Original Article / Оринални рад

Bojan Ristivojević¹, Nikola Kotur¹, Biljana Stanković¹, Vladimir Gašić¹, Jelena Lazić²,³, Sonja Pavlović¹, Branka Zukić¹,⁎

The pharmacogenomics of vincristine-induced peripheral neuropathy in pediatric acute lymphoblastic leukemia patients in Serbia – a single center experience

Фармакогеномика винкристином индукуване периферне неуропатије код деце са акутном лимфобластном леукемијом у Србији – искуство једног центра

¹University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Laboratory for Molecular Biomedicine, Belgrade, Serbia;
²University of Belgrade, University Children’s Hospital, Belgrade, Serbia;
³University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Received: August 13, 2021
Revised: November 21, 2021
Accepted: November 25, 2021
Online First: December 7, 2021
DOI: https://doi.org/10.2298/SARH210813099R

⁎Correspondence to:
Branka ZUKIĆ
Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, Belgrade 11042, Serbia
E-mail: branka.zukic@imgge.bg.ac.rs

*Accepted papers are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the Serbian Archives of Medicine. They have not yet been copy-edited and/or formatted in the publication house style, and the text may be changed before the final publication.

Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author’s last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. Srp Arh Celok Lek. Online First, February 2017.

When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.
The pharmacogenomics of vincristine-induced peripheral neuropathy in pediatric acute lymphoblastic leukemia patients in Serbia – a single center experience

Summary

Introduction/Objective Vincristine (VCR) is one of the key drugs in current treatment protocols for pediatric acute lymphoblastic leukemia (ALL). By destabilization of microtubules, VCR arrests cells in metaphase, inducing apoptosis of malignant cells. VCR also causes axonal degradation and impairment of axonal transport, which leads to vincristine-induced peripheral neuropathy (VIPN).

This study aimed to investigate association of five variants in pharmacogenes involved in VCR metabolism with VIPN in Serbian ALL children. We wanted to discover candidate pharmacogenomic markers of VIPN in Serbian population, too.

Methods PCR and sequencing-based methodology was used to detect variants in CYP3A5, CEP72, ACTG1, MIR3117 and MIR4481 genes. Statistical analyses were performed for investigation of their association with VIPN in 56 pediatric ALL patients. Population VCR pharmacogenomics analysis of 17 pharmacogenes from in-house next-generation sequencing data was also done. Data on allele frequency distribution for European population were extracted from public databases.

Results During the treatment, 17.86% of patients developed VIPN. Association analyses have shown that none of the genetic variants contributed to the occurrence of VIPN in our study. Population pharmacogenomics study didn’t reveal valid candidate pharmacovariants for VIPN. Our results suggested that pre-emptive pharmacogenetic testing for VCR is not applicable presently.

Conclusion More comprehensive approaches are needed to identify panel of genes that could explain the VIPN development after VCR administration in ALL patients. Utilizing better designed GWAS studies and more robust artificial intelligence-based tools would provide a panel of pharmacogenes for pre-emptive tests of VIPN to individualize therapy for ALL in children.

Keywords: acute lymphoblastic leukemia; pharmacogenomics; vincristine; vincristine-induced peripheral neuropathy (VIPN)
INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, comprising about one-fourth of all cancers in children. The cure rate for childhood ALL reached 85%, but about 75% of all patients experience treatment side effects, and 1–3% of all children with ALL die due to the treatment toxicity [1]. Application of the principles of pharmacogenomics could lower the number and intensity of drug induced side effects.

One of the key drugs in treatment protocols for pediatric ALL is vincristine (VCR). VCR binds to the tubulin, preventing the polymerization of microtubules and inducing apoptosis in cancer cells. However, the affinity of VCR for the tubulin makes the microtubules in nerve fibers likely target of VCR action, leading to axonal degradation, impairing axonal transport and causing the development of vincristine-induced peripheral neuropathy (VIPN). VIPN is a major side effect of VCR administration, manifesting as muscle weakness, areflexia, neuropathic pain, sensory loss or autonomic polyneuropathies [2]. VIPN often results in dose reduction, treatment delays and further withdrawal. The occurrence of VIPN in children is determined by multiple factors [3, 4], with most of the recent studies focusing on genetic influences [5].

