Pharmacogenomics of Vincristine-Induced Peripheral Neuropathy Implicates Pharmacokinetic and Inherited Neuropathy Genes

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Vincristine is an effective chemotherapeutic drug for various cancers, including acute lymphoblastic leukemia (ALL). Unfortunately, clinical utility is restricted by dose-limiting vincristine-induced peripheral neuropathies (VIPN). We sought to determine the association of VIPN with a recently identified risk variant, *CEP*72 rs924607, and drug absorption, distribution, metabolism, and excretion (ADME) gene variants in pediatric ALL. This was followed by a meta-analysis of pharmacogenomic data from over 500 patients. *CEP*72 rs924607 was significantly associated with VIPN ($P = 0.02$; odds ratio (OR) = 3.4). ADME analyses identified associations between VIPN and *ABCC*1 rs3784867 ($P = 5.34 \times 10^{-5}$; OR = 4.9), and *SLC*5A7 rs1013940 ($P = 9.00 \times 10^{-4}$; OR= 8.6); genes involved in vincristine transport and inherited neuropathies, respectively. Meta-analysis identified an association with a variant related to *TTPA* (rs10504361: $P = 6.85 \times 10^{-4}$; OR = 2.0), a heritable neuropathy-related gene. This study provides essential corroboratory evidence for *CEP*72 rs924607 and highlights the importance of drug transporter and inherited neuropathy genes in VIPN.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Dose-limiting VIPNs have been shown to have a genetic component. Pharmacogenomic studies have begun to identify genetic factors that can help explain these adverse reactions, but more research is required in this regard.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ To assess whether the *CEP72* biomarker and drug ADME pharmacogenomic variants are associated with VIPN in pediatric patients with ALL.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ We confirmed the association of *CEP72* rs924607 with VIPN, providing additional evidence for the potential clinical utility of this biomarker. Further, we showed that genetic variation within the vincristine drug transporter (*ABCC1*) was also associated with VIPN. Last, we uncovered the first evidence that genes that cause inherited neuropathies (*SLC5A7* and *TTPA*) may contribute to VIPN susceptibility.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ These findings will help guide the development of predictive markers and neuroprotective strategies for VIPN in order facilitate precision medicine and optimal care on an individualized basis for oncology patients.

Vincristine is an antineoplastic agent that is used to treat various cancers. This includes the most common pediatric form, acute lymphoblastic leukemia (ALL), a cancer that has survival rates that have increased to over 90% in certain world regions in recent decades.[1,2] Vincristine-induced neurotoxicity is a common and severe adverse drug reaction (ADR) that can occur in over 50% of

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vincristine-treated patients.[3–5] This potentially life-threatening and debilitating toxicity includes vincristine-induced peripheral neuropathy (VIPN), which can cause loss of motor function, abnormal sensation, and pain.[6] This reaction severely affects the quality of life of patients, as well as influences the dosing and clinical utility of the drug.[7] Genetic causes are thought to play an important role in this ADR because VIPN-susceptibility varies across ancestries[8] and the trait has been shown to be heritable in model organisms.[9]

Pharmacogenomics provide an attractive approach to mitigate VIPN – predictive testing would allow patients to be stratified according to risk before treatment, allowing for either increased monitoring or dose changes in at-risk patients. Furthermore, the identification of genetic risk factors may provide novel insights into the mechanisms underlying variability in VIPN-susceptibility. Recently, a genome-wide association study (GWAS) in two cohorts reported that variation linked to the microtubule-organizing centrosomal protein, *CEP72*, significantly contributes to the risk of VIPN in pediatric ALL.[10] This is in line with the mechanism of action of vincristine, which perturbs mitosis by interfering with microtubule formation.[7,11] An initial replication study failed to detect a significant association between this *CEP72* risk genotype and VIPN,[12] which may have been driven by differences in the therapy stage during which VIPN was assessed (i.e., continuation vs. induction).[13] Subsequently, members of the research group involved in the discovery of the *CEP72*-VIPN association replicated this finding in a clinical trial of adult patients with ALL,[14] but confirmation in an independent pediatric cohort is still warranted.

We, therefore, assessed the relationship between VIPN and *CEP72* genotype in a retrospective cohort of pediatric patients with ALL. This was followed by an exploratory analysis of pharmacogenomic variants from absorption, distribution, metabolism, and excretion (ADME) genes and a meta-analysis of ADME genetic variants that overlapped variants from the previous GWAS.[10] Studies such as these provide essential corroboratory clinical evidence for previously identified drug safety biomarkers, while also identifying additional variants for future study.

