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ORIGINAL RESEARCH

Association of NR1I2 Polymorphism with Midazolam Clearance in Mechanically Ventilated ICU Patients: A Population Pharmacokinetic and Pharmacogenetic Study

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Background: Significant variability in the metabolism of midazolam (MDZ) exists among mechanically ventilated (MV) patients in the intensive care unit (ICU) due to complex clinical conditions and genetic factors. The NR1I2 gene (PXR), which encodes a nuclear receptor that regulates drug-metabolizing enzymes like CYP3A4, plays a critical role in MDZ metabolism. Polymorphisms in NR1I2, along with variations in genes such as CYP3A4, CYP3A5, and ABCB1, may influence enzyme activity and MDZ pharmacokinetics (PK). Understanding these factors is essential for optimizing MDZ dosing in high-risk patient populations.

Methods: We studied 61 MV ICU patients receiving continuous MDZ infusion. A population pharmacokinetic (PopPK) model was used to assess MDZ PK, with genetic factors (NR1I2 rs2461817, CYP3A4, CYP3A5, ABCB1, and other PXR polymorphisms) and clinical covariates (body weight (BW), aspartate aminotransferase (AST) levels) evaluated for their impact on MDZ clearance (CL).

Results: The PK of MDZ and its metabolite, 1-hydroxymidazolam (1-OH-MDZ), were accurately described using a one-compartment model. The estimated population means for MDZ and 1-OH-MDZ CL were 22.6 L/h (inter-individual variability [IIV] 59.4%) and 67.1 L/h (IIV 57.7%), respectively. MDZ CL was significantly associated with the NR1I2 rs2461817 polymorphism and AST levels, accounting for 11.3% of the variability. MDZ CL decreased by 32.7% as AST increased from 22 IU/L to 60 IU/L, and by 40.7% in patients homozygous for the NR1I2 rs2461817 variant. BW also influenced the CL of 1-OH-MDZ, demonstrating a 34.2% increase as weight increased from 54 kg to 65 kg. Simulations confirmed the significant impact of NR1I2 rs2461817 on MDZ CL.

Conclusion: The PopPK model highlights the significant impact of NR1I2 rs2461817 polymorphism on MDZ CL in Chinese MV patients, emphasizing the need to consider genetic and clinical factors for optimizing MDZ dosing in ICU settings.

Keywords: midazolam, 1-hydroxymidazolam, mechanically ventilated, population pharmacokinetics, NR1I2 polymorphism

Introduction

Sedation is crucial in managing mechanically ventilated (MV) patients in the intensive care unit (ICU), as it reduces restlessness and facilitates invasive procedures.[1,2] Inadequate sedation occurs in over 20% of ICU patients, with some studies reporting rates as high as 75%.[3] Insufficient and excessive sedation pose considerable risks: inadequate sedation can cause patient-ventilator asynchrony, restlessness, and unplanned extubation, whereas excessive sedation may lead to prolonged mechanical ventilation, extended ICU stays, and an increased incidence of delirium.[1,4]

Midazolam (MDZ), a commonly used sedative in the ICU, is favored for its rapid onset, especially in scenarios requiring quick sedation, such as anesthesia induction or acute seizure management.[5–7] Although it is typically considered to have

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a short half-life ($t_{1/2}$),[7] midazolam's pharmacokinetics (PK) can vary significantly in ICU patients due to factors such as organ failure.[5,8,9] For instance, its $t_{1/2}$ can extend from 2.2 h to 35.5 h, and in cases of liver or renal dysfunction, it may even reach up to 140 h.[10] While a prolonged $t_{1/2}$ can help maintain stable sedation and reduce the frequency of dose adjustments, it also raises the risk of drug accumulation, over-sedation, delayed awakening, slow cognitive recovery, and extended ICU stays.[5] In patients with morbid obesity, an increased volume (V_d) can further prolong drug clearance (CL), necessitating careful dose adjustments to prevent both under- and over-sedation.[11]

Given these PK challenges, an individualized drug management strategy is essential when using MDZ. Clinical decisions should not only rely on monitoring sedation depth and physiological parameters but should also account for the patient's specific pathophysiological state, genetic factors influencing drug metabolism, and other PK changes. These factors collectively contribute to MDZ's PK variability, emphasizing the need for personalized dosing strategies to optimize therapeutic outcomes, reduce side effects, and improve patient prognosis.

The PK of MDZ is influenced by both genetic and physiological factors, underscoring the necessity of understanding these variables for personalized dosing strategies.[12–14] Traditional statistical methods often fail to capture the complex interactions among these factors. In contrast, population pharmacokinetics (PopPK) offers a more effective approach to identify clinical factors contributing to MDZ PK variability. Recent PopPK studies on MDZ predominantly focused on pediatric groups[11,15–21] with limited studies conducted on adults.[18,22–25] Key predictors of MDZ CL and its metabolite, 1-hydroxymidazolam (1-OH-MDZ), include alcohol misuse, the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, age, albumin levels, interleukin-6 (IL-6) levels, body weight (BW), and creatinine clearance (CL_{cr}).[18,23,25] CYP3A4 and CYP3A5, Phase I enzymes, primarily metabolize MDZ to 1-OH-MDZ.[22] However, the impact of CYP3A4 and CYP3A5 polymorphisms on MDZ PK remains unclear.

The Pregnane X receptor (PXR; NR1I2) plays a crucial role in drug metabolism and significantly influences MDZ metabolism.[23,26–29] Variations in PXR expression or activity, along with single nucleotide polymorphisms (SNPs) in the PXR gene, contribute to inter-individual variability in drug metabolism.[29] While SNPs in the PXR locus have been shown to affect the PK of drugs like atazanavir, cyclosporine, and carbamazepine, their impact on MDZ metabolism, especially in Chinese ICU patients, remains underexplored and warrants further investigation.[29–31]

This study aims to develop the first PopPK model specifically tailored for MDZ in Chinese ICU patients, integrating both physiological covariates and NR1I2 genetic polymorphisms. By integrating these variables, the model will offer insights into optimizing MDZ dosing, minimizing inadequate sedation, and improving patient safety in critically ill populations.

Materials and Methods

Study Design and Study Population

The study was conducted in the ICU of Fujian Medical University Union Hospital, Fuzhou, China, between April 2020 and August 2022. The study received approval from the hospital's Institutional Review Board (IRB No.: 2021YF003-01). Written informed consent was obtained from the patient or their legal representative, ensuring compliance with ethical protocols. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting criteria. The criteria for inclusion were: (1) Administered a continuous intravenous infusion of MDZ; (2) Participants aged 18 years or older, irrespective of gender; (3) Required mechanical breathing for a minimum duration of 24 hours. The exclusion criteria consisted of the following: (1) Hepatic coma or cirrhosis; (2) Inability to evaluate sedation levels due to underlying neurological problems; (3) Hemodynamic instability necessitating frequent modifications of sedative medicine doses; (4) Pregnant and lactating women, or anyone with an allergy to MDZ.

