






The Effect of *UGT1A9*, *CYP2B6* and *CYP2C9* Genes Polymorphism on Propofol Pharmacokinetics in Children

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Purpose: This study was conducted to determine the effect of *UGT1A9* 98T>C, *CYP2B6* 516G>T and *CYP2C9* 430C>T genetic polymorphisms on the pharmacokinetics of propofol in children of different sexes and ages who undergone total intravenous anesthesia (TIVA) and deep sedation during diagnostic and therapeutic procedures.

Patients and Methods: The prospective study included 94 children, ASA I-II status, 1 to 17 years of age, who undergone standard anesthetic protocol for TIVA, which implied the continuous use of propofol. Before the administration of propofol, venous blood was sampled to determine the presence of genetic variations in *UGT1A9*, *CYP2B6* and *CYP2C9* gene using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). From each patient included in the study blood samples were taken: 10 mins after the induction of anesthesia, immediately before the discontinuation of the propofol infusion, 10 mins after discontinuation of the propofol infusion and 20 mins after discontinuation of the propofol infusion to determine the pharmacokinetics of the drug in the plasma of the subjects. The plasma propofol concentration was determined by HPLC analytical technique.

Results: *UGT1A9* genotype is an independent predictor of the propofol concentration in children immediately after the end of the continuous infusion and 10 mins afterwards. In the carriers of the polymorphic *UGT1A9* C allele, the propofol distribution constant was higher. The carriers of the polymorphic *CYP2B6* T allele received a significantly lower overall and initial dose of propofol. Unlike polymorphism of the *UGT1A9* gene, the tested *CYP2C9* and *CYP2B6* gene polymorphisms are not independent predictors of the pharmacokinetics of propofol.

Conclusion: Further investigations of *UGT1A9*, *CYP2B6* and *CYP2C9* and other genes that participate in propofol metabolism as well as detailed analyses of the general conditions, administered therapies and associated diseases could explain the large interindividual variability of propofol metabolism in children.

Keywords: polymorphism, gene, propofol, metabolism, children

Introduction

Propofol (2,6 di-isopropylphenol) is the most commonly used intravenous anesthetic in modern medicine and is used for inducing and maintaining of anesthesia, as well as for procedural sedation during diagnostic and therapeutic procedures. It gained wide application primarily due to a favorable induction in anesthesia and rapid clearance. However, there are data on the individual variations of its pharmacokinetics potentially imposing the need for the application of different doses of anesthetics to achieve effective general anesthesia.¹ Most likely, these variables are

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the result of differences in the kinetics of its metabolism and the inactivation processes under the action of the corresponding enzymes, and due to the existence of their various genetically conditioned isoforms.

Propofol is mainly metabolized by hepatic and extra-hepatic cytochrome P450 2B6 (*CYP2B6*) and cytochrome P450 2C9 (*CYP2C9*),² as well as by UDP-glucuronosulfotransferase 1A9 (*UGT1A9*). UDP-glucuronosulfotransferase 1A9 (*UGT1A9*) catalyzes the formation of propofol glucuronide.³ The cytochrome P450 enzymes (*CYP2B6* and *CYP2C9*) are responsible for the formation of a hydroxyl derivative of propofol-4-hydroxypropofol, which can further be transformed into 4-hydroxypropofol-1-ObD-glucuronide (Q1G) and 4-hydroxypropofol-4-ObD-glucuronide (Q4G).^{4,5} About 70 to 90% of propofol is eliminated by urine in the form of the glucuronide metabolite.^{3,4} It is postulated that the polymorphism of single nucleotide polymorphisms in the genes encoding these enzymes can be responsible for the formation of individual variables of the metabolic products of propofol in infant age, which may result in unpredictable effects of the usual doses of anesthetics as well as prolonged waking time (recovery) from anesthesia.

The objective of this study was to determine the effect of genetic polymorphisms *CYP2C9 430C>T*, *CYP2B6 516G>T* and *UGT1A9 98T>C* on the pharmacokinetics of propofol in children of different sexes and ages who undergo total intravenous anesthesia and deep sedation during diagnostic and therapeutic procedures.

Patients and Methods

Patients

The study included ninety-four children (N = 94), 1 to 17 years old, who underwent total intravenous anesthesia (TIVA) and sedation for diagnostic and therapeutic procedures in plastic, abdominal surgery, orthopedics, urology and gastroenterology. The material used for the research was collected at the Department of Anesthesia and Reanimation of the Clinic for Anesthesia and Reanimation at the Clinic for Children's Surgery and Orthopedics at the Clinical Center in Nis and at the University Children's Hospital, Faculty of Medicine, University of Belgrade. The parents were informed about the objectives of the research and gave their consent to the use of the taken material and data by signing an informed consent. The whole study was approved by the Ethics Committee of the Medical Faculty in Nis, no. 12-8765/9 as well as by the Ethics Committee of the University Children's Hospital in Belgrade,

no. 26/340 and the Ethics Committee of the Clinical Center in Nis, no. 27771/11. The clinical study was conducted in accordance with the ethical principles of Good Clinical and Laboratory Practices and the Helsinki Declaration.⁶ The study did not include children with allergies to propofol, soybeans, peanuts, eggs; for which anamnestic data on the consumption of tobacco, psychoactive substances and alcohol had been obtained; which had a hemoglobin values of less than 7 g/dl.

Anesthesia Protocol

After premedication with atropine (0.01 mg/kg) and midazolam (0.1 mg/kg), anesthesia was induced by bolus dose of propofol (1% Fresenius Kabi) from 2.5 to 3.5 mg/kg body weight, and after was maintained by continuous infusion of propofol using infusion pump (3–15 mg/kg/h). For analgesia, fentanyl was administered at a dose of 1–2 mcg/kg during the induction, and, if needed, it was added at the dose of 0.5 to 1 mcg/kg during anesthesia. Muscle relaxation was provided by rocuronium bromide in a bolus dose of 0.6–1 mg/kg, followed by 0.2 to 0.3 mg/kg every 20 mins. Atropine 0.02 mg/kg and prostigmine 0.05 mg/kg were given for the reversal of the neuromuscular block. Patients are ventilated by oxygen and air (35: 65%; 50: 50%) or oxygen and nitrous oxide (35: 65%). During anesthesia, vital parameters were monitored (systolic and diastolic pressure, heart rate, oxygen saturation, end-tidal carbon dioxide), and, in individual patients, the depth of sedation and anesthesia was measured by analyzing the value of the bispectral index (BIS).

Methods

Material Sampling

From each patient included in the study, five blood samples were taken:

- (a) 2mL of blood before propofol administration to determine the presence of gene mutations in enzymes that participate in propofol metabolism
- (b) 1mL (max 2mL) of blood:
 - 10 mins after the induction of anesthesia
 - immediately before the discontinuation of the propofol infusion
 - 10 mins after discontinuation of the propofol infusion
 - 20 mins after discontinuation of the propofol infusion

to determine the pharmacokinetics of the drug in the plasma of the subjects.