The most comprehensive candidate gene and genome-wide association studies (GWAS) pointed to the possible involvement of following pharmacogenes: CYP3A5, CEP72, ACTG1 [6–10]. Also, several variants in genes encoding miRNAs were shown to be potential pharmacogenomic markers of VIPN [11]. CYP3A5 is the most important metabolizer of VCR. The rs776746 variant in the third intron introduces a premature stop codon which leads to low or no expression of this enzyme [6]. CEP72 gene encodes a centrosomal protein important for microtubule formation and stability of the centrosome. Variant rs924607 in the promoter region of this gene could be associated with a higher risk of development and severity of VIPN [11] by possibly introducing a binding site for transcriptional repressor NKX-6.3, lowering expression on CEP72 mRNA [7]. Alterations in the interactions between gamma isoform of actin (ACTG1) and microtubules are involved in signal transduction to the actin cytoskeleton. The variant ACTG1 rs1135989 was associated with higher risk and more frequent episodes of high-grade neurotoxicity, as well as a lower tolerated VCR dose [10]. A recent study identified variants in genes encoding miRNAs related to VIPN [11]. The variant MIR3117 rs12402181 reduces the risk of VIPN by decreasing the degradation and increasing expression of ABCC1.
and RALBP1 mRNAs, thus increasing the efflux of VCR from the axons. The variant MIR4481 rs7896283 increases the stability of the premature miR-4481, lowering expression of proteins in the axon guidance pathway that may affect peripheral nerve regeneration [11]. Several studies have indicated additional pharmacogenes potentially involved in development of VIPN [8, 9, 12–15].

The aim of this study was to determine if the selected genetic variants CYP3A5 rs776746, CEP72 rs924607, ACTG1 rs1135989, MIR3117 rs12402181 and MIR4481 rs7896283 are associated with the development of VIPN in ALL children treated with VCR in Serbia. Additionally, we aimed to perform an analysis of clinical exome sequencing data for population pharmacogenomics study to discover candidate pharmacogenomic markers of VIPN in Serbian population.

METHODS

Subjects

This study included 56 children diagnosed with ALL between 2010 and 2018 at the University Children’s Hospital, Belgrade, Serbia. It was approved by the University Children's Hospital Ethics Committee and performed according to the Declaration of Helsinki. Informed consent was obtained from the parents or legal guardians of each patient.

All patients were treated according to the ALL IC-BFM 2009 protocol, divided in usual phases: remission induction and early intensification, consolidation, reinduction and maintenance. The patients were stratified into three risk groups: standard risk (SR), intermediate risk (IR) and high risk (HR). Patients in IR and HR groups were randomized during the early intensification phase (IB) in arm 1 (IR-1 and HR-1) and arm 2 (IR-2 and HR-2). Stratification and randomization of the patients resulted in patients receiving different number of VCR doses (1.5 mg/m² per dose) during the treatment (Table 1) [16].
Patients were assessed for VIPN using the National Cancer Institute Common Toxicity Criteria [17]. A patient was diagnosed with VIPN if exhibiting one or more symptoms of vincristine-related neurotoxicity at the end of reinduction phase.

Data for the European population frequencies of the investigated variants were extracted from Genome Aggregation Database, GnomAD.

**Genetic variants detection**

Detection of variants investigated in this study (ACTG1 rs1135989, CEP72 rs924607, MIR3117 rs12402181, MIR4481 rs7896283 and CYP3A5 rs776746) was performed using PCR and sequencing-based methodology as described elsewhere [18]. Primer sequences and annealing conditions used for amplification of each variant are available upon request. Allele frequency for CEP72 rs924607 in healthy controls of Serbian descent was determined using the same methodology.

**Population pharmacogenomics study**

We have searched the literature on the PubMed database before April 2021, using the terms “vincristine” AND “pharmacogenomics” OR “pharmacogenetics” AND “GWAS” OR “candidate gene” OR “vincristine-induced peripheral neuropathy”. Studies were identified from the titles and abstracts by the primary (BR) and the secondary reviewer (BZ).

In-house database of 154 TruSight One Illumina sequenced clinical exomes belonging to individuals of Serbian descend was used to search for variants in 17 genes found during literature search that could be related to VCR metabolism: CYP3A4, CYP3A5, ABCB1, PON1, ABCA4, ABCG1, CY51A1, SLCO1C1, ABCC1, SLC5A7, TTPA, ABCC2, SYNE2, COCH, TUBB1, TUBB2B and TUBB3.

