Sufentanil and Midazolam Dosing and Pharmacogenetic Factors in Pediatric Analgosedation and Withdrawal Syndrome

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Summary

Our aim was to describe the effect of dosing and genetic factors on sufentanil- and midazolam-induced analgosedation and withdrawal syndrome (WS) in pediatric population. Analgosedation and withdrawal syndrome development were monitored using COMFORT-neo/-B scores and SOS score. Length of therapy, dosing of sufentanil and midazolam were recorded. Genotypes of selected candidate polymorphisms in *CYP3A5*, *COMT*, *ABCB1*, *OPRM1* and *PXR* were analysed. In the group of 30 neonates and 18 children, longer treatment duration with midazolam of 141 h (2 – 625) vs. 88 h (7 – 232) and sufentanil of 326.5 h (136 – 885) vs. 92 h (22 – 211) (median; range) was found in the patients suffering from WS vs. non-WS group, respectively. Median midazolam cumulative doses were in the respective values of 18.22 mg/kg (6.93 – 51.25) vs. 9.94 mg/kg (2.12 – 49.83); $P=0.03$, and the respective values for sufentanil were 88.60 µg/kg (20.21 – 918.52) vs. 21.71 µg/kg (4.5 – 162.29); $P<0.01$. Cut off value of 177 hours for sufentanil treatment duration represented predictive factor for WS development with 81 % sensitivity and 94 % specificity. SNPs in the candidate genes *COMT*, *PXR* and *ABCB1* affected the dosing of analgosedative drugs, but were not associated with depth of analgosedation or WS. Cumulative dose and length of analgosedative therapy with sufentanil significantly increases the risk of WS in critically ill neonates and children.

Key words

Analgosedation • Withdrawal • Sufentanil • Midazolam

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Introduction

Sufentanil is a strong sedative opioid indicated for analgosedation monotherapy in ventilated patients. Sufentanil administration may be accompanied by known opioid-related adverse effects-nausea, vomiting, respiratory depression, constipation and withdrawal syndrome (Schug *et al.* 1992). Clinical effect is mediated by polymorphic µ-opioid receptor 1 (OPRM1). Variant of the major *OPRM1* nonsynonymous SNP *118A>G* (*rs1799971*) decreased efficiency of G-protein coupling to 58 % in comparison with wt homozygotes (Oertel *et al.* 2009). The clinical relevance of this SNP for the sufentanil treatment in pediatric population has been indicated by Mamie *et al.* (2013), who described higher Faces Pain Scale (FPS) scores across the 24 postoperative hours in heterozygotes compared with wt homozygotes. In another study, infants (n=140) carrying variant allele suffered from shorter duration of neonatal abstinence syndrome (NAS) and were less likely to receive any treatment than wt homozygotes (Wachman *et al.* 2013).

Catechol-O-methyl transferase (COMT) plays a central role in the degradation of extracellular dopamine and norepinephrine in the CNS. Variant allele homozygotes for the SNP *COMT*rs4680 had enzymatic activity decreased by 38 % compared to wild-type homozygotes in post mortem collected brain samples (Chen *et al.* 2004, Lachman *et al.* 1996). Higher levels of

dopamine in *COMTrs4680* SNP carriers were associated with lower levels of enkephalins, leading to down regulation of μ -opioid receptor (Zubieta *et al.* 2003). Wild-type homozygous children had significantly higher sufentanil-induced analgesia after orthopedic or abdominal surgery (Mamie *et al.* 2013). Variant allele carriers stayed shorter time in the hospital and the treatment with 2 or more medications was used less frequently than in wild-type homozygous neonates with NAS (18 % vs. 56 %), (Wachman *et al.* 2013).

