ARTICLE

Variant-based heritability assessment of dexmedetomidine and fentanyl clearance in pediatric patients

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Abstract

Despite complex pathways of drug disposition, clinical pharmacogenetic predictors currently rely on only a few high effect variants. Quantification of the polygenic contribution to variability in drug disposition is necessary to prioritize target drugs for pharmacogenomic approaches and guide analytic methods. Dexmedetomidine and fentanyl, often used in postoperative care of pediatric patients, have high rates of inter-individual variability in dosing requirements. Analyzing previously generated population pharmacokinetic parameters, we used Bayesian hierarchical mixed modeling to measure narrow-sense (additive) heritability (h^2_{SNP}) of dexmedetomidine and fentanyl clearance in children and identify relative contributions of small, moderate, and large effect-size variants to h^2_{SNP}. We used genome-wide association studies (GWAS) to identify variants contributing to variation in dexmedetomidine and fentanyl clearance, followed by functional analyses to identify associated pathways. For dexmedetomidine, median clearance was 33.0 L/h (interquartile range [IQR] 23.8–47.9 L/h) and h^2_{SNP} was estimated to be 0.35 (90% credible interval 0.00–0.90), with 45% of h^2_{SNP} attributed to large-, 32% to moderate-, and 23% to small-effect variants. The fentanyl cohort had median clearance of 8.2 L/h (IQR 4.7–16.7 L/h), with estimated h^2_{SNP} of 0.30 (90% credible interval 0.00–0.84). Large-effect variants accounted for 30% of h^2_{SNP}, whereas moderate- and small-effect variants accounted for 37% and 33%, respectively. As expected, given small sample sizes, no individual variants or pathways were significantly associated with dexmedetomidine or fentanyl clearance by GWAS. We conclude that clearance of both drugs is highly polygenic, motivating the future use of polygenic risk scores to guide appropriate dosing of dexmedetomidine and fentanyl.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

There is wide inter-individual variation in pharmacokinetics of dexmedetomidine and fentanyl. Genomic contribution to this inter-individual pharmacokinetic variation for both drugs remains poorly understood, as candidate gene analyses have failed to identify specific genes explaining a significant portion of the variability.

WHAT QUESTION DID THIS STUDY ADDRESS?

How much of the inter-individual variability in dexmedetomidine and fentanyl clearance can be attributed to common single nucleotide variation in the genome? In addition, within this heritable component, what proportion can be attributed to variants with individually small, medium, or large effects?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Dexmedetomidine and fentanyl clearance are moderately heritable and highly polygenic traits, with small- and moderate-effect variants accounting for the majority of estimated heritability.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Our findings of polygenic contribution to dexmedetomidine and fentanyl clearance highlight the importance of using genome-wide approaches for the investigation of pharmacologic phenotypes. Our study motivates the application of heritability analyses to additional pharmacokinetic datasets. Our findings demonstrate the need to assemble much larger cohorts that will enable the development, validation, and eventual implementation of polygenic risk scores for genetically-informed drug dosing in clinical practice.

INTRODUCTION

Precision medicine, and specifically the individualization of treatment decisions based on genetic variation (pharmacogenomics), is a rapidly evolving area of clinical care. Clinical pharmacogenomics is generally limited to the use of well-studied genetic variants with high impact on drug outcomes. For example, the enzyme thiopurine S-methyltransferase plays a vital role in metabolism of thiopurine drugs, and up to 10% of individuals in different populations are characterized as poor or intermediate metabolizers. In many clinical settings, genetic testing is performed to identify those with decreased enzyme activity prior to initiating therapy with a thiopurine agent, as alternate drugs or greatly reduced doses may be warranted to avoid severe toxicity.[1–3] More recently, a genome-wide association study (GWAS) identified variants in a second gene, *NUDT15*, contributing to thiopurine toxicity, leading to the addition of *NUDT15* variants to pharmacogenomic tests.[1,4] However, variants in these two genes are not fully predictive of thiopurine toxicity.[3] To more fully predict outcomes for drugs with residual variability or where no single pharmacogene has been identified, there is increasing focus on developing polygenic predictors of drug disposition and response.[5,6]

Dexmedetomidine, an α_2-agonist, and fentanyl, an opioid, are frequently used post-operatively in pediatric intensive care units (ICUs) to achieve sedation and analgesia.[7–10] Both are commonly dosed via continuous intravenous infusions using fixed weight-based rates, which is suitable for some patients but might require titration due to over- or undersedation for others. Importantly, both

over- and undersedation can be harmful to pediatric ICU patients. Oversedation has been associated with bradycardia, respiratory depression, increased need for mechanical ventilation, and prolonged ICU stays, whereas undersedation increases the risk for agitation, patient self-injury, and anxiety. Both over- and undersedation have been reported to lead to development of delirium, withdrawal syndromes, neuromuscular weakness and atrophy, and post-traumatic stress disorder.[11]