The criteria for pharmacogenomics relevance of a variant were allele frequency higher than 5% and assigned annotation in PharmGKB database [19].
Assignment of a Level of Evidence by the PharmGKB annotation scoring system for clinical and variant annotations enables easier identification of significant pharmacovariants. The clinical annotation score represents the sum of the scores of all attached variant, guideline and drug label annotations. Variant annotations are scored depending on: phenotype category, p-value, cohort size, effect size and weighting by study type or by association and significance. [19].

**Statistical analysis**

All variants were tested for the Hardy–Weinberg equilibrium (HWE) using an exact test.

The association of age of the patients and occurrence of VIPN was tested using logistic regression. The associations of immunophenotype, gender and number of doses administered were tested for association with VIPN using Fisher’s exact test.

The association between the genotyped variants and VIPN was tested using multiplicative genetic model. Multivariate analysis was adjusted for number of VCR doses or gender.

Association analyses were performed using SPSS (version 21, IBM, Armonk, NY, USA).

Probabilities lower than 5% were considered statistically significant.

**RESULTS**

**Demographic and clinical characteristics of the subjects**

This study encompassed 56 children with ALL, with slight predominance of male gender (N=29; 51.8%). The median age at diagnosis was 5.3 years, ranging from 0.7 to 17.9 years. Risk stratification revealed following distribution: SR – 11 (19.6%), IR – 28 (50%) and HR – 17 (30.4%).
Ten patients (17.86%) developed VIPN during the therapy, with high predominance of girls (70%). The median age at diagnosis of the patients that developed VIPN was 3.7 years, ranging from 1 to 17.1 years. Nine out of ten patients were stratified into the IR and HR groups (IR – 5, HR – 4).

Occurrence of VIPN was higher in girls, though statistical significance hasn’t been demonstrated (0.171, Fisher’s exact test, OR=3.03). A trend of higher occurrence of VIPN in patients who received more VCR doses was observed, but without statistical significance. However, patients who received more that 10 doses of VCR were 3.6 times more likely to develop VIPN than patients who received less than 10 doses (OR = 0.363; CI = 0.83 – 15.89; p = 0.092; Fisher’s exact test) (Table 1).

**Association of pharmacogenetic markers with VIPN**

The HWE testing showed that genotype frequencies corresponding to all variants investigated in this study were in the equilibrium. Frequencies of investigated variants in Serbian pediatric ALL patients and control group of European origin and results of HWE testing are presented in Figure 1.

We have analysed the association between the investigated variants and VIPN. For each variant, we evaluated the contribution associated with each additional minor allele to the probability of developing the neuropathy using a multiplicative genetic model [20]. In univariate analysis, no variant showed a statistically significant association with the VIPN. Applying logistic regression, the following p-values associated with variants in *ACTG1, CEP72, MIR3117, MIR4481* and *CYP3A5* genes were obtained: 0.917, 0.898, 0.788, 0.310, and 0.577, respectively. In univariate or multivariate analysis (adjusted for number of VCR doses or gender), no variant showed a statistically significant association with VIPN (Table 2).
Population pharmacogenomics study

In-house database of clinical exome sequences of 154 Serbian individuals was searched for variants in 17 genes with possible influence to VCR metabolism. Ten variants in 6 genes have been detected with allele frequency higher than 5%. Allele frequencies for most of them were similar to the ones detected in European population (Table 3).

Only two variants were worthy to be further analyzed as potential pharmacogenetic markers of VIPN in Serbian population. Although a variant \textit{CYP3A4} rs4986910 is assigned to have a level of evidence 2A, its allele frequency in Serbian population is very low (0.97%), similarly to European populations (0.73%). Therefore, it is not considered to be an appropriate candidate for pre-emptive pharmacogenomic testing.

Variant \textit{CEP72} rs924607, considered to be the best candidate pharmacogenomic marker for VCR, is present in Serbian population with 60% compared to 43.4% in European populations. Despite high allele frequency in Serbian population, this marker is not an applicable pharmacogenetic marker in pediatric ALL since our study demonstrated that it has the same distribution in pediatric ALL patients with and without VIPN.