The starting dosage and method of administration were set according to the manufacturer's package insert for MDZ. 50 mg MDZ solution was diluted into 40 mL of 0.9% normal saline or 5% glucose solution, forming a final concentration of 1 mg/mL. It was administered through a pump system with a rate of 2–4 mL per hour. Nurses evaluated the level of sedation using the Richmond Agitation-Sedation Scale (RASS), adjusting the infusion rate to achieve a specific RASS score, with each dose adjustment being documented. Data collected during the treatment with MDZ were extracted from the hospital's electronic medical record system in a standardized format. The variables collected in this study included demographic information such as age, sex, body weight (BW), and height. Other data points recorded were the date of

MDZ initiation, disease diagnosis, RASS, APACHE II score, serum creatinine (SCr), C-reactive protein (CRP), IL-6, alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), albumin (ALB), prothrombin time (PT), and co-medications such as propofol and methylprednisolone. The body mass index (BMI) and creatinine clearance (CL_{cr}) were calculated using their respective formulas.

$$BMI = weight(kg) \div height^2(m^2)$$

CL_{cr} were estimated by Cockcroft and Gault equation:

$$CL_{cr}(mL/min) = [(140 - age(years) \times weight(kg))]/[(72 \times SCr)/88.4] \times [0.85\ (if\ female)]$$

Blood Sampling and Analytical Assays

The arterial blood sampling schedule included the following time points: before the initial MDZ dose (t = 0), followed by sampling at t = 0–0.5 h, t = 1–3 h, t = 4–6 h, and t = 10–12 h. The samples were subsequently centrifuged at 15,000 × g for 10 minutes at 4°C, and the plasma was stored at −80°C until required.

The concentrations of MDZ and 1-OH-MDZ in plasma were measured using a validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The analysis was performed on a Shimadzu LC-20 system (Nishinokyo-Kuwabaracho, Japan) coupled with an AB SCIEX Triple Quad 4500 MD mass spectrometer (Framingham, MA, USA). Midazolam-d4 maleate was used as the internal standard. Gradient elution was carried out at a flow rate of 0.6 mL/min. The linear ranges for MDZ and 1-OH-MDZ were 0.5–1000 ng/mL and 0.25–500 ng/mL, respectively. The method demonstrated intra-day and inter-day precision below 9%, and accuracy ranged from 92.11% to 111.52%. The limits of quantification for MDZ and 1-OH-MDZ were 0.5 ng/mL and 0.25 ng/mL, respectively.

Furthermore, the handling of missing data and outliers was addressed in the following manner:[32] Missing covariate data was imputed using the median value of the population. Concentration values that fell below the lower limit of quantitation (<5%) were excluded from the pharmacokinetic evaluation.

Genotyping

Genomic DNA was obtained from whole blood using the TIANamp Blood DNA Kit (Tiangen, Beijing, China). Research has demonstrated that variations in NR1I2 can impact the expression of other genes, including CYP3A and ABCB1. To explore these variations, we analyzed 23 loci across four genes (CYP3A4, CYP3A5, ABCB1, and NR1I2) . The specific loci analyzed included rs2242480 and rs2246709 (CYP3A4); rs776746 and rs15524 (CYP3A5); rs2032582, rs1128503 and rs1045642 (ABCB1); and rs1464603, rs1464602, rs3732357, rs6785049, rs2276707, rs10934498, rs3814055, rs2472677, rs3732359, rs3814058, rs3732360, rs4688040, rs2276706, rs1523130, rs2461817, and rs1523127 (NR1I2).

Population Pharmacokinetic Modelling

Structural Model and Choice of Statistical Model

The PopPK of MDZ and its active metabolite (1-OH-MDZ) were analyzed using NONMEM 7.5.0 (ICON Development Solutions, MD, USA). A simultaneous modeling approach was applied, in which MDZ disposition was described by a one-compartment model, and 1-OH-MDZ was characterized by a separate one-compartment model for its formation and elimination. The dose, infusion rate, and plasma concentrations of MDZ (M_w = 325.77 g/mol) and 1-OH-MDZ (M_w = 341.77 g/mol) were converted to molar equivalents, and the conversion rate of MDZ to 1-OH-MDZ was fixed at 0.6 based on prior literature.[23] Individual variability (IIV) was described using an exponential model, while residual variability (RUV) was evaluated using additive, proportional, and combined error models. The final model was selected based on the objective function value (OFV), parameter precision, error estimates, shrinkage values, and goodness-of-fit (GOF) plots. Model outputs and diagnostics were analyzed using R (version 3.6.1) and Perl-Speaks-NONMEM (PsN, version 5.0.0) within RStudio.

Covariate Analysis

Covariate selection was performed using stepwise forward inclusion and backward elimination, with OFV reductions >3.84 ($p < 0.05$) for inclusion and >6.63 ($p < 0.01$) for elimination. In addition, multicollinearity among the continuous covariates was evaluated using the variance inflation factor (VIF), with a threshold of VIF < 10 indicating no significant multicollinearity.[33] Covariates included demographic factors (eg, age, sex, BW), biochemical parameters (eg, AST, ALT, CRP), concomitant medications, and genetic polymorphisms. Concomitant medications included drugs commonly used in the ICU, such as propofol and methylprednisolone, with a usage rate exceeding 10%.

Model Evaluation

The accuracy and consistency of the final PopPK model were evaluated using GOF plots, bootstrap resampling, and visual prediction checks (VPC). Model adequacy was assessed using GOF plots. The final model's internal stability and validity were assessed using a non-parametric bootstrap method, which involved generating 1000 bootstrap datasets. The median and 95% confidence intervals (ranging from 2.5% to 97.5%) of the parameter estimations were compared between the 1000 bootstrap replicates and the original dataset. The predictive performance was assessed using VPC on 1000 simulated datasets. The plots were used to compare the observed data with the 5th, 50th, and 95th percentiles of the simulated data.

Simulation

Simulations were conducted to assess the impact of key covariates, including AST levels and NR1I2 rs2461817 genotypes, on the CL of MDZ and its metabolite, 1-OH-MDZ. To simulate plasma concentrations in a typical patient, IIV and RUV were set to zero. A standard dosing regimen of MDZ was applied, consisting of a loading dose of 3 mg and a maintenance dose of 4 mg/h, derived from the recommended dose range (0.01–0.05 mg/kg for loading and 0.02–0.1 mg/(kg·h) for maintenance) based on the median weight (62 kg) of the study population. AST levels were stratified at the 25th, 50th, and 75th percentiles (22, 37, and 60 IU/L), and patients were grouped by the NR1I2 rs2461817 genotype into wild-type homozygous, mutant heterozygous, and mutant homozygous categories.