The need for dosing adjustments of dexmedetomidine and fentanyl to maintain appropriate sedation is due in part to well-described inter-individual variation in pharmacokinetics. For both drugs, previous studies have shown wide variation among individuals in drug absorption, bioavailability, and clearance.[8,12–15] Specifically, inter-individual variations in drug clearance rates in pediatric ICU patients have been reported as high as three-fold for dexmedetomidine[16] and 10-fold for fentanyl.[17] Pharmacogenomics research on dexmedetomidine and fentanyl pharmacokinetics has focused primarily on genes encoding enzymes known to play important roles in drug metabolism; these include cytochrome P450 (CYP) 2A6 and glucuronidation enzymes UGT1A4 and UGT2B10 for dexmedetomidine and CYP3A4 and CYP3A5 for fentanyl. To date, genes that have been identified explain little of the inter-individual variability observed for clearance of both drugs.[18–20] The broader polygenic contribution to dexmedetomidine and fentanyl clearance has not been explored.

Genome-wide approaches, including GWAS and Bayesian nonlinear modeling, take a broader approach by looking at the contribution of variants across the genome to a phenotype of interest. GWAS analyses individually

examine millions of common genetic variants to identify those associated with a given phenotype.[21] Bayesian non-linear models can quantify the collective contributions of large numbers of single nucleotide polymorphisms (SNPs) and estimate narrow-sense heritability (h^2_{SNP}), the proportion of phenotypic variation that can be attributed to additive influences of SNPs.[22] In contrast to GWAS-based estimations of h^2_{SNP}, Bayesian modeling can account for linkage disequilibrium by assuming that not all variants will have a non-zero effect on the phenotype. The estimated h^2_{SNP} can also be subdivided into relative contributions from genes with individually small, moderate, or large effects on the phenotype of interest. These genome-wide approaches are complementary and frequently used in assessment of nonpharmacologic phenotypes, but their application within pharmacogenomics has been limited to date. We previously demonstrated validity of using one Bayesian method to explore pharmacologic outcomes, even when applied to small sample sizes,[23] and GWAS approaches have successfully detected pharmacogenomic signals despite small cohorts in a few examples where effect size of the variant(s) is very large,[24,25] including the *NUDT15* association to thiopurine toxicity, as described above.[1,4]

In this study, we used the results of previously performed population pharmacokinetic analyses[16,17] and deployed polygenic approaches to assess genomic contribution to dexmedetomidine and fentanyl clearance in children after cardiac surgery. We used BayesR,[26–28] an established Bayesian hierarchical analysis method, to estimate h^2_{SNP} of dexmedetomidine and fentanyl clearance and assess the relative contribution of small-, moderate-, and large-effect variants. We hypothesized that common genomic variation contributes substantially to inter-individual variation in these phenotypes and that many variants with small effects on the phenotypes would cumulatively account for a greater proportion of heritability than few variants with large effects. We additionally assessed for specific variants that may be associated with dexmedetomidine or fentanyl clearance in our datasets using GWAS.

METHODS

Study design and data collection

The parent study supporting participant recruitment, specimen collection, and data collection was approved by the Vanderbilt University Medical Center Institutional Review Board and has been previously described in detail.[29] Pediatric patients undergoing surgery for congenital heart disease were voluntarily enrolled in this study via written parental informed consent and, when appropriate,

informed assent from the patient. Enrollment of participants into the parent study began in 2007 and is ongoing; the genomic analyses presented here used data collected from April 2013 to October 2017. All participants were admitted to the pediatric cardiac ICU following surgery and underwent routine clinical care as determined by the primary clinical team, including selection and dosing of all medications. Study participants provided a blood or saliva sample for DNA extraction, and drug concentrations were measured from remnant blood samples collected during the course of clinical care. Participants were excluded from analysis if their surgery was canceled, if they had no genotype data available, if they did not survive to hospital discharge, or if they required extracorporeal membrane oxygenation postoperatively.

Study data were collected and stored using REDCap, a secure web application hosted at Vanderbilt University.[30] Medical history and demographic data were documented by the study team upon study enrollment. Surgical and clinical data were extracted from the electronic health record (EHR) by the study team during the hospital stay. Drug data, including dosing and times of administration, were extracted from the EHR and the Vanderbilt Enterprise Data Warehouse and standardized using the EHR2PKPD system.[31]

This study was reviewed by the Institutional Review Board at Vanderbilt University Medical Center and determined to constitute non-human subject research.

