Genetic Variants Associated With Vincristine-Induced Peripheral Neuropathy in Two Populations of Children With Acute Lymphoblastic Leukemia

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Vincristine is one of the core chemotherapy agents used in the treatment of pediatric acute lymphoblastic leukemia (ALL). However, one of the major toxicities resulting from vincristine exposure is vincristine-induced peripheral neuropathy (VIPN). When VIPN results in significant morbidity, the vincristine dose may need to be reduced, thus potentially decreasing the effectiveness of treatment. To date, there are no robust biomarkers used clinically to determine which patients will be at risk for worse neuropathy. The current study included genomewide association study (GWAS) in two independent cohorts: Pediatric Oncology Group (POG) ALL trials and a multicenter study based at Indiana University in children with ALL. A meta-analysis of the cohorts identified two single-nucleotide polymorphisms (SNPs), rs1045644 and rs7963521, as being significantly (*P* value threshold 0.05/4749 = 1.05E-05) associated with neuropathy. Subsequently these SNPs may be effective biomarkers of VIPN in children with ALL.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ There are limited published data on validated genomic predictors of chemotherapy-induced peripheral neuropathy and none published with the comprehensive and validated phenotyping that was accomplished in this study.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ What genetic mutations are associated with vincristine-induced peripheral neuropathy (VIPN) in children with (acute lymphoblastic leukemia (ALL).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The results from this study provide two additional therapeutic targets for addressing neuropathy in children diagnosed with ALL and receiving vincristine.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Neuropathy has a significant impact on the health trajectory of the children with ALL and developing novel treatments to alleviate or eliminate this side effect would greatly increase the quality of life for these survivors. Furthermore, having the ability to predict children at highest risk for significant neuropathy could eventually provide an opportunity for balancing disease risk with risk for severe, irreversible adverse side effects to optimize outcomes in children with curable cancers.

Leukemia is the most commonly diagnosed pediatric cancer (ages 0–19 years: 26% of pediatric cancers) with acute lymphocytic leukemia (ALL) being the most common subtype (75%).[1] Due to improved multidrug treatment regimens developed over the past 40 years, the 5-year survival rate for pediatric ALL has increased from 57% to 90%.[1] Vincristine is one of the core chemotherapeutic agents used in the standard multidrug treatment plan.[1] However, due to vincristine exposure, ~ 78% of these children develop clinically significant neuropathy (vincristine-induced peripheral neuropathy (VIPN)).[2] The development of VIPN is characterized by progressive motor, sensory, and autonomic nerve dysfunction[3–5] and may result in vincristine dose

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reductions during treatment, which increased the risk for an overall loss of therapeutic efficacy.

Vincristine is metabolized by the cytochrome P450 (CYP)3A family of phase I drug-metabolizing enzymes.[6] Previous work from our laboratory revealed that children with the active form of the *CYP3A5* (high CYP3A5 protein expresser genotype) had a five-fold greater clearance of the drug and experienced less VIPN than the CYP3A5 low expressers. Furthermore, subjects in the CYP3A5 high-expresser genotype group had lower neuropathy scores over the course of ALL treatment.[6,7] In addition, low expressers experienced more persistent neuropathy after completion of vincristine therapy.[8] Further studies have demonstrated it is specifically the *CYP3A5*1* allele that allows for expression of the active CYP3A5 enzyme. As such, in our prior study, subjects carrying at most one *CYP3A5*1* allele (CYP3A5 high expressers) are less likely to experience severe VIPN.[9]

The development of VIPN not only jeopardizes therapy but also may negatively affect the function and quality of life for children long after the completion of treatment.[10–12] Ness *et al.*[13] evaluated a large cohort of adult survivors of pediatric cancer and found that exposure to vincristine during childhood was associated with a higher risk for motor impairment later in life. Motor impairment can lead to decreased physical activity, obesity, type 2 diabetes, metabolic syndrome, and cardiovascular disease.[14–17] A recent study by Ou *et al.*[18] found that pediatric survivors 5–10 years post-ALL diagnosis and only receiving chemotherapy had significantly more hospitalizations after completion of ALL treatment than that of the general population, thus suggesting the presence of late-effect health burdens in cancer survivors.

Because the survival rate is so high and the long-term sequelae of vincristine exposure so prevalent in children with ALL, it is imperative that we elucidate the key factors underlying the vulnerability of some children to develop severe and/or persistent neurotoxicity. This is an important step toward not only maximizing efficacy but also minimizing toxicities in the treatment of pediatric ALL. In this study, we conducted a genomewide association study (GWAS) to investigate genetic biomarkers in two vincristine-treated cohorts of children with precursor B-cell ALL.

