ORIGINAL ARTICLE

Novel associations between FAAH genetic variants and postoperative central opioid-related adverse effects

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Opioid effects are potentiated by cannabinoid agonists including anandamide, an endocannabinoid. Inter-individual variability in responses to opioids is a major clinical problem. Multiple deaths and anoxic brain injuries occur every year because of opioid-induced respiratory depression (RD) in surgical patients and drug abusers of opioids and cannabinoids. This study aimed to determine specific associations between genetic variants of fatty acid amide hydrolase (FAAH) and postoperative central opioid adverse effects in children undergoing tonsillectomy. This is a prospective genotype-blinded observational study in which 259 healthy children between 6 and 15 years of age who received standard perioperative care with a standard anesthetic and an intraoperative dose of morphine were enrolled. Associations between frequent polymorphisms of FAAH and central postoperative opioid adverse effects including, RD, postoperative nausea and vomiting (PONV) and prolonged stay in Post Anesthesia Recovery Room (postoperative anesthesia care unit, PACU) due to RD and PONV were analyzed. Five specific FAAH single nucleotide polymorphisms (SNPs) had significant associations with more than twofold increased risk for refractory PONV (adjusted \( P < 0.0018 \)), and nominal associations \( (P < 0.05) \) with RD and prolonged PACU stay in white children undergoing tonsillectomy. The FAAH SNP, rs324420, is a missense mutation with altered FAAH function and it is linked with other FAAH SNPs associated with PONV and RD in our cohort; association between PONV and rs324420 was confirmed in our extended cohort with additional 66 white children. Specific FAAH polymorphisms are associated with refractory PONV, opioid-related RD, and prolonged PACU stay due to opioid adverse effects in white children undergoing tonsillectomy.

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INTRODUCTION

Opioids are commonly used analgesics to manage surgical pain. However, effective and safe postoperative pain management with opioids is an unmet perioperative clinical need. This is mainly because of narrow therapeutic indices and large inter-individual variations in opioid responses. Morphine is one of the commonly used perioperative opioids. Similar to other opioids, clinical doses of morphine can cause significant respiratory depression (RD), along with other adverse effects such as postoperative nausea and vomiting (PONV). Genetic factors contribute to significant variability in opioid-induced RD, nausea and analgesia in twin studies.1,2 Important genetic risk factors for increased opioid-induced postoperative RD and other adverse effects are currently not well known. Endocannabinoids play a significant role in pain modulation and inflammation.3 Anandamide, an endogenous cannabinoid, has been demonstrated to have analgesic properties in several different models of pain mostly by activation of cannabinoid receptors, CB1 and CB2. However, the intense analgesic actions of anandamide are short-lived because of its rapid catabolism by fatty acid amide hydrolase (FAAH).4–6 The current literature suggests that FAAH inhibition enhances analgesia by increasing the bioavailability of anandamide,7 and this is a promising strategy to treat certain types of pain and inflammation.8–13 Considering remarkable regulation of anandamide’s duration of action and amplitude by FAAH and tight control of fast catabolism of fatty acid amides by a single enzyme, inhibitors of FAAH have been targeted as valuable pharmaceutical agents for the treatment of pain and inflammation.6,14 In addition, evidence suggests that human FAAH genetic variants modulate pain,15 but their clinical role in surgical pain management is not well studied.

Endogenous cannabinoid receptors are widely distributed throughout the central nervous system, including the brainstem, and modulate a variety of functions, including breathing. In addition to effects on pain sensitivity, endogenous cannabinoids have been shown to mediate the antinociceptive effects of opioids.16 It had been shown that the cannabinoid receptor CB1 is involved in morphine’s nociception and mediates the influence via \( \mu \)-opioid receptor agonistic action.17 In addition, anandamide, if protected from degradation by FAAH, acts via the CB1 receptor and modulates morphine’s analgesia by interactions with kappa opioid receptors (Supplementary Figure 1).18 In neonatal mice, the activation of cannabinoid CB1 receptor with anandamide had been shown to depress the medullary respiratory rhythm generator, probably via the catecholaminergic system.19 This could potentially explain increased mortality20 and morbidity21,22 in infants exposed to substance abuse including cannabinoid during the perinatal period and opioid/marijuana abusers.