Drug concentration measurement and clearance calculations

The primary outcomes used for analysis were the clearances estimated per individual for each of the two target drugs, as previously described.[16,17] Briefly, dexmedetomidine and fentanyl dosing data were extracted from the EHR and drug concentrations were measured in remnant plasma specimens collected for research purposes using high-throughput tandem mass spectrometry. Measured drug concentrations, drug dosing data, and covariate data collected for the parent study were used to generate a population pharmacokinetic model to estimate clearance of each drug, using Monolix 2021R. A two-compartment model was selected as the base model for both drugs, and covariate modeling was performed using prespecified covariates.

For dexmedetomidine, the following covariates were considered in the population pharmacokinetic modeling based on previous research and biologic plausibility: *UGT1A4*, *UGT2B10*, and *CYP2A6* variants, body weight, postnatal age, postmenstrual age, sex, Society of Thoracic Surgeons-European Association for Cardio-Thoracic

Surgery (STAT) Congenital Heart Surgery Mortality score, cardiac bypass time, length of ICU stay, and serum creatinine. Of these, only body weight and postmenstrual age significantly improved model fit and remained in the final covariate model.[16]

For fentanyl, CYP3A metabolizer status (based on *CYP3A45* genotype), body weight, postnatal age, postmenstrual age, sex, patient-reported race, serum creatinine, STAT score, cardiac bypass time, concomitant CYP3A inducers and inhibitors, and the first five principal components of ancestry were considered in the pharmacokinetic model. Body weight, postnatal age, STAT score, and CYP3A metabolizer status were included in the final covariate model.[17]

For the final model for each drug, patient covariates, estimated model parameters, and the conditional mode of the individual random effect on clearance were used to calculate the estimated clearances used in this analysis.

Genotyping, quality control, and processing

Genome-wide SNPs were derived from either the Affymetrix Axiom Precision Medicine Research Array or Precision Medicine Diversity Array (Thermo Fisher Scientific) using Genome Reference Consortium Human Build 37 (GRCh37/hg19). The BayesR method is sensitive to population substructure, meaning that inclusion of individuals across different ancestries could confound analyses and result in spurious findings. Therefore, analyses were restricted to individuals of European ancestry as this population was the most prominent in our cohorts and the only population for which sample size was sufficient for BayesR. To do this, within each cohort (dexmedetomidine and fentanyl), genotype data from participants was combined with that of Hapmap European (Utah residents with Northern and Western European ancestry from the CEPH collection), Asian (Han Chinese in Beijing, China and Japanese in Tokyo, Japan), and African (African ancestry in Southwest USA; Luhya in Webuye, Kenya; Maasai in Kinyawa, Kenya; and Yoruba in Ibadan, Nigeria) populations to assess ancestry. Principal component (PC) analyses were performed, and, because individuals with self-reported white race overlapped heavily with the HapMap European population and segregated from Asian and African HapMap populations, final analyses were restricted to participants within three standard deviations of the mean of PC1 and PC2 of self-reported white participants (Figure S1). This approach effectively restricted our analyses to participants of European ancestry.

Genotype data were imputed to the TOPMed reference panel R2 using the TOPMed Imputation Server. Imputed gene dosages were converted to hard calls using PLINK2 and filtered by info scores greater than 0.8. SNPs were included if they met the following quality control (QC) parameters:

minor allele frequency greater than 1% within the cohort, SNP genotyping rate greater than 98%, and Hardy–Weinberg equilibrium greater than 10^{-6}. Sex chromosomes were excluded from analysis. Individuals were removed if they had a genotype call rate less than 98%, discrepancy between the genetically-estimated sex and the sex assigned in the database, or a high degree of genetic relatedness to another sample using identity-by-descent analysis (Figure S2).

For all individuals who passed QC, any clearance values greater than the median $+ 3 \times$ interquartile range [IQR] or less than the median $- 3 \times$ IQR were removed as outliers from the data set for analysis.

BayesR analysis

Following QC of data sets as described above, SNPs in high linkage disequilibrium ($r^2 > 0.9$) were removed. Residuals were then calculated from clearance values incorporating the first 20 PCs of ancestry. Residuals were used in the final Bayesian analysis because the BayesR software is not able to include covariates (Figure S2).