Results

Patients and Samples

We assessed 69 patients for eligibility based on the inclusion and exclusion criteria. Eight patients were excluded due to incomplete data (n=3) or failure to meet inclusion criteria (n=5) (Figure 1). Table 1 provides a concise summary of the clinical characteristics exhibited by these patients. Out of the 61 patients, 237 blood samples were analyzed, with both MDZ and 1-OH-MDZ measured in each sample. Samples with concentrations below the quantification limit (3 for MDZ and 3 for 1-OH-MDZ, each amounting to 1.3%) were excluded. The plasma concentrations of MDZ ranged from 1.53 to 1622.36 ng/mL, while the plasma concentrations of 1-OH-MDZ ranged from 0.28 to 166.19 ng/mL. The initial infusion rate for all patients ranged from 2 to 6 mg/h. The genotyping findings for the patients can be found in Supplementary Table 1. All the 22 SNP genotypes, except for NR1I2 rs1464603, had P-values larger than 0.05, indicating that they all adhered to Hardy-Weinberg equilibrium.

Development of the Population Pharmacokinetic Model

Figure 2 illustrates the structure of the PopPK model that describes the pharmacokinetic connection between MDZ and 1-OH-MDZ. Both MDZ and 1-OH-MDZ were most accurately characterized by a one-compartment model. IIV was characterized utilizing an exponential model for IIV, whereas for MDZ and 1-OH-MDZ, intra-individual variability was characterized using a combination model and an additive model, respectively. The covariate screening process is provided in Supplementary Table 2. The specifications of the primary model are displayed in Table 2.

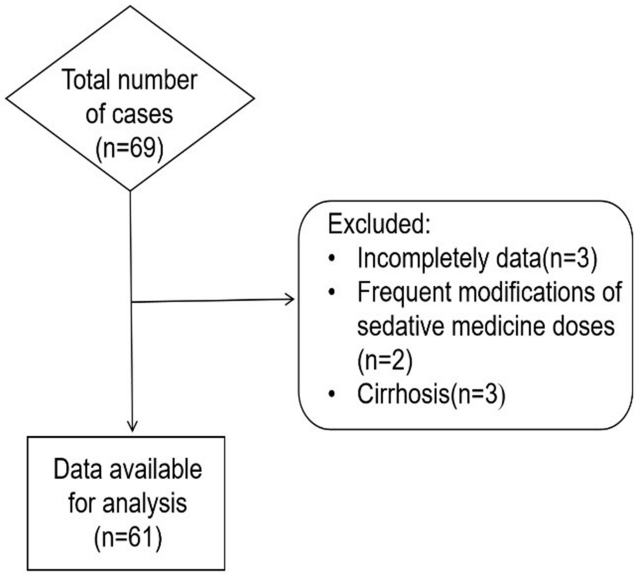

Figure 1 Flowchart of patient inclusion for the modeling population.

Final Model Evaluation

The GOF plots of the final model exhibited effective data characterization, as seen in Figure 3.The model's ability to estimate individual concentrations was demonstrated by the uniformly distributed individual predicted values for MDZ and its metabolite 1-OH-MDZ when plotted against observed concentrations, aligning closely with the line of unity. Nevertheless, the population estimate of MDZ, and its metabolites failed to accurately account for elevated concentrations. It is probable that the limited number of patient cases with varying dosing and inconsistent sampling times contributed to this outcome. Supplementary Figure 1 provides additional GOF plots. The evaluation of VPC predictive performance showed high levels of accuracy. Figure 4 displays the median concentrations and 95% confidence intervals for MDZ and 1-OH-MDZ as shown by VPC.

Table 1 Demographic and Clinical Characteristics of the Patient

Characteristics	Median (Range) or N (%)
Weight (kg)	62.0 (38.0–87.6)
Height (cm)	169.0 (143.0–180.0)
BMI (kg/m^2)	22.34 (16.23–29.96)
Age (y)	67 (29–90)
Sex (Male/Female)	42 (68.9%)/ 19 (31.1%)
APACHE II	16 (2–24)
TBIL (μmol/L)	15.4 (3.5–169.7)
ALB (g/L)	31.7 (21.7–65.1)
ALT (IU/L)	22.0 (2.0–391.0)
AST (IU/L)	37.0 (10.0–362.0)
ALP (IU/L)	75.0 (12.0–463.0)
GGT (IU/L)	41.0 (3.0–507.0)
IL-6 (pg/mL)	109.6 (4.9–5000.0)
CRP (mg/L)	82.14 (1.95–295.74)
SCr (μmol/L)	71.0 (30.0–507.0)
CL_{cr} (mL/min)	70.77 (8.97–203.4)
Initial infusion rate (mg/h)	4 (2–6)

(*Continued*)

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Table 1 (Continued).

Characteristics	Median (Range) or N (%)
Plasma concentration (ng/mL)	
MDZ	149.26 (1.53–1622.36)
1-OH-MDZ	18.66 (0.28–166.19)
Samples	
MDZ	237
1-OH-MDZ	237
Co-medications	
Propofol	22 (36.1%)
Remifentanil	16 (26.2%)
Fentanyl	9 (14.8%)
Sufentanil	8 (13.1%)
Methylprednisolone	7 (11.5%)

Abbreviations: MDZ, midazolam; 1-OH-MDZ, 1-hydroxymidazolam; BMI, body mass index; APACHE II, acute physiology and chronic health evaluation II score; TBIL, total bilirubin; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; IL-6, interleukin-6; CRP, C-reaction protein; SCr, serum creatinine; CL_{cr}, creatinine clearance.

Simulations

The simulation results indicate that MDZ plasma concentrations are affected by the AST levels of patients and the NR1I2 rs2461817 genotype. As patients' AST levels increase, so do MDZ plasma concentrations. As an illustration, the increase in AST levels from 22 IU/L to 60 IU/L leads to a decrease in MDZ CL from 27.8 L/h to 18.7 L/h (Figure 5). Furthermore, various genotypes of NR1I2 rs2461817 have an impact on MDZ plasma concentrations. Patients with the rs2461817 mutant homozygous genotype had higher MDZ plasma concentrations compared to those with wild-type homozygous or mutant heterozygous genotypes. In addition, the CL value decreases from 22.6 L/h to 13.4 L/h in these patients (Figure 6).

Moreover, Figure 7 demonstrates the influence of BW on 1-OH-MDZ plasma levels. The simulation results demonstrate that plasma concentrations of 1-OH-MDZ exhibit variability in relation to BW. As BW increases from 54 kg to 65 kg, the CL of 1-OH-MDZ increases from 53.9 L/h to 72.3 L/h.