RESULTS

Pediatric Oncology Group GWAS analysis

Starting with 1,696 patients from the Pediatric Oncology Group (POG) 9904 and 9905 studies, 1,103 patients were classified as white based on the population stratification classification using the GWAS data. Genotype data quality control (QC) analysis required a minimum sample call rate of 95%, a minimum single-nucleotide polymorphism (SNP) call rate of 95%, a Hardy-Weinberg P value > 0.0001, and a minor allele frequency > 0.05. This QC analysis reduced the total number of SNPs to 587,014 among 1,068 patients. The overlapping POG GWAS SNPs with the ADVANCE Trial GWAS SNPs further reduced the number of SNPs to 4,749. A consort plot (**Figure 1**) summarizes these data-filtering steps. Bonferroni correction

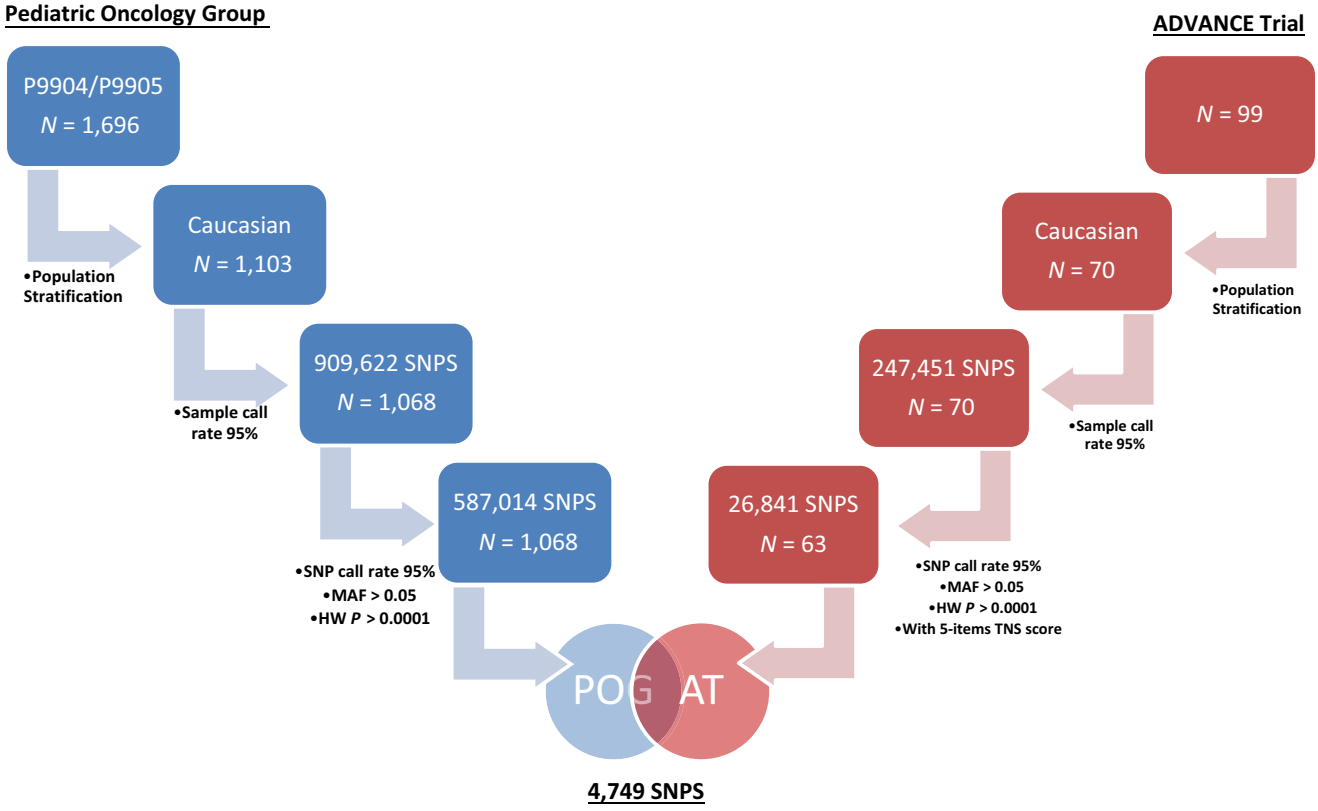

Figure 1 Patient and single-nucleotide polymorphism (SNP) selection flowchart for Pediatric Oncology Group (POG) and ADVANCE trial (AT). HW, Hardy-Weinberg equilibrium; MAF, minor allele frequency; TNS, total neuropathy score. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 Demographic variable and neuropathy score for POG and ADVANCE trial