Midazolam is another drug used for analgesedation in pediatric and neonatal intensive care units (Anderson and Larsson 2011). Midazolam is metabolized in the liver via CYP3A (Wong *et al.* 2004) and the clinical effects of midazolam are prolonged in neonates due to immature cytochrome P450 enzymes. Polymorphisms in CYP3A as well as in nuclear receptors regulating its transcription potentially influence midazolam effects (De Jonge *et al.* 2015, Oleson *et al.* 2010, Seng *et al.* 2014). The effect of the common polymorphism *CYP3A5**3 is unclear, as the results of the studies are contradictory (Shih and Huang 2002, Wong *et al.* 2004). Further, in *CYP3A5* non-expressers, some role of *CYP3A4**22 polymorphism on midazolam clearance has been observed in allograft recipients, but this SNP has a very low allele frequency in Caucasian population (De Jonge *et al.* 2015).

Midazolam is a substrate of efflux transporter ABCB1 that could affect the distribution of the drug in the body and subsequently also alter its efficacy (Tolle-Sander *et al.* 2003).

Patients at pediatric intensive care units suffer frequently from iatrogenic withdrawal syndrome (WS) related to opioid and/or benzodiazepine that develops in up to 57 % children (Katz *et al.* 1994). Traditionally fentanyl is used as first-line opioid (66 %) and midazolam as the first-line benzodiazepine (86 %) for analgesedation at PICU. The WS was dependent on the length and total exposure to fentanyl and midazolam with the incidence increasing after 5 days of continuous infusion or when round-the-clock administration schedule was used (Arnold *et al.* 1990, Dominguez *et al.* 2003, French and Nocera 1994, Grant *et al.* 2013, Katz *et al.* 1994).

The aim of our study was to describe the impact of candidate gene polymorphisms on sufentanil- and midazolam- induced analgesedation and/or WS. Another aim of our study was to describe the relation between sufentanil dosing or treatment duration and development of WS.

Methods

Mechanically ventilated full-term neonates and children at the Pediatric Intensive Care and Resuscitation Unit of the General Faculty Hospital in Prague were prospectively recruited into the study after obtaining informed consent from their legal representatives from January 2010 to September 2013. The study was approved by the Ethics Committee of the General Faculty Hospital in Prague and it was conducted in accordance with the Declaration of Helsinki.

Inclusion criteria encompassed neonates ≥ 36 gestational weeks and children ≥ 3 months to 18 years of age, with length of continuous intravenous infusion of sufentanil and/or midazolam ≥ 48 h. Neonates and children with severe hepatic impairment and acute renal failure were excluded. Subjects were treated according to PICU sedation recommendation (Poh *et al.* 2014).

Postmenstrual age (PMA), weight/birth weight, sex, ethnicity, main and secondary diagnoses were recorded for all subjects, while the Apgar score, way of delivery (spontaneous/Caesarian section) were registered for neonates only.

Organ functions were evaluated using Goldstein score for cardiovascular, respiratory, neurological, hematological and hepatic dysfunction (Goldstein *et al.* 2005). Renal functions were recorded according to pRIFLE criteria (Akcan-Arikan *et al.* 2007). Length of therapy (h) and cumulative dose of tramadol (mg/g) were recorded as the median (range). Concomitant therapy, length of PICU and overall hospital stay were also registered.

Validated COMFORT-B and COMFORT-NEO scale was used for monitoring of analgesedative effects. COMFORT score of < 11 was considered indicative of oversedation, COMFORT score of > 22 for insufficient sedation (Van Dijk *et al.* 2000, Van Dijk *et al.* 2009). Symptoms of withdrawal syndrome were systematically evaluated after fifth day of stay at PICU. The Sophia Observational Scale (SOS) was used for WS assessment. The total score was noted every 3 h, with the count equal or more than 4 for two consecutive measurements indicative for WS occurrence (Ista *et al.* 2013, Ista *et al.* 2009, Ista *et al.* 2009). Both SOS and COMFORT score were systematically registered by trained personnel (linear weighted kappa median 0.80), (Pokorná *et al.* 2016).