RESULTS

A total of 167 cases (i.e., VIPN grade ≥ 2) and 57 controls (i.e., VIPN grade 0) met clinical and genomic quality control and were included in the discovery pharmacogenomic association studies, after excluding 29 patients with grade 1 VIPN and 12 patients with only cranial or autonomic neurotoxicities (**Figure S1**, **Table S1**). This study design is classified as a nonmatched case-control association study, in which significantly different clinical and demographic factors between cases and controls were corrected for during pharmacogenomic association analyses. The majority of VIPN cases were classified as grade 2 ($n = 100$), followed by grade 3 ($n = 66$), and grade 4 ($n = 1$). The median days from first vincristine dose to VIPN were 57 (interquartile range: 18–127 days). Patients were predominantly of European genetic ancestry (78%, **Figure S2**) and treatment protocol information is listed in **Table S2**. **Table 1** depicts differences in clinical and demographic factors between VIPN cases and controls. Cases were more likely to be male and received vincristine for a longer duration compared to controls (median of 812 days vs. median of 757 days). All controls, however, received vincristine for at least 16 months, a period of time in which 94% of cases had developed VIPN (**Figure S3**), indicating sufficient follow-up time. The cumulative vincristine dose was not significantly associated with case status, whereas duration of treatment was. This observation may have resulted from the fact that many VIPN cases had either vincristine doses held or reduced during treatment as a result of the experienced toxicities, which may explain the difference in therapy duration and the lack of association with cumulative dose. Cases were also more likely to have received nifedipine compared to controls. To help reduce the influence of potential confounders on the genetic association analyses, all significantly associated demographic/clinical variables (i.e., sex, vincristine duration, and nifedipine use) were included as covariates in regression models along with genetic ancestry.

Table 1 Clinical and demographic characteristics of the CPNDS pediatric patients with ALL

Patient characteristics	Cases ($n = 167$) VIPN grade ≥ 2	Controls ($n = 57$) VIPN grade = 0	*P* value
Age, years (median (IQR))	4.8 (3.3–9.0)	5.4 (3.3–9.0)	0.63
Sex (male *n*, (%))	101 (60.4%)	23 (40.4%)	**0.009**
Vincristine duration, days (median (IQR))	812 (759–1137)	757 (745–825)	**0.0004**
Vincristine cumulative dose, mg/m^2 (median (IQR))	61.4 (48.0–72.0)	66.0 (51.0–74.8)	0.17
Vincristine doses, number (median (IQR))	39 (32–45)	38 (37–39)	0.47
Concomitant medication (*n*, (%))			
Strong cytochrome *P450* 3A inhibitors[a]	59 (35.3%)	17 (29.8%)	0.52
Antifungal azoles	54 (32.3%)	14 (24.6%)	0.32
Nifedipine	22 (13.2%)	1 (1.8%)	**0.01**
Genetic ancestry (median (IQR))			
PC 1 (African from non-African)	0.011 [0.010–0.012]	0.010 [0.008–0.012]	**0.03**
PC 2 (European from Asian)	0.020 [0.017–0.022]	0.019 [0.011–0.021]	0.17
PC 3 (South Asian from non-South Asian)	0.008 [0.005–0.012]	0.006 [0.002–0.010]	**0.02**
PC 4 (Amerindian from non-Amerindian)	0.010 [0.006–0.013]	0.010 [0.005–0.012]	0.37

ALL, acute lymphoblastic leukemia; CPNDS, Canadian Pharmacogenomics Network for Drug Safety; IQR, interquartile range; PC, principal component.The Wilcoxon rank-sum test or Fisher's exact test where appropriate. $P < 0.05$ regarded as significant and is displayed in bold in the table.
[a]Classified as inhibitors according to the Flockhart Table[35]