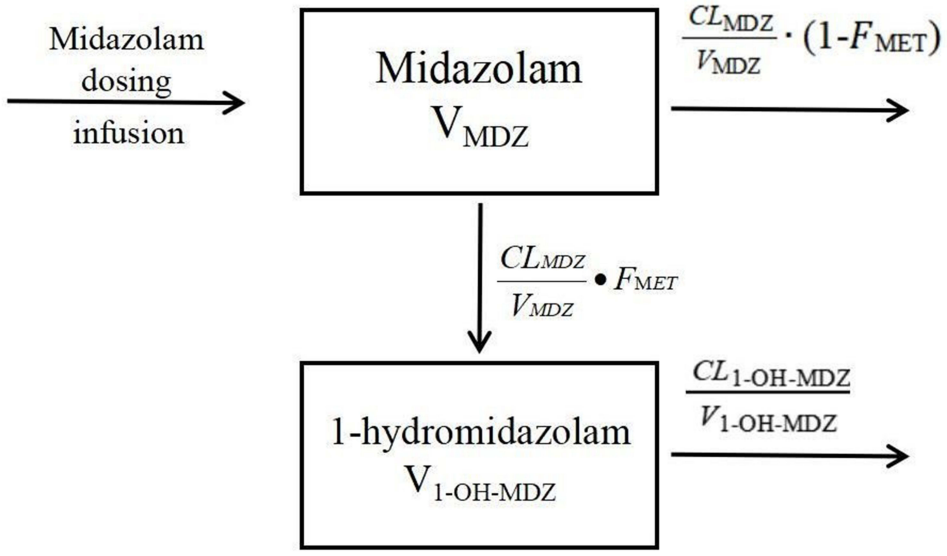

Figure 2 The structural model for MDZ and 1-OH-MDZ.

Table 2 The Specifications of the Primary Model

Parameter	Base Model (n = 61)		Final Model (n =61)		Bootstrap (n=1000)
	Estimate	RSE %	Estimate	RSE%	Median (95% CI*)
MDZ					
CL_{MDZ} (L/h)	17.50	11	22.6	16	22.15 (12.55–30.99)
V_{MDZ} (L)	15.8	43	16.00	42	15.58 (5.44–44.61)
1-OH-MDZ					
$CL_{1\text{-}OH\text{-}MDZ}$ (L/h)	62.30	9	67.10	10	65.79 (52.43–79.36)
$V_{1\text{-}OH\text{-}MDZ}$ (L)	86.20	34	86.20	35	86.32 (1.39–149.375)
F_{MET}	0.6	/	0.6	/	/
Covariate effect					
AST on CL_{MDZ}	/	/	−0.397	47	−0.385 (−0.783 – −0.0151)
rs2461817 on CL_{MDZ}	/	/	−0.405	29	−0.389 (−0.591 – −0.0659)
BW on $CL_{1\text{-}OH\text{-}MDZ}$	/	/	1.58	30	1.54 (0.52–2.58)
IIV					
CL_{MDZ} (CV%)	70.70	19	59.40	20	58.18% (35.90–74.54%)
$CL_{1\text{-}OH\text{-}MDZ}$ (CV%)	62.00	10	57.70	11	56.31% (40.70–68.66%)
RUV					
MDZ					
Proportional (CV%)	40.60	38	40.00	38	41.14% (13.47–52.69%)
Additive (ng/mL)	3.38	56	3.43	56	3.22 (1.15–5.02)
1-OH-MDZ					
Additive (ng/mL)	0.6	22	0.6	22	0.58 (0.41–0.76)

Note: *2.5th and 97.5th percentiles of bootstrap parameter estimate.

Abbreviations: MDZ, midazolam; 1-OH-MDZ, 1-hydroxymidazolam; CL_{MDZ}, clearance of MDZ; V_{MDZ}, volume of distribution of MDZ; $CL_{1\text{-}OH\text{-}MDZ}$, clearance of 1-OH-MDZ; $V_{1\text{-}OH\text{-}MDZ}$, volume of distribution of 1-OH-MDZ; F_{MET}, the proportion of MDZ converted to 1-OH-MDZ; IIV, inter-individual variability; RUV, residual variability; AST, aspartate aminotransferase; BW, body weight; rs2461817 is the PXR gene; CI, confidence interval; RSE, relative standard error; CV%, coefficient of variation (CV%= sqrt (exp(OMEGA) − 1) × 100); Proportional, proportional residual; Additive, additive residual.

Discussion

The first prospective PopPK investigation of MDZ in Chinese ICU patients receiving MV is presented in this work. We looked at PK and pharmacogenetic data from 61 MV patients on continuous MDZ therapy. By combining the AST levels and the NR1I2 rs2461817 polymorphism, our covariate analysis explained 11.3% of the IIV in MDZ CL. Incorporating BW as a covariate for the CL of 1-OH-MDZ led to a decrease in IIV from 62.0% to 57.7%, explaining 4.5% of the IIV in 1-OH-MDZ CL.

Our data indicate that one-compartment models accurately reflect the PK characteristics of MDZ and 1-OH-MDZ. This is consistent with the PK analyses published for MDZ. However, some studies have suggested two-compartment models for MDZ.[18] It is important to note that there is limited research on the PK of continuous MDZ infusion in adult patients. These studies primarily focused on patients with acute renal failure (ARF) and acute respiratory distress syndrome (ARDS) related to COVID-19.[22,25] To date, no analysis has been conducted on Chinese ICU patients using PopPK. The population mean values of MDZ CL in our study showed significant variations compared to those reported by Swart and Smeets, with our values being 22.6 L/h, 5.47 L/h, and 6.7 L/h, respectively.[22,24] Although our study included patients with various disease types, most participants were postoperative patients in relatively good health. This may explain why the estimated MDZ CL was similar to that of healthy individuals.[34–36]

Although we did not observe a significant correlation between MDZ CL and APACHE II scores in our study, further research on the relationship between APACHE II scores and drug CL or V_d in critically ill patients suggests a potential link. For instance, in the MDZ PopPK model by Swart et al, patients with an APACHE II score ≥26 had a significantly increased V_d.[37] Additionally, a prospective observational study in ICU patients treated for tuberculosis found that subtherapeutic rifampin concentrations were often associated with higher APACHE II scores (P=0.03).[38] In a propofol