		Total sample
ADVANCE trial sample (*N*)		63
POG sample (*N*)		1,068
Age—ADVANCE trial only	Mean (SD)	8.22 (4.68)
	Range	1–19
Gender		*n* (%)
ADVANCE trial	Male	29 (46.0)
POG	Male	559 (52.3)
ADVANCE trial	Female	34 (54.0)
POG	Female	509 (47.7)
ADVANCE trial – only TNS 5-item score (12-month average)	Mean (SD)	3.78 (2.61)
	Range	0–11
ADVANCE trial—only TNS 5-item score (12-month maximum)	Mean (SD)	6.95 (4.11)
	Range	0–20
POG—only CTC NE		*n* (%)
	NE = 3, 4	51 (4.8)
	NE = 2, 3, 4	87 (8.1)
	NE or NE pain = 2, 3, 4	135 (12.6)

CTC, common terminology criteria; NE, neuropathy event; TNS, total neuropathy score; POG, Pediatric Oncology Group.

for multiple comparisons with this number of SNPs requires a *P* value of 0.0000105 for genomewide significance (0.05/4749).

Among these 1,068 patients, 4.8% of patients experienced National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) grade 3 or higher neuropathy (see **Table 1**). As gender was not associated with the time to onset of grade 3 or 4 neuropathy, the GWAS was analyzed in a univariate SNP association analysis. The POG GWAS Manhattan plots (**Figure 2a**) revealed only one SNP, rs7792939, meeting the minimum genomewide association level for significance ($P = 2.39E-06$). Patients with one or more copies of the minor allele for this SNP incurred a hazard ratio (HR) of 2.83 for developing grade 3 or 4 neuropathy (95% confidence interval (CI) 1.82–4.39).

ADVANCE trial GWAS analysis

The ADVANCE trial had data from 99 eligible patients available at the time of this GWAS analysis. After population stratification classification, 70 white patients were selected for inclusion in this analysis. The QC analysis required a minimum sample call rate of 90%, a minimum SNP call rate of 90%, a Hardy-Weinberg *P* value > 0.001, and a minor allele frequency > 0.05. It reduced the number of SNPs to 26,825. The overlapping SNPs between the genotyping platforms utilized in the POG and ADVANCE cohorts further reduced the number of SNPs for analysis to 4,749. The consort plot (**Figure 1**) summarizes these data-filtering steps.

Among these 70 patients, 46% were men and 54% were women (see **Table 1**); the average age was 8.2 years (SD = 4.7), which ranged from 1–19 years; the average Total Neuropathy Score-Pediatric Vincristine (TNS-PV) 5-item neuropathy score during the first 12 months of vincristine treatment was 3.78 (SD = 2.61) and ranged from 0–11; the maximum 5-item neuropathy score during the first 12 months was 6.95 (SD = 4.11) and ranged from 0–20. Neither gender nor age was associated with the average or maximum neuropathy score. None of the 4,749 SNPs showed significance in predicting either the average or maximum neuropathy scores. In particular, the SNP rs7792939, which showed significance in the POG trial data analysis, was not significant ($P = 0.37$) in the ADVANCE trial (**Figure 2b**).

Integrated GWAS analyses

Two integrated GWAS analyses were conducted to test and validate the SNPs in their associations with the neuropathy among the two study populations. The first one was a meta-analysis, in which the association test statistics from the two studies (POG trials and ADVANCE trial) were weighted by their standard errors, and summarized into one statistic. The meta-analysis was conducted based on the same genetic model (additive or dominance), and the same direction of SNP effects between two studies. In addition, the statistics of two different neuropathy scores (average and maximum) from the ADVANCE trial were integrated with the statistics of the POG neuropathy phenotype separately.

Using the same genomewide *P* value threshold 0.05/4749 = 1.05E-05, we identified two SNPs that reached the genomewide significance level (**Table 2**). The meta-statistics comparing the average ADVANCE trial neuropathy and POG neuropathy scores showed SNP rs1045644 had a dominance effect with a *P* value = 2.36E-06. This SNP rs1045644 effect remains significant (*P* value = 3.78E-06) after multivariate analyses of both SNPs rs1045644 and rs7963521. Both additive and dominance genetic model meta-analyses evaluating maximum ADVANCE trial neuropathy and POG neuropathy scores were statistically significant, $P = 8.66E-06$ and 8.65E-07, respectively. In particular, in the POG trials, rs1045644 subjects with one or two copies of the minor allele ($G = 0.4113$: 1000 Genomes) had a protective effect against developing neuropathy. These subjects had an HR of 0.27 ($\exp(-1.28) = 0.27$) for developing grade 3 or 4 neuropathy compared with subjects with two major alleles (95% CI 0.16–0.50). In the ADVANCE trial, patients with one or two copies of the minor allele for the same SNP rs1045644 had significantly lower maximum neuropathy scores than subjects with two major alleles (3.56 units smaller; 95% CI −5.45 to −1.67).