For each cohort, BayesR (version 1), a hierarchical Bayesian mixture model using Markov Chain Monte Carlo estimation, was used to fit all SNPs simultaneously to a model predicting drug clearance in order to give unbiased estimates of SNP effect sizes.[26,27] BayesR uses k different normal distributions to model the prior distribution of SNP effects, where the sum of the mixture proportions is constrained to unity. As in previous analyses, we set $k = 4$,[27] where each component was modeled as a normal distribution with a mean of 0 and a variance of 0, 0.01%, 0.1%, and 1% of the additive genetic variance, respectively, as shown: $p\left(\beta | \pi, \sigma_g^2\right) = \pi_1 N\left(0, 0 \times \sigma_g^2\right) + \pi_2 N\left(0, 0.0001 \times \sigma_g^2\right) + \pi_3 N\left(0, 0.001 \times \sigma_g^2\right) + \pi_4 N\left(0, 0.01 \times \sigma_g^2\right)$. In this model, β signifies a mixture of four zero-mean normal distributions of SNP effects with a fixed relative variance for each mixture component, π represents mixture proportions constrained to sum to unity, and σ_g^2 is additive genetic variance explained by SNPs. The mean and variance of the first component is set to zero to account for sparseness in the model. The components *k1*, *k2*, *k3*, and *k4* are referred to as no-effect, small-effect (each SNP explaining 0.01% of the variance), moderate-effect (each SNP explaining 0.1% of the variance), and large-effect SNPs (each SNP explaining 1% of the variance), respectively. To estimate σ_g^2, the algorithm uses a Gibbs scheme to sample values from each unknown parameter's posterior distribution.[26,27]

Narrow-sense heritability (h_{SNP}^2) was calculated as $h_{SNP}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$ where σ_g^2 and σ_e^2 (residual variance) were estimated by BayesR. The number of iterations was increased from the default 20,000 to 60,000 in our analyses to allow for convergence given the small sample sizes in our data

sets. Default settings were used for all other prior distribution parameters. The 90% highest density credible intervals were calculated. Results were processed using custom R scripts.

Genomewide association studies

Dexmedetomidine and fentanyl cohorts used for GWAS were identical to those input into BayesR, and the same quality control steps were performed (Figure S2). SNPs in high linkage disequilibrium ($r^2 > 0.7$) were removed to reduce computational time, leaving 986,408 and 1,080,019 SNPs for final analysis for dexmedetomidine and fentanyl, respectively. PLINK software (versions 1.9 and 2.0) using linear regression was utilized to test each SNP for association with the continuous drug clearance phenotype. PLINK software was also used to extract the first 10 PCs, which were used as covariates.[32] Results were processed using custom R scripts, and Manhattan and quantile-quantile (Q-Q) plots were generated using the qqman package (version 0.1.8).

We performed post hoc functional analyses of variants with association p values less than or equal to 1×10^{-4} by GWAS using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) functional annotation tool.[33] Gene lists for each data set (dexmedetomidine and fentanyl) included all genes within 100,000 base pairs of those GWAS SNPs with association p values less than or equal to 1×10^{-4}. Functional analyses using DAVID were restricted to gene ontology (molecular function, biological process, and cellular component), pathways (KEGG pathway, BioCarta, and biological biochemical image database), and diseases (OMIM and UniProt).

RESULTS

Study population characteristics

Characteristics of the dexmedetomidine and fentanyl cohorts are shown in Table 1 and summaries of surgical procedures incurred by participants in each cohort are shown in Table S1. The dexmedetomidine cohort included 354 participants, as described previously.[16] Due to sensitivity of the BayesR method to population substructure, 70 individuals were removed due to genotypes consistent with non-European ancestry, classified as individuals with PCs outside three standard deviations of the mean PC1 or PC2 of self-reported white participants (Figure S1) and one individual was removed due to high relatedness. Three additional individuals were excluded from analysis due to identification of log-transformed

TABLE 1 Dexmedetomidine and fentanyl cohort characteristics.

	Dexmedetomidine ($n = 280$)	Fentanyl ($n = 346$)
Age (months)	20.7 (5.2–72.8)	8.5 (3.7–60.1)
Weight (kg)	9.6 (6.1–19.6)	7.7 (5.2–17.1)
Male sex	145 (51.7%)	184 (53.2%)
Self-reported white race	277 (98.9%)	340 (98.3%)
Length of ICU stay (days)	3 (2–7)	4 (2–7)
Cardiac bypass time (h)	1.6 (1.1–2.4)	1.7 (1.1–2.5)
STAT score	2 (1–3)	2 (1–3)
Clearance (L/h)	33.4 (23.5–47.8)	8.1 (4.6–16.6)

Note: Data are median (interquartile range) for continuous variables and NN (%) for categorical variables.