Venous blood was taken from a previously placed intravenous cannula in an amount corresponding to previously established ethical standards.⁶

All blood samples were taken with EDTA as an anticoagulant. The first full blood sample was used for DNA isolation and gene testing. The second, third, fourth and fifth samples were taken at pre-defined intervals - these samples were centrifuged at 3000 rpm after which plasma was extracted for the determination of propofol pharmacokinetics.

Full blood as well as plasma samples were stored at -80°C until DNA isolation, or until the concentration of propofol was determined.

Drug clearance was calculated using the following formula: $Cl = R_0 / C_2$ clearance; R_0 propofol infusion rate; C_2 propofol concentration at the end of infusion

Isolation of DNA and Examination of Gene Polymorphisms

All genetic tests were carried out at the Laboratory for Functional Genomics and Proteomics of the Scientific

Research Center for Biomedicine, Faculty of Medicine, University of Nis, Serbia.

The genomic DNA was isolated from full blood previously sampled with EDTA, using the commercial QIAamp DNA Blood Mini Kit (Quiagen GmbH, Hilden, Germany).

Detection of gene polymorphism assay *CYP2C9*2*, *UGT1A9 98T>C* and *CYP2B6 516G>T* was performed using DNA chain replication reactions followed by polymorphisms analysis of restriction fragment length (polymerase chain reaction-restriction fragment length polymorphism, PCR-RFLP).

Amplification of the DNA segment by polymerase chain reaction was carried out in a reaction mixture of 25 µL to which 50ng/µL DNA was added using an appropriate primer set. The primer sequence and the temperature response profile are shown in Table 1.

(rs1799853) After verification of the PCR product size 454bp on horizontal electrophoresis using 2% agarose gel, restriction digestion with *AvaII* restriction enzyme was performed at a temperature of 37°C. The obtained digestion was performed on a 8% polyacrylamide gel, and the obtained results were interpreted as follows: the presence of the

Table 1 Sequences of the Primer and Temperature Profile of the Chain-Replication Reaction for *CYP2C9 * 2* Polymorphism, *UGT1A9 98T>C* and *CYP2B6 516G>T*

Gene Polymorphism	Primers	PCR Reaction Conditions	Restriction Enzymes
<i>CYP2C9*2</i> (rs1799853)	F 5'-GTA TTT TGG CCT GAA ACC CAT A-3' R 5'-GGC CTT GGT TTT TCT CAA CTC-3'	Initial denaturation 95°C(2 min) 32 cycles: Denaturation 95°C(15s) Annealing 62°C(15s) Elongation 72°C(15s) Terminal elongation 72°C(30s) Termination 4°C	<i>AvaII</i>
<i>UGT1A9</i> (rs72551330)	F 5'-GTT CTC TGA TGG CTT GCA CA-3' R 5'-ATG CCC CCT GAG AAT GAG TT-3'	Initial denaturation 95°C(2min) 32 cycles: Denaturation 95°C(15s) Annealing 62°C(15s) Elongation 72°C(15s) Terminal elongation 72°C(30s) Termination 4°C	<i>StyI</i>
<i>CYP2B6</i> (rs 3745274)	F 5'-TAG GTG ACA GCC TGA TGT TC-3' R 5'-TCA TCC TTT TCT CGT GTG TTC T-3'	Initial denaturation 95°C(2min) 32 cycles: Denaturation 95°C(15s) Annealing 62°C(15s) Elongation 72°C(15s) Terminal elongation 72°C(30s) Termination 4°C	<i>BsrI</i>

restriction site (*1/*1) on both alleles yielded fragments of size 397 and 57bp, while the absence of a restriction site (*2/*2) gives a fragment of size 454bp. The heterozygous *1/*2 genotype is characterized by the presence of three fragments of size 454, 397 and 57bp (Figure 1).

(rs72551330) After verification of the 167 bp PCR product on horizontal electrophoresis using 2% agarose gel, restriction digestion with *StyI* restriction enzyme was performed at a temperature of 37 °C. The obtained digestion was performed on 8% polyacrylamide gel, and the obtained results were interpreted as follows: two fragments of size 113 and 54bp were obtained in wild type, three fragments of 167, 113 and 54bp in heterozygote and 167bp for polymorphic homozygote (Figure 2).

(rs3745274) After verification of PCR product size 526bp on horizontal electrophoresis using 2% agarose gel, restriction digestion was carried out using *BsrI* restriction enzyme at a temperature of 60 °C. The assay of digestion obtained was carried out on 8% polyacrylamide gel, and the obtained results were interpreted as follows: three fragments of size 241, 268 and 17bp were obtained in wild type, 509 and 17bp in polymorphic homozygotes (Figure 3).

Determination of the Concentration of Propofol in Plasma by HPLC Analytical Technique

All chemicals used were HPLC purity. The standard of propofol was procured from Sigma Aldrich (USA). The manufacturer of acetonitrile was J.T.Baker (Netherlands), and trifluoroacetic acid Merck (Germany). Deionized

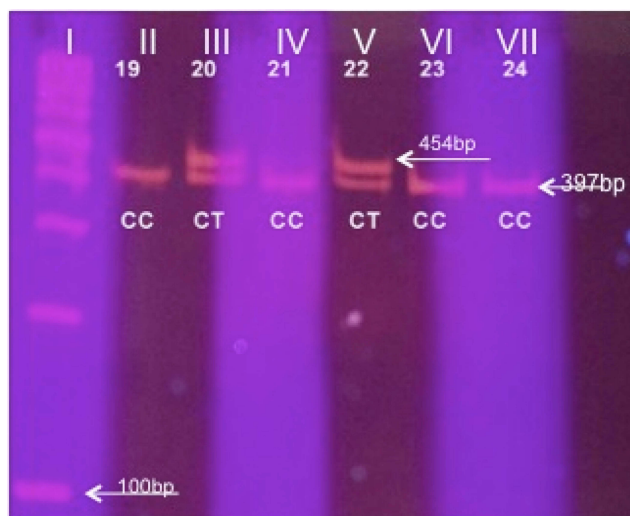


Figure 1 Results of restrictive digestion using 8% acrylamide gel for the *CYP2C9* gene. Column I DNA standard (ladder) of 100bp. Columns II, IV, VI and VII, C/C (wild type) (397 + 57bp); Column III, V C/T, Arginine/Cysteine - heterozygote (454 + 397 + 57bp).

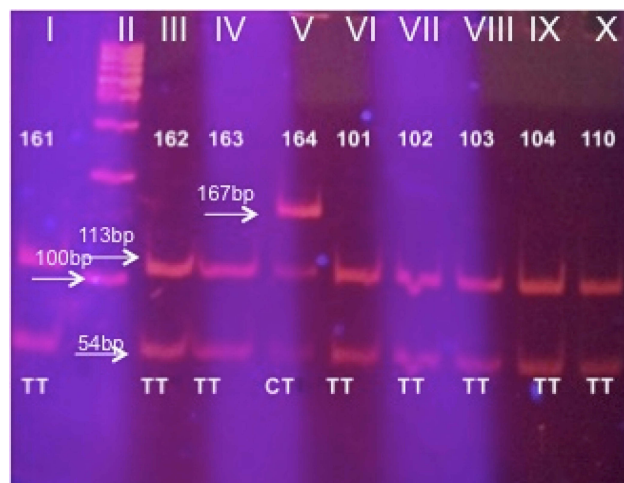


Figure 2 Results of restrictive digestion using 8% acrylamide gel for *UGT1A9* gene. Column II DNA standard of 100 bp. Column I, III, IV, VI, VII, VIII, IX, X T/T (wild type) (113 + 54 bp); Column V C/T - heterozygote (167 + 113 + 54bp).