The other SNP showing genomewide significance was rs7963521. In this case, only the additive meta-analysis for maximum neuropathy scores for both the ADVANCE trial and the POG trials were statistically significant, $P = 1.05E-05$. A second copy of rs7963521 minor allele ($c = 0.3822$: 1000 Genomes) led to increased risk of grade 3 or 4 neuropathy in both study populations (POG trial: HR 2.23 (1.49, 3.35); ADVANCE trial: HR 2.16 (0.53, 3.70)). Taking two SNPs together, rs1045644 and rs7963521, into the multivariate analysis and meta-analysis, rs1045644 remains significant (*P* value = 3.78E-06). In the ADVANCE trial, both SNPs can predict 26% of variation in the VIPN among patients.

In a second meta-analysis, top SNPs were first selected from the POG GWAS, and only the top SNPs were validated in the

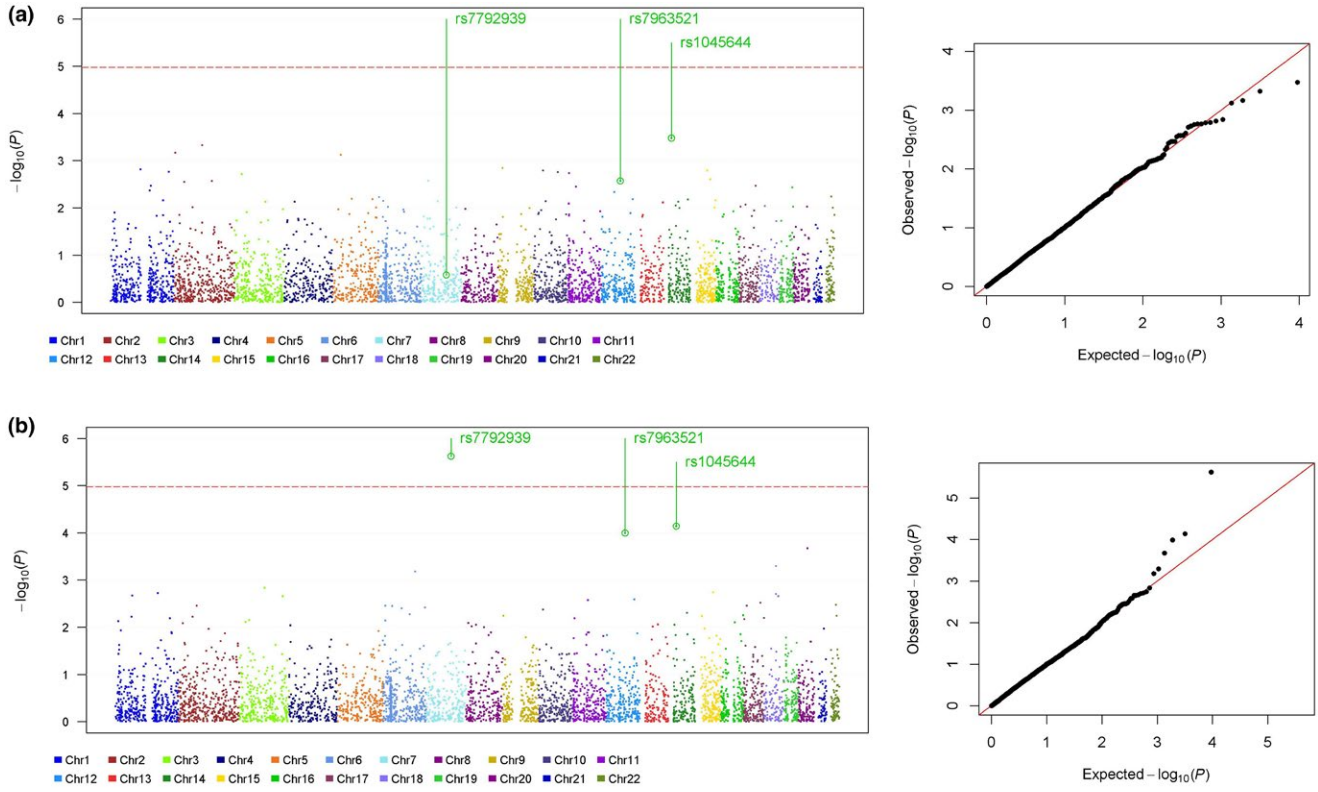

Figure 2 (**a**) Manhattan plot and Quantile-Quantile plot for Pediatric Oncology Group study. (**b**) Manhattan plot and Quantile-Quantile plot for ADVANCE trial. [Colour figure can be viewed at wileyonlinelibrary.com]