Abbreviations: ICU, intensive care unit; STAT, The Society of Thoracic Surgeons-European Association for Cardio-Thoracic Surgery.

clearance values as outliers; therefore, a total of 280 subjects were included for final analysis of dexmedetomidine clearance. The median postnatal age at the time of surgery was 20.7 months (IQR 5.2–72.8) and the median body weight was 9.6 kg (IQR 6.1–19.6). Median length of ICU stay was 3 days (IQR 2–7), and median cardiac bypass time was 1.6 h (1.1–2.4). Median individual dexmedetomidine clearance, as estimated by population pharmacokinetic modeling,[16] was 33.4 L/h (IQR 23.5–47.8).

The previously described fentanyl cohort included 434 participants[17]; 87 subjects were removed due to being outside of three standard deviations of the first two PCs of self-reported white participants (Figure S1) and one individual was removed due to high relatedness, leaving a total of 346 individuals for final analysis. The median postnatal age at the time of surgery was 8.5 months (IQR 3.7–60.1) and the median body weight was 7.7 kg (IQR 5.2–17.1). Median length of ICU stay was 4 days (IQR 2–7), and median cardiac bypass time was 1.7 h (1.1–2.5). Median fentanyl clearance, as estimated by population pharmacokinetic modeling,[17] was 8.1 L/h (IQR 4.6–16.6).

Heritability estimates of dexmedetomidine and fentanyl clearance

Dexmedetomidine clearance values from 280 individuals were log-transformed and adjusted for the first 20 PCs (Figure S3). Covariates that were considered for inclusion in the population pharmacokinetic models used to calculate drug clearance values were not considered for inclusion in the BayesR model to estimate heritability. Residual values and 1,121,432 genetic variants were

input into the BayesR model. The estimated additive genetic variance (σ_g^2) was 0.13 with a residual variance (σ_e^2) of 0.24, resulting in a calculated h_{SNP}^2 of 0.35 with a 90% highest density credible interval of 0.00–0.90 (Figure 1). As described above, the estimated h_{SNP}^2 was further subdivided into relative contributions from small-, moderate-, and large-effect variants, which account for 0.01%, 0.1%, and 1% of σ_g^2, respectively. For dexmedetomidine, 36% of the estimated h_{SNP}^2 was accounted for by 2997 small-effect variants, 26% was accounted for by 264 moderate-effect variants, and 38% was accounted for by 57 large-effect variants. Therefore, over half of the estimated h_{SNP}^2 of dexmedetomidine clearance was attributed to small and moderate effect-size variants (Figure 1).

For fentanyl, clearance values from 346 individuals were adjusted using the first 20 PCs (Figure S3) and analyzed with 1,222,265 genetic variants using BayesR. The estimated σ_g^2 was 0.25, σ_e^2 was 0.61, and calculated h_{SNP}^2 was 0.29 with a 90% highest density credible interval of 0.00–0.84 (Figure 1). Relative contributions from small-, moderate-, and large-effect variants were as follows: 29% of estimated h_{SNP}^2 was accounted for by 2420 small-effect variants, 42% was accounted for by 365 moderate-effect variants, and 38% was accounted for by 55 large-effect variants. As with dexmedetomidine, more than half of the estimated h_{SNP}^2 of fentanyl clearance was attributed to small and moderate effect-size variants (Figure 1).

Genome-wide association analysis of dexmedetomidine and fentanyl clearance

Given the substantial heritability of clearance for both drugs and small number of large-effect variants for each drug, we performed GWAS to identify any variants significantly associated with the outcomes. To our knowledge, these are the first GWAS of dexmedetomidine and fentanyl clearance performed to date. Q-Q plots demonstrated

mild inflation of smaller p values observed for dexmedetomidine and a relatively uniform distribution for fentanyl (Figure S4). For both dexmedetomidine and fentanyl clearance, GWAS failed to identify any variants meeting the genome-wide statistical significance threshold of 5×10^{-8} (Figure 2).

Results of the GWAS analysis are reported in Tables S2 and S3. For dexmedetomidine clearance, the variants within 100,000 base pairs of coding regions with the strongest statistical associations (smallest p values) were: *CACNB2*, a voltage-gated calcium channel; *LRRC8C*, which facilitates cAMP-cGMP import across plasma membranes; and *RAP1A*, a member of the RAS oncogene family (Table S2). For fentanyl clearance, the variants within coding regions with the smallest p values were: *CD58*, involved in adhesion and activation of T cells; *DYSF*, which contributes to calcium-mediated muscle contraction; and *ORAI2*, which plays a role in store-operated calcium channel activity (Table S3).