Samples of peripheral venous blood for DNA isolation were collected in tubes containing EDTA and

immediately frozen and stored at -20°C until further processing. Genomic DNA was extracted from blood leukocytes using QIAamp DNA Blood Mini Kit (Quiagen Ltd.). Detection of the candidate gene polymorphisms in *COMT* (*rs4633*, *rs4680*, *rs4818*) *ABCB1* *G2677T/A* (*rs2032582*), *ABCB1* *C3435T* (*rs1045642*), *OPRM1A118G* (*rs1799971*) and *PXR* *10799 G/A*, *PXR* *-25385 C/T*, *PXR* *-24113 C/T*, *PXR* *7635 A/G* (*rs1054191*, *rs3814055*, *rs2276706*, *rs6785049*) was performed as published previously (Bartosova *et al.* 2015, Buzkova *et al.* 2008, George *et al.* 2008, Pechandova *et al.* 2006, Polívková 2006, Redden *et al.* 2005, Van Schaik *et al.* 2002).

Mann-Whitney test was used for between group median comparison of cumulative doses, doses per kg and hour and the length of therapy. Chi-square test was used for categorical variables. A *P*-value of less than 0.05 was considered statistically significant. In order to explain the development of WS, a multivariate logistic regression model was used with the total dose of sufentanil, total dose of midazolam, duration of sufentanil treatment and duration of midazolam treatment as explanatory variables in subgroups of neonates and children over 3 months of age, who received both drugs concomitantly. Logistic regression analysis was carried out with Wald forward stepwise method, *P* for inclusion and exclusion of a parameter was set to 0.05 and 0.15, respectively. The ROC curve analysis was performed to identify the value offering the best sensitivity and specificity. The statistical package Dell STATISTICA version 12 was used.

Results

Baseline characteristics

Baseline characteristic parameters of neonates and children are summarized in Table 1. Twenty-five neonates were admitted with respiratory failure (due to perinatal asphyxia, RSV pneumonia, or aspiration of amniotic fluid), 4 neonates with sepsis and one with meningoencephalitis. Totally 24 neonates were discharged with no or minor health disturbance, 2 were transferred to another department, 2 patients were discharged with moderate/severe health disturbance and 2 died. Seven children were admitted with pneumonia/respiratory failure, 5 children suffered from burns, 2 from myocarditis, 2 from brain atrophy, one from sepsis and dehydration. Seven children were discharged with no or minor consequences, 10 were transferred to another department and one child died. Table 2 presents observed allelic frequencies in the whole study population. Except sufentanil and midazolam, the patients received median (range) of 0.25 mg/kg/h (0.03 – 0.34 mg/kg/h) hourly dose i.e. median (range) of 6.0 mg/kg/day (0.72 – 8.16 mg/kg/day) of tramadol (13 neonates, 14 children) and median (range) of 0.24 mg/kg/h (0.03 – 1.06) of hourly dose i.e. median (range) of 5.76 mg/kg/day (0.72 – 25.4 mg/kg/day divided in two or three doses) of phenobarbital (28 neonates, 15 children). Some subjects received also clonidine (2 neonate and 6 children), ketamine (5 children) or chloralhydrate (4 children).

Table 1. Baseline characteristic parameters of neonates and children, F-female, M-male, n-number, PMA-postmenstrual age.

Parameter	Neonates (N=30)	Children (N=18)
Sex (M/F)	17/13	13/5
PMA (weeks), median (range)	40 (37 – 42)	124 (48 – 730)
Weight (kg), median (range)	3.37 (2.45 – 4)	12 (3.4 – 50)
Apgar score 1 st min, median (range)	4.5 (0 – 10)	-
Apgar score 5 th min, median (range)	7 (1 – 10)	-
Apgar score 10 th min, median (range)	8 (3 – 10)	-
Spontaneous labor (n/N)	13/30	-

Analgesedative therapy, COMFORT score, SOS score

Neonates received sufentanil for significantly shorter period than children while their median cumulative sufentanil dose was numerically lower,

although not reaching the level of significance ($P=0.11$). No significant difference was found between neonates and children with respect to dosing and duration of midazolam therapy (Table 3). Hourly dose of sufentanil

Table 2. Allelic frequencies in the study population.