ADVANCE trial. This testing and validation analysis was only conducted within the same genetic model. In selecting the top three SNPs from the POG trial GWAS, only rs1045644 was validated in the ADVANCE trial, wherein the P value was $< 0.05/3 = 0.017$ (see **Table 2**). All the other testing and validation SNP selection strategies led to the same results.

DISCUSSION

Treatment for ALL pediatric patients includes vincristine as part of the standard multidrug plan.[1] However, due to vincristine exposure, ~ 78% of these children will develop VIPN.[2] Even though many of these children will recover from VIPN, there is a subgroup who will continue to experience long-term effects well into adulthood.[19] Furthermore, there are significant concerns that late effects or chronic effects of cancer treatment, such as irreversible neuropathy, may be associated with decreased physical activity, which may ultimately increase the risk of obesity in cancer survivors as well as a multitude of downstream negative health effects.[20] As such, developing therapeutic approaches that avoid neuropathy in children at highest risk for neuropathy, particularly those with lower-risk cancers, is crucial to optimizing long-term outcomes in survivors of childhood cancers. The best possible solution is to find a chemotherapy agent that avoids neuropathy; however, until one is developed and successfully incorporated into care for childhood ALL, it is critical that we elucidate the key factors underlying the vulnerability of some children to significant neuropathy. This may ultimately allow eliminating or decreasing vincristine dosing in children at highest risk for severe neuropathy, particularly in those with low-risk disease.

The objective of the current study was to utilize the patients from our limited institution ADVANCE trial as a validation cohort for a larger POG population in a GWAS for VIPN. The results from the meta-analysis for the ADVANCE and POG trials revealed two SNPs, which met genomewide significance for association with the maximum TNS in year 1 but only in the additive model. The first SNP was rs1045466, located on chromosome 14 and part of the coagulation factor C homology gene. The second SNP was rs7963521, located on chromosome 12 and associated with the regulation of chemerin plasma levels.[21]

Specifically, rs1045466 is part of the coagulation factor C homology gene, which encodes the secreted protein cochlin[22] and is associated with progressive hearing loss and vestibular imbalance.[23] It seems that a disruption in this gene can lead to loss of cellularity and aggregation of acellular eosinophilic deposits in the cochlear and vestibular labyrinths. In addition, there is aggregation of cochlin in the areas that normally express the protein.[23] These deficits can begin to appear at any time in adulthood: early or late.[24]

Although similar to the characteristics of Meniere disease, the current literature shows that this gene does not seem to be involved

Table 2 POG and ADVANCE neuropathy *P* values

	rs1045644			rs7963521		
Study	POG	ADVANCE	POG and ADVANCE combined	POG	ADVANCE	POG and ADVANCE combined
Sample size (*n*)	1,065	63		1,046	63	
MAF	0.38	0.33	–	0.41	0.43	–
Average in year 1 (ADD)						
P value	7.32E-05	2.15E-03	1.85E-05	1.02E-04	1.32E-02	2.56E-05
Effect size	−1.02	−1.48		0.80	1.26	
95% CI	[−1.52, −0.51]	[−2.40, −0.56]		[0.4, 1.21]	[0.27, 2.25]	
Average in year 1 (DOM)						
P value	1.72E-05	3.33E-04	2.36E-06[a]	7.82E-03	2.69E-03	2.92E-03
Effect size	−1.28	−2.28		1.03	2.133	
95% CI	[−1.86, −0.69]	[−3.48, −1.08]		[0.27, 1.78]	[0.77, 3.50]	
Maximum in year 1 (ADD)						
P value	7.32E-05	1.79E-03	8.66E-06[a]	1.02E-04	6.57E-03	1.05E-05[a]
Effect size	−1.02	−2.36		0.80	2.161	
95% CI	[−1.52, −0.51]	[−3.80, −0.91]		[0.4, 1.21]	[0.63, 3.70]	
Maximum in year 1 (DOM)						
P value	1.72E-05	3.80E-04	8.65E-07[a]	7.82E-03	2.66E-03	1.69E-03
Effect size	−1.28	−3.56		1.03	3.36	
95% CI	[−1.86, −0.69]	[−5.45, −1.67]		[0.27, 1.78]	[1.21, 5.50]	

