; Maasai in Kinyama, Kenya) compared with cells from individuals of European ancestry.

Because rs924607 is an imputed SNP, we validated the genotype in a subset of 30 patients randomly chosen to represent the 3 diplotypes for *CEP72*. This documented com-

Figure 1. Regional Association Plot for the rs924607 Locus on Chromosome 5

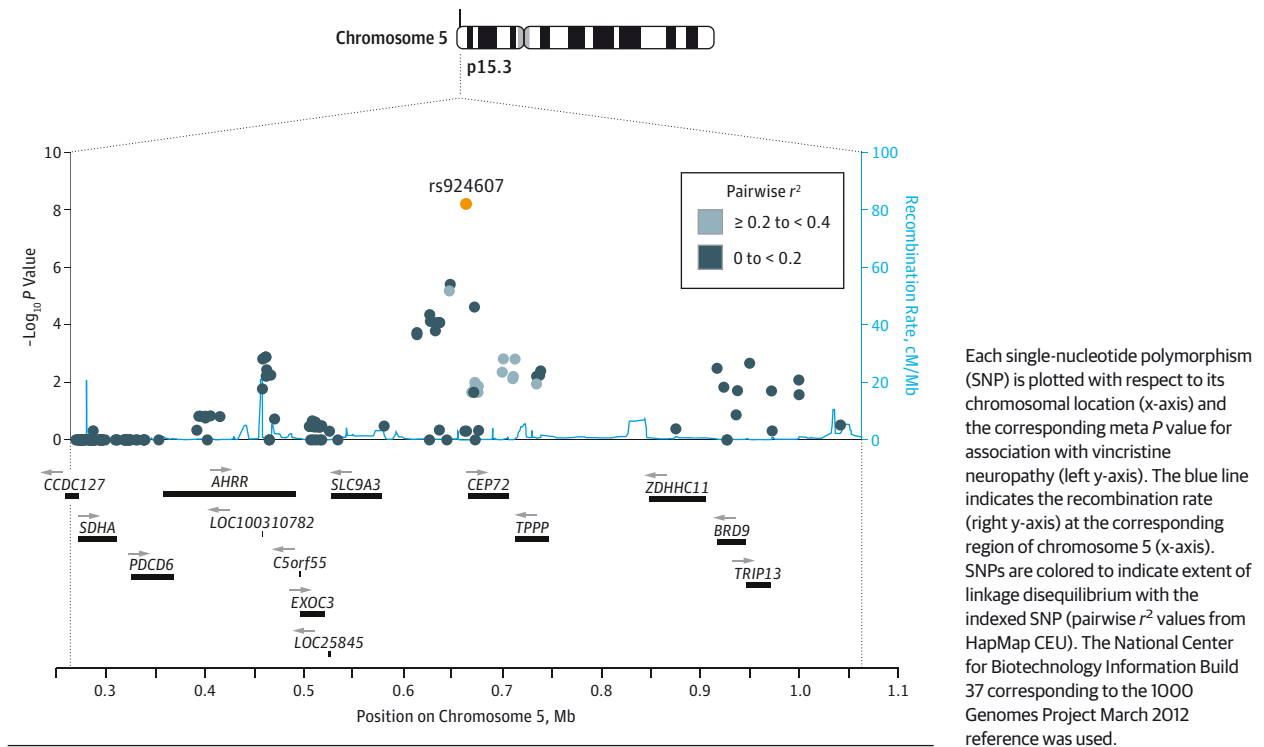


Table 3. Vincristine-Induced Peripheral Neuropathy and rs924607 Genotype

Genotype	No. of Patients ^a					Total	Grade 2-4 Vincristine Neuropathy, %
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4		
St Jude Total XIII B							
CC	65	0	17	8	1	91	28.6
CT	81	0	9	9	0	99	18.2
TT	12	0	13	7	0	32	62.5
Total	158	0	39	24	1	222	
COG AALL0433							
CC	37	1	4	3	0	45	15.6
CT	28	1	4	3	0	36	19.4
TT	9	1	3	5	0	18	44.4
Total	74	3	11	11	0	99	
Combined cohort of St Jude Total XIII B and COG AALL0433							
CC	102	1	21	11	1	136	24.3
CT	109	1	13	12	0	135	18.5
TT	21	1	16	12	0	50	56.0
Total	232	3	50	35	1	321	