We subsequently used the DAVID functional annotation tool to perform post hoc functional analyses on the variants with GWAS p values less than or equal to 1×10^{-4} within 100,000 bp of known genes (Tables S4 and S5). These analyses did not identify any statistically significant pathways or functions enriched for clearance of either drug. For dexmedetomidine, the top three gene ontology categories were anchoring junction, specific granule membrane, and integral component of plasma membrane (Table S6). Exogenous drug catabolism was among the top 10 pathways identified, which was associated with a total of four genes: *CYP2A13*, *CYP2A7*, *CYP2F1*, and *NOS1*. *CYP2A13*, *CYP2A7*, and *CYP2F1* were all located within 100,000 base pairs of a single SNP on chromosome 19. For the fentanyl clearance data set, membrane, protein destabilization, and positive regulation of protein serine/threonine kinase activity were the top three gene ontology categories that emerged. No pathways related to drug metabolism were enriched for the fentanyl data set (Table S7).
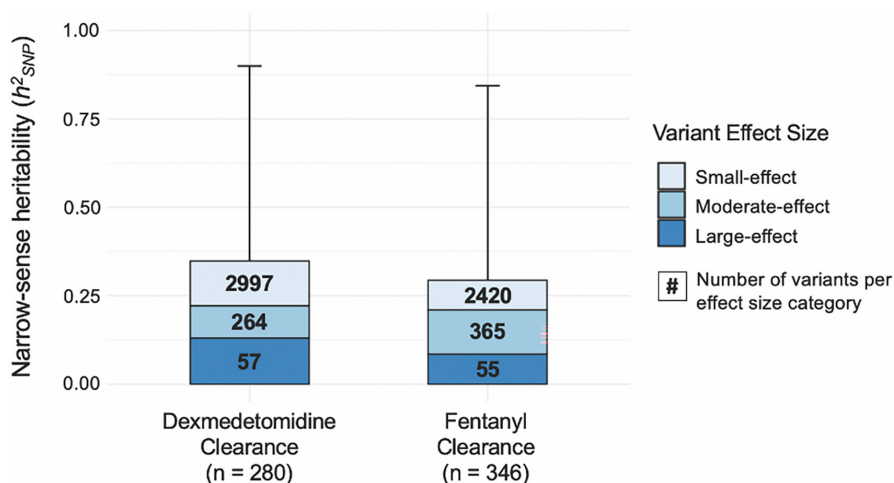

FIGURE 1 Narrow-sense heritability (h_{SNP}^2) estimates of dexmedetomidine and fentanyl clearance. Total h_{SNP}^2 is further subdivided into relative contributions from large-effect (dark blue), moderate-effect (medium blue), and small-effect (light blue) variants. Numerical values within each effect size box represent the number of variants that comprise each effect size category. Error bars represent 90% high-density credible intervals for h_{SNP}^2.
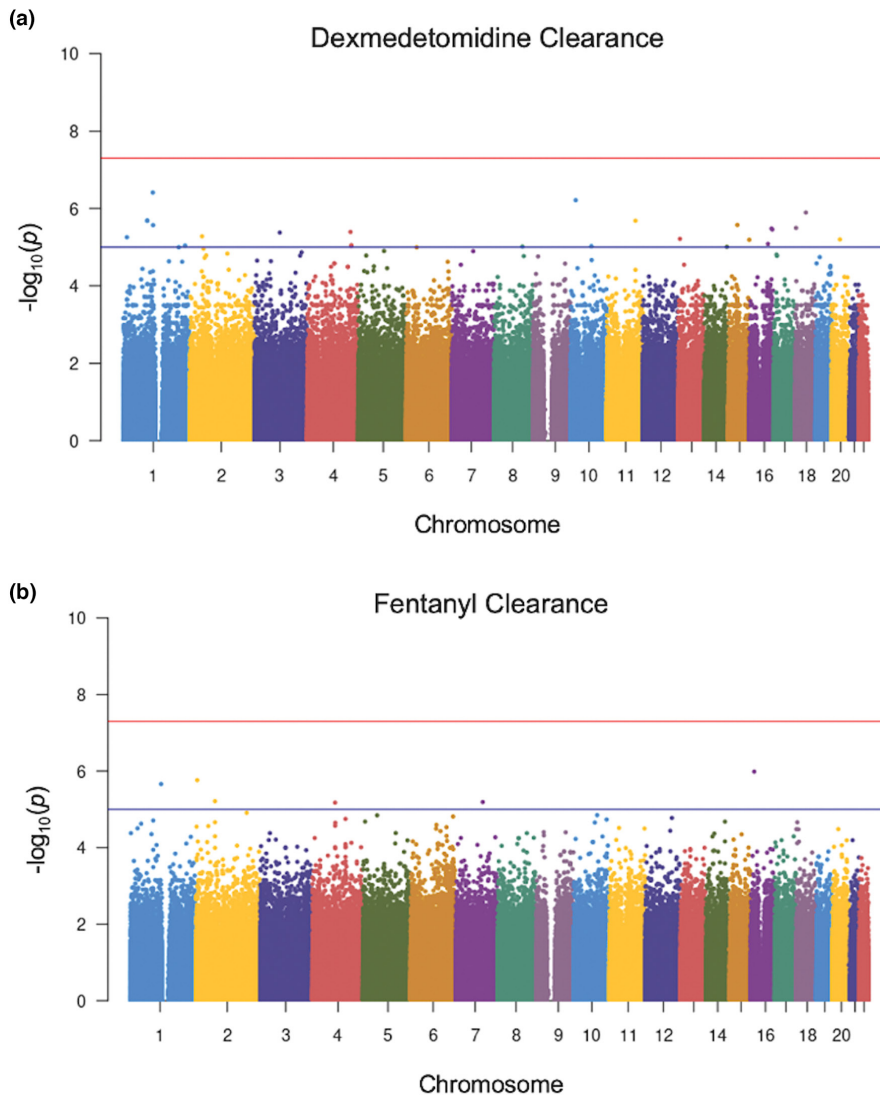
(a)