The 5-item Total Neuropathy Score–Pediatric Vincristine (TNS-PV) score includes sensory symptoms, temperature sensibility, vibration sensibility, strength, and tendon reflexes. The POG and ADVANCE combined score is the combined *P* values from the POG and ADVANCE 5-item TNS-PV score.
ADD, additive model; CI, confidence interval; DOM, dominant model; MAF, minor allele frequency; POG, Pediatric Oncology Group.
[a]Sample statistically significant after meta-analysis.

in risk for developing Meniere's[25] and is a syndrome unto itself. In a large retrospective study of childhood cancer survivors, Oeffinger *et al.*[26] found that survivors had a relative risk of 6.3 (3.3–11.8) compared with their healthy siblings for developing severe or disabling hearing loss, which was not correctable with the use of an aid. Although it is not clear how many of the cases were in individuals treated with vincristine, the demographics do show that 29.5% of the surveyed population had a diagnosis of leukemia. Another large more recent study found similar results of hearing loss in survivors as compared with their peers.[27] Our findings regarding SNP rs1045466 and the role it plays in deafness provides us with a clear starting point to find a potential biomarker for predicting which survivors of childhood cancers may develop severe hearing loss. Furthermore, cochlin is involved in embryonic stem cell self-renewal via stimulation by bone morphogenetic protein (BMP). More importantly, if overexpressed, cochlin can substitute for BMP.[28] This is a key point as BMP plays critical roles throughout neural stem cell maturation and neurogenesis well into adulthood.[29] In a recent study by Heggeness *et al.*,[30] they demonstrated that direct injection of BMP into a mouse model of sciatic injury could induce marked proliferation of previously quiescent cells within peripheral nerves. Given our findings that show SNP rs1045466 is associated with lower neuropathy scores in our subjects, we hypothesize that the cochlin protein is being overexpressed and facilitating recovery from the toxicity of vincristine. Mechanistic studies linking cochlin expression to VIPN should be considered.

The second SNP, rs7963521, is located on chromosome 12 and is associated with plasma/serum levels of chemerin.[21] Chemerin is a versatile protein that acts on the chemokine-like receptor 1, G protein-coupled receptor 1, and the C-C chemokine-like receptor 2.[31,32] Depending on how the protein is processed from its preprochemerin form, it can be involved in angiogenesis,[33] adipogenesis,[34] osteoblastogenesis,[35] diabetes,[36] or inflammatory processes.[37–39] It is clear that many of these processes, if disrupted from normal functioning, could lead to long-term or chronic disorders. Findings from the pediatric cancer survivor studies mentioned previously indicate that many survivors have long-term disorders associated with these physiologic pathways.[26,27] It is possible that, in the setting of chemotherapy-induced peripheral neuropathy, the impact of variability in chemerin levels could lead to impaired myelin repair via the role chemerin plays in adipogenesis. Specifically, chemerin is involved with cell differentiation of preadipocytes into adipocytes.[34] Subsequently in the presence of growth factors, adipocytes can differentiate into Schwann cells which remyelinate peripheral nerves.[40] Additionally, chemerin levels have been identified as an independent predictor for 5-year mortality in other types of cancers, such as gastric cancer.[41] Thus, in addition to being a predictor of long-term sequelae of chemotherapy treatment, SNP rs7963521 may

also be useful as a predictor of long-term survival for pediatric patients with cancer.

Diouf *et al.*[42] found that an SNP in the *CEP72* gene, which is involved in microtubule formation, is also associated with VIPN in children with ALL. However, in our current study, this SNP was not associated with severe VIPN in either of our independent cohorts.

Our findings are consistent with those of Gutierrez-Camino *et al.*,[43] who also did not find an association with CEP72 in pediatric patients with ALL. The difference in our results may be due to the use of the TNS-5-item TNS-PV as the main measure of our neuropathy score as compared to the NCI CTCAE version 4.0 scale. The TNS-5-item TNS-PV is a more granular neuropathy assessment tool, and it is likely the subpopulation of patients with ALL we used for analysis was phenotypically different.

Overall, the GWAS results provide two biologically interesting SNPs that may be useful as biomarkers for prediction of severe VIPN in children with ALL. The next critical step will be to elucidate the functional consequences of these SNPs as well as to understand their potential relevance in predicting risk for irreversible VIPN in pediatric cancer survivors. This understanding will be important in optimizing long-term outcomes of survivors of childhood cancer as well as beginning to move toward individualized treatment strategies based on risk of disease and risk for significant neuropathy.