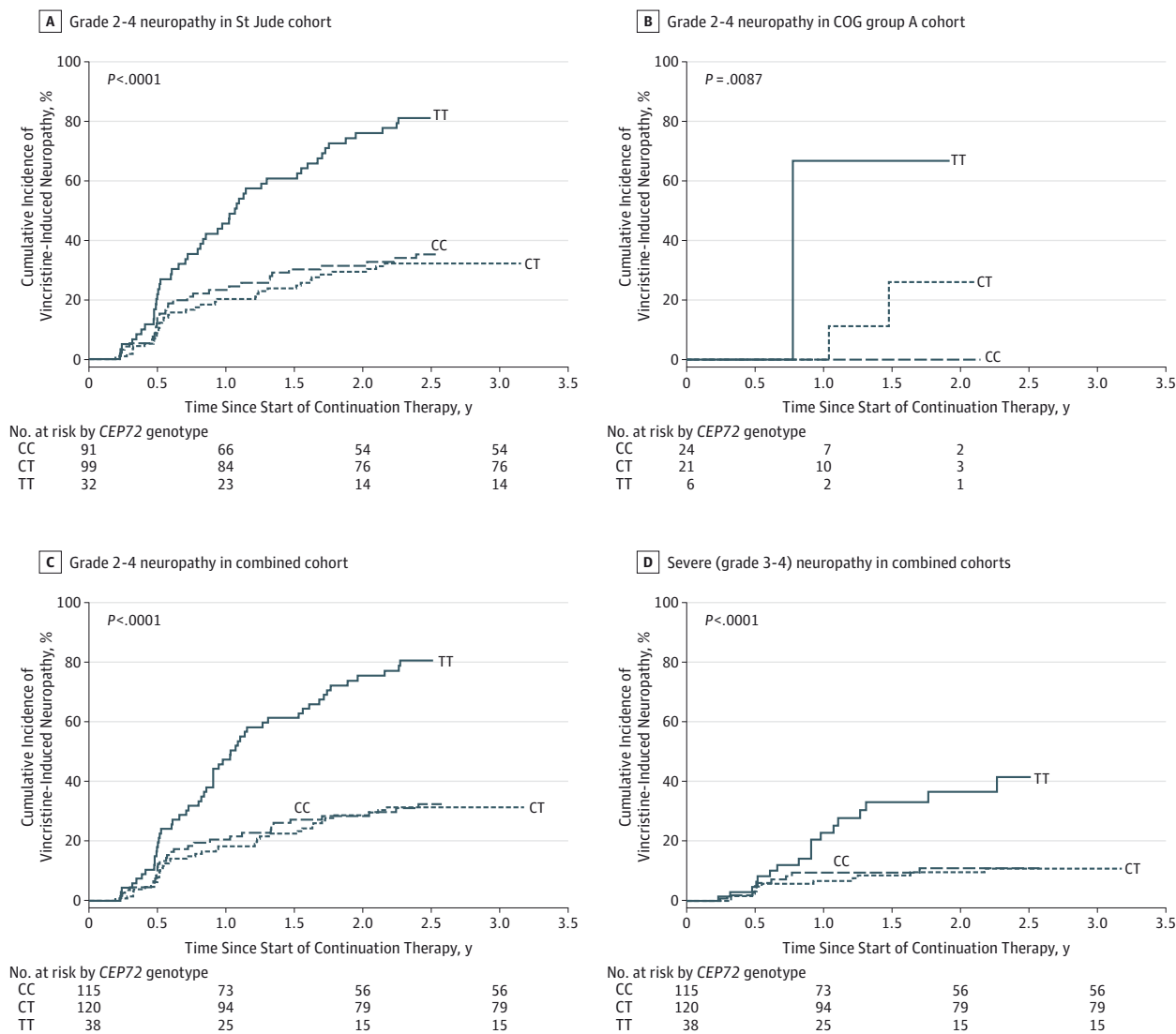
Abbreviation: COG, Children's Oncology Group.