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Opioid and cannabinoid systems reciprocally and synergistically modulate functions at multiple levels. However, the effects of genetic variants of FAAH on clinical pain management with opioids are not well studied. We hypothesized that genetic variations in FAAH significantly influence the safety and efficacy of morphine in children undergoing surgery. The purpose of this study was to investigate the associations between common genetic polymorphisms of FAAH and opioid-related effects and adverse effects following tonsillectomy in a large pediatric population. Such knowledge will help advance the ultimate goal of individualizing perioperative pain management in children.

**PATIENTS AND METHODS**

**Study design and setting**

This is a prospective, genotype-blinded, clinical observational study in a large cohort of children undergoing outpatient adenotonsillectomy with standard perioperative anesthetic, surgical and nursing care. The study is part of a larger ongoing clinical study, entitled Personalizing Perioperative Morphine Analgesia in Children, which is registered with clinicaltrials.gov, NCT01140724. This large prospective clinical study with standard perioperative care (with the clinical care team blinded to patients’ genotypes) evaluates factors contributing to inter-individual variations in analgesic and adverse effect responses to perioperative opioids in children. The study was approved by the institutional review board and written informed consent was obtained from parents and assent obtained when appropriate from children > 7 years of age before enrollment.

**Participants**

Children aged 6–15 years undergoing elective outpatient tonsillectomy or adenotonsillectomy were recruited for the study on the day of surgery. Sample inclusion criteria were children designated to have an American Society of Anesthesiologists (ASA) physical status 1 or 2 scheduled for tonsillectomy or adenotonsillectomy because of recurrent tonsillitis, adenotonsillar hypertrophy or obstructive sleep apnea (OSA). Sleep-disordered breathing with a history of snoring plus respiratory pauses during sleep lasting more than 10 s or daytime drowsiness was considered to constitute the clinical diagnosis of OSA. Accordingly, the indication for tonsillectomy in these children was documented as OSA. In addition, the Pediatric Sleep Questionnaire, 23,24 was used to assess children for sleep disorders. If the parent of study child reports ‘yes’ to 8 or more of the 22 questions in the Pediatric Sleep Questionnaire, the child was considered to have OSA.

**Clinical outcome measures**

Metrics for analgesic effectiveness and opioid-related adverse effects were recorded for each participant. For this paper, we focused on two opioid-related adverse effect outcomes: clinical RD and refractory PONV. Total morphine requirement (mg kg$^{-1}$ of body weight) was also examined as a measure of analgesic effectiveness. In our study, we defined clinical RD as a persistent (more than a minute) oxygen desaturation < 90% or respiratory rate < 8 breaths per minute or oxygen desaturation < 94% along with respiratory rate < 10 per minute requiring supplemental oxygen to maintain SpO2 > 94% in the absence of clinically obvious upper airway obstruction. We defined PONV as an actual episode of emesis and/or episode of self-reported persistent nausea needing an antiemetic intervention. Prolonged PACU stay (> 90 min) secondary to RD and refractory PONV were assessed consistently by the research coordinator. Total morphine dose was total amount of morphine used (in mg kg$^{-1}$) intraoperatively and immediate postoperative period in PACU.

**Genotyping**

Blood was drawn for DNA in the operating room upon intravenous line placement under anesthesia for genotyping of FAAH single nucleotide polymorphisms (SNPs). DNA was isolated on the same day and frozen at − 20°C. Six previously studied common SNPs were selected to be genotyped using TaqMan allelic discrimination system assays (Life Technologies, Applied Biosystems, Grand Island, NY, USA). These included rs932816, rs4141964, rs3766246, rs324420, rs324419 and rs2295632. In addition, a genome-wide genotyping was performed on the Illumina Human Omni-1 Genotyping array using the iScan System (Illumina, San Diego, CA, USA) and Infinium2 chemistry. Genotypes were called using the GenTrain2 algorithm within Illumina Genome Studio. Samples with call rates below 95% and SNPs with call rates below 95% or a Hardy Weinberg equilibrium
(HWE) P-value less than 0.001 were dropped from the study. We identified 39 SNPs in the FAAH gene location and within 5 kb upstream or downstream of FAAH. Two of the SNPs, rs3766246 and rs324420, were included in TaqMan and Illumina Omni5 GWAS assays with 100% concordance in genetic result reports. In addition, we used 244 validated ancestry informative markers (AIMs) for population stratification. As genotyping was done after clinical care and clinical data collection, perioperative care providers and researchers were blinded to genotypes when clinical care was delivered.

Statistical analysis
To assess whether self-reported white and black races match well to genetic ancestry, we used 1397 HapMap subjects as our reference populations. Out of the 244 AIMs genotyped, 218 were found in the HapMap data. Therefore, we performed principal component analysis with 218 AIMs using SVD 7.7.6 (Golden Helix, Bozeman, MT, USA). Up to 10 principal components were also used in the assessment of the potential confounding by population stratification. The genomic inflation factor (λ) was estimated from the median χ2 statistic in PLINK.

Other statistical analyses were performed using Statistical Analysis Software (SAS), version 9.3, JMP Genomics, version 6.0 (SAS Institute Inc., Cary, NC, USA); and R.

Prior to analyses, quality of the data was checked. Characteristics of the patients and properties of the SNPs were examined in African American and Caucasian children respectively. HWE was tested. To analyze binary outcomes RD and PONV, logistic regressions were performed. To analyze total morphine requirement, linear regression was used. Prior to evaluation of FAAH variants, the effects of covariates were tested. For total morphine dose, age, sex, body mass index (BMI) z scores and OSA were evaluated. For adverse effect outcomes RD and PONV, total morphine was considered as an additional covariate. To select the best fitting model, log likelihood, Akaike and Bayesian Information criterion were compared, and residuals were examined. Covariates that significantly improved model fitting (P < 0.05) were retained for subsequent genetic analyses. To assess the single SNP association with the outcomes, we used additive models, in which the genotypes were recoded and tested as continuous variables. Genotypes were recoded to 0, 1 and 2 according to the number of minor alleles of the entire cohort. Statistical modeling was conducted with white and black patients separately.

In this study, we focused on the association of two adverse outcomes RD and PONV, with genetic ancestry. We compared self-reported races with genetic ancestry estimated from 218 AIMs. In 250 out of the total of 259 patients (> 95%), self-reported races clustered well with European and African ancestry; the remaining 9 subjects had genetic marker admixture. Principal component 1 and 2 successfully separated and African ancestry; the remaining 9 subjects had genetic marker admixture. Principal component 1 and 2 successfully separated and African ancestry; the remaining 9 subjects had genetic marker admixture. Principal component 1 and 2 successfully separated black and white races (data not shown). In this study, we stratified the analyses by self-reported races, as race differences in opioid effects have been reported in children,26 and self-reported races are readily available to clinicians compared with genetic AIMs.

RESULTS
Demographics
A consort diagram illustrates eligible, approached and enrolled study subjects (Figure 1). Participants were primarily white with slightly more girls. Compared with white children, black children were slightly heavier and had higher OSA frequencies (Table 1).

FAAH SNPs description
A total of 39 SNPs in the FAAH gene were genotyped by TaqMan and/or Illumina Human Omni 5 array techniques. Two FAAH SNPs, rs3766246 and rs324420, were genotyped by both methods; both methods yielded identical genotypes for all subjects, suggesting high reliability of our genotype data. Among the 39 SNPs, 14 had minor allele frequency of 5% or more in both white and black children. Tests on HWE showed that these 14 SNPs were all in HWE at alpha = 0.004 level (Bonferroni correction of 14 tests). Therefore, 14 SNPs were included in genetic association analyses.

We compared self-reported white and black races with genetic ancestries estimated from 218 AIMs. In 250 out of the total of 259 patients (> 95%), self-reported races clustered well with European and African ancestry; the remaining 9 subjects had genetic marker admixture. Principal component 1 and 2 successfully separated black and white races (data not shown). In this study, we stratified the analyses by self-reported races, as race differences in opioid effects have been reported in children,26 and self-reported races are readily available to clinicians compared with genetic AIMs.

Table 1. Characteristics of participants

|                  | Whites (N = 216) | Blacks (N = 43) |
|------------------|-----------------|----------------|
| Age (year) (median (IQR)) | 8.4 (7.1–11.0) | 8.8 (7.1–11.2) |
| Weight (kg) (median (IQR)) | 33.8 (25.8–46.3) | 34.7 (26.2–54.1) |
| BMI z scores | 0.7 (−0.2–1.6) | 1.2 (0.0–2.0) |
| Intra-operative morphine requirement (mg kg−1) (median (IQR)) | 0.19 (0.17–0.21) | 0.20 (0.16–0.20) |

Sex (N, %)
- Male 105 (49%) 19 (44%)
- Female 111 (51%) 24 (56%)

OSA (N, %)
- Yes 93 (43%) 29 (67%)
- No 123 (57%) 14 (33%)

PONV (N, %)
- Yes 36 (17%) 3 (7%)
- No 180 (83%) 39 (93%)

Respiratory depression (N, %)
- Yes 68 (32%) 17 (40%)
- No 148 (68%) 26 (60%)

Total morphine requirement (mg kg−1)
- Yes 0.25 (0.08) 0.30 (0.09)
- No 0.21 (0.05)

Abbreviations: BMI, body mass index; IQR, inter-quartile range; OSA, obstructive sleep apnea; PONV, postoperative nausea and vomiting.

Genetic association with clinical outcomes
Black children required higher total morphine dose (P < 0.05, t test) and tended to have lower incidence of PONV (P = 0.159, Fisher’s exact test), but the incidences of RD were comparable between black and white children (P = 0.376) (Table 1). Overall, the incidence of RD was more than PONV in our study population (Table 1).

Before testing the genetic effect, we evaluated the effects of co-variables on PONV or RD. For PONV, significant sex and morphine dose effects were detected; for RD, significant effects of morphine dose and BMI z score were detected. No significant OSA effect was detected for either PONV or RD. The significant co-variables were then included in the genetic models, in which single SNP association with PONV or RD was tested in whites and blacks, respectively. The results were summarized in Table 2 and Figures 2a and b.

Genetic association with PONV
In white patients, statistically significant association was detected between PONV and five SNPs (rs4141964, rs3766246, rs324420, rs4141964, and rs324420).
One additional copy of the minor allele of rs4141964, rs3766246, rs324420, rs2295632 and kgp12517369 increased the odds of PONV by 2.42-, 2.42-, 2.73-, 2.61- and 2.61-fold, respectively (Table 2). In addition, two nominal associations were observed (Table 2). However, in black children, no association was detected with any of the SNPs (Table 2).

Prolonged PACU stay due to refractory PONV represent a severe form of PONV. When we tested genetic association with prolonged stay due to PONV in white children only (because no black child had prolonged PACU stay due to refractory PONV), we observed significant associations with rs4141964 ($P = 0.0097$), rs3766246 ($P = 0.0097$), rs324420 ($P = 0.0089$), rs2295632 ($P = 0.0016$) and kgp12517369 ($P = 0.0016$).

Genetic association with RD

We detected nominal association of RD with rs324420, rs4141964, rs2295632, rs3766246 and kgp12517369 in white children (Table 2). No association was detected for any of the SNPs in black children.

Table 2. Single FAAH SNP associations with PONV and respiratory depression

| Outcome | SNP | Location | White | Black |
|---------|-----|----------|-------|-------|
|         |     |          | Minor allele (%) | P  | P  | OR (95% CI) | Minor allele (%) | P  | P  | OR (95% CI) | Putative Function |
|         |     |          | HWE | Association | Beta ± s.e. | HWE | Association | Beta ± s.e. | Function |
| PONV    | rs4141964 | 46865040 | A (0.39) | 0.127 | 0.0014 | 2.42 (1.41, 4.16) | G (0.27) | 0.472 | intron |
|         | rs3766246 | 46865671 | T (0.39) | 0.127 | 0.0014 | 2.42 (1.41, 4.16) | C (0.27) | 0.134 | intron |
|         | rs324420  | 46870761 | A (0.23) | 0.008 | 0.0003 | 2.73 (1.58, 4.73) | A (0.41) | 0.939 | missense |
|         | rs2295633 | 46874383 | A (0.38) | 0.103 | 0.0028 | 2.26 (1.32, 3.85) | G (0.43) | 0.980 | intron |
|         | rs11576941 | 46875067 | A (0.31) | 0.867 | 0.0311 | 0.49 (0.26, 0.94) | A (0.10) | 0.389 | intron |
|         | rs2295632 | 46879562 | A (0.29) | 0.029 | 0.0005 | 2.61 (1.52, 4.47) | C (0.35) | 0.606 | downstream |
|         | kgp12517369 | 46882118 | A (0.29) | 0.029 | 0.0005 | 2.61 (1.52, 4.47) | G (0.42) | 0.359 | N/A |

| RD      | rs4141964 | 46865040 | A (0.39) | 0.127 | 0.0402 | 1.57 (1.02, 2.41) | G (0.27) | 0.472 | intron |
|         | rs3766246 | 46865671 | T (0.39) | 0.127 | 0.0402 | 1.57 (1.02, 2.41) | C (0.27) | 0.134 | intron |
|         | rs324420  | 46870761 | A (0.23) | 0.008 | 0.0473 | 1.61 (1.01, 2.59) | A (0.41) | 0.939 | missense |
|         | rs2295632 | 46879562 | A (0.29) | 0.029 | 0.0343 | 1.62 (1.04, 2.54) | C (0.35) | 0.606 | downstream |
|         | kgp12517369 | 46882118 | A (0.29) | 0.029 | 0.0343 | 1.62 (1.04, 2.54) | G (0.42) | 0.359 | N/A |

Abbreviations: CI, confidence interval; HWE, Hardy Weinberg equilibrium; OR, odds ratio; PONV, postoperative nausea and vomiting; RD, respiratory depression; SNP, single nucleotide polymorphism. Note: effects were shown as OR and 95% CI for PONV and RD. OR indicated the odds ratio when minor allele increased by one copy; effect for total morphine was shown as dose increase for one copy increase of the minor allele. In white children, tests on genetic association with PONV were adjusted for sex and total morphine dose; tests on genetic association with RD were adjusted for total morphine dose and BMI z score. In black children, no statistically significant co-variables were detected; therefore no co-variables were included in the genetic association tests.

Figure 2. FAAH genotypes associated with RD, PONV and morphine requirement. (a) The y axis shows the –log10$P$ values and the x axis shows the chromosomal positions of the FAAH SNPs. Results are shown for whites (red dots) and blacks (blue dots) separately. P values of the genetic association of the 39 FAAH SNPs with PONV (top) and RD (bottom). The reference lines represent the thresholds of $P = 0.0018$ (shot dash line) and $P = 0.05$ (dotted line), respectively. (b) The y axis shows the –log10$P$ values and the x axis shows the chromosomal positions of the of the 11 FAAH SNPs between 46.86 and 46.89 Mb of Chromosome 1 with PONV (top panel) and RD (bottom panel). Results are shown for whites (red dots) and blacks (blue dots) separately. The reference lines represent the thresholds of $P = 0.0018$ (shot dash line) and $P = 0.05$ (dotted line), respectively. FAAH, fatty acid amide hydrolase; PACU, Post Anesthesia Care Unit; PONV, postoperative nausea and vomiting; RD, respiratory depression.

rs2295632 and kgp12517369. One additional copy of the minor allele of rs4141964, rs3766246, rs324420, rs2295632 and kgp12517369 increased the odds of PONV by 2.42-, 2.42-, 2.73-, 2.61- and 2.61-fold, respectively (Table 2). In addition, two nominal associations were observed (Table 2). However, in black children, no association was detected with any of the SNPs (Table 2). Prolonged PACU stay due to refractory PONV represent a severe form of PONV. When we tested genetic association with prolonged stay due to PONV in white children only (because no black child had prolonged PACU stay due to refractory PONV), we observed significant associations with rs4141964 ($P = 0.0097$), rs3766246 ($P = 0.0097$), rs324420 ($P = 0.0089$), rs2295632 ($P = 0.0016$) and kgp12517369 ($P = 0.0016$).
Genetic association with perioperative morphine requirement
No genetic association was detected with total intraoperative and postoperative morphine use in white or black children.

Linkage disequilibrium analysis
On the basis of single SNP association tests, an interesting region was identified in FAAH gene ranging from 46865040 to 46882118 bp of chromosome 1 (Human Genome, version HG37.5), with 11 SNPs harbored in this region. Out of these 11 SNPs, 7 were associated with PONV and 5 associated with RD in whites (Figures 2a and b) with high linkage disequilibrium between these 11 SNPs (Supplementary Figure 2).

Sex-specific SNP effect
For significant associations between rs4141964, rs3766246, rs324420, rs2295632 and kpg12517369 and PONV, there were no sex-specific SNP effects (P > 0.05).

Genomic inflation
We detected statistically significant genetic association with PONV in white. To evaluate the effect of population structure on the association, we assessed the genomic inflation factor (λ) using all FAAH SNPs and ancestry informative markers genome wide. We found that λ is 1, suggesting no strong confounding by population stratification exists on the SNP association with PONV. When adjusted with up to 10 principal components, the genetic association with PONV in whites remained unchanged (data not shown).

Reliability of FAAH genetic association with PONV
Because of biological and significant statistical significant associations between FAAH SNP, rs324420 and PONV, to validate associations, we reanalyzed associations with additional 66 white children who had tonsillectomy with similar protocol. The bigger cohort (original cohort of 216 white children plus 66 additional white children) reproduced following consistent and significant associations. The FAAH SNP, rs324420, was significantly associated with PONV (P = 0.0053) with addition of one copy of minor allele (A) increasing OR by 2.0-fold; and it was also associated with PONV leading to prolonged PACU stay (P = 0.0209) with addition of one copy of minor allele (A) increasing OR by 2.2-fold. Though not statistically significant, the rs324420 AA genotype children overall stayed in PACU longer (97.9 (84.3–113.6) min) than the CC and CA genotype children (88.2 (79.9–88.2 min, P = 0.072)), which is clinically and economically relevant following a common outpatient surgery.

DISCUSSION
Our study showed significant associations between FAAH polymorphisms and refractory PONV following a common outpatient surgery, tonsillectomy. In addition, nominal associations with opioid-induced RD, and prolonged recovery room stays due to PONV with specific FAAH SNPs were identified in a group of white children. Specifically, in white children, addition of one copy of the minor allele of rs4141964, rs3766246, rs324420, rs2295632 and kpg12517369 increased the odds of PONV by 2.42-, 2.42-, 2.73-, 2.61- and 2.61-fold, respectively (P < 0.0018, Table 2). These five FAAH SNPs including a missense polymorphism, rs324420, had nominal associations with opioid-related RD and prolonged stays in PACU due to refractory PONV, highlighting possible biological synergistic interactions between opioid and endocannabinoid pathway.

The FAAH-1 gene, located on chromosome 1, codes for FAAH which degrades anandamide. After sequencing all 15 exons of the FAAH gene, a human study identified the FAAH SNP, rs324420, as a significant polymorphism; the minor allele of this relatively common missense mutation (385C>A) converts a conserved proline to threonine, resulting in a FAAH variant that has an enhanced sensitivity to proteolytic degradation and reduced cellular stability,27 potentially resulting in high anandamide levels. As can be seen from Supplementary Figure 3, P129 is located in an exposed and highly variable loop, away from the active site and FAAH dimerization interface. This position and the lack of evolutionary conservation make direct functional effect on enzymatic activity unlikely, supporting the alternative hypothesis of a lower number of active copies for the mutant protein with higher sensitivity to proteolytic degradation. In our study population, this particular SNP is in strong linkage disequilibrium with other SNPs of a haplotype of in FAAH gene region (Supplementary Figure 2) and is significantly associated with both PONV and RD (Figure 3). Two different central opioid adverse effects, PONV and RD, are associated with multiple FAAH SNPs with high linkage; seven SNPs were associated with PONV and five SNPs were associated with RD (Table 2). When sequential modeling (type 1 analysis) in whites was performed to test whether other significantly associated FAAH SNPs would explain PONV and RD in addition to rs324420, no other FAAH provided additional information to the association between rs324420 and PONV or RD.

Interestingly, this particular missense FAAH SNP, rs324420, is strongly associated with both street drug and alcohol abuse and dependence.7–32 In the USA, opioid overdose/RD-related deaths are more frequent than motor-vehicle-related injury deaths.13 Our finding of association of RD with FAAH gene (especially AA genotype of rs324420) may have potential significance for millions of patients prescribed and individuals abusing opioid agonists and/or cannabinoids (for example, marijuana, synthetic cannabinoids22,34–39) every year, for both the abuse potential and the potential for life-threatening RD.

RD related to opioids is a serious, potentially life-threatening, however, preventable complication. When we used a clinically relevant, widely accepted definition for RD, we found associations with FAAH SNPs (Table 2). Genetic risk factors (for example, codeine in ultrarapid metabolizers) can increase the risk of RD and death.40,41 Proactive risk identification and prevention are