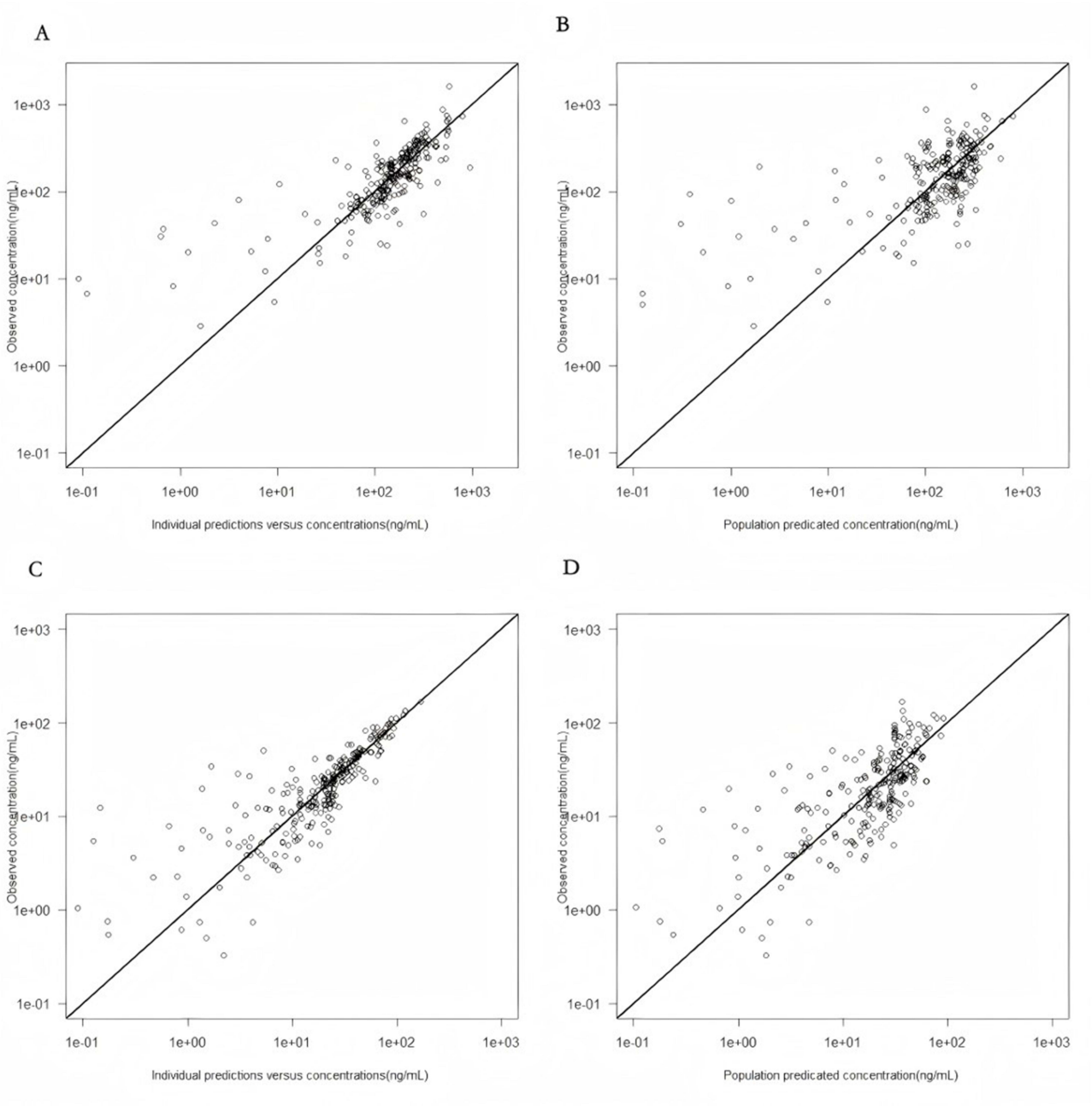

Figure 3 Goodness-of-fit plots of the final model. Individual predictions (**A**) and population predictions (**B**) vs observations of midazolam, individual predictions (**C**) and population predictions (**D**) vs observations of 1-hydroxymidazolam.

study, patients with an APACHE II score ≤20 had an average CL of 92 L/h (95% CI, 81–104), while those with an APACHE II score >20 had an average CL of 117 L/h (95% CI, 89–144).[37] These findings suggest that APACHE II scores may influence PK characteristics, particularly CL and V_d, in critically ill patients. This could help explain the relatively higher MDZ CL observed in our study, potentially due to the lower APACHE II scores and the relatively mild conditions of our cohort.

Furthermore, the population mean value of 1-OH-MDZ CL in our study closely matched that of Swart et al (67.1 L/h vs 62 L/h), but it differed significantly from the value reported by Smeets et al (132 L/h).[22,24] The MDZ V_d estimate in our study was 15.80 L, which aligns with the lower range of V_d values observed for MDZ in healthy subjects (0.4–2.0 L/kg).[22,24,39] The calculated V_d for MDZ, excluding compartmentalization, is closer to the central compartment volume