Gene	SNP	RS number	Observed variant allele frequency
<i>CYP3A5</i>	<i>C27289A</i> (<i>CYP3A5</i> *2)	<i>rs28365083</i>	0.00
	<i>A6986G</i> (<i>CYP3A5</i> *3)	<i>rs776746</i>	0.95
<i>OPRM1</i>	<i>A118G</i>	<i>rs1799971</i>	0.11
<i>ABCB1</i>	<i>C3435T</i>	<i>rs1045642</i>	0.50
<i>ABCB1</i>	<i>G2677T/A</i>	<i>rs2032582</i>	0.45
<i>PXR</i>	<i>G10799A</i>	<i>rs1054191</i>	0.15
<i>PXR</i>	<i>C-25385T</i>	<i>rs3814055</i>	0.37
<i>PXR</i>	<i>C-24113T</i>	<i>rs2276706</i>	0.37
<i>PXR</i>	<i>A7635G</i>	<i>rs6785049</i>	0.66
<i>COMT</i>	<i>C186T</i>	<i>rs4633</i>	0.49
<i>COMT</i>	<i>C408G</i>	<i>rs4818</i>	0.36
<i>COMT</i>	<i>G472A</i>	<i>rs4680</i>	0.50

Table 3. Analgosedative therapy in neonates and children, MDZ-midazolam, values in media (range), * $P < 0.05$ vs. children.

AS drug	Neonates (N=30)	Children (N=18)
<i>Patients on sufentanil</i>	26/30	17/18
<i>Patients on MDZ</i>	23/30	14/18
<i>Cumulative sufentanil dose (µg/kg)</i>	28.23 (5.10 – 280.40)	63.44 (4.50 – 918.52)
<i>Cumulative MDZ dose (mg/kg)</i>	12.63 (2.12 – 51.25)	12.47 (2.83 – 41.36)
<i>Duration of sufentanil perf. (h)</i>	109 (55 – 790)*	182 (22 – 885)
<i>Duration of MDZ perf. (h)</i>	105 (7 – 625)	111 (2 – 398)
<i>Sufentanil dose µg/kg/h</i>	0.24 (0.07 – 1.96)	0.35 (0.10 – 1.04)
<i>MDZ dose mg/kg/h</i>	0.11 (0.05 – 0.35)	0.14 (0.07 – 0.29)

Table 4. COMFORT and SOS score values in neonates and children, values in median (range), * $P < 0.05$ vs. children.

Scale evaluation	Neonates (N=30)	Children (N=18)
<i>SOS score</i>	1 (0 – 17)*	2 (0 – 12)
<i>COMFORT NEO/B score</i>	11 (2 – 26)	16 (5 – 31)
<i>COMFORT NEO/B >22/n</i>	2/2278	99/2008
<i>COMFORT NEO/B <11/n</i>	1117/2278	46/2008

was also similar in both populations.

Higher SOS score values were observed in the subgroup of children. COMFORT score <11 was registered in almost half of the neonates, whereas oversedation was relatively rare in children over 3 months. On the contrary, only exceptionally COMFORT score over 22 was registered in neonates (Table 4).

Genetic polymorphisms

Wild-type allele carriers in *COMT*rs4680 received significantly higher hourly dose of sufentanil [(0.25 µg/kg/h (0.07 – 1.18)], than variant homozygotes [(0.15 µg/kg/h (0.10 – 0.23); $P=0.04$]. There was a numerical trend towards higher hourly dose of sufentanil in *PXR*A7635G wt allele carriers [0.33 µg/kg/h (0.23 – 0.45)] compared with variant allele carriers

[0.21 µg/kg/h (0.07 – 1.18); $P=0.10$].