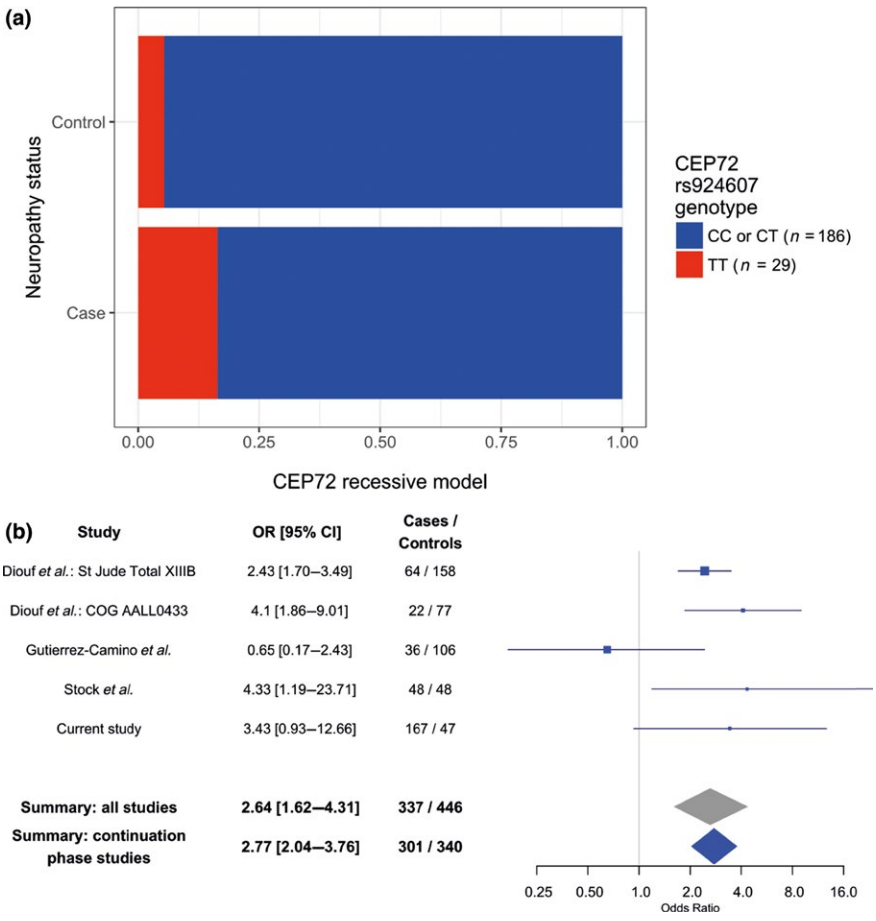

Figure 1 *CEP72* is significantly associated with vincristine-induced peripheral neuropathies (VIPN) in pediatric patients with acute lymphoblastic leukemia. (**a**) Recessive genotype frequencies for *CEP72* rs924607 differed significantly between VIPN cases and controls ($P = 0.02$). The TT risk genotype occurred in 16.0% of cases (VIPN grade ≥2) and 5.4% of controls (VIPN grade 0). (**b**) Meta-analysis of the association of *CEP72* rs924607 and VIPN confirms the role of this variant with vincristine-related adverse effects. The gray summary diamond indicates meta-analysis results including all studies, whereas the blue summary diamond shows the results for studies where VIPN was assessed throughout continuation therapy (i.e., excluding the Gutierrez-Camino *et al.*[12] study). Genotype 95% confidence intervals (CIs) are represented for consistency across all studies, as opposed to the 90% CI that was used to determine *CEP72* rs924607 replication evidence for the Canadian Pharmacogenomics Network for Drug Safety cohort in the current study.[10,13,14]

The *CEP72* rs924607 TT genotype was significantly associated with VIPN in the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) cohort (one-sided $P = 0.02$; odds ratio (OR) = 3.43; 90% confidence interval (CI) = 1.15–10.3). The TT risk genotype frequencies for *CEP72* rs924607 were 16.0% in VIPN cases and 5.4% in controls (**Figure 1a**, **Table S3**), displaying a specificity of 0.95 (95% CI = 0.85–0.99) and sensitivity of 0.16 (95% CI = 0.11–0.22). The association between the *CEP72* rs924607-TT genotype and VIPN was further confirmed in a meta-analysis of the CPNDS results and published-*CEP72*-VIPN studies (**Figure 1b**; n studies = 4; $P = 8.02 \times 10^{-11}$; OR = 2.77; 95% CI = 2.04–3.76; no heterogeneity: $I^2 = 0.0\%$).

Subsequent analysis of 4,522 ADME variants meeting the inclusion criteria for association testing revealed that no other previously identified variant was significantly associated with VIPN (i.e., $P < 0.05$; **Table S4**). Further, no variants assessed within the vincristine metabolism-related gene cluster (i.e., *CYP3A5-CYP3A7-CYP3A4*) displayed $P < 0.05$. An intronic *ABCC1* polymorphism (**Table S5**) was the top ADME variant with the strongest evidence of association (rs3784867 $P = 5.34 \times 10^{-5}$; OR = 4.91; 95% CI = 1.99–12.10; specificity = 0.88; sensitivity = 0.36; **Table S6**). The frequency of rs3784867 genotypes correlated with VIPN grade upon the inclusion of grade 1 patients (**Figure 2a**; $P = 7.12 \times 10^{-4}$). We assessed *ABCC1* rs3784867 for replication in the previously published VIPN-GWAS summary statistics,[10] and, although the risk allele was more frequent in cases in both the St Jude Total XIIIB and COG AALL0433, only suggestive evidence for replication in the combined XIIIB-COG cohorts was observed (one-sided $P = 0.08$). Interestingly, the third most significant finding from the ADME analyses was a missense variant in *SLC5A7* (rs1013940 $P = 9.00 \times 10^{-4}$; OR = 8.60; 95% CI = 1.68–44.15; specificity = 0.96; sensitivity = 0.18; **Table S7**), a gene that encodes a choline transporter, where mutations are known to cause a form of hereditary motor neuropathy.[15] Of note, all homozygous risk variant carriers presented with the most severe VIPN grade (**Figure 2b**). This variant is also an *SLC5A7* expression quantitative trait locus (eQTL and Genotype-Tissue Expression (GTEx) multitissue $P = 3.62 \times 10^{-7}$).[16] Unfortunately,