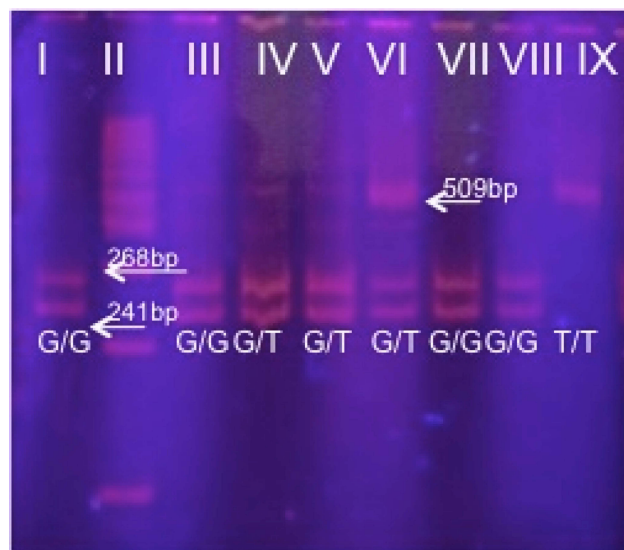


Figure 3 Results of restrictive digestion using 8% acrylamide gel for the *CYP2B6* gene. Column II DNA standard (ladder) of 100bp. Column I, III, VII, VIII G/G (wild type) (268 + 241 bp); Column IV, V, VI G/T - heterozygote (509 + 268 + 241 bp); Column IX, T/T (509 bp).

water was obtained by the MicroPure Ultra Thin Water System (Thermo Scientific, Germany).

HPLC analytical techniques were applied to measure the content of propofol in plasma, based on the methods described by Moghaddam et al⁷ and Mikstacki et al.²

For analysis, 200 µL of plasma was used, which was first deproteinized with 200 µL of trifluoroacetic acid (TFA) solution in acetonitrile (0.3%). After a short vortex, centrifugation was followed at 12,000 rpm for 10 min at 4 °C. Subsequently, the supernatant was isolated, of which 20 µL was injected onto the HPLC instrument Agilent 1200

(Agilent, USA), equipped with DAD and fluorescence detectors. For the chromatographic analysis, the analytical reverse-phase column Pursuit C18, 150 x 3 mm particle size of 3 micrometers (Agilent, USA), was maintained at 30 °C. The mobile phase consisted of a 0.1% aqueous trifluoroacetic acid solution (component A) and acetonitrile (component B). A gradient analysis system for the flow rate of 1 mL/min was used. From the initial 40% B share of the component B it grew to 100% to 10 mins, in order to return to the initial value for the next 1.5 mins and stay on it for up to 14 mins, which ended the analysis. With an additional 3 mins of equilibrium, the total duration of the analysis was 17 mins.

For the detection of propofol, two detectors were used: DAD which monitored changes in the UV-Vis region at 275nm and FLD whose excitation and emission wavelength were adjusted to 276nm and 310nm, respectively. The identification of propofol in the test samples was done by comparison with the standard solution of propofol. Using known concentrations of propofol standards, standard curves in UV-Vis and the fluorescent region were made, by which the concentration of propofol in the samples was calculated. The precision of the assay was 8.7%.

Statistical Analysis

Statistical analysis of the obtained results was performed using a software package for statistical data processing (SPSS, v.21). The obtained results were presented in the form of a mean value with standard deviation, or a median with an interquartile difference in the case of linear variables, while the category variables are represented by an absolute number with the corresponding percentage share. In order to determine the statistically significant relationship between the variables, the parametric (Student's *t*-test) and non-parametric test (Mann-Whitney *U*-test) were used depending on the data distribution normality. The method of linear regression analysis, univariate and multivariate, was used to identify predictors of dependent variables, doses, concentrations and clearance of propofol, and then to obtain a statistically significant prediction model. Statistical significance was determined at $p < 0.05$.

Results

The study included 94 children aged 1 to 17 years (average age 9.13 ± 5.32 years old), of which 53 were male (56.4%) and 41 females (43.6%). The characteristics of the examined group are given in Table 2. The highest

Table 2 Demographic Data of Patients Included in the Study

		$\bar{x} \pm SD$ and M (IQ) or N(%)
Age (years)		9.13 ± 5.32 9.0 (5.0–14.0)
Age group	1–3 years 4–6 years 7–10 years 11–14 years 15–18 years	20 (21.3%) 14 (14.9%) 20 (21.3%) 21 (22.3%) 19 (20.2%)
Gender (male)		53 (56.4%)
Body weight (kg)		37.53 ± 23.29 34.0 (16.5–20.2)
Body height (cm)		137.21 ± 33.80 140.0 (111.5–165.2)
Body mass index (kg/m^2)		18.16 ± 4.87 16.7 (14.2–22.3)
Nutritional status	Underweight Normal Overweight Obese	25 (26.6%) 45 (47.9%) 13 (13.8%) 11 (11.7%)
Congenital anomalies		33 (35.1%)
Comorbidities		20 (21.3%)
Type of surgical intervention	Abdominal Urological Plastic Orthopedics	16 (17.0%) 9 (9.6%) 51 (54.3%) 16 (17.0%)
Total dose of propofol (mg)		318.73 ± 217.59 277.5 (160.0–396.2)
Induction dose of propofol (mg)		97.45 ± 57.03 85.0 (47.5–150.0)
Maintenance dose of propofol (mg)		221.18 ± 182.19 160.0 (100.0–300.0)
Anesthesia duration (min)		61.38 ± 33.93 57.5 (40.0–75.0)

number of patients had normal nutritional status (47.9%). In 33 cases (35.1%) anatomic malformations were noted; in 20 (21.3%) comorbidities were present, most of which were respiratory tract infections (45.0%) and epilepsy (30.0%). The majority of interventions were from the domain of plastic surgery (54.3%). The average duration of anesthesia was 58 mins. The average total dose of propofol was 278 mg, an initial dose 85 mg and a maintenance dose 160 mg.

Gene Variations - *UGT1A9*, *CYP2C9* and *CYP2B6*

All 94 patients were tested for polymorphisms for *UGT1A9*, *CYP2C9* and *CYP2B6* enzymes that participate in the metabolism of propofol. The frequency of different genotypes is given in Table 3. The allele and genotypic frequencies of the studied polymorphisms were similar in cases and controls and followed the Hardy-Weinberg equilibrium

Due to a small number of samples with a polymorphic *UGT1A9* allele, it was not possible to determine all the differences in the characteristics of patients with different genotypes. However, all four carriers of the polymorphic allele belonged to younger age groups, therefore they had less body weight ($p < 0.001$) and height ($p < 0.01$), but also a lower body mass index ($p < 0.05$), with the trend of poorer nutrition ($p = 0.060$). When it comes to *CYP2C9**2 polymorphism, there were no statistically significant differences between the two groups of patients. Carriers of *CYP 2B6 GG* genotypes were older ($p < 0.01$), had higher body weight ($p < 0.01$) and height ($p < 0.05$).