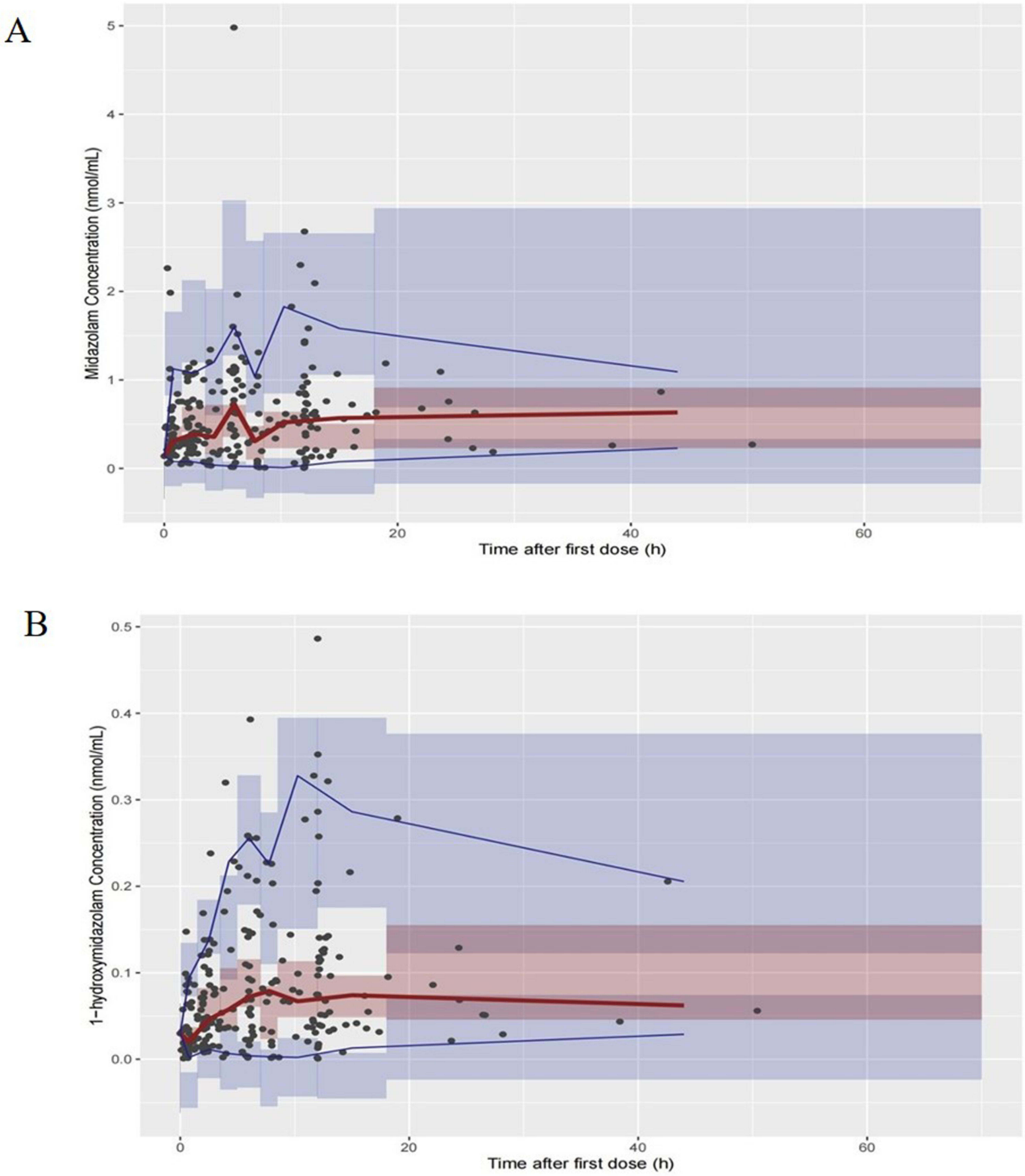

Figure 4 Visual predictive check (shaded areas) and observed data (solid dots) of midazolam (**A**) and 1-hydroxymidazolam (**B**) serum concentration versus time for the final model. Solid dots represent the observed concentrations. The solid lines represent the 5th (lower blue), 50th (red), and 95th (upper blue) percentiles of the observed data. The shaded areas represent the 95% confidence intervals of the 5th, 50th, and 95th percentiles of the simulated concentrations.

(27.2 L) reported by Seng et al.[23] Variations in patient disease types, study duration, and ALB levels may explain the significant differences in V_d observed between our study and previous research. Swart and Smeets included patients with COVID-19-related ARDS and ARF, with observation periods of 11 to 611 hours and 24 to 715 hours, respectively.[22,25] In contrast, our study was conducted over a shorter duration (0–24 hours) and included ICU patients with diverse

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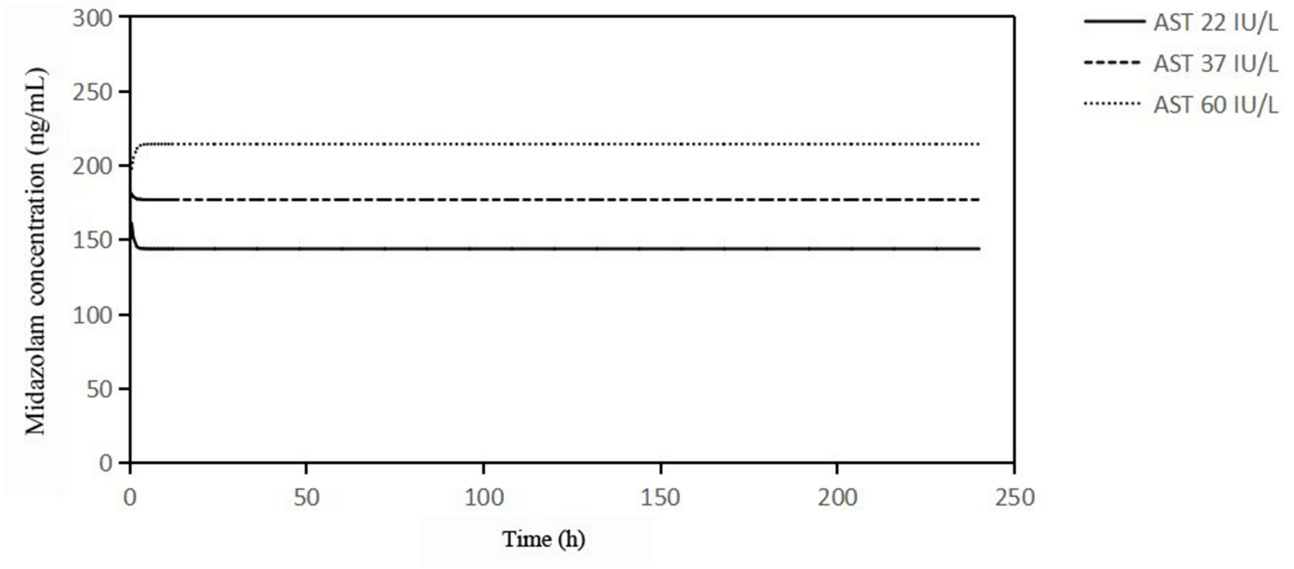

Figure 5 Simulated plasma profiles of midazolam (MDZ) after a 3 mg MDZ loading dose followed by 4 mg/h continuous infusion for 10 days in patients with different aspartate aminotransferase (AST) levels.