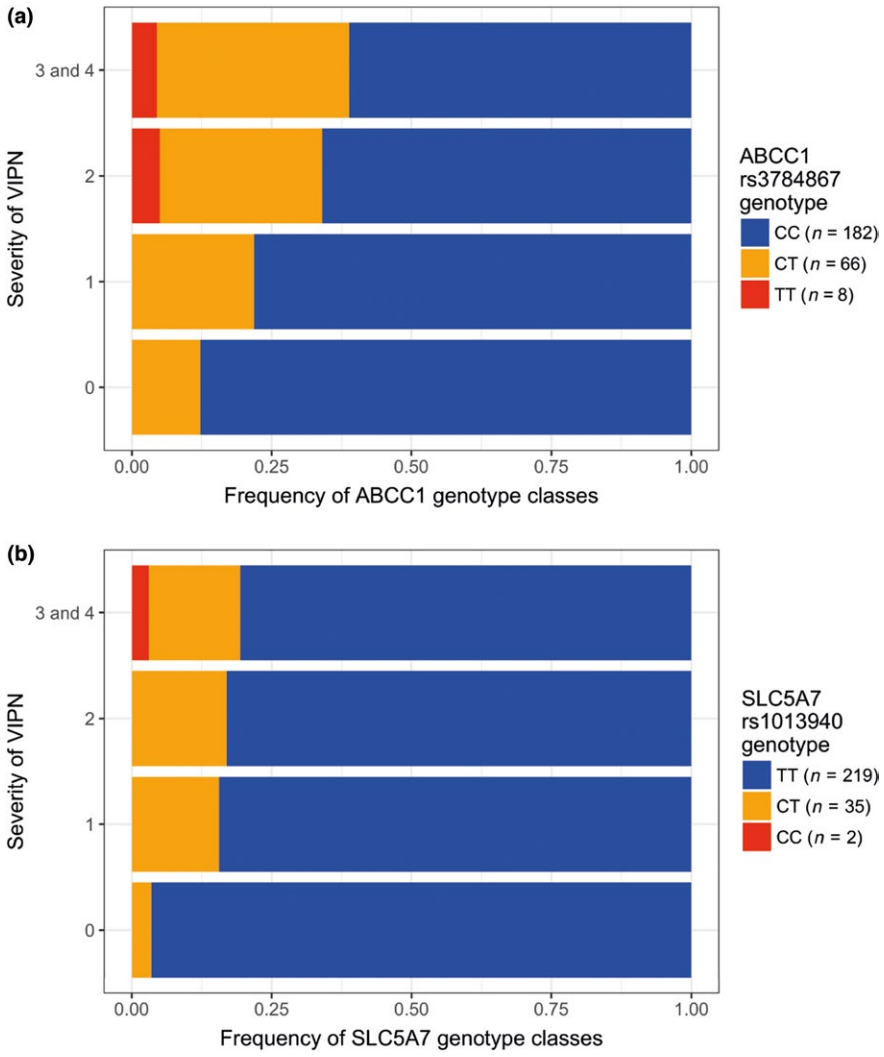

Figure 2 Top absorption, distribution, metabolism, and excretion (ADME)-vincristine-induced peripheral neuropathies (VIPN) variant genotypes stratified by severity of the adverse event. (**a**) *ABCC1* rs3784867 was the most significantly associated pharmacogenomic variant in the VIPN-ADME analyses ($P = 5.34 \times 10^{-5}$; odds ratio (OR) = 4.9). Genotype frequency was also correlated with severity of the adverse event ($P = 7.12 \times 10^{-4}$). (**b**) *SLC5A7* rs1013940 is a missense variant in a peripheral neuropathy gene that was among the most significantly associated ADME variants ($P = 9.00 \times 10^{-4}$; OR = 8.6). The variant was also moderately associated with VIPN grade severity ($P = 0.01$), with all homozygous carriers of the risk allele presenting with severe VIPN (i.e., grade 3 and 4). Because only one case of grade 4 VIPN was observed in this cohort, this patient was combined with the grade 3 group.

no data were available for this variant or those in linkage disequilibrium in the GWAS summary statistics, so we were unable to assess the *SLC5A7* variant for replication or in the meta-analysis.

We performed annotation of the *ABCC1* rs3784867 variant to delineate its potential functional role. No variants in strong LD (i.e., $r^2 > 0.8$ and no missense variants with $D' > 0.8$) with the marker were identified in the 1,000 Genomes Project. Annotation of rs3784867 with DNAse and histone ChIP-Seq data indicated the presence of enhancer and histone marks in five tissues/cell types (i.e., embryonic stem cells, induced pluripotent stem cells, hematopoietic stem cells, smooth muscle, and the pancreas). Additionally, rs3784867 was predicted to alter homeobox transcription factor motifs (**Figure S4**).

The most significant association detected in the VIPN-ADME-GWAS meta-analysis (**Table S8**) of 379 variants was rs10504361 ($P = 6.85 \times 10^{-4}$; OR = 1.98; 95% CI = 1.34–2.94; **Figure 3**). This variant is a GTEx-eQTL[16] for the *TTPA* and *GGH* genes. Because *TTPA* mutations are known to cause ataxia with isolated vitamin E deficiency, a neurologic condition associated with neuropathies,[17] we assessed differences in predicted gene expression of *TTPA* in the tibial nerve with S-PrediXcan, which collapses eQTL data by gene, in the GWAS-array genotyped cohorts. These analyses revealed positive gene-level *Z*-scores in both cohorts, indicating that VIPN cases were predicted to have increased expression compared to controls ($P = 6.1 \times 10^{-5}$).