Propofol Dose

The average total administered dose of propofol was 318.73 ± 217.59 mg, with an initial dose of 97.45 ± 57.03 mg and a maintenance dose 221.18 ± 182.19 mg. Considering the large variations in the body weight of patients, as well as the duration of anesthesia, the total dose corrected by body weight (9.18 ± 4.68 mg/kg) and a dose of propofol administered as a continuous infusion (6.63 ± 2.62 mg/kg/h) were calculated.

Carriers of polymorphic *UGT1A9 C* allele received significantly lower overall ($p < 0.05$) and initial dose of

Table 3 Distribution of Genotypes *UGT1A9* (98T>C), *CYP2C9* (430C>T) and *CYP2B6* (516G>T) in Patients Anesthetized with Propofol

SNP (rs number)	Genotype	Patients N (%)	Alleles	N (%)
<i>UGT1A9</i> (rs72551330)	TT	90 (95.74)	T	184 (97.9)
	TC	4 (4.3)	C	4 (2.1)
	CC			
<i>CYP2C9</i> (rs1799853)	CC	74 (78.7)	C	166 (88.3)
	CT	18 (19.1)	T	22 (11.7)
	TT	2 (2.1)		
<i>CYP2B6</i> (rs 3745274)	GG	30 (31.9)	G	119 (63.3)
	GT	59 (62.8)	T	69 (36.7)
	TT	5 (5.3)		

propofol ($p < 0.05$), with a lower maintenance trend ($p = 0.051$). There was also a statistically significant difference in the dose of propofol administered as a continuous infusion ($p < 0.05$). None of the propofol doses was statistically significant in relation to *CYP2C9**2 polymorphism. The induction dose of propofol ($p < 0.05$), as well as the total dose ($p < 0.01$) were higher in the *CYP2B6 GG* genotype (Table 4).

Linear regression analysis of the total dose of propofol per kilogram of body weight as well as linear regression analysis of propofol dose in the form of continuous infusion (mg/kg/h) are presented in Tables 5 and 6.

Concentration of Propofol

Concentration of propofol was measured at 4 different times: 10 mins after introduction into anesthesia, at the end of infusion of propofol, as well as 10 and 20 mins after the completion of the propofol infusion. The mean measured values were: 35.19 ± 54.72 mg/L, 44.82 ± 82.84 mg/L, 3.33 ± 4.96 mg/L and 1.77 ± 3.90 mg/L, respectively. The propofol concentration dropped by 70% after 10 mins from the completion of the propofol infusion, and by an additional 7% in the next 10 mins.

A statistically significant difference was found in the measured propofol concentrations corrected with the total given dose in 3rd ($p < 0.05$) and 4th time ($p < 0.05$). In addition, there was a trend of significantly higher concentrations of propofol in all 4 times in patients with *UGT1A9* polymorphism, however a trend of faster elimination of propofol in the first 20 mins in these patients was noticed (Table 7). None of the propofol doses was statistically significant in relation to *CYP2C9**2 polymorphism. None of the propofol doses was statistically significant in relation to the polymorphism of the *CYP2B6* gene, but there was a trend of a higher percentage reduction in the concentration of propofol between 10 and 20 mins after cessation of the infusion ($p = 0.072$).

By the method of univariate linear regression, predictors of propofol concentration in blood immediately after the discontinuation of the propofol infusion were identified: *UGT1A9* gene polymorphism and propofol dose administered as a continuous infusion. A statistically significant model of multivariate linear regression was obtained ($F = 4.951$, $p < 0.01$), which predicted 7.9% of the variance of the dependent variable, whereby, as independent predictors, they participated with 4.9% ($p < 0.05$) and 4.2% ($p < 0.05$), respectively.

By the method of univariate linear regression, predictors of a percentage reduction in propofol blood concentrations were

Table 4 Propofol Dose Dependent on Genotype *UGT1A9*, *CYP2C9* and *CYP2B6*

<i>UGT1A9</i>	TT (N=90)	CT (N=4)	Z (p)
Total dose of propofol (mg)	327.18±218.38 168 (100–290)	128.75±52.02 74 (55–145)	2.201 (0.028*)
Induction dose of propofol (mg)	100.0±56.90 50 (30–95)	40.0±14.14 30 (30–30)	2.217 (0.027*)
Total dose of propofol for anesthesia maintenance (mg)	227.07±183.83 100 (60–170)	88.75±44.79 44 (25–100)	1.951 (0.051)
Total dose of propofol corrected by body weight (mg/kg)	9.22±4.75 6.5 (5.2–8.5)	8.25±3.17 5.1 (4.6–8.5)	0.066 (0.948)
Dose of propofol for anesthesia maintenance (mg/kg/h)	6.57±2.66 5.0 (2.8–7.0)	7.94±0.59 7.3 (7.1–8.2)	2.336 (0.019*)
<i>CYP2C9</i>	CC (N=74)	CT+TT (N=20)	Z (p)
Total dose of propofol (mg)	313.49±224.19 265 (156–392)	338.15±195.30 335 (180–473)	0.836 (0.403)
Induction dose of propofol (mg)	96.89±57.40 80 (40–150)	99.50±57.08 100 (50–142)	0.2247 (0.820)
Total dose of propofol for anesthesia maintenance (mg)	216.46±186.26 160 (100–300)	238.65±169.62 188 (112–356)	0.731 (0.465)
Total dose of propofol corrected by body weight (mg/kg)	8.94±3.46 8.8 (6.6–10.0)	10.08±7.76 7.9 (5.9–11.6)	0.559 (0.576)
Dose of propofol for anesthesia maintenance (mg/kg/h)	6.74±2.50 7.1 (5.4–8.2)	6.21±3.04 7.0 (3.2–7.5)	1.021 (0.307)
<i>CYP2B6</i>	GG (N=30)	GT+TT (N=64)	Z (p)
Total dose of propofol (mg)	354.83±170.57 340 (216–508)	301.81±235.77 240 (132–380)	1.959 (0.050*)
Induction dose of propofol (mg)	119.67±57.58 110 (70–170)	87.03±54.13 70 (40–130)	2.571 (0.010**)
Total dose of propofol for anesthesia maintenance (mg)	235.17±136.86 185 (144–368)	214.62±200.60 185 (144–368)	1.547 (0.122)
Total dose of propofol corrected by body weight (mg/kg)	8.29±2.58 8.0 (6.2–10.0)	9.60±5.36 8.0 (6.2–10.0)	0.767 (0.443)
Dose of propofol for anesthesia maintenance (mg/kg/h)	6.07±2.55 6.7 (3.3–8.0)	6.89±2.63 8.9 (6.5–10.6)	0.994 (0.320)

Notes: *p<0.05, **p<0.01.

identified for 10th minute after completion of the infusion: body mass index, nutrition status, dose of propofol administered as a continuous infusion, and appropriate concentrations measured in blood samples at 4 time points. A statistically significant model of multivariate linear regression ($F = 16.904$, $p < 0.001$) was obtained which predicted 42.0% of the variation of the dependent variable. Independent predictors of a decrease in concentration in the first 10 mins were: body

mass index, as well as initial concentrations of propofol (in the first two measurements). Patients with a lower body mass index showed a higher percentage of concentration reduction in the first 10 mins (partial $R^2=10.6\%$, $p < 0.01$). Also, higher initial concentrations (after 10 mins of infusion and at the end of the infusion of propofol) predicted a greater reduction in the first 10 mins (partial $R^2=9.5\%$, $p < 0.01$, partial $R^2=22.4\%$, $p < 0.001$; respectively) (Table 8).