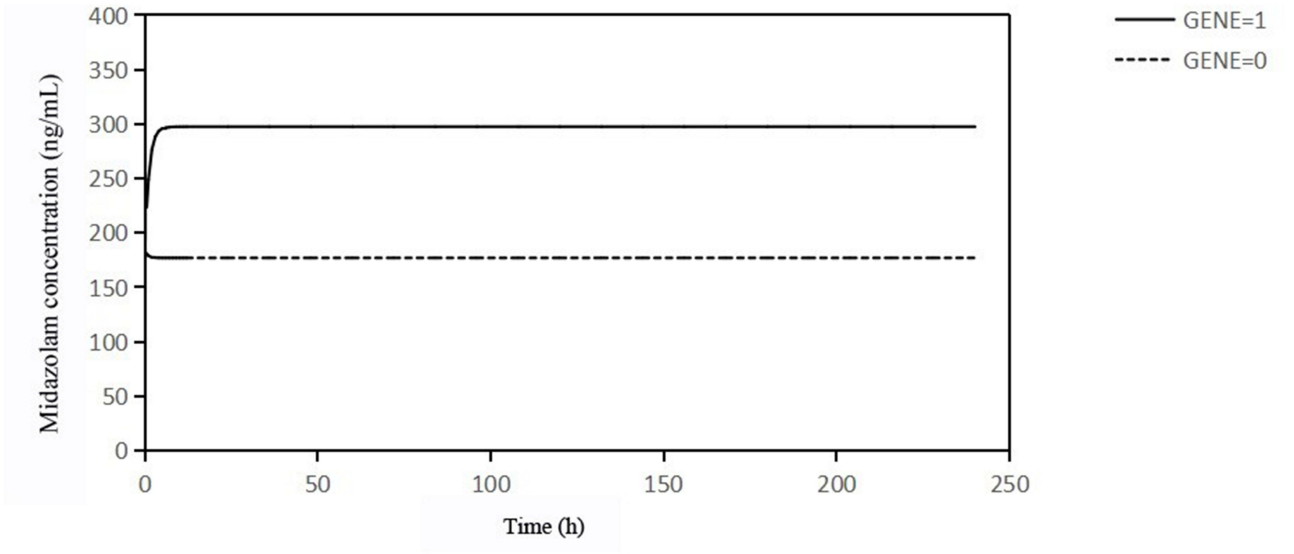

Figure 6 Simulated plasma profiles of midazolam (MDZ) after a 3 mg MDZ loading dose followed by 4 mg/h continuous infusion for 10 days in patients with different NR1I2 rs2461817 genotypes. GENG = 1 indicates that the NR1I2 rs2461817 genotype reflects a homozygous mutation. GENE = 0 indicates that the NR1I2 rs2461817 genotype is wild-type homozygous or mutant heterozygous.

conditions. Vree et al demonstrated that a decrease in serum ALB levels (<25 g/L) is associated with increased V_d and slower MDZ elimination.[40] However, the majority of patients in our study had ALB levels within the normal range. Additionally, previous studies suggest that fat mass can significantly impact PK parameters.[41] Although most of our patients had a body mass index (BMI) within the normal range, we did not measure fat mass, which may have contributed to variability in V_d. Future PK studies should extend the trial duration and explore the effects of fat mass, patient stratification, and other variables on V_d.

The identifiability of the model structure is essential in constructing a PopPK model. This guarantees the calculation of all PK parameters based on specified input, system, and output circumstances. Studies examining parent pharmaceuticals and

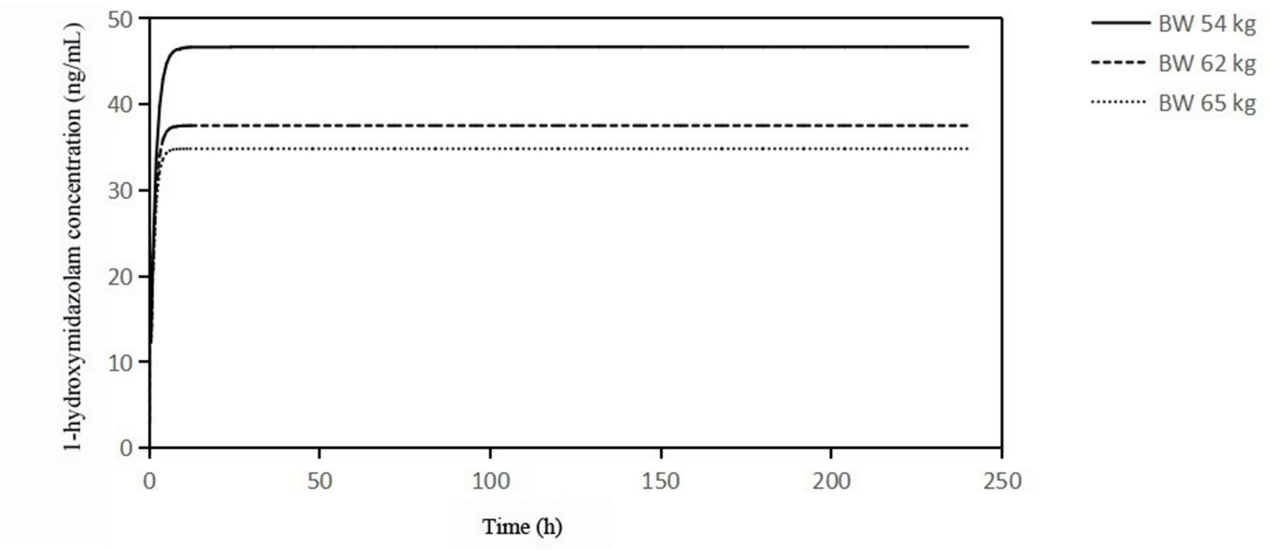

Figure 7 Simulated plasma profiles of 1-hydroxymidazolam (1-OH-MDZ) after a 3 mg midazolam (MDZ) loading dose followed by a 4 mg/h continuous infusion for 10 days in patients with different body weights (BW).

their metabolites have discovered that it is not possible to directly compute the fraction of the parent drug metabolized to its metabolite (F_{MET}) and the V_d of the metabolite. However, it is possible to determine their ratio.[42,43] In order to make the model less complex and easier to understand, it is sometimes customary to set either the F_{MET} or the V_d of the metabolite.[44,45] When examining studies on the PK of MDZ and 1-OH-MDZ, the majority have established a constant value for F_{MET}. According to the study conducted by Seng et al, the FMET value indicates that 60% of MDZ is transformed to 1-OH-MDZ.[23]

This study revealed a notable correlation between the NR1I2 rs2461817 SNP and MDZ CL. Specifically, individuals with the homozygous mutation of rs2461817 exhibited a 41% decrease in MDZ CL compared to those with the wild-type and heterozygous variants. This discovery suggests that genetic differences in the NR1I2 gene can influence the production and activity of CYP3A, thereby affecting MDZ metabolism. Our study highlights this noteworthy genetic relationship.

We investigated the factors contributing to the limited influence of the CYP3A4 and CYP3A5 genotypes on MDZ CL in our study. We propose that MDZ metabolism in ICU patients is influenced by multiple factors. Beyond genetic factors, drug-drug interactions (DDIs), inflammatory cytokines (such as TNF-α),and other clinical factors may inhibit or diminish the regulatory effects of PXR on CYP3A4 and CYP3A5.[6,46] Therefore, while PXR gene regulation does play a role, clinical factors may obscure or attenuate the genetic influence on MDZ metabolism, which may explain the minimal effects of CYP3A4 and CYP3A5 genotypes on MDZ CL in our study. In conclusion, while genetic factors related to CYP3A4 and CYP3A5 may have some impact, further research is needed to better understand how these factors interact with clinical variables to optimize drug metabolism and pharmacotherapy.

Our study also found a negative correlation between AST levels and MDZ CL. Simulation results indicated that when AST levels increased from 22 IU/L to 60 IU/L, MDZ CL decreased by approximately 33%. This is consistent with the known role of CYP3A4 and CYP3A5 in MDZ metabolism. Similar findings were reported by Seng et al, who showed a reduction in MDZ CL as TBIL levels increased.[23] Both AST and TBIL are biomarkers of hepatic function, with AST reflecting hepatocellular injury.[47–49] Elevated AST levels typically indicate liver damage, particularly under acute stress conditions common in critically ill patients. While ALT is more sensitive for detecting liver injury in chronic liver diseases, AST may be more relevant in acute conditions, such as in ICU patients undergoing metabolic stress. Notably, AST is also a marker of mitochondrial damage, with approximately 80% of AST located in mitochondria, which play a critical role in liver function and drug metabolism.[50] This finding aligns with a PopPK study of linezolid in critically ill pediatric patients, where AST was similarly identified as a key covariate for drug CL.[51] This may explain the closer association between AST levels, MDZ metabolism, and liver function in ICU patients, where acute liver damage is more pronounced.