A trend towards significance was observed in midazolam dosing in *ABCB1C3435T* both wild-type allele carriers [*CC* + *CT*, 0.10 mg/kg/h (0.05 – 0.29)] and *CC* carriers [0.10 mg/kg/h (0.05 – 0.20)] and variant allele homozygotes [0.13 (0.08 – 0.35); $P=0.09$, $P=0.10$, respectively].

No association was observed between the candidate gene polymorphisms and COMFORT score values.

Withdrawal syndrome (WS)

Overall, 16 subjects developed withdrawal syndrome, 7 neonates and 9 children. All WS subjects received sufentanil. Significantly more analgesedative drugs per patient were used in the subjects, who suffered from WS compared with non-WS group. The analgesedative therapy, artificial ventilation and hospital stay were also significantly longer in the WS patients (Table 5).

Table 5. Results of therapy according to withdrawal syndrome. AS-analgesedation, AV-artificial ventilation, MDZ-midazolam, perf.-perfusion, values in median, range, * $P<0.05$ vs. WS group, ** $P<0.001$ vs. WS group.

Parameter	WS group (N=16)	Non-WS group (N=32)
Both sufentanil and MDZ (n/N)	15/16	20/32
Only sufentanil (n/N)	1/16	7/32
Only MDZ (n/N)	0/16	5/32
AS drug (n/patient)	4 (2 – 7)	3 (1 – 4)**
Days of AS therapy	21 (9 – 60)	6 (2 – 13)**
Days of AV	13.5 (7 – 81)	5 (3 – 12)**
Days of hospitalization	24 (10 – 81)	13 (3 – 29)**
Cumulative sufentanil dose (µg/kg)	88.60 (20.21 – 918.52)	21.71 (4.50 – 162.29)**
Cumulative MDZ dose (mg/kg)	18.22 (6.93 – 51.25)	9.94 (2.12 – 49.83)*
Duration of sufentanil perf. (h)	327 (136 – 885)	92 (22 – 211)**
Duration of MDZ perf. (h)	141 (2 – 625)	88 (7 – 232)*
Sufentanil dose (µg/kg/h)	0.33 (0.10 – 1.18)	0.23 (0.70 – 1.96)
MDZ dose (mg/kg/h)	0.105 (0.06 – 0.35)	0.12 (0.05 – 0.24)

Significantly higher median cumulative doses of both sufentanil and midazolam were received by WS patients, both in the overall population as well as in the neonate and children subgroups. Median sufentanil cumulative doses in neonates with and without WS were 81.58 µg/kg/h (33.83 – 208.40) and 20.55 µg/kg/h (5.10 – 162.29); $P<0.01$, respectively. The respective values for children were 95.61 µg/kg/h (20.21 – 918.52) and 34.20 µg/kg/h (4.50 – 91.69); $P<0.05$. Similar pattern was noted for median midazolam cumulative doses 20.38 mg/kg/h (10.63 – 51.25) and 10.57 mg/kg/h (2.12 – 49.83); $P<0.05$ in WS and non-WS groups, respectively. The respective values for children were 16.73 mg/kg/h (6.93 – 41.36) and 9.40 mg/kg/h (2.83 – 35.45); $P=0.13$.

Duration of therapy was also higher with WS compared with non-WS group. Median sufentanil treatment duration in neonates in WS and non-WS patients was 378 h (177 – 790) and 88.5 h (0 – 211);

$P<0.01$, respectively, while the median midazolam treatment duration was 184 h (105 – 625) and 82 h (7 – 219); $P<0.01$, respectively. The respective values for the children groups were 234 h (136 – 885) and 142 h (22 – 184); $P<0.01$ for sufentanil and 113.5 h (2 – 398) and 111 h (24 – 232); $P<0.45$ for midazolam. The multivariate analysis showed that the duration of sufentanil treatment gave the best explanation of development of withdrawal syndrome (0.81 sensitivity and 0.94 specificity), while the other explanatory variables were not significant. ROC curve analysis was performed on the duration of sufentanil treatment, the AUC was 0.96, $P=0.0039$. The cut-off value (risk value) of sufentanil treatment duration for ROC was not a single unambiguous value in case of Youden index, while smallest distance from ROC point with sensitivity and specificity equaled 1 gave the risk value of 177 h (7 days and 9 hours).