^a Numbers of patients for each rs924607 genotype and at each grade of vincristine-induced neuropathy are presented for the St Jude Total XIII B

cohort, for the COG AALL0433 cohort, and for the combined cohort. Patients who experienced more than 1 episode of neuropathy at different grades were included at the highest grade reported.

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Figure 2. Cumulative Incidence of Vincristine-Induced Neuropathy Among Patients With Different *CEP72* Genotypes



The cumulative incidence of vincristine-induced neuropathy (grades 2-4) differed by *CEP72* promoter SNP (rs924607) genotype in the St Jude cohort (A) ($P < .0001$), the Children’s Oncology Group (COG) group A cohort (B) ($P = .0087$), and the combined St Jude and COG group A cohort (C) ($P < .0001$). Likewise, the cumulative incidence of severe neuropathy (grades 3-4) was also significantly higher in patients homozygous for the *CEP72* risk allele (TT) (D).

P values were computed based on weighted logistic regression adjusting for ancestry and accounting for multiple episodes of neuropathy. The number of patients evaluable and at risk of neuropathy is provided for each *CEP72* genotype (diplotype). The x-axis represents time since the start of continuation therapy for ALL.

plete concordance between the imputed genotypes and those obtained by direct genotyping (eFigure 4 in the Supplement).

Association of *CEP72* rs924607 Genotype With Development, Onset Time, and Severity of Vincristine-Induced Peripheral Neuropathy

The cumulative incidence of all neuropathy episodes (grade 2-4) differed significantly by *CEP72* rs924607 genotype (diplotype) in both patient cohorts treated with 1.5 mg/m² of vincristine, with *P* values in the St Jude, COG, and combined cohorts of $P < .0001$, $P = .0087$, and $P < .0001$, respectively, by Gray test (Figure 2, A-C). Likewise, the cumulative incidence of severe neuropathy episodes (grades 3-4) was also signifi-

cantly higher in patients homozygous for the *CEP72* risk allele ($P < .0001$) (Figure 2D). Based on binomial proportions, 21 of 38 (55.3%; 95% CI, 39.7%-69.9%) patients with the *CEP72* TT genotype experienced grade 2 to 4 neuropathy during the monitoring period compared with 46 of 235 (19.6%; 95% CI, 14.8%-25.3%) patients with the CC/CT genotype ($P < .001$). The cumulative incidence of the first neuropathy episode (grade 2-4) was 60.8% (95% CI, 43.9%-77.6%) among patients with the *CEP72* TT genotype compared with 23.4% (95% CI, 17.4%-29.4%) among patients with the CC/CT genotype ($P < .001$). The risk of developing neuropathy was significantly higher in patients with the *CEP72* TT genotype (hazard ratio for TT vs CT/CC, 3.58; 95% CI, 2.1-6.1) in a Cox regression model adjusting

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for cumulative vincristine dose and genetically determined ancestry (eTable 7 in the Supplement). This *CEP72* SNP was not significantly associated with the incidence of vincristine-induced neuropathy among patients randomized to treatment with the higher (experimental) dose of vincristine (eFigure 5 in the Supplement).

