SUPPLEMENTAL SECTION

ABCC3 Genetic Variants are Associated with Postoperative Morphine-induced Respiratory Depression and Morphine Pharmacokinetics in Children

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Morphine Pharmacokinetic Model Development and Evaluation

A population pharmacokinetic model was developed for morphine using nonlinear, mixed effects modeling approach (NONMEM; version 7.2, ICON Dev. Soln., MD, USA) with PsN-Toolkit (version 3.5.3) as the interface. Data pre-processing, post-processing and visualization were performed using the statistical package R (version 2.15). A two compartment structural model, parameterized in terms of clearance (*CL)*, central volume of distribution (V_1), inter-compartmental clearance (Q), peripheral volume (V_2) of distribution, was used to describe the morphine concentration–time profiles. A delay compartment was incorporated in the model to describe the delay metabolite formation. The metabolite pharmacokinetics was modeled using an additional compartment for each metabolite and was parameterized in terms of formation clearance (FCL_{M3G} & FCL_{M6G}), volume of distribution (V_{M3G} & V_{M6G}) and clearance (CL_{M3G} & CL_{M6G}).

The effect of body size on the PK parameters was normalized using power law based weight scaling as below:

$$CL_{mean,WT} = CL_{popmean,70Kg}\left(\frac{WT}{70}\right)^{\gamma 1}$$

$$V_{mean,WT} = V_{popmean,70Kg}\left(\frac{WT}{70}\right)^{\gamma 2}$$

where $CL_{mean,70Kg}$ and $CL_{mean,70Kg}$ are population mean clearance and volume for individuals weighing 70 Kg. Allometric weight exponents were fixed to 0.75 and 1.0 for clearances and volumes of distribution except for parameters of interest (morphine CL, CL_M and metabolite formation clearances, FCL_{M3G} & FCL_{M6G}). Between-subject variability was described by a log-normal parameter distribution. A proportional error model and a linear additive error model on log transformed concentrations were evaluated to account for unexplained residual variability with $\boldsymbol{\varepsilon}^2_{M}$, $\boldsymbol{\varepsilon}^2_{M3G}$ and $\boldsymbol{\varepsilon}^2_{M6G}$ representing the corresponding variances for morphine, M3G and M6G concentrations respectively. Initial comparison between different models was based on a drop of Objective Function Value (OFV). A drop in OFV of

3.84 ($p<0.05$, Degree of freedom =1) between nested models was considered statistically significant. In addition to OFV improvement, models were evaluated using diagnostic goodness-of-fit plots examined to identify possible trends suggestive of model misspecification, η-distribution histograms were examined to ensure unimodality. The final model stability was evaluated by refitting the model to 1000 randomly sampled bootstrap datasets.

Morphine and metabolite pharmacokinetic model

Morphine and metabolite PK were described using a multi-compartment model, details of which have been described in detail in an earlier report (1). Briefly, morphine PK for the tonsillectomy cohort were described using a 2 compartmental model with inter-individual variability on morphine clearance (CL). Morphine metabolite formation PK was described using a single compartment each with inter-individual variability on the formation clearances (FCL_{M3G} and FCL_{M6G}). A similar structural model sufficiently described the PK profiles from the Spine surgery cohort. A proportional error model best captured intra-individual error in the tonsillectomy cohort while a linear additive error model on log transformed concentration best captured intra-individual error for the spine surgery cohort.

Model parameters of the PK models from the two studies are presented in the Table S1. Inter-individual variability for morphine clearance was not included in the model for spine study as it was estimated with high uncertainty (76% Relative Standard error (RSE)) and high shrinkage (46%). Across both studies, plots of post-hoc estimates of individual metabolite formation clearances with weight suggested there was substantial over-prediction at lower weights and under-prediction at higher weights when formation clearances were scaled using an exponent of 0.75. Exponent estimates for scaling formation clearances to body weight for both metabolites were found to be significantly higher than 0.75, similar in both studies; and hence was kept as the part of the final model.

References

(1) Venkatasubramanian, R. *et al.* ABCC3 and OCT1 genotypes influence pharmacokinetics of morphine in children. *Pharmacogenomics* **15**, 1297-309 (2014).