Limitations

The main limitation of this study is that neuropathy assessments varied in the POG and ADVANCE samples. The NCI CTCAE version 2.0 (National Institutes of Health, Division of Cancer Treatment & Diagnosis, Washington, DC) was used as the primary tool for quantifying VIPN in the POG studies. Yet, the CTCAE has been criticized as a neuropathy assessment measure due to concerns regarding limited inter rater reliability and floor effects, which result in most scores falling in the low range (CTCAE 1 or 2). Thus, although the CTCAE can underestimate neuropathy severity, we attempted to compensate for this concern by using CTCAE scores ≥ 3 as indicative of severe neuropathy. Second, CTCAE and 5-item TNS-PV scores are not directly comparable because TNS-based scores arise from validated, standardized neurologic assessment procedures and scoring criteria.[44,45] However, we justify our comparisons based on our prior work, which demonstrates moderately strong correlations among TNS-PV scores and CTCAE sensory ($r = 0.52$) and motor scores ($r = 0.48$).[44] Given these correlational data, and that the CTCAE was the only neuropathy measure available from the POG studies, the use of data from these two different neuropathy measures is justified.

METHODS

Patients

POG studies 9904 and 9905. DNA samples were analyzed from patients enrolled in the POG trials P9904 and P9905. A total of 1,696 children with precursor B-cell ALL were included in the initial sample set. The purpose of the POG 9904 and 9905 trials was to compare the efficacy of short and long infusion times of methotrexate with or without multidrug intensification.

Patients received 18–23 doses of vincristine (1.5 mg/m^2 per dose) depending on study arm over the course of treatment along with other standard chemotherapy agents used in the treatment of childhood ALL. The complete treatment duration was 2.5 years from the date of diagnosis, and patients remained on the study until completion, relapse, or death. The data for these studies were frozen as of September 30, 2011. For the purposes of this neuropathy-focused study, patients with the following conditions were excluded: Down syndrome, Charcot Marie Tooth disease, baseline peripheral neuropathy, and history of liver disease with chronic elevation in serum liver transaminases and bilirubin to greater than five-times the upper limit of normal based on normal values for age.

ADVANCE trial. The ADVANCE trial was a four-institution study with the goal to validate previous findings that children expressing the CYP3A5 genotype develop less severe VIPN as compared with nonexpressers, as well as evaluating for other potential predictors of VIPN. In summary, children with newly diagnosed precursor B-cell ALL ($N = 99$) were recruited from four academic medical centers: Indiana University School of Medicine/Riley Hospital for Children, the University of Michigan Comprehensive Cancer Center/Mott Children's Hospital, Vanderbilt University Medical Center/Monroe Carell Jr. Children's Hospital, and George Washington University/Children's National Medical Center. Participants were between the ages of 1 and 18 years at the time of diagnosis and received vincristine according to POG treatment trials (including POG studies 9904 and 9905). The standard vincristine dosage received was 1.5 mg/m^2 (capped at 2-mg maximum dose). Toxicity-based dose modifications were defined according to the specific POG protocol guiding the individual child's leukemia treatment. Patients were excluded if they had any of the following criteria: baseline peripheral neuropathy score greater than grade 1 per the NCI CTCAE version 4.0; currently receiving erythropoietin, itraconazole, or vitamin supplement > 100% of the recommended daily allowance; Down syndrome; pregnancy; or a history of coexisting serious illness that would limit neurological assessments. All procedures were reviewed by the Indiana University Internal Review Board and approved (protocol #1105005420).

Combined meta-analysis

Initially, each group was analyzed separately utilizing their maximal neuropathy score during the first year—NCI CTCAE for the POG patients and TNS-PV for the ADVANCE trial patients. The overlap in SNPs between the groups was examined for those who had the same minor and major allele. The *P* value from each group was combined using the equation below. This allowed for a comparison between the two groups regardless of the neuropathy assessment tool utilized.

$$\text{Equation: } Z \sim \frac{n_1 \times \text{est}_1 + n_2 \times \text{est}_2}{\sqrt{n_1^2 \times \text{se}_1^2 + n_2^2 \times \text{se}_2^2}}.$$

Neuropathy phenotype

POG studies 9904 and 9905. As part of the required clinical trial adverse event monitoring, children enrolled in the POG trials were clinically assessed by physical examination for peripheral neuropathy by their treating oncologists throughout treatment at each clinic visit. Based on NCI CTCAE version 2.0, the VIPN events are defined when patients experienced symptomatic neurotoxicity with neuropathy grade ≥ 3 in either motor or sensory neurons.