DISCUSSION

Pediatric oncology has seen a dramatic improvement in the prognosis for patients over recent decades, yet ADRs from these drugs have become an increasing concern. In this study, we independently

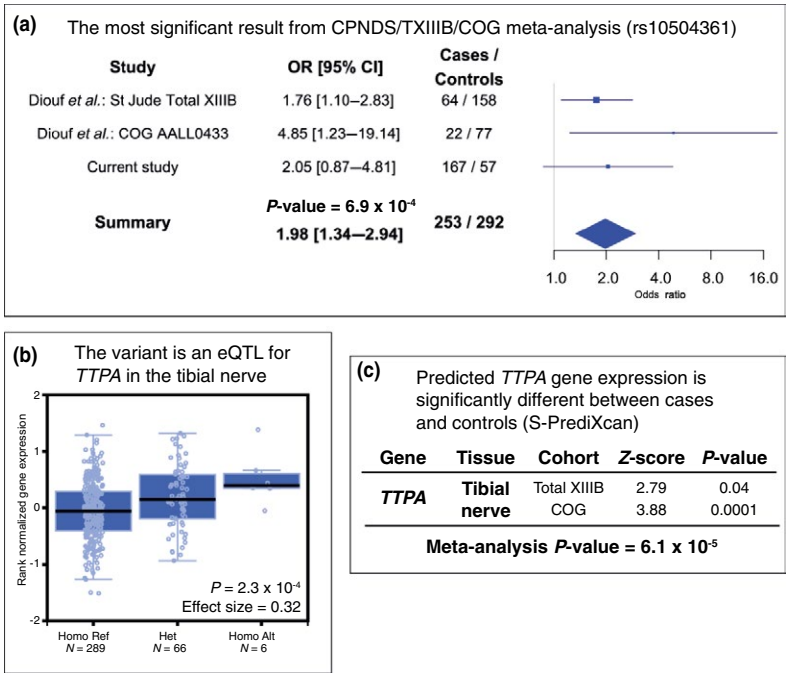

Figure 3 Association of *TTPA*-related variation and gene expression with vincristine-induced peripheral neuropathy (VIPN). (**a**) Meta-analysis of overlapping absorption, distribution, metabolism, and excretion (ADME)-genomewide association study (GWAS) variants in the Canadian Pharmacogenomics Network for Drug Safety (CPNDS)/St Jude Total XIIIB/COG AALL0433 cohorts identified rs10504361 as the most significantly associated variant. (**b**) This variant has been shown to be associated with differential expression of the *TTPA* gene in the tibial nerve in GTEx. (**c**) Imputing expression of *TTPA* using genome wide array data revealed that VIPN cases were predicted to have higher levels of *TTPA* expression compared to controls as reflected by gene-level S-PrediXcan *Z*-scores (i.e., positive scores indicate increased expression is associated with increased risk for VIPN). CI, confidence interval; OR, odds ratio.

confirmed a role of *CEP72* rs924607 in VIPN susceptibility for the first time in a retrospective cohort of pediatric patients. This finding validates the importance of this pharmacogenomic variant for the prediction of a serious ADR caused by an essential chemotherapeutic agent. Our exploratory pharmacogenomic analyses also brought to light the potential VIPN-related importance of the transporter, *ABCC1*, and highlighted genes associated with inherited neuropathy conditions, such as *SLC5A7* and *TTPA*.

Confirmation of *CEP72* as a pharmacogenomic risk factor for VIPN

CEP72 rs924607 is a promising VIPN biomarker given its potential role in the mechanism of action of this antimitotic agent. However, prior to the current study, all evidence supporting this finding was obtained from a single research group, although for the most recent replication study,[14] these investigators collaborated on the study by performing genotyping of *CEP72* rs924607 while blinded to VIPN phenotype. Of note, this the only other external study refuting the role of this variant in VIPN. Our positive results, supported by a meta-analysis of published VIPN-*CEP72* studies,[10,12,14] provide the first external validation of this finding in pediatric patients. In particular, the replication of *CEP72* in a diverse pediatric ALL cohort confirms the generalizability of this pharmacogenomic marker. Our data also improve the precision and accuracy of estimates regarding the effect of this variant (meta-analysis: $P = 8.02 \times 10^{-11}$; OR = 2.77; 95% CI = 2.04–3.76) in studies assessing VIPN from vincristine continuation therapies. Concurrently, these results corroborate the validity of our grading scheme that retrospectively assessed VIPN based on medical records of patients recruited via active surveillance.

