Challenges and cautions with small and retrospective postoperative pain genome-wide association studies with TAOK3

To the Editor:

As investigators working on perioperative opioid pharmacogenetics in children, we read with interest the article by Cook-Sather et al. [2]. The authors are to be congratulated for their effort. Indeed, they are addressing a very important problem, variability in response to morphine for pain management in children.

Using a genome-wide association approach and retrospective chart review, the study by Cook-Sather et al. identified variants (rs795484 and rs1277441) in TAOK3 encoding the serine/threonine-protein kinase TA03 that were associated with increased morphine requirements in children of European Caucasian (EC) ancestry and with increased acute postoperative pain in both EC and African-American (AA) subjects [2]. Although these results are promising, the authors failed to achieve statistical significance for genome-wide association studies or to provide biological support for a TAOK3 role in morphine response.

Caution is needed in interpreting this study’s findings because they are further weakened by the following methodological concerns.

1. The study’s retrospective discovery cohort spanned 10 years; anesthesia and perioperative pain management varied, and all clinical measures relied on what could be extracted from charts. Including surgical patients over a large timeframe without a specific practice protocol introduces bias due to changing clinical practices. Administered total morphine doses can vary by different clinical care providers’ preferences. The impact of secular trends needs to be tested explicitly to ensure no systematic bias. Variability in preoperative acetaminophen administration and prophylactic oxycodone use would be expected to influence postoperative pain and total morphine dose requirements. The effect size of this difference due to use of oxycodone was more than that of the study’s primary finding’s effect size. Inconsistent use of these adjunct analgesics that directly affected the primary outcome measure further confounded the phenotype. An additional source of the heterogeneity was the underlying health status of the participants [American Society for Anesthesiologists (ASA) physical status 1 to 3] which can confound postoperative pain and morphine requirements with varying severity of different coexisting morbidities. Not all ASA status 3 subjects have similar underlying disease, which may further affect the outcome measures. Finally, pain measures were scores from 3 different pain scales normalized with linear correlation assumption among the pain scales, which weakens confidence in the second study outcome, pain.

2. This study was a small subset of a larger group of participants, with little information on how these participants differed from those not included in the analysis. There was a huge selection bias, with exclusion of many children who did not have DNA samples (about 3700 of 4600 children were excluded) and those potentially had opioid-related complications (such as respiratory depression, postoperative nausea and vomiting, and intractable pain) and unanticipated hospital admissions. Clearly, this significant selection bias limits the study’s generalizability.

3. Morphine response phenotyping is incomplete. The primary outcome measure is morphine dose requirement, yet opioid adverse effects are not addressed. Titrating morphine postoperatively based on pain scores alone [1] is a risky and inappropriate clinical practice [6]. The presence of respiratory depression, oxygen desaturation, and airway obstruction limits morphine administration, and ultimately the total morphine dose may be lower without reflecting analgesia effectiveness.

4. There were also serious issues regarding the secondary outcome of pain. In this study, recovery nurses documented pain scores. Three different pain scales were used and were normalized with linear correlation assumption among the pain scales. The validity of this approach to documenting pain is highly questionable. There is no mention of the length of stay in the recovery room (or time to meet discharge readiness) because it is clinically and economically more important than clinically negligible differences in total morphine doses and pain scores alone that do not negatively affect clinically meaningful outcomes and length of hospital stay.

5. Lastly, the genetic data used in this study were generated from multiple genetic platforms (Illumina HumanHap550 Single Nucleotide Polymorphism (SNP) array, Illumina Human610-Quad version 1, in addition to TaqMan SNP genotyping). Although using multiple platforms often cannot be avoided, specific care needs to be taken to demonstrate that these platforms are not creating systematic bias. This can be especially problematic if those who had high pain scores and morphine requirements were run on different arrays or even processed in different batches or at different institutions. Indeed, if there are platform differences in clinical practice of morphine consumption, then associated variants could be spurious. In addition, imputations of the TAOK3 region were used, which further complicates the reliability of genotyping.

In addition to methodological concerns, it should be noted that the findings of this study contradicted previously reported associations of morphine consumption with race [4,5] and sex [3]. Specifically, Cook-Sather et al. found that children from EC ancestry had a significantly higher morphine requirement than children of AA ancestry, a finding that contradicts previous reports in a similar pediatric surgical population [4,5] and a systematic meta-analysis of studies that showed that sex differences exist in morphine-induced analgesia in both experimental pain studies and clinical studies, with greater morphine efficacy in female patients.