DISCUSSION

Efforts towards treatment individualization of patients experiencing side effects of drugs are made constantly. Previous studies analysed pharmacogenomics of some essential drugs used for treatment of pediatric ALL patients in Serbia [18, 21, 22].

We analysed the correlation of five variants in pharmacogenes involved in VCR metabolism in pediatric ALL patients with VIPN. Total of 56 patients treated according to BFM protocol were included in the study. Ten patients (17.86%) developed VIPN during the treatment. Association analyses have shown that none of the genetic variants were significant for the occurrence of VIPN in our cohort.

Our results have shown a trend of higher occurrence of VIPN in girls as in several studies [7], while others reported no influence of patients’ sex to VIPN development [12, 23]. We have
also observed a trend of higher occurrence of VIPN in patients who received more than 10 doses of VCR during the therapy, as they were 3.6 times more likely to develop VIPN. Several studies reported significant association between VCR dose and VIPN [7, 14] in contrast to other ones [12, 24]. Assessment of cumulative dose effect of VCR to VIPN development has also shown conflicting results. There are reports of the absence of association between VIPN and cumulative VCR dose [24], as well as the reports showing that cumulative dose of VCR is associated with VIPN [25].

Variant CEP72 rs924607 was identified in GWAS study as key pharmacogene relevant for VIPN in ALL children [7], and those findings were confirmed in meta-analysis of pharmacogenomic data from over 500 patients [8]. However, several studies did not confirm this association [9, 23, 26]. Our results do not support the association of CEP72 rs924607 with VIPN in pediatric ALL patients. The frequency of rs924607 T allele in our group of ALL patients with VIPN was almost the same as in ALL patients without VIPN (45.0% and 43.5%, respectively). The frequency of the same allele in our healthy population was rather high (60.0%), similar to frequency of this variant in European population (43.4%). Therefore, we conclude that CEP72 rs924607 pharmacomarker wasn’t shown to be of pharmacogenomic relevance for Serbian population.

Variants ACTG1 rs1135989 [10] and CYP3A5 rs776746 [6] have also been reported to contribute to VIPN susceptibility. Further, analysis of the SNPs in miRNAs which could regulate VCR-related genes in a large cohort of pediatric ALL patients identified the MIR3117 rs12402181 and MIR4481 rs7896283 as variants significantly associated with VIPN [11]. In our study, none of the aforementioned variants has shown statistically significant association with VIPN in pediatric ALL patients.

As additional variants have been indicated to contribute to development of VIPN, we have performed the pilot population pharmacogenomics study in order to assess if the molecular genetics study of additional potential pharmacogenes would be beneficial and informative. The population pharmacogenomics study encompassed 17 relevant, literature reviewed pharmacogenes present in in-house NGS database sequences of Serbian individuals: CYP3A4, CYP3A5, ABCB1, PON1, ABCA4, ABCG1, CY51A1, SLCO1C1, ABCC1, SLC5A7, TTPA, ABCC2, SYNE2, COCH, TUBB1, TUBB2B and TUBB3 [8,9,12–15]. Also, for these pharmacogenes our population pharmacogenomics study didn’t reveal valid candidate
pharmacovariant for VIPN. A GWAS study identified PNPLA3 rs735409 as potential pharmacovariant relevant for VCR use [27]. Further study confirmed that the PNPLA3 rs735409 variant was associated with hepatotoxicity induced by asparagine administration [28], and we decided not to include it in our study. Our results have shown that pre-emptive pharmacogenetic testing for vincristine is not presently applicable either in pediatric ALL patients or in patients of Serbian descent whom VCR needs to be administered.

VCR metabolic pathway is complex. Many transporters and enzymes have important role in the VCR pharmacokinetics and pharmacodynamics and so far, data have not shown a single universal VCR pharmacogenomic marker [29]. This is another example of the failure of predictions that have resulted from GWAS studies. Lack of consistency in neuropathy assessment, grading systems, and the choice of end points make it difficult to interpret results between studies [4, 29]. Treatment regiments, including number of VCR doses administered and lengths of VCR treatment, differ between treatment protocols used in different studies [30]. Furthermore, population-specific genetic variants could have relevance or other expression quantitative trait loci of the genes in question for VIPN assessment.