The failure of the first rs924607-VIPN replication attempt[12] brings to light important phenotypic considerations for pharmacogenomic studies. Unlike the discovery study, which included monitoring during induction and continuation phases of vincristine therapy, Gutierrez-Camino *et al.*[12] assessed VIPN solely during the 4-week induction phase of vincristine therapy. Of note, the majority (63%) of the VIPN cases in our cohort developed toxicity after this 4-week time frame (**Figure S3**). Our meta-analysis was highly significant when excluding this study[12] assessing VIPN only during induction therapy from analysis with no evidence for heterogeneity between studies. It has further been suggested that *CEP72* rs924607 may be primarily relevant to patients receiving vincristine doses of <2 mg/m^2 because higher doses of vincristine may overcome the protective influence of the ancestral genotypes.[13] Indeed, when excluding the CPNDS patients that received vincristine doses ≥ 2 mg/m^2 (i.e., 34.3% of the cohort), the risk genotype was carried exclusively in cases (i.e., 16.1% of 112 cases; Fisher's exact $P = 0.007$). These findings emphasize the importance of carefully matching drug-induced phenotypes in pharmacogenomic replication studies.[18]

Exploratory analyses of ADME genetic variation

Because numerous pharmacogenes belong to ADME-related pathways,[19] we conducted an exploratory association analysis of

ADME variants with regards to VIPN using a custom genotyping panel that extensively captures variation within these regions. Initial pharmacogenomic studies investigating VIPN hypothesized that *CYP3A* genetic variation may be relevant for this ADR because vincristine is chiefly metabolized by these enzymes.[8] Our results, however, indicate that pharmacogenomic variation in *CYP3A* genes did not contribute significantly toward this phenotype in our patient cohort.

Our analyses revealed that the ADME variant with the strongest evidence for an association with VIPN, rs3784867 ($P = 5.34 \times 10^{-5}$; OR = 4.91; 95% CI = 1.99–12.10), occurs in the intron of *ABCC1*. This gene encodes multidrug resistance protein 1, a protein, which is involved in both transport of, and resistance to, vincristine.[20,21] *ABCC1* has also been implicated in anthracycline-related and methotrexate-related pharmacogenomic phenotypes.[22] Rs3784867 is predicted to alter binding sites for homeobox transcription factors (**Figure S4**), which are involved in hematopoiesis and are dysregulated in leukemia,[23] indicating that rs3784867 could affect the expression of *ABCC1*. Notably, previous pharmacogenomic studies have detected associations with *ABCC1* and the paralogous transporter *ABCC2* and VIPN-risk,[24,25] although we did not replicate associations of the variants that were implicated in these studies. As discussed previously, heterogeneity in VIPN assessment may contribute toward the lack of transferability of pharmacogenomic markers between studies and because our study used an intervention-based VIPN grading scheme, this may have contributed toward these discrepant results. The correlation between rs3784867 and ADR severity adds support for this variant's role in VIPN risk. However, because only suggestive evidence for replication was observed in the GWAS summary statistics for rs3784867, it is, important to further investigate this variant in additional VIPN cohorts.

Overlap between the genomics of VIPN and inherited neuropathy conditions

It has been suggested that a continuum exists between the genetic etiology of Mendelian disorders and drug-induced traits that present with similar clinical phenotypes.[26] This is in line with what has been observed for GWAS of chemotherapy-induced peripheral neuropathies from paclitaxel (*FGD4* gene)[27] and docetaxel (*VAC14* gene),[28] whereas both genes have also been implicated in inherited neuropathy conditions. Phenotype overlaps are further substantiated by the fact that vincristine is contraindicated in patients with hereditary neuropathies (e.g., Charcot-Marie-Tooth disease), because VIPN can be particularly severe in these patients.[29] Our VIPN-ADME-GWAS meta-analysis identified a variant (rs10504361) that is associated with the expression of *TTPA* in the peripheral nervous system. This protein influences bioavailability of alpha-tocopherol, a form of vitamin E. Deleterious *TTPA* mutations cause the recessive neurodegenerative condition ataxia with isolated vitamin E deficiency, which is associated with peripheral neuropathy.[30] *In silico* gene expression analysis indicated that cases were predicted to have increased levels of *TTPA* in the tibial nerve when compared to controls. This finding warrants further research to delineate the potential mechanism of action of the variant in the context of VIPN. Sensory conduction in the tibial nerve has been shown to be impaired in vincristine treated patients with ALL[31] and it has motor functions through innervating the muscles of the leg, including the tibialis posterior muscle, which is involved in plantar flexion.[32] This indicates that the tibial nerve may be an appropriate model to study gene expression in VIPN. Finally, our third most highly associated VIPN-ADME gene was *SLC5A7*, a choline transporter that is implicated in distal hereditary motor neuropathy type 7A [15] and congenital myasthenic syndromes, which are associated with muscle weakness.[33] Given that the association with VIPN was observed for a missense *SLC5A7* variant (rs1013940) with a large effect size (OR > 8), and the similarity of the ADR and inherited phenotypes related to this gene, this candidate variant also warrants further investigation.