By fitting an accelerated failure time model²² with *CEP72* genotype, cumulative vincristine dose, and genetically determined ancestry as explanatory variables, the estimated ratio of median time to the first episode of neuropathy in patients with a TT genotype compared with patients with a CT or CC genotype was approximately 20% shorter in TT patients of any race and at any cumulative vincristine dose level ($P = 6.19 \times 10^{-6}$ for the regression coefficient of the SNP) (eTable 8 in the Supplement). Without adjusting for covariates, among those who developed grade 2 to 4 neuropathy, the average time to development of neuropathy was 225 days (95% CI, 169-281 days) in patients with the *CEP72* genotype TT compared with 307 days (95% CI, 244-370 days) in patients with the CT/CC genotype.

In unadjusted analysis comparing the mean grade of neuropathy observed at the first episode (assigning grade 0 to those who did not develop neuropathy) in patients treated with 1.5 mg/m² of vincristine, the mean grade of neuropathy in white patients with the *CEP72* high-risk genotype (TT) was significantly higher (2.7-fold) than the mean grade of neuropathy in patients with the *CEP72* CC or CT genotypes (1.23 [95% CI, 0.74-1.72] vs 0.45 [95% CI, 0.3-0.6]; $P = .004$ by *t* test). The numbers of patients in other ancestry groups were too small to make meaningful subgroup comparisons. Using the Poisson regression model to assess the severity (grade) of neuropathy at first episode, with genetically defined ancestry and cumulative vincristine dose as covariates in the combined cohort, the average grade (by CTCAE) of neuropathy was 2.4-fold greater (95% CI, 1.59-3.69) in patients homozygous for the *CEP72* risk allele (TT) compared with all other patients ($P < .0001$) (eTable 9 in the Supplement).

Rs924607 and *CEP72* mRNA Expression

Because rs924607 is located in the promoter region of *CEP72*, we examined *CEP72* messenger RNA (mRNA) expression levels for association with rs924607 genotype in HapMap samples (CEU), using the publicly available gene expression data set.²⁴ Patients who were homozygous for the variant T allele had significantly lower expression of *CEP72* mRNA compared with those who were heterozygous or homozygous for the wild-type C allele ($P = .03$) (eFigure 6A in the Supplement). The effect of rs924607 genotype on *CEP72* expression was confirmed using a luciferase reporter assay in 2 different human cell lines, documenting significantly lower expression with the T allele compared with the C allele in both SH-SY5Y neuroblastoma cells (eFigure 6B in the Supplement) and Nalm6 leukemia cells (eFigure 6C in the Supplement). The rs924607 T variant creates a consensus binding site for the NKX-6.3 transcription factor (repressor) (eFigure 6, D-H and eTable 10 in the Supplement), markedly enhancing NKX-6.3 binding to the risk allele, leading to lower *CEP72* mRNA expression.

Low *CEP72* Expression and Vincristine-Induced Toxicity

The relationship between *CEP72* expression and vincristine-induced neurotoxicity was assessed in neurons derived from human induced pluripotent stem cells (iPSCs), revealing significantly greater sensitivity to vincristine when *CEP72* expression was reduced (eFigure 7, A-B in the Supplement).

Likewise, reducing expression of *CEP72* in 2 human ALL cells lines, Nalm6 (B-cell lineage) (eFigure 7C in the Supplement) and CEM (T-cell lineage) (eFigure 7E), was associated with increased sensitivity to vincristine (eFigure 7, D and F). Moreover, primary leukemia cells from patients with newly diagnosed ALL who were homozygous for the *CEP72* risk allele (TT) (mean LC_{50} , 0.9 μ M; 95% CI, -0.5 to 2.3 μ M) were more sensitive to vincristine compared with patients with either the CT (mean LC_{50} , 2.6 μ M; 95% CI, 0.6-4.7 μ M) or CC (mean LC_{50} , 11.9 μ M; 95% CI, 3.0-20.7 μ M) *CEP72* genotype ($P = .02$) (eFigure 7G).