Table 5 Linear Regression Analysis of the Total Dose of Propofol per Kilogram of Body Weight

	Univariate		Multivariate	
	B (95% CI for B)	p	B (95% CI for B)	p
Gender (female)	2.691 (0.828–4.555)	0.005	0.337 (–1.029–1.702)	0.625
Age (years)	–0.215 (–0.391–0.038)	0.018	0.295 (–0.157–0.746)	0.198
Age group	–0.726 (–1.386–0.066)	0.032		
Body weight (kg)	–0.049 (–0.090–0.009)	0.017	–0.058 (–0.132–0.015)	0.117
Body height (cm)	–0.044 (–0.072–0.017)	0.002	–0.057 (–0.113–0.001)	0.045*
Congenital anomalies	2.798 (0.862–4.734)	0.005	1.694 (0.312–3.075)	0.017*
Anesthesia duration (min)	0.088 (0.067–0.110)	0.000	0.093 (0.074–0.113)	0.000***

Notes: * $p < 0.05$, *** $p < 0.001$.

Table 6 Linear Regression Analysis of Propofol Dose in the Form of Continuous Infusion (Mg/Kg/h)

	Univariate		Multivariate	
	B (95% CI for B)	p	B (95% CI for B)	p
Age (years)	–0.239 (–0.328–0.150)	0.000	0.099 (–0.220–0.417)	0.540
Age group	–0.871 (–1.204–0.538)	0.000		
Body weight (kg)	–0.059 (–0.079–0.039)	0.000	–0.054 (–0.107–0.002)	0.043*
Body height (cm)	–0.037 (–0.051–0.023)	0.000	–0.015 (–0.054–0.024)	0.444
Body mass index (kg/m ²)	–0.173 (–0.279–0.068)	0.002		
Congenital anomalies	1.873 (0.812–2.935)	0.001	1.240 (0.267–2.213)	0.013*

Note: * $p < 0.05$.

By the method of univariate linear regression, predictors of propofol concentrations reduction in percentage were identified for 20th minute after the infusion was complete: body mass index, nutrition status, propofol dose administered as a continuous infusion, and corresponding concentrations in the first 3 times. A statistically significant model of multivariate linear regression ($F=12.894$, $p < 0.001$) was obtained, which predicted 34.8% of the variation of the dependent variable. Independent predictors of a decrease in concentration in the first 20 mins were: the dose of propofol administered as a continuous infusion, as well as the initial propofol concentrations (in the first two times). Patients who received a higher corrected dose of propofol showed a higher percentage of concentration reduction in the first 20 mins (partial $R^2 = 4.5\%$, $p < 0.05$). Also, higher initial concentrations (after 10 mins of infusion and at the end of infusion of propofol) predicted a greater reduction in the first 20 mins (partial $R^2=10.7\%$, $p < 0.01$, partial $R^2=14.7\%$, $p < 0.001$; respectively) (Table 9).

The average distribution constant of the drug was $7.39 \pm 4.68/h$. It was higher in carriers of the polymorphic *UGT1A9C* allele. There was no difference in relation to the *CYP2C9* genotype, nor the *CYP2B6* genotype.

The average clearance of the drug was 742.6 mL/min. Drug clearance was lower in polymorphic *UGT1A9 C* allele ($p=0.088$) carriers, while there was no difference in relation to *CYP2C9 *2* and *CYP2B6* genotype (Table 10). Propofol concentrations in association with *UGT1A9 98T>C*, *CYP2C9*2* and *CYP2B6 516G>T* polymorphisms are presented in Figures 4–6. By the method of univariate linear regression, the predictors of propofol clearance had been identified after cessation of infusion: age and age group, body weight, body mass index, nutritional status, and the presence of malformations (Table 11). A statistically significant model of multivariate linear regression was obtained ($F=6.500$, $p < 0.001$), which predicted 15.2% of variation of dependent variables. The only independent predictor was the body mass index ie increase of BMI increased the propofol clearance (partial $R^2 = 8.8\%$, $p < 0.01$).

Discussion

Genetic variation can affect drug response in multiple ways, although it remains unclear how rare genetic variants affect drug response⁸ and to what extent they are clinically relevant. To improve pharmacogenetic utility and human health, researches are conducted to identify variants that provide cost-effective targets for pharmacogenetic testing in future.⁹

Table 7 Concentrations of Propofol in Four Times, Depending on the Genotype *UGT1A9*, *CYP2C9* and *CYP2B6*

<i>UGT1A9</i>	TT (N=90)	CT (N=4)	t or Z (p)
C1 (mg/L)	34.26±52.76 6.6 (1.5–53.4)	56.12±98.10 9.0 (3.9–155.4)	0.491 [#] (0.121)
C2 (mg/L)	40.66±74.58 4.5 (1.8–48.4)	13.75±187.84 67.8 (9.9–334.9)	1.487 [#] (0.137)
C3 (mg/L)	3.28±5.02 1.5 (0.8–3.3)	4.47±3.63 4.1 (1.3–8.0)	1.274 [§] (0.203)
C4 (mg/L)	1.78±3.99 0.9 (0.6–1.7)	1.61±0.75 1.7 (0.8–2.2)	1.152 [#] (0.249)
C2 corrected by total dose of propofol (mg/L/mg)	0.19±0.41 0.02 (0.00–0.18)	2.12±3.59 0.59 (0.06–5.82)	1.932 [#] (0.053)
C3 corrected by total dose of propofol (mg/L/mg)	0.01±0.02 0.00 (0.00–0.01)	0.06±0.07 0.03 (0.00–0.13)	2.060 [#] (0.037*)
C4 corrected by total dose of propofol (mg/L/mg)	0.01±0.01 0.00 (0.00–0.01)	0.02±0.02 0.01 (0.00–0.03)	2.135 [#] (0.033*)
C3 reducing versus C2 (%)	69.11±25.25 71.8 (50.0–93.0)	85.01±21.11 94.3 (63.5–97.2)	1.237 [§] (0.219)
C4 reducing versus C2 (%)	75.30±4.59 82.0 (30.3–96.9)	91.01±12.53 95.9 (77.8–99.3)	1.527 [#] (0.127)
C4 reducing versus C3 (%)	44.53±21.60 43.6 (27.9–71.8)	65.19±20.95 73.4 (41.4–73.4)	1.627 [§] (0.108)
Additional reducing C3-C4 (%)	9.80±9.10 7.1 (2.5–14.4)	8.64±9.37 5.2 (1.5–5.2)	0.237 [#] (0.813)
<i>CYP2C9</i>	CC (N=74)	CT+TT (N=20)	t or Z (p)
C1 (mg/L)	34.04±48.94 8.6 (1.7–55.4)	39.46±73.70 5.4 (1.4–26.8)	0.556 [#] (0.578)
C2 (mg/L)	47.94±88.55 7.2 (1.9–53.6)	33.44±57.80 3.3 (1.6–46.1)	0.610 [#] (0.542)
C3 (mg/L)	3.68±5.49 1.6 (0.8–3.4)	2.02±1.53 1.3 (0.7–3.4)	0.656 [#] (0.512)
C4 (mg/L)	1.43±1.40 0.9 (0.6–1.7)	2.83±8.10 0.9 (0.6–1.7)	0.645 [#] (0.519)
C2 corrected by total dose of propofol (mg/L/mg)	0.32±0.96 0.03 (0.01–0.27)	0.10±0.16 0.02 (0.01–0.14)	0.748 [#] (0.454)
C3 corrected by total dose of propofol (mg/L/mg)	0.02±0.03 0.01 (0.0–0.02)	0.01±0.01 0.01 (0.0–0.01)	0.693 [#] (0.488)
C4 corrected by total dose of propofol (mg/L/mg)	0.01±0.01 0.00 (0.00–0.01)	0.01±0.02 0.00 (0.00–0.01)	0.426 [#] (0.673)
C3 reducing versus C2 (%)	70.77±25.04 80.5 (48.1–93.7)	66.59±26.09 68.6 (52.6–91.2)	0.651 [§] (0.517)