ADVANCE trial. Vincristine-induced neuropathy was assessed weekly for the first month of ALL treatment, followed by monthly for the remainder of the first year, followed by quarterly for the duration of ALL treatment using the TNS, NCI CTCAE version 4.0 and the Modified "Balis" Pediatric Scale of Peripheral Neuropathy.[45] We used a TNS

variant, the TNS-PV, revised and validated by our team for use in children receiving vincristine.[44] VIPN-associated pain was measured using the Pediatric Neuropathic Pain Scale–Five (PNPS-5). The FACES pain scale[46,47] was used to assist children to select a PNPS-5 pain severity rating. If the child did not understand a question, parents/guardians estimated the pain scores based on observations of their children. Based on our previous findings of the five TNS items most associated with VIPN,[44] a 5-item total neuropathy score (TNS-PV) was used to summarize the VIPN in this genetic association data analysis. The 5-item score includes sensory symptoms (i.e., numbness, tingling, and neuropathic pain), temperature sensibility, vibration sensibility, strength, and tendon reflexes. TNS and PNPS-5 scores were assigned by trained evaluators. Neuropathy assessment training was completed as previously described.[44] NCI-CTCAE neuropathy scores were assigned by treating oncologists and advanced practice providers as part of clinical care. Medical caregivers and trained neuropathy evaluators were blinded to each other's neuropathy scores.

Sample genotyping

POG studies 9904 and 9905. *POG ALL trial genotyping.* DNA (500 ng) was digested with restriction enzymes, amplified, labeled, and hybridized to the Affymetric GeneChip Human Mapping 6 set for P9904 and 9905. The genotypic data used for analysis are in the PLINK format, which includes genotype calls and on-calls for each SNP per sample.

ADVANCE trial. DNA (750–1,500 ng) was amplified, fragmented, precipitated, and hybridized to the Human Exome Bead Chip at the Center for Inherited Disease Research at Johns Hopkins University. The genotypic data used for analysis are in the PLINK format, which includes genotype calls and on-calls for each SNP per sample.

Genotype quality control analysis

QC was performed to remove both samples and markers, which were unreliable using the criteria described in this section. Samples with > 5% missing rate were excluded.

Furthermore, SNPs with a study-wide missing data rate of > 5% and/or evidence of Hardy-Weinberg disequilibrium ($P \leq 0.0001$) were discarded. SNPs with minor allele frequency of < 0.05 were also removed from the analysis because previous studies have shown that these SNPs have little power to detect association and are more prone to genotypic errors resulting in false-positive evidence of association (see **Figure 1**). PLINK was utilized to manipulate the data by generating both per sample and per SNP metrics to assess the quality of the genotypic data.[48] Upon completion of the QC assessment, the final patient sample number for analysis was 1,068.

GWAS analysis

A principal components approach was applied to correct for any population stratifications.[49] Using the first two principle components, the white patient samples were selected for GWAS analysis.

In the POG trials, the association between genotype and the time to the first neuropathy event (neuropathy grade ≥ 3 in either motor or sensory neurons) was analyzed through the Cox proportional hazard regression model. In the ADVANCE trial, the association between genotypes and neuropathy scores was analyzed through linear regression. Other clinical, demographic, and population stratification variables (i.e., genomic data–derived race) were tested as covariates in the regression analyses. The genetic effect of any individual SNP was tested in dominant and additive (gene-dose) models. These analyses were performed in R coxph and lm packages.[50] Both univariate and multivariate analyses were conducted. The corresponding *P* value of each SNP was summarized across chromosomes in Manhattan and QQ plots. Genetic analysis was only conducted among SNPs included in genotyping platforms utilized in both the POG trials and the ADVANCE trial. A meta-analysis was implemented between two sets of genetic association analysis results. In this meta-analysis, the directionality of the SNPs was first confirmed by the minor allele frequency. The summary statistic was a weighted mean of two regression coefficients by their corresponding standard errors, respectively. The Bonferroni correction was used to justify the genomewide significant *P* values. Furthermore, in multivariate analysis of ADVANCE trial data, the *R*-square of the SNP combination predictive effect on VIPN is reported.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

J.L.R., L.L., T.S., E.M.L.S., C.W.C., and C.L. wrote the manuscript. J.L.R., E.M.L.S., and C.W.C. designed the research. J.L.R., E.M.L.S., R.H.H., R.H., E.W., and N.W. performed the research. L.L., C.W.C., C.L., and R.H. analyzed the data.

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