(b)

FIGURE 2 Manhattan plots showing genome-wide association study results for dexmedetomidine (a) and fentanyl (b) clearance. For both plots, all genetic variants are shown as individual data points ordered by chromosome across the x-axis. The y-axis represents the negative log of the associated p value for each variant. The red line represents the standard genome-wide significance threshold of 5×10^{-8}. The blue line at 1×10^{-5} is suggestive of association. No variants in either the dexmedetomidine or fentanyl clearance analysis crossed this threshold to reach statistical significance.

DISCUSSION

In this study, we sought to explore the genomic contribution to variation among pediatric patients in clearance of two drugs commonly used after cardiac surgery: dexmedetomidine and fentanyl. Our data verify the clinical observation of large inter-individual variability in drug clearance, as our pharmacokinetic analyses demonstrated an approximately two-fold difference between the first and third quartile of estimated dexmedetomidine clearance and an almost four-fold difference for fentanyl clearance, consistent with previous estimates.[16,17] We used the clearance data, coupled with genome-wide genotype data, to employ a Bayesian hierarchical modeling methodology. Given that both h^2_{SNP} estimations were greater than 0.25, inter-individual variation in clearance of dexmedetomidine and fentanyl in this population is moderately heritable. Further, the observation that less than 40% of h^2_{SNP} is attributable to large-effect variants suggests that these traits are highly polygenic. The credible intervals for our observations are wide, which may be improved with larger sample sizes. These results suggest that, for both drugs, many genes with individually smaller effect sizes combine to comprise a substantial genomic contribution to drug clearance. Therefore, polygenic approaches combined with large sample sizes are necessary to gain a comprehensive understanding of genetic contribution to the individual pharmacokinetics of these drugs, which may ultimately be utilized, for example in the form of polygenic risk scores, in clinical practice to individualize therapy.

Although less than 40% of the estimated heritability for both dexmedetomidine and fentanyl clearance was attributed to variants with large effect sizes, their estimated contribution to heritability of both drugs was substantial. We therefore utilized GWAS in an attempt to identify any SNPs that were significantly associated with dexmedetomidine or fentanyl clearance. These analyses failed to identify any Bonferroni-corrected statistically significant SNPs for clearance of either drug. Secondary functional analyses of genes with p values smaller than 1×10^{-4} using

the DAVID functional annotation tool also did not pinpoint specific pathways or processes that were enriched within our data sets. For dexmedetomidine clearance, exogenous drug catabolism and the epoxygenase P450 pathway were among the top functional hits. However, neither of these pathways reached statistical significance and each involved only a few genes, all of which were located near a single variant identified by GWAS. Furthermore, these enzymes have not been previously reported to play a role in dexmedetomidine metabolism. For fentanyl clearance, no pathways or processes related to drug metabolism were enriched. In agreement with the results of the Bayesian analysis, these findings also suggest a highly polygenic contribution of the genome to dexmedetomidine and fentanyl clearance and support continued efforts toward utilization of polygenic approaches to investigate the pharmacokinetics of both drugs. It is also important to consider that drug metabolism and distribution are dependent on pathways across many tissue types[34,35] and are highly variable across the lifespan.[36,37] Therefore, future efforts aimed at defining pathways and predictive models across different tissue types and different ages may aid in the identification of novel genetic associations with drug outcomes.