(Continued)

Table 7 (Continued).

C4 reducing versus C2 (%)	76.55±25.22 85.5 (60.3–97.2)	74.05±21.55 74.5 (61.7–94.5)	0.806 [#] (0.421)
C4 reducing versus C3 (%)	46.63±22.17 45.5 (31.2–65.4)	40.38±20.30 42.2 (24.2–61.0)	1.049 ^{\$} (0.298)
Additional reducing C3-C4 (%)	9.39±9.19 6.1 (2.5–13.2)	11.06±8.68 11.2 (2.5–15.2)	0.823 [#] (0.411)
CYP2B6	GG (N=30)	GT+TT (N=64)	t or Z (p)
C1 (mg/L)	40.22±52.77 13.0 (2.2–72.0)	32.84±55.86 4.5 (1.5–31.7)	1.054 [#] (0.292)
C2 (mg/L)	42.51±67.93 10.4 (2.1–59.7)	45.92±89.55 4.0 (1.8–41.0)	0.538 [#] (0.590)
C3 (mg/L)	4.26±5.93 2.3 (0.8–6.7)	2.89±4.42 1.3 (0.8–2.8)	1.626 [#] (0.104)
C4 (mg/L)	2.71±6.63 1.1 (0.7–1.8)	1.34±1.29 0.8 (0.6–1.7)	1.103 [#] (0.190)
C2 corrected by total dose of propofol (mg/L/mg)	0.17±0.30 0.03 (0.00–0.23)	0.21±1.02 0.3 (0.01–0.19)	0.140 [#] (0.889)
C3 corrected by total dose of propofol (mg/L/mg)	0.01±0.02 0.01 (0.00–0.02)	0.02±0.03 0.1 (0.0–0.01)	0.406 [#] (0.685)
C4 corrected by total dose of propofol (mg/L/mg)	0.01±0.01 0.00 (0.00–0.01)	0.01±0.01 0.00 (0.00–0.01)	0.373 [#] (0.709)
C3 reducing versus C2 (%)	69.62±26.23 72.9 (46.7–94.6)	69.92±24.92 74.1 (51.9–93.5)	0.052 ^{\$} (0.959)
C4 reducing versus C2 (%)	77.53±24.33 85.1 (62.0–97.3)	75.30±24.54 83.8 (59.6–96.9)	0.418 [#] (0.676)
C4 reducing versus C3 (%)	51.81±20.42 53.8 (32.9–67.7)	42.35±21.95 42.2 (24.0–61.4)	1.824 ^{\$} (0.072)
Additional reducing C3–C4 (%)	12.65±12.24 9.1 (3.2–17.6)	8.45±6.91 6.3 (2.3–14.0)	1.232 [#] (0.218)

Notes: *p<0.05, #Mann–Whitney U-test, \$Student's t-test.

Table 8 Linear Regression Analysis of the Reduction in the Concentration of Propofol in Percentage 10 mins After Completion of the Infusion

	Univariate Analysis		Multivariate Analysis	
	B (95% CI for B)	p	B (95% CI for B)	p
Body mass index (kg/m ²)	–1.831 (–2.870–0.793)	0.001	–1.389 (–2.267–0.511)	0.002 ^{**}
Nutritional status	–8.398 (–13.906–2.890)	0.003		
Propofol (mg/kg/h)	3.170 (1.192–5.148)	0.002	1.235 (–0.458–2.928)	0.151
C1 (mg/L)	0.167 (0.078–0.257)	0.000	0.112 (0.037–0.187)	0.004 ^{**}
C2 (mg/L)	0.157 (0.103–0.211)	0.000	0.125 (0.075–0.176)	0.000 ^{***}
C3 (mg/L)	2.054 (0.824–3.285)	0.001		
C2 corrected by total dose(mg/L/mg)	0.009 (0.003–0.015)	0.002		
C3 corrected by total dose (mg/L/mg)	0.342 (0.147–0.538)	0.001		
C4 corrected by total dose (mg/L/mg)	0.847 (0.330–1.364)	0.002		

Notes: **p<0.01, ***p<0.001.

Table 9 Linear Regression Analysis of the Reduction in the Concentration of Propofol in Percentage 20 mins After Completion of the Infusion

	Univariate Analysis		Multivariate Analysis	
	B (95% CI for B)	p	B (95% CI for B)	p
Body mass index (kg/m ²)	-1.359 (-2.387-0.331)	0.010	-0.857 (-1.754-0.041)	0.061
Nutrition status	-6.180 (-11.460-0.901)	0.022		
Propofol (mg/kg/h)	3.233 (1.343-5.123)	0.001	1.738 (0.010-3.466)	0.049*
C1 (mg/L)	0.171 (0.086-0.257)	0.000	0.124 (0.047-0.201)	0.002**
C2 (mg/L)	0.135 (0.080-0.189)	0.000	0.100 (0.048-0.152)	0.000***
C3 (mg/L)	2.383 (1.225-3.541)	0.000		
C2 corrected by total dose (mg/L/mg)	0.008 (0.002-0.014)	0.007		
C3 corrected by total dose (mg/L/mg)	0.335 (0.146-0.523)	0.001		

Notes: *p<0.05, **p<0.01, ***p<0.001.