The main limitation of our study is a small number of patients included. However, we have tried to overcome the limitations of GWAS studies by analysing the patients treated with the same protocol and by establishing the comparative groups with similar VCR administration.

CONCLUSION

Our results have shown that pre-emptive pharmacogenetic testing for VCR is not presently applicable either in pediatric ALL patients or in patients from Serbian descent whom VCR needs to be administered. Association analyses have shown that none of the genetic variants were significant for the occurrence of VIPN in our cohort.

More comprehensive approaches are needed to identify a panel of genes that could explain the VIPN development in ALL patients. Extending genome-wide research to larger, well-characterized and more diverse patient cohorts and development of more robust artificial intelligence bioinformatics tools, including machine learning, statistical learning, and soft-
computing approaches, should be done. This would provide a panel of pharmacogenes that could be used for pre-emptive tests of VCR side effects leading to therapy individualization in pediatric ALL patients.

ACKNOWLEDGMENT

This work was supported by Ministry of Education, Science and Technological Development Republic of Serbia, EB: 451-03-9/2021-14/200042.

Conflict of interest: None declared.
REFERENCES

1. Pavlovic S, Kotur N, Stankovic B, Zukic B, Gasic V, Dokmanovic L. Pharmacogenomic and Pharmacotranscriptionomic Profiling of Childhood Acute Lymphoblastic Leukemia: Paving the Way to Personalized Treatment. Genes (Basel). 2019;10(3):191. DOI: 10.3390/genes10030191 PMID: 30832275

2. van de Velde ME, Kaspers GL, Abbink FCH, Wilhelm AJ. Ket JCF, van den Berg MH. Vincristine-induced peripheral neuropathy in children with cancer: A systematic review. Vol. 114, Critical Reviews in Oncology/Hematology. Elsevier Ireland Ltd; 2017. p. 114–30. DOI: 10.1016/j.critrevonc.2017.04.004. PMID: 28477739

3. Triarico S, Romano A, Attinà G, Capozza MA, Maurizi P, Mastrangelo S, et al. Vincristine-Induced Peripheral Neuropathy (VIPN) in Pediatric Tumors: Mechanisms, Risk Factors, Strategies of Prevention and Treatment. Int J Mol Sci. 2021 Apr 16;22(8):4112. DOI: 10.3390/ijms2204112 PMID: 33923421

4. Madsen ML, Due H, Ejskjær N, Jensen P, Madsen J, Dybkær K. Aspects of vincristine-induced neuropathy in hematologic malignancies: a systematic review. Cancer Chemother Pharmacol. 2019 Sep 18;84(3):471–85. DOI: 10.1007/s00280-019-03884-5. PMID: 31214762

5. Pozzi E, Fumagalli G, Chiorazzi A, Canta A, Cavaletti G. Genetic factors influencing the development of vincristine-induced neurotoxicity. Expert Opin Drug Metab Toxicol. 2021 Feb 1;17(2):215–26. : 10.1080/17425255.2021.1855141 PMID: 33283553

6. Egbelakin A, Ferguson MJ, MacGill EA, Lehmann AS, Topletz AR, Quinney SK, et al. Increased risk of vincristine neurotoxicity associated with low CYP3A5 expression genotype in children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2011 Mar;56(3):361–7. DOI: 10.1002/pbc.22845. PMID: 21225912

7. Diouf B, Crews KR, Lew G, Pei D, Cheng C, Bao J, et al. Association of an inherited genetic variant with vincristine-related peripheral neuropathy in children with acute lymphoblastic leukemia. JAMA. 2015 Feb 24;313(8):815–23. DOI: 10.1001/jama.2015.0894. PMID: 25710658

8. Wright GEB, Amstutz U, Drögemöller BI, Shih J, Isserholz SR, Hayden MR, et al. Pharmacogenomics of Vincristine-Induced Peripheral Neuropathy Implicates Pharmacokinetic and Inherited Neuropathy Genes. Clin Pharmacol Ther. 2019;105(2):402–10. DOI: 10.1002/cpt.1179. PMID: 29999516

9. Li L, Sajdik T, Smith EMLL, Chang CW, Li C, Ho RH, et al. Genetic Variants Associated With Vincristine-Induced Peripheral Neuropathy in Two Populations of Children With Acute Lymphoblastic Leukemia. Clin Pharmacol Ther. 2019 Jun 21;105(6):1421–8. DOI: 10.1002/cpt.1324. PMID: 30566763