Taken together, our data motivate collection of larger and more ancestrally diverse cohorts of dexmedetomidine- and fentanyl-exposed individuals to develop and validate polygenic predictive models for these drugs, incorporating effects of many genes with a spectrum of effect size. Such predictors are now available for nonpharmacologic phenotypes such as type 2 diabetes,[38] coronary artery disease,[39,40] neurodegenerative disease,[41,42] and some cancers.[43–45] Polygenic risk predictors have been demonstrated to be especially useful for those individuals at the tail ends of the distribution for a given phenotype.[46] Applied to dexmedetomidine and fentanyl clearance, polygenic risk scores may be most useful for identifying individuals at the highest risk for undersedation or oversedation with standard drug dosing regimens secondary to differences in drug clearance. These approaches may be useful across a wide range of drugs with complex pharmacokinetic and/or pharmacodynamic pathways. Estimations of h^2_{SNP} for drug traits may help identify the best target drugs for genomic approaches (i.e., those with high heritability).

Our study was limited by the application of modeling methodologies traditionally used with large sample sizes, such as Bayesian modeling and GWAS, to our small cohorts. This limitation likely resulted in decreased precision and wide credible intervals in our Bayesian analyses; however, Bayesian modeling has been previously used for pharmacogenomic analyses with similarly sized data sets.[23] Additionally, it has been demonstrated that in polygenic phenotypes accounted for by primarily small-effect variants, as is true for both of our phenotypes, large sample sizes are required to detect significant variants with GWAS.[47] Therefore, given the small sample sizes, our GWAS analyses may be underpowered to detect the statistical association of some genes with our phenotypes and may have missed potentially significantly associated variants. Furthermore, development of clinically useful polygenic risk scores requires data sets much larger than those commonly available for pharmacologic outcomes. To combat this persistent limitation of pharmacogenomic research, we advocate for continued efforts toward curation of large data sets. To do so would require building scalable, sharable data structures and collaborative efforts both within and across international borders. In today's era, with the ubiquity of EHRs and advent of high throughput analytic techniques, such collaboration is both achievable and worthwhile.

Another limitation inherent in heritability studies is the need to restrict analyses to individuals of a single ancestry. Studies conducted in the United States and Europe often restrict analyses to European ancestry, and, as a result, the majority of publications in genomics include only individuals of European descent.[48,49] Our study was limited in a similar manner as our cohort was relatively small and currently lacks sufficient numbers of individuals of non-European ancestries to perform analyses in other ancestries. It is paramount that we prioritize diversity within pharmacogenomics research; doing so will ensure applicability of findings across populations and foster new discoveries. We are continuously enrolling subjects in this cohort and hope to soon build large enough sample sizes to repeat these analyses for individuals of other ancestries. Additionally, methodological developments in datasets of diverse and complex ancestries would enable more ancestrally inclusive studies that may reveal important differences in inter-individual drug variability.

Finally, both a limitation of our current study and an opportunity for future investigation is the application of these same methodologies to diverse pharmacodynamic outcomes. For dexmedetomidine and fentanyl, such outcomes may include standardized sedation scores. These analyses may identify a unique set of genes that influence the response to dexmedetomidine and fentanyl in this population. In order to be successful, these strategies must account for differences in pharmacokinetics, either through the use of robust modeling or through the measurement of drug concentrations.

In summary, our study demonstrates that the genome contributes substantially to inter-individual variation in dexmedetomidine and fentanyl clearance in the pediatric cardiac surgery population. Clearance of both drugs was polygenic, with small and moderate effect-size variants

comprising the majority of estimated heritability, whereas no individual variant had a significant association with clearance of either drug. Taken together these findings motivate the development of polygenic predictors of drug responses with the ultimate goal of implementing genomically informed drug dosing into clinical practice.

AUTHOR CONTRIBUTIONS

M.L.S., A.M., N.T.J., M.L.W., L.C., and S.L.V.D. wrote the manuscript. M.L.S., A.M., N.T.J., M.L.W., T.E., J.D.M., L.C., P.K., and S.L.V.D. designed the research. M.L.S., A.M., N.T.J., M.L.W., J.B., and S.L.V.D. performed the research. M.L.S. analyzed the data.

CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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