Given the small sample size and lack of statistical significance of the genome-wide association study by Cook-Sather et al., its highly problematic and retrospective phenotyping, different genotyping platforms with imputations, lack of robust biological support, and external validations from a similar population, these results must be viewed with a high degree of caution. Because of wider total morphine dose ranges, possibly from different clinical care provider preferences for different subject populations (differences in ASA physical statuses, age, pain scales, intensity of Sleep Disordered Breathing (SDB), use of preoperative acetaminophen, and postoperative oxycodone use), the observed nonsignificant study findings could be by chance alone and/or partly explained by secular trends in morphine use. Further research is essential to determine the possible role of TAOK3 or other genes nearby in postoperative morphine analgesia. Only biologically relevant and clinically meaningful associations with external validations can be considered for genotype-guided personalized pain management in children.

Conflict of interest statement

All authors declare no conflicts of interest.
Modulatory effects of TAOK3 variants on morphine requirement in acute postoperative pain: An early genome wide association study contribution to the field of pediatric pain

Moving beyond biologic plausibility theory, a recent independent publication provides biologic support. In both Drosophila and mice Tao/TAok3 was shown to control JNK pathway activation and modulate ethanol sensitivity [6]. Mice heterozygous for a disrupted allele of the homologous Taok3 gene (Taok3Gt) were resistant to the acute sedative effects of ethanol. Although morphine was not studied and individual experiments did not address analgesia, the sedative/analogesic–resistant phenotype is similar, invokes pain pathways proven to mediate morphine effects, and is evolutionarily conserved over 2 species.

There were no temporal trends in clinical practice save the later unavailability of sodium pentothal. Importantly, Pearson correlations for date and morphine dose were insignificant and average morphine doses for individual years were within 0.25 SD of discovery/replication cohort averages. Preoperative acetaminophen seemed not to influence outcomes. For the 3.8% of children in the combined cohorts who received no preoperative acetaminophen, morphine dose was consistent with discovery/replication cohort averages. Routine gastric evacuation after tracheal intubation may have altered acetaminophen exposure, however, potentially contributing to pain/morphine dose variability and thereby diminishing GWAS signal significance.

Likewise, oxycodone was not a factor in morphine consumption during the study period. The phenomenon we saw was this: some children were more comfortable after intraoperative morphine and needed little if any additional further analgesics, intravenous or enteral. Oxycodone would likely reduce morphine requirement were morphine titrated for pain control beyond the postanesthesia care unit period. Dr. Sadhasivam et al. [13] make erroneous assertions regarding causality and “oxycodone effect size.” The 4.0% of discovery cohort children who had no oral opioids received 17.9 µg/kg less morphine in recovery and 12.9 µg/kg less total morphine. That is, the claimed effect direction [13] is opposite that of our findings. Further, the absolute value of the purported “effect size” (12.9 µg/kg) is clearly less than that for the top minor alleles at TAOK3 (17.6 µg/kg/minor allele, hence 35.2 µg/kg for homozygotes).

At reviewer request, we conducted a separate GWAS analysis for total morphine in European Caucasian (EC) subjects who did not receive oral opioids (n = 267). The significance of rs795484 (β 18.15; 95% confidence interval [CI] 11.23 to 25.07; P = 5.32 × 10⁻⁷) and rs1277441 (β 17.55, 95% CI 10.56 to 24.54; P = 1.54 × 10⁻⁶) improved. By excluding more comfortable children, we concentrated the discovery cohort with a less comfortable/higher dose morphine phenotype and would have introduced a biased post hoc analysis. To maintain strict study methodology and conservative interpretation, however, we did not change the discovery cohort selection process, even though it might have strengthened the TAOK3 significance level.

Although not all children have the same coexisting diseases leading to physical status assignments of 2 or 3, regression analyses mandated the use of physical status as a covariate. Perhaps a specific measure of sleep-disordered breathing (SDB) would have been a better covariate, but not all children had polysomnography to quantify it. Physical status likely functions as a surrogate marker for SDB severity in this population with high SDB prevalence.

We acknowledge a potential selection bias associated with humanitarian intent in those who had previously donated samples for the Center for Applied Genomics (CAG) database. Otherwise we believe the genotyped subjects reflect an unbiased sampling of our larger pool of day surgery tonsillectomy and adenoidectomy patients. Consistent with this are dose statistics for n = 3899 subjects who had tonsillectomy and adenoidectomy with intraoperative morphine—average 91.9 µg/kg; SD 29.4 µg/kg; range 9.6 to 283.0 µg/kg compared with the final discovery cohort with an average of 88.2 µg/kg; SD 26.9 µg/kg; and range 24.8 to 272.7 µg/kg. Any