10. Ceppi F, Langlois-Peltier C, Gagné V, Rousseau J, Ciolino C, Lorenzo S De, et al. Polymorphisms of the vincristine pathway and response to treatment in children with childhood acute lymphoblastic leukemia. Pharmacogenomics. 2014 Jun 1;15(8):1105–16. DOI: 10.2217/pgs.14.1468. PMID: 25084203

11. Gutierrez-Camino A, Umeoro M, Martin-Guerrero I, García de Andoin N, Santos B, Sastre A, et al. Mitopharmacogenetics of Vincristine and peripheral neurotoxicity in childhood B-cell acute lymphoblastic leukemia. Pharmacogenomics J. 2018 Dec 27;18(6):704–12. DOI: 10.1007/s41397-017-0003-3. PMID: 29282364

12. Lopez-Lopez E, Gutierrez-Camino A, Astigarraga I, Navajas A, Echebarria-Barona A, Garcia-Miguel P, et al. Vincristine pharmacokinetics pathway and neurotoxicity during early phases of treatment in pediatric acute lymphoblastic leukemia. Pharmacogenomics. 2016 May 1;17(7):731–41. DOI: 10.2217/pgs-2016-0001. PMID: 27180762

13. Abaji R, Ceppi F, Patel S, Gagné V, Xu CJ, Spinella J-F, et al. Genetic risk factors for VIPN in childhood acute lymphoblastic leukemia patients identified using whole-exome sequencing. Pharmacogenomics. 2018 Oct;19(15):1181–93. DOI: 10.2217/pgs-2018-0093. PMID: 30191766

14. Guilhaumour R, Solas C, Bourgarel-Rey V, Quarta S, Rome A, Simon N, et al. Impact of plasma and intracellular exposure and CYP3A4, CYP3A5, and ABCB1 genetic polymorphisms on vincristine-induced neurotoxicity. Cancer Chemother Pharmacol. 2011 Dec 4;68(6):1633–8. DOI: 10.1007/s00280-011-1745-2. PMID: 21968951

15. Martin-Guerrero I, Gutierrez-Camino A, Echebarria-Barona A, Astigarraga I, García de Andoin N, Navajas A, et al. Variants in vincristine pharmacodynamic genes involved in neurotoxicity at induction phase in the therapy of pediatric acute lymphoblastic leukemia. Pharmacogenomics J. 2019; 10.1038/s41397-019-0081-5. PMID: 30723315

16. ALL IC-BFM 2009. A Randomized Trial of the I-BFM-SG for the Management of Childhood non-B Acute Lymphoblastic Leukemia Final Version of Therapy Protocol from August-14-2009. 2015.

17. COMMON TOXICITY CRITERIA MANUAL Common Toxicity Criteria, Version 2.0. 1999.

18. Kotur N, Lazic J, Ristivojevic B, Stankovic B, Gasic V, Dokmanovic L, et al. Pharmacogenomic markers of methotrexate response in the consolidation phase of pediatric acute lymphoblastic leukemia treatment. Genes (Basel). 2020;11(4):1–17. DOI: 10.3390/genes11040468. PMID: 32344632

19. Whirl-Carrillo M, McDonagh EM, Hebert JM, Gong L, Sangkuhl K, Thorn CF, et al. Pharmacogenomics knowledge for personalized medicine. Clin Pharmacol Ther. 2012 Oct 1;92(4):414–7. DOI: 10.1038/clpt.2012.96. PMID: 22992668

DOI: https://doi.org/10.2298/SARH210813099R Copyright © Serbian Medical Society
20. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT. Basic statistical analysis in genetic case-control studies. Nat Protoc. 2011 Feb;6(2):121–33. DOI: 10.1038/nprot.2010.182. PMID: 21293453

21. Gasic V, Zukic B, Stankovic B, Janic D, Dokmanovic L, Lazic J, et al. Pharmacogenomic markers of glucocorticoid response in the initial phase of remission induction therapy in childhood acute lymphoblastic leukemia. Radiol Oncol. 2018;52(3):296–306. DOI: 10.2478/raon-2018-0034. PMID: 30210047