Context of current VIPN pharmacogenomic findings

With the availability of high throughput genotyping platforms, pharmacogenomic studies are beginning to uncover novel biology that contributes toward ADRs in a clinically relevant manner.[19] Although the current investigation provides valuable findings, there are caveats. Despite the fact that the sample size of the current study is relatively large for a pharmacogenomic study, including more patients in future cohorts would be beneficial. This is reflected by the fact that our exploratory analysis of ADME variants yielded no associations reaching statistical significance after stringent Bonferroni multiple testing correction. Additional confirmatory evidence should also be pursued in the future through functional experiments for the novel candidate genes identified in the ADME/GWAS analyses. Nonetheless, given the known involvement of the highlighted genes in vincristine pharmacokinetics and inherited neuropathy conditions, this study provides interesting avenues for future research. The retrospective nature of this study is also a potential limitation, because milder forms of VIPN may be poorly captured in standard medical records. We took great care in developing a slightly augmented Common Terminology Criteria for Adverse Events (CTCAE) grading scheme that can be applied in this context. Indeed, our results support the appropriateness of this grading scheme through the: (i) replication of the VIPN *CEP72* GWAS variant with a similar effect size as previously reported; (ii) identification of candidate genes that have a clear biological mechanism pertaining to the ADR; and (iii) the correlation of the genotype frequencies of top candidate variants with VIPN severity.

Conclusions and future directions

VIPNs are important clinical concerns, especially given the increased survival rates of pediatric patients with cancer and potential long-term effects of this ADR. The confirmatory results obtained in the current study highlight the *CEP72* rs924607 risk marker for further evaluation of its clinical utility in the context of potential pharmacogenomic risk predictions and preventive interventions. Specifically, predicting in whom debilitating VIPN is likely to occur would allow clinicians to discuss other therapeutic options, additional physiotherapy interventions, and, importantly, inform patients and parents in advance what toxicity may occur, further enhancing the family's role in cancer management.

Given the current level of evidence for the *CEP72* risk genotype, increased clinical monitoring and earlier initiation of physiotherapy interventions for VIPN in rs924607 TT patients could potentially be beneficial. Oncologists may also consider ALL treatment protocols with lower doses of vincristine and/or shorter vincristine treatment durations in high-risk genotypes. It is important to note that alternative protocols should fall within current standards of care and display similar efficacy and survival outcomes. The results of an ongoing clinical trial investigating the influence of vincristine dose and duration reductions in *CEP72* rs924607 TT patients (ClinicalTrials.gov: NCT03117751) will also be highly informative in determining whether pharmacogenomic genotyping can minimize VIPN risk while maintaining treatment efficacy.

Additional VIPN pharmacogenomic markers, if confirmed through future replication, may further improve VIPN risk stratification. Finally, it will also be beneficial for future studies to use high-throughput sequencing approaches to delineate extreme cases of VIPN in future studies, given the importance of rare variation for pharmacogenomic traits.[19] In particular, resequencing studies would enable the investigation of rare variants in candidate genes for VIPN, such as the ones identified in this study. Comprehensive risk assessments that take different clinical, demographic, and pharmacogenomic factors into account may, therefore, ultimately lead to safer and more effective use of vincristine-based chemotherapy in children.

METHODS

Patients and clinical assessment

Pediatric patients with ALL (age at diagnosis ≤18 years) receiving treatment protocols, including vincristine, were recruited from seven pediatric hospitals across Canada (Calgary, London, Ottawa, Montréal, Toronto, Vancouver, and Winnipeg). Written informed consent and/or assent was obtained from all participants or their parents/legal guardians. The study was approved by ethics committees at participating universities and hospitals. Relevant clinical and demographic data were collected using the CPNDS active surveillance methodology.[34] This included capturing information for relevant concomitant medications (e.g., CYP3A inhibitors, because these enzymes represent the major metabolic path of vincristine).[7,35]

Patients were assessed for vincristine-related neurotoxicities using a refined National Cancer Institute (NCI) CTCAE version 4.0 Grading System (**Table S1**). Specifically, to facilitate grading of the retrospective clinical data and standardize grading across multiple sites, this system included definitions of neurotoxicity-related clinically based interventions, such as pharmacotherapy, rehabilitative therapy, and vincristine dose reductions, to complement the relatively broad definitions (e.g., "noninvasive intervention" or "limiting activities of daily living") used in the CTCAE. To be considered for grading, any intervention had to be used specifically for the treatment for vincristine-related neurotoxicities, and any symptom had to be attributed to vincristine-related neurotoxicities. We excluded patients with grade one (minor) VIPN from our initial pharmacogenomic nonmatched case-control association analyses (**Figure S1**, cases were defined as grade ≥2, whereas controls were defined as patients not developing neuropathy, referred to as grade 0), because the need for a predictive biomarker is greater in those patients with neurotoxicity of sufficient severity to warrant a modification in clinical care (increased monitoring and drug therapy changes). In addition, patients presenting only with symptoms of cranial or central nervous system neurotoxicities without motor or sensory neurotoxicity were not included in the analysis.