Table 10 The Effect of Gene Variation for *UGT1A9*, *CYP2C9* and *CYP2B6* on the Average Drug Clearance Value

<i>UGT1A9</i>	TT (N=90)	CT (N=4)	t (p)
Clearance (mL/min)	1986.78±3175.66 915.2 (109.4-2366.7)	1565.56±1860.62 63.8 (11.9-1570.5)	1.704 (0.088)
<i>CYP2C9</i>	CC (N=74)	CT+TT (N=20)	Z (p)
Clearance (mL/min)	1986.78±3175.66 724.0 (99.2-2366.7)	1565.56±1860.62 828.9 (157.0-2181.1)	0.374 (0.708)
<i>CYP2B6</i>	GG (N=30)	GT+TT (N=64)	t (p)
Clearance (mL/min)	1975.55±2758.10 851.0 (94.9-2822.3)	1858.41±3042.13 742.6 (27.7-2243.0)	0.179 (0.859)

It is assumed that the etiology of individual variability in the response to anesthetics may also be influenced by genetic polymorphisms in metabolic and functional pathways.

Propofol is the most popular intravenous anesthetic used in children. As is commonly known, children are not “small adults”, and in that regard, it should be noted that numerous developmental changes, particularly in neonates and infants, affect the metabolism and pharmacokinetics of propofol.^{10,11} EC₅₀ in children was found to be 3.71–3.85µg/mL¹² in contrast to adults in which 2.19 to 3.07µg/mL was found.¹³ The half-life of propofol is not reliably established in children, and the interaction of propofol with opioids during TIVA and TCI (target controlled infusion) makes the prediction of recovery even more complicated.¹⁴ The loss of consciousness occurs at a concentration of 2±0.9µg/mL, until the return of consciousness and awakening from anesthesia can occur at concentrations of 1.8±0.7µg/mL. According to studies published in the literature there is no difference between children and adults.^{14,15}

In the available literature that presented pharmacogenetic testing and the examination of the gene polymorphism

influence on the metabolism of propofol, three genes are most often listed. Based on our findings, this is the first study in our country to investigate the effect of *CYP2C9*2*, *CYP2B6 516G>T* and *UGT1A9 98C>T* genetic polymorphisms on the pharmacokinetics of propofol in children of different sexes and ages who undergo total intravenous anesthesia and deep sedation during diagnostic and therapeutic procedures. *CYP2C9* and *CYP2B6* participate in the first stage of biotransformation of many clinically significant drugs, including anesthetic propofol, catalyzing hydroxylation of propofol in humans.

For the *CYP2C9* gene, over 65 haplotypes are described, which result from insertion, deletion, and replacement of individual base pairs. In several studies,^{16,17} two non-synonymous changes of individual nucleotides (SNPs) p.R144C (*c.430C>T*, rs1799853, *CYP2C9*2*) and p.I359L (*c.1075A>C*, rs1057910, *CYP2C9*3*), which are responsible for slower substrate metabolism in certain phenotypes are being analyzed intensively. The effect of these polymorphisms is, first of all, examined on examples of warfarin and non-steroidal anti-inflammatory drugs. Both allele with reduced function, *2 and

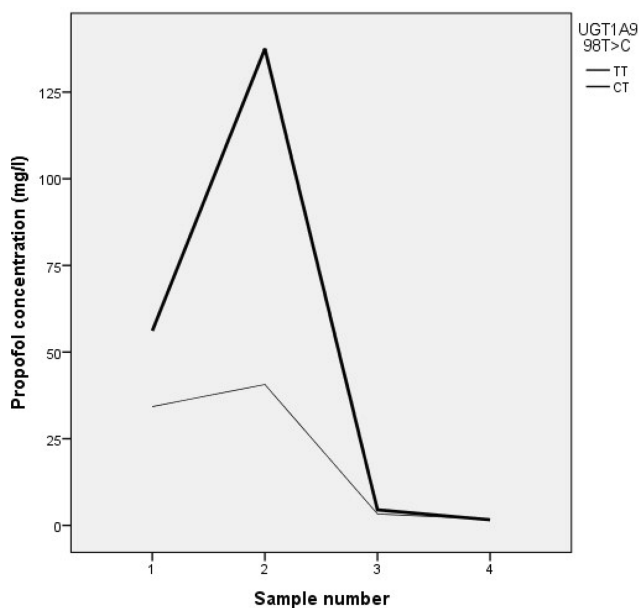


Figure 4 Propofol concentrations in association with *UGT1A9 98T>C* polymorphism.

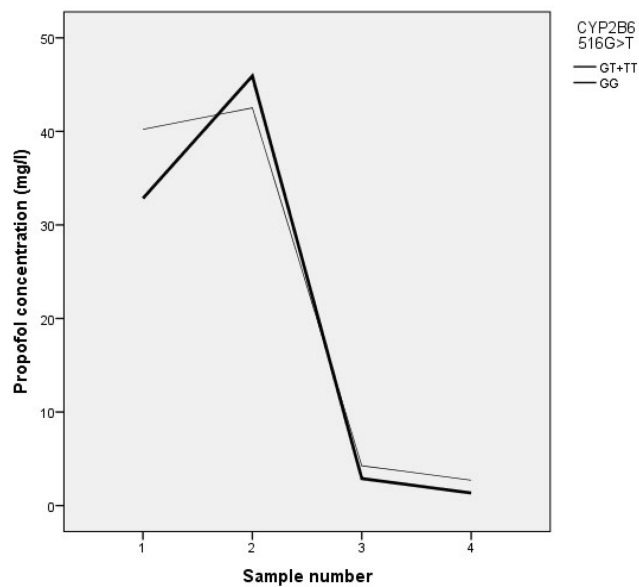


Figure 6 Propofol concentrations in association with *CYP2B6 516G>T* polymorphism.

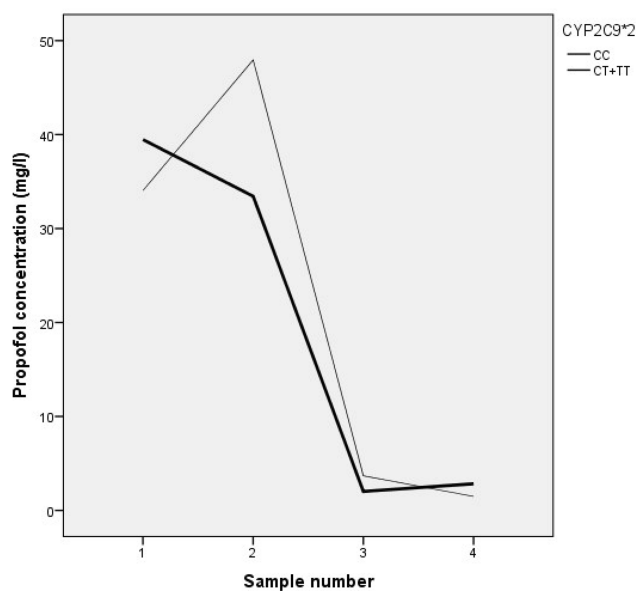


Figure 5 Propofol concentrations in association with *CYP2C9*2* polymorphism.