22. Dokmanovic L, Milosevic G, Peric J, Tosic N, Krstovski N, Janic D, et al. Next generation sequencing as a tool for pharmacogenomic profiling: Nine novel potential genetic markers for targeted therapy in childhood acute lymphoblastic leukemia. Srp Arh Celok Lek. 2018;146(7–8):407–11. DOI: https://doi.org/10.2298/SARH171003194D

23. Gutierrez-Camino A, Martin-Guerrero I, Lopez-Lopez E, Echebarria-Barona A, Zabalza I, Ruiz I, et al. Lack of association of the CEP72 rs924607 TT genotype with vincristine-related peripheral neuropathy during the early phase of pediatric acute lymphoblastic leukemia treatment in a Spanish population. Pharmacogenet Genomics. 2016 Feb;26(2):100–2. DOI: 10.1097/FPC.0000000000000191. PMID: 26618658

24. Gilchrist LS, Tanner L. The pediatric-modified total neuropathy score: A reliable and valid measure of chemotherapy-induced peripheral neuropathy in children with non–CNS cancers. Support Care Cancer. 2013 Mar;20(1):235. DOI: 10.1007/s00520-012-1591-8 PMID: 22993026

25. Lavoie Smith EM, Li L, Chiang C, Thomas K, Hutchinson RJ, Wells EM, et al. Patterns and severity of vincristine-induced peripheral neuropathy in children with acute lymphoblastic leukemia. J Peripher Nerv Syst. 2015 Mar;20(1):37–46. DOI: 10.1111/jn.12114 PMID: 25977177

26. Zečkanović A, Jazbec J, Kavčič M. Centrosomal protein72 rs924607 and vincristine-induced neuropathy in pediatric acute lymphocytic leukemia: meta-analysis. Futur Sci OA. 2020 Aug;6(7):FSO582. DOI: 10.2144/fsoa-2020-0044 PMID: 32802391

27. Diouf B, Evans WE. Pharmacogenomics of Vincristine-Induced Peripheral Neuropathy: Progress Continues. Vol. 105, Clinical Pharmacology and Therapeutics. Nature Publishing Group; 2019. p. 315–7. DOI: 10.1002/cpt.1209. PMID: 30277559
**Table 1.** Characteristics of pediatric acute lymphoblastic leukemia patients

| Characteristics          | Patients without VIPN | Patients with VIPN | Total | p¹ |
|--------------------------|-----------------------|--------------------|-------|----|
| Age (years)              |                       |                    |       |    |
| Average                  | 7                     | 6.8                | 7     | 0.913² |
| Median                   | 5.5                   | 3.7                | 5.3   |    |
| Range                    | 0.7–17.9              | 1.0–17.1           | 0.7–17.9 |    |
| Sex (N/%)                |                       |                    |       | 0.171³ |
| Male                     | 26 (56.5%)            | 3 (30%)            | 29 (51.8%) |    |
| Female                   | 20 (43.5%)            | 7 (70%)            | 27 (49.2%) |    |
| Immunophenotype (N (%))  |                       |                    |       | 1³ |
| B-lineage                | 42 (91.3%)            | 10 (100%)          | 52 (49.2%) |    |
| T-lineage                | 4 (8.7%)              | 0 (0%)             | 4 (7.1%) |    |
| Risk group (N %)         |                       |                    |       | 0.346¹ |
| SR; 8 VCR doses          | 10 (21.7%)            | 1 (10%)            | 12 (21.4%) |    |
| IR-1; 8 VCR doses        | 18 (39.1%)            | 2 (20%)            | 19 (33.9%) |    |
| IR-2; 12 VCR doses       | 5 (10.9%)             | 3 (30%)            | 8 (14.3%) |    |
| HR-1; 12 VCR doses       | 10 (21.7%)            | 3 (30%)            | 13 (23.2%) |    |
| HR-2; 16 VCR doses       | 3 (6.5%)              | 1 (10%)            | 4 (7.2%) |    |

VIPN – vincristine-induced peripheral neuropathy; VCR – vincristine;

¹p-value refers to statistical testing the difference between groups of patients with and without VIPN;

²Logistic regression;