BW was found to be a significant covariate for the CL of 1-OH-MDZ, consistent with the results reported by Seng et al.[23] In the ICU setting, drug CL is often closely associated with liver and renal function, and the workload of these organs may be more directly related to the patient's total body weight rather than just fat mass.[52] ICU patients' weight can fluctuate significantly due to factors such as nutritional status, fluid resuscitation, and disease severity.[53] Since BW is a dynamic indicator, it is better able to capture these fluctuations and may more accurately reflect the overall factors affecting drug PK. For this reason, BW appears to have a more direct relationship with 1-OH-MDZ CL compared to BMI. While BMI reflects the ratio of fat to lean mass, BW encompasses all body components, including muscle and fluids, which may have a greater impact on metabolic capacity. Moreover, the effect of BW on 1-OH-MDZ V_d might be harder to capture given the substantial weight fluctuations in ICU patients. Thus, BW's influence on 1-OH-MDZ CL may be more pronounced in our study due to its more direct relationship with metabolic capacity and drug CL.

The primary pathway for 1-OH-MDZ CL involves the process of glucuronidation by uridine diphosphate glucuronosyltransferases (UGT) 1A4 and 2B4/7, resulting in the formation of hydroxy midazolam glucuronide.[54] Prior research has demonstrated that obesity can enhance UGT activity in both mice and humans.[47,55] Xu et al discovered that in obese mice, fatty liver caused an increase in hepatic UGT expression. This was accompanied by elevated mRNA levels and binding activity of many nuclear receptors, including PXR.[55] These findings suggest that higher BW may affect the expression and function of UGT by stimulating the production of nuclear factors such as PXR. This, in turn, may speed up the metabolism of 1-OH-MDZ. Although our study did not assess UGT genes, which could help clarify this connection, further research should consider incorporating UGT genes and other body composition factors, such as fat mass, to enhance our understanding of MDZ metabolism in ICU patients.

Clinicians are also concerned about DDIs involving MDZ. When MDZ is administered alongside CYP3A enzyme inducers, inhibitors, or competitive inhibitors, these drugs can affect the metabolism of MDZ, resulting in alterations in its plasma concentration. Some commonly co-administered drugs include carbamazepine (an antiepileptic), olanzapine (an antipsychotic), itraconazole, fluconazole, voriconazole (azole antifungals), propofol, and fentanyl (an opioid analgesic). Interactions with anesthetics can lead to an increase in MDZ concentrations. Propofol-induced reduction in MDZ CL may be attributed to both hemodynamic suppression and enzyme inhibition caused by propofol.[56] It is important to mention that the hemodynamic effects of propofol may also impact the distribution of MDZ to peripheral tissues, thus influencing its central volume of distribution.[56] However, in a small subset of cases (36.1%), the co-administration of propofol did not have a significant effect on the V_d of MDZ as a clinical factor. In addition, the co-administration of fentanyl and MDZ may competitively inhibit CYP3A4, resulting in a reduction of MDZ CL.[57] Regrettably, our study did not observe a noteworthy influence of fentanyl on MDZ plasma concentrations. This outcome may be attributed to the limited size of our cohort. In addition, the use of methylprednisolone was infrequent, and the connection between corticosteroids and MDZ PK parameters is still uncertain.[58,59] Additional PK studies involving larger groups of participants are necessary to gain a comprehensive understanding of the clinical importance of these factors. This will allow for a more comprehensive understanding of the diverse characteristics of ICU patients.

Many restrictions apply to this study. Firstly, the reported V_d and CL of 1-OH-MDZ are "apparent" values because we could not determine the proportion of MDZ metabolized to 1-OH-MDZ. More samples collected after injection should be the goal of future research to better comprehend this conversion ratio. Secondly, this study did not develop a population pharmacokinetic/ pharmacodynamic (PK/PD) model connecting MDZ plasma concentration with sedation scores (RASS scores) due to the inadequate data and its subjectivity. Future research will involve a larger cohort and objective PD indicators, such as EEG, to establish a robust PK/PD model, thereby optimizing MDZ dosing and enhancing clinical outcomes. Thirdly, we did not quantify 1-hydroxymidazolam glucuronide, a Phase II metabolite. 1-OH-MDZ reacts with glucuronic acid to produce 1-OH-MG, which is a less potent form and is eliminated by the kidneys. However, in ICU patients with renal failure, there is a risk of 1-OH-MG buildup, which could result in minor sedative effects.[18,60] To improve the applicability of the PopPK model, future studies should include 1-OH-MG and perform external validation.

Furthermore, the absence of known plasma concentration ranges for MDZ and 1-OH-MDZ complicates the assessment of existing dosing regimens, as it hinders the ability to attain targeted sedation levels. There is no recommended range for 1-OH-MDZ. However, a Ramsay score of 2–4 corresponds to a median MDZ plasma concentration of 200–300 ng/mL.[60] Since the Ramsay and RASS scales correlate highly in ICU settings,[61] 200–300 ng/mL may be a good target dosage for mild to

moderate sedation. Our MCS data suggest that patients with the NR1I2 rs2461817 wild-type homozygous or heterozygous genotype and AST ≤37 IU/L may not be appropriate candidates for the usual dosage regimen. Improving efficacy and lowering side effects will be possible with the use of model-based MCS and the establishment of pharmacodynamic markers.

Conclusions

In this diverse ICU population, we successfully developed a PopPK model for MDZ. Our findings confirm the significant impact of the NR1I2 rs2461817 genotype on MDZ CL. Additionally, we identified several important factors influencing the CL of MDZ and 1-OH-MDZ, including AST levels and BW. These findings highlight the importance of incorporating genetic and clinical factors into MDZ dosing strategy. Specifically, for a patient with NR1I2 rs2461817 genotype homozygous variant, lower dose should be considered when MDZ is used for sedation in ICU patients. Future work should focus on refining these PK models and integrating PD factors to improve dose prediction and optimize therapeutic outcomes.

Data Sharing Statement

The relevant author can be contacted with any request for any of the data used in this work.

Ethics Approval

The ethical committee of Fujian Medical University Union Hospital gave its approval to the study, which was carried out in compliance with the Declaration of Helsinki (No. 2021YF003-01).

Informed Consent Statement

All participants to the study gave their informed permission.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors assert that they have no conflicts of interest.

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