*3, pose a significant risk factor for warfarin-induced hemorrhage, or bleeding in the upper parts of the gastrointestinal tract after the administration of non-steroidal anti-inflammatory drugs.¹⁸ Homozygotes for *3 allele show only 25% of the activity, while in heterozygotes this is between 40% and 75%, depending on the substrate.^{19,20} The allele frequency *2 (*rs1799853, 430C>T*) is about 6.8%. In our study, the representation of this heterozygous *CYP2C9*2* (C/T) allele in children was (19.1%), while the representation of homozygous

form (T/T) was 2.1%. No single dose of propofol that was used in pediatric patients in the course of this clinical study in relation to body weight and duration of anesthesia was not statistically significant in relation to polymorphism *CYP2C9*2*. As found in the Mikstacki et al² study, the obtained results of our study indicate that the polymorphism of the *CYP2C9* gene did not affect the pharmacokinetic profile of propofol.

The average relative contribution of *CYP2B6*, which participates in the first phase of biotransformation of xenobiotics, in relation to the total proportion of the liver cytochrome P450 family (CYP), ranges from 2 to 10%. The *CYP2B6* gene is one of the most polymorphic CYP superfamily genes in humans, with more than 100 known variants of DNA.²¹ However, the most widely analyzed variant in pharmacogenetic studies are *c.516G>T* (*rs3745274, Gln172His*) polymorphism, found on various *CYP2B6* haplotypes. This variation causes the emergence of an alternative splicing on iRNA which results in loss of exons from 4 to 6, which leads to a serious reduction in the activity of the protein and the enzyme *CYP2B6*.²²

The effect of *CYP2B6 c.516 G>T* on the pharmacokinetic profile of propofol in the available studies has not been fully clarified.^{1,23,24} However, *CYP2B6* plays an important role in the process of biotransforming this anesthetic through hydroxylation. A recent study conducted by Mourao et al¹ on 108 adult patients during TIVA, however, suggests that the present variant of the *c.516G>T CYP2B6* gene allele reduces the required dose of propofol versus

Table 11 The Predictors of Propofol Clearance After Cessation of Infusion

	Univariate Analysis		Multivariate Analysis	
	B (95% CI for B)	p	B (95% CI for B)	p
Age (years)	0.150 (0.040–0.260)	0.008	0.032 (–0.94–0.158)	0.614
Age group	0.492 (0.079–0.906)	0.020		
Body weight (kg)	0.041 (0.016–0.066)	0.001		
Body mass index (kg/m ²)	0.233 (0.118–0.348)	0.000	0.199 (0.063–0.334)	0.004**
Nutritional status	0.885 (0.259–1.511)	0.006		
Congenital anomalies	–1.392 (–2.640–0.144)	0.029	–0.964 (–2.176–0.249)	0.118

Note: **p<0.01.

body weight and length of anesthesia by about 7%, due to slower metabolism of propofol. As no statistically significant correlation were found in our study between the amount of propofol and the variation of the *CYP2B6 c.516G>T* gene, the possible difference in the obtained results may be due to the low frequency of *c.516G>T* variants of the *CYP2B6* gene.

Since *UGT1A9* plays a major role in the metabolism of propofol, it can be considered that all polymorphisms in this gene have a high significance and impact on the metabolism of propofol. Apart from the liver, super families of this enzyme (*UGT1*, *UGT2*, *UGT3*, *UGT8*) are present in the kidneys, colon, ovary, and testis,⁵ where they participate in the second phase of biotransformation and elimination of propofol but also in biotransformation and elimination of other important drugs, such as some anti- medicines (irinotecan, sorafenib, epirubicin).²⁵ Among the most basic variants of the *UGT1A9* gene, which leads to decreased enzyme activity, are three known amino acid changes: p.M33T, p.D256N and p. I242Ks. The variations of sequences in codon 33 (*c.98T>C*, *rs72551330*, *UGT1A9*3*) affect the pharmacokinetics of the propofol by changing or decreasing the affinity of the *UGT1A9*3* for binding substrate, which makes this gene variant as a factor that determines persons as poor metabolizers.²⁶ These variants of the genes may be responsible for a lower degree of inactivation of propofol by the glucuronidation process, and its slower urinary clearance, as well as hepatotoxic effects that can lead to liver failure in patients on irinotecan chemotherapy.²⁷ In our study, 4 polymorphic variants of this gene were found in the form of heterozygote (CT), which makes 4.3%. Although this is a relatively small proportion of this polymorphism in the examined population of children, the obtained results indicate that carriers of the polymorphic *UGT1A9 C* allele received significantly lower total (p < 0.05) and the initial dose of propofol (p < 0.05), with a lower maintenance dose (p = 0.051). In addition, there is a

trend of significantly higher concentrations of propofol in all 4 times in *UGT1A9* polymorphism carriers.

The average clearance of the drug was 742.6 mL/min. There was a lower clearance curve for polymorphic *UGT1A9 C* allele (p = 0.088) carriers, while there was no difference in the *CYP2C9*2* genotype and *CYP2B6* genotype. In the available literature there is a small number of papers related to the effects of the variant of the *c.98T>C*, *rs72551330*, *UGT1A9*3* genes on the metabolism of propofol and the clinical evaluation of the effects of anesthesia and analgesia. Recently, a group of Chinese researchers in their study examined the effects of this gene polymorphism in the induced abortion procedure using propofol in 156 women. No significant differences were observed in patients with –440C/T, –1818T/C and –1887T/G regarding the onset of nausea, vomiting and/or respiratory depression, and it was concluded that the polymorphisms of these *UGT* super families do not affect metabolic rate and not potentiate adverse effects of propofol.²⁸ However, Fukuda et al²⁹ found significant reduction in the process of glucuronidation of mycophenolic acid used in suppressive therapy in kidney transplantation in children, for which metabolism was found to be responsible the UDP-glucuronosulfotransferase enzyme 1A9 (*UGT1A9*).

Limitations

The sample size was relatively small and only three polymorphisms were analyzed so additional studies are required with a larger number of patients whereby more genes would be analyzed.

Conclusion

This study showed that *UGT1A9* genotype is an independent predictor of the propofol concentration in children immediately after the end of the continuous infusion and 10 mins afterwards. In the carriers of the polymorphic *UGT1A9C* allele, the propofol distribution constant was higher. The

carriers of the polymorphic *CYP2B6* T allele received a significantly lower overall and initial dose of propofol. Unlike polymorphism of the *UGT1A9* gene, the tested *CYP2C9* and *CYP2B6* gene polymorphisms are not independent predictors of the pharmacokinetics of propofol. As independent predictors of the achieved concentrations of propofol in children, in addition to the *UGT1A9* polymorphism examined, the following are distinguished: the duration of anesthesia, the induction dose of propofol and the administered dose of propofol in the form of a continuous infusion. As an independent predictor of the decrease in propofol concentration in children, in addition to the *UGT1A9* polymorphism examined, the following are distinguished: body mass index, propofol dose administered in the form of continuous infusion, as well as drug concentration in all four measured times.

Abbreviations

ASA, American Society of Anesthesiologists; TIVA, total intravenous anesthesia; BIS, bispectral index; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; HPLC, high performance liquid chromatography; DAD, diode-array detection; FLD, fluorescence detection; BMI, body mass index.

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Disclosure

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