No association was found between genotypes

and WS development.

Discussion

Our prospective naturalistic study indicated that although the analgosedative therapy was closely monitored using validated scaling systems for both therapeutic efficacy and withdrawal syndrome, achievement of optimal analgosedation is challenging in many patients. Especially the neonate subgroup represented difficult to treat patient population. The COMFORT NEO scale values indicated that in half of the scoring times the neonates were oversedated, while insufficient sedation was rare. On the other hand the sedation in the children subgroup was well maintained since only 7 % of the score values indicated either insufficient sedation or oversedation. The incidence of withdrawal syndrome in both patient groups was high; it was diagnosed in 23 % of neonates and 50 % of children. The neonates received similar hourly doses of both drugs as children, while the treatment duration with sufentanil and sufentanil cumulative dose was lower in the neonate group. This dosing pattern likely explain the different clinical outcome with respect of sedative effect and WS between neonates and children, since the immaturity of the elimination pathways in neonates were likely responsible for the episodes of acute oversedation, when comparable doses were administered to neonates as to the children.

The duration of opioid administration, cumulative dose and number of medications used represented risk factors for the development of withdrawal syndrome. All the subjects, who received sufentanil therapy over 9 days, and, on the contrary, no subject with less than 5.5 days of the sufentanil therapy duration developed WS. Patients with WS also spent significantly longer period on artificial ventilation and in the hospital overall. We followed the symptoms of WS first after 5 days of analgosedation in agreement with previous studies. Previously, the incidence of WS approached 100 % in two studies in patients treated for more than 5 days (Bicudo *et al.* 1999, Fernandez-Carrion *et al.* 2013), whereas Franck *et al.* (2004) reported 86 % incidence. In the other published study by Sfoggia *et al.* (2013), the global incidence of 34 % for WS was reported, while the incidence in highest drug dose group reached 49 %. A recent study suggests that iatrogenic WS after only 3 days of sedation or analgesia may also occur in minority of patients (Da Silva *et al.* 2016). Our

estimate of cut off value of 7 days (177 h) of sufentanil treatment duration as a risk for development of WS may be considered as rather conservative estimate since we based our observation on relatively strict scoring system of Sophia Observational Scale.

The median sufentanil cumulative doses in our study were approximately 4 times higher in the WS group when compared with the non-WS patients and this difference was similar in neonates and children. The impact of sufentanil cumulative doses on the development of WS has not been studied so far, but since the opioid potency of sufentanil is considered 7-10 times higher in comparison with fentanyl (Li *et al.* 2015), it could be assumed, that the cumulative dose as a risk factor of WS shall be correspondingly lower. Our median values of 95.61 µg/kg (20.21 – 918.52) and 81.58 µg/kg (33.83 – 208.4) in the WS group in children and neonates, respectively, compare well with the mean cumulative fentanyl dose 0.98 mg/kg reported previously in patients with WS (Fernandez-Carrion *et al.* 2013). Further, Dominguez *et al.* (2003) found that fentanyl total dose ≥ 0.415 mg/kg (415 µg/kg) predicted withdrawal with 70 % sensitivity and 78 % specificity. In an earlier study in older children, higher fentanyl cumulative doses, but shorter treatment duration as a risk factor than in our study was reported (Katz *et al.* 1994). Cumulative fentanyl dose of 1.5 mg/kg or duration of perfusion for more than 5 days appeared to be a risk for the development of WS, whereas a cumulative fentanyl dose of >2.5 mg/kg (2,500 µg/kg) and >9 days of therapy was 100 % predictive (Katz *et al.* 1994). This difference may be probably attributed to different scoring systems used in both studies that may have different sensitivity to detect WS.