³Fisher’s exact test
Table 2. Association of analyzed variants and vincristine-induced peripheral neuropathy (VIPN)

| Gene    | dbSNP       | Genotype | N (%) | Patients without VIPN | Patients with VIPN | p     | p¹     | p²     |
|---------|-------------|----------|-------|------------------------|--------------------|-------|--------|--------|
| ACTG1   | rs1135989 C>T | CC       | 25 (44.6%) | 21 (45.7%) | 4 (40%) | 0.917 | 0.837  | 0.886  |
|         |             | CT       | 24 (42.9%) | 19 (41.3%) | 5 (50%)  |       |        |        |
|         |             | TT       | 7 (12.5%)  | 6 (13%)    | 1 (10%)   |       |        |        |
| CEP72   | rs924607 C>T | CC       | 17 (30.4%) | 14 (30.4%) | 3 (30%)  | 0.898 | 0.909  | 0.791  |
|         |             | CT       | 29 (51.8%) | 24 (52.2%) | 5 (50%)  |       |        |        |
|         |             | TT       | 10 (17.9%) | 8 (17.4%)  | 2 (20%)   |       |        |        |
| MIR3117 | rs12402181 G>A | GG      | 38 (67.9%) | 31 (67.4%) | 7 (70%)  | 0.788 | 0.963  | 0.605  |
|         |             | GA       | 17 (30.4%) | 14 (30.4%) | 3 (30%)  |       |        |        |
|         |             | AA       | 1 (1.8%)   | 1 (2.2%)   | 0 (0%)   |       |        |        |
| MIR4481 | rs7896283 T>C | TT      | 20 (35.7%) | 15 (32.6%) | 5 (50%)  | 0.310 | 0.188  | 0.500  |
|         |             | TC       | 27 (48.2%) | 23 (50%)   | 4 (40%)  |       |        |        |
|         |             | CC       | 9 (16.1%)  | 8 (17.4%)  | 1 (10%)  |       |        |        |
| CYP3A5  | rs776746 A>G | AA       | 0 (0%)     | 0 (0%)     | 0 (0%)   | 0.577 | 0.702  | 0.360  |
|         |             | AG       | 13 (23.2%) | 10 (21.8%) | 3 (30%)  |       |        |        |
|         |             | GG       | 43 (76.8%) | 36 (78.2%) | 7 (70%)  |       |        |        |

¹ adjusted for the number of vincristine doses;

² adjusted for sex
Table 3. Allele frequencies of pharmacogenes related to vincristine-induced peripheral neuropathy in Serbian population (MAF > 5%)

| Gene | dbSNP 1 | PharmGKB LoE 2 | MAF in Serbian population (%) | MAF in European population (GnomAD) (%) |
|------|---------|---------------|-------------------------------|----------------------------------------|
| CEP72 | rs924607 | 3             | 60.0                          | 43.3                                   |
| ABCC2 | rs3740066 | 3             | 27.27                         | 37.02                                  |
| ABCC2 | rs2273697 | 3             | 17.85                         | 19.75                                  |
| ABCC2 | rs17222723 | 3           | 5.19                          | 5.61                                   |
| SLC5A7 | rs1013940 | VA            | 8.44                          | 8.01                                   |
| PON1  | rs854560 | 4             | 37.01                         | 36.7                                   |
| PON1  | rs662   | 3             | 22.4                          | 28.06                                  |
| COCH  | rs1045644 | VA            | 54.87                         | 63.49                                  |
| TUBB1 | rs6070697 | VA            | 15.26                         | 17.94                                  |
| TUBB1 | rs463312 | VA            | 6.17                          | 5.26                                   |
| ABCC1 | rs246221 | VA            | 31.82                         | 30.5                                   |

MAF – minor allele frequencies;

1reference single nucleotide polymorphism (SNP) ID number (rs number) of SNPs that map an identical location assigned by the National Center for Biotechnology Information;

2PharmGKB level of evidence, score of pharmacogenomics clinical (level 1: the highest, level 4: the lowest evidence association) and variant relevance (VA –variant annotation)
Figure 1. Minor allele frequencies (MAFs) of analyzed genetic variants in the study group and European population; all investigated variants were in Hardy–Weinberg equilibrium (HWE) (for rs1135989 HWE was 0.768, for rs924607 0.790, and for the other variants 1); The data for MAF in the European population was extracted from GnomAD database