Pharmacogenomic genotyping and quality control

Genomic DNA from participants was genotyped for *CEP72* rs924607 using a TaqMan assay (C___8292459_20; ThermoFisher, Waltham, MA) and for 7,907 ADME variants with a custom Infinium Panel (Illumina, San Diego, CA).[36] ADME genotype clusters were extracted from GenomeStudio Software (Illumina) and underwent standard quality control procedures. Specifically, array data were assessed for variant (≥95%) and sample call rates (≥90%), and deviations from Hardy Weinberg equilibrium (Fisher's exact $P < 0.001$ in controls).

Bioinformatic and statistical analyses

Statistical and bioinformatic analyses were performed using either R or SNP & Variation Suite (SVS) version 8.4 (Golden Helix, Bozeman, MT), unless otherwise stated. Genetic ancestry was determined via principal component analyses using pruned ADME genotypes (50-variant window, shifted by 5-variants after each assessment, pruning $r^2 > 0.5$) and minor allele frequency <0.01 with EIGENSOFT version 5.0, incorporating the 1,000 Genomes Project[37] samples as a reference. The first four principal components, along with clinical factors that differed significantly ($P < 0.05$) between cases and controls were included in subsequent case-control association analyses to correct for possible population stratification and clinical confounders. Sensitivity and specificity of prioritized variants were calculated with epiR (https://cran.r-project.org/web/packages/epiR).

To investigate the *CEP72* rs924607 VIPN risk genotype, we decided *a priori* to first test for an association between this marker and VIPN using a recessive model, as reported in the initial study. For this analysis, a one-sided P value < 0.05 was considered statistically significant. A random-effects meta-analysis was performed on *CEP72* rs924607, incorporating the results from this study along with published data, using the GWAMA software[38]; related plots were generated in the R package, forestplot (https://CRAN.R-project.org/package=forestplot).

These analyses were followed by exploratory association analyses of ADME variants passing quality control, and displaying minor allele frequency >0.05, using logistic regression and an additive genetic model. Due to the exploratory nature of this analysis, multiple testing corrections using a conservative Bonferroni threshold was not applied. Instead, variants with $P < 0.001$ were prioritized. To provide corroboratory evidence for top VIPN-associated variants, ordinal regression of VIPN grade was also performed using the R package ordinal.

An Embase literature search (further details are provided in **Table S4**) was completed to identify previously identified VIPN-related variants and assess their association with the ADR in the CPNDS cohort. Further, VIPN-GWAS summary statistics were obtained for the two pediatric ALL cohorts from Diouf *et al.*[10] and a meta-analysis of nonambiguous overlapping ADME variants with evidence for association ($P < 0.05$) in at least one cohort was performed as specified above.

To examine predicted functional effects of variants prioritized from the association analyses, Ensembl Variant Effect Predictor,[39] HaploReg version 4.1,[40] GTEx,[16] and the JASPAR Core Database[41] were used to annotate variants. Differences in predicted gene expression profiles in the tibial nerve[16] between GWAS-genotyped VIPN cases and controls were calculated using S-PrediXcan,[42] where specified, with P values being combined using Fisher's method in metap (https://cran.r-project.org/web/packages/metap).

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Figure S1. CONSORT diagram of pediatric ALL patients included in the CPNDS VIPN study.

Figure S2. Principal component analysis of the CPNDS VIPN cohort (cases, $n = 167$; controls, $n = 57$; grade 1 VIPN, $n = 29$), including

the Phase 3 1,000 Genomes Project samples as a reference (AMR, Admixed American; AFR, African; EAS, East Asian; EUR, European; SAS, South Asian).

Figure S3. Cumulative percentage of vincristine-induced peripheral neuropathy cases over time, indicating that 94% of cases developed in the first 16 months after initiating vincristine therapy (blue dashed line).

Figure S4. Human transcription factor binding site analysis of the *ABCC1* rs3784867 variant (C>T).

Table S1. Refined CTCAE vincristine-induced neurotoxicity grading scale that specifies clinical interventions related to neurotoxicity.

Table S2. Summary of the treatment protocols for the CPNDS pediatric ALL cohort included in the pharmacogenomic association analyses.

Table S3. *CEP72* rs924607 genotype counts in pediatric patients with ALL stratified by VIPN status.

Table S4. Literature search strategy for identification of previously identified VIPN pharmacogenomic variants and assessment of these variants in the CPNDS ALL cohort.

Table S5. Top ADME variants associated with VIPN ($P < 0.001$) in the pediatric CPNDS ALL cohort.

Table S6. *ABCC1* rs3784867 genotype counts in pediatric patients with ALL stratified by VIPN status.

Table S7. *SLC5A7* rs1013940 genotype counts in pediatric patients with ALL stratified by VIPN status.

Table S8. Most significant associations from the VIPN-ADME-GWAS meta-analysis of the St Jude Total XIIIB, COG AALL0433, and CPNDS cohorts.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

G.E.B.W. wrote the manuscript. G.E.B.W., U.A., S.R.R., B.C.C., and C.J.D. designed the research. G.E.B.W., U.A., B.I.D., J.S., S.R.R., M.R.H., B.C.C., and C.J.D.R. performed the research. G.E.B.W., U.A., B.I.D, B.C.C., and C.J.D.R. analyzed the data.

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