Discussion

This study found that an inherited variant in the promoter region of the *CEP72* gene was associated with a higher incidence and severity of vincristine-related peripheral neuropathy in children with ALL, with the cumulative incidence of neuropathy significantly higher and the mean grade of neuropathy significantly greater in patients homozygous for the *CEP72* risk allele (TT) compared with all other patients. The *CEP72* gene encodes a centrosomal protein that is essential for microtubule formation, and vincristine exerts its pharmacologic effects by inhibiting microtubule formation. We found that the *CEP72* variant associated with vincristine neuropathy (T allele at rs924607) creates a binding site for a transcriptional repressor, leading to lower *CEP72* mRNA expression, and we showed that reducing the expression of *CEP72* in human iPSC neurons or leukemia cells increased their sensitivity to vincristine. Because vincristine-induced toxicity can be influenced by cumulative dose of vincristine and can differ by race, we included both cumulative dose and ancestry as covariates in our analyses, revealing that the *CEP72* rs924607SNP was the only SNP reaching genome-wide significance ($< 5 \times 10^{-8}$) in its association with vincristine-induced neuropathy. The frequency of the risk allele (T) was lower in African American patients compared with other racial groups, consistent with its lower frequency in African ancestry samples in the HapMap collection. This finding is also consistent with the reported lower incidence of vincristine-induced neuropathy in black patients.²⁵ We found that children who inherited 2 copies of the *CEP72* risk allele (homozygous for T at rs924607) had a significantly higher incidence of vincristine-induced peripheral neuropathy (60.8% [95% CI, 43.9%-77.6%]) in patients with the TT genotype compared with 23.4% [95% CI, 17.4%-29.4] in patients with the CC/CT genotype). We also found that patients with *CEP72* TT had increased severity (grade) of toxicity at first episode (2.4-fold [95% CI 1.6-3.7] higher grade of neuropathy for TT vs CT/CC) at any given cumulative dose of vincristine.

Vincristine is widely used to treat leukemia, lymphoma, various solid tumors, and brain tumors in children and adults.²⁶

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Vincristine-induced peripheral neuropathy, characterized by sensory and motor dysfunction, causes extensive morbidity and often disrupts treatment. In children with ALL, the reported incidence of vincristine-induced neuropathy differs across treatment protocols, in part because of the grade of toxicity considered,^{27,28} the vincristine dose and number of doses given,²⁹ and the types of symptoms captured.⁹ In the current study, patients received uniform treatment according to prospective clinical trial protocols, with neuropathy assessed by NCI criteria to prospectively identify all patients who developed grades 2 to 4 neuropathy during continuation therapy with vincristine. Although a genetic component of vincristine-induced neuropathy would be consistent with previously reported racial differences in the incidence of vincristine-induced neuropathy,²⁵ previous candidate gene studies have not yielded reproducible findings. We therefore took an agnostic genome-wide approach to identify genetic variants influencing vincristine-induced peripheral neuropathy, revealing the previously unrecognized association with *CEP72* genotype.

The current study has limitations, including the small sample size (n = 321), the small number of patients homozygous for the *CEP72* risk allele (n = 50; approximately 16% of pa-

tients), and our assessment of children only in the context of ALL chemotherapy. However, our clinical findings are corroborated by multiple lines of laboratory evidence in human iPSC neurons and leukemia cells and, if verified in additional patient populations, could lead to a new approach for identifying patients at high risk of vincristine-related neuropathy. Because our data suggest that the leukemia cells of patients who are homozygous for the *CEP72* risk allele are more sensitive to vincristine, it may be possible to treat these patients with a lower dose of vincristine to decrease the risk or severity of neuropathy without compromising the antileukemic effects of vincristine, a possibility that merits assessment in future clinical trials.

Conclusions

In this preliminary study of children with ALL, an inherited polymorphism in the promoter region of *CEP72* was associated with increased risk and severity of vincristine-related peripheral neuropathy. If replicated in additional populations, this finding may provide a basis for safer dosing of this widely prescribed anticancer agent.

ARTICLE INFORMATION

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