Median midazolam cumulative doses in the WS and non-WS groups of 18.22 (6.93 – 51.25) and 9.94 (2.12 – 49.83) mg/kg, in our study were within the range of previously reported values. In an early small study, cumulative MDZ dose of 60 mg/kg was reported to constitute a risk factor for WS (Fonsmark *et al.* 1999). However, in another study the median of midazolam equivalents per kg of body weight was 7.6 mg/kg (4.2 – 10.4) vs. 2.3 mg/kg (1.0 – 3.5) in patients at risk vs. patients at no risk for WS development; $P < 0.001$, respectively (Franck *et al.* 2008). In the SOS evaluation study (concomitant opioid-morphine), the cumulative dose of midazolam was 77.9 mg/kg (34.6 – 169.6) with 17 (9 – 27) days of exposure in the WS group and 34.8 mg/kg (16.9 – 71.8) with 9 days (6 – 14) of exposure

in non-WS group ($P < 0.0001$) (Ista *et al.* 2013). High variability of the midazolam dosing data with respect to WS development is likely to be due to differences in co-administered medications, especially from the analgesedative class, which is largely inconsistent among the published studies.

We have not found any significant association between candidate gene polymorphisms and clinical scoring either for the depth of analgesedation (COMFORT scale) or withdrawal syndrome. The *rs4680* SNP in *COMT* seems to affect the dosing of study drugs in our patients to achieve equal clinical response. Homozygotes for the variant allele received lower hourly dose than wild-type allele carriers. There are no data on relationship between *COMT* SNPs and sufentanil efficacy, however, this SNP has been frequently considered in patients treated with other opioids. Variant *COMT*rs4680 has shown to be associated with increased pain intensity scores but did not affect morphine or oxycodone consumption in postoperative studies (Kambur *et al.* 2013, Kolesnikov *et al.* 2011, Nielsen *et al.* 2015). In studies focused on chronic pain the variant allele was associated with higher morphine requirements when compared with wild-type homozygous subjects (Rakvag *et al.* 2005, Reyes-Gibby *et al.* 2007), but contradictory results have been obtained in other studies (Klepstad *et al.* 2011, Nielsen *et al.* 2015, Rakvag *et al.* 2008, Walter and Lotsch 2009).

We have observed a trend of lower hourly dose of sufentanil in *PXR A7635G* variant carriers compared to wild-type homozygotes. Experimental data suggest, that *PXR* activation increases P-glycoprotein (*ABCB1*) activity *in vivo* and tightens the blood-brain barrier to methadone, reducing the drug's CNS efficacy (Bauer *et al.* 2006). In theory, variant *PXR* with lower activity would alter the pain perception or enable decreased opioid dose.

ABCB1 3435TT homozygotes in our study

population received significantly higher mean hourly midazolam dose compared with wild-type homozygotes. Midazolam belongs among known *ABCB1* substrates, but one study did not find association between clinical response to midazolam, midazolam plasma levels and *ABCB1* SNPs (Byon *et al.* 2012, Tolle-Sander *et al.* 2003).

We acknowledge the basic limitation of our study, which are the rather limited number of patients and the non-interventional study design. It should be, however, emphasized that this is the first study directly focused on sufentanil-induced analgesedation and withdrawal syndrome in critically ill neonates and children and as such the descriptive nature of the study is highly relevant. Moreover, the study adds preliminary data of the pharmacogenetic background for sufentanil and midazolam related clinical effects in pediatric patients, although these need to be further confirmed.

Conclusions

Cumulative sufentanil and midazolam doses as well as duration of treatment represent risk factors for the development of WS. The sufentanil treatment duration of 177 h or higher is an independent risk for the development of WS. SNPs in the candidate genes of *COMT*, *PXR* and *ABCB1* may affect the dosing of analgesedative drugs, but this needs to be further investigated.

Conflict of Interest

There is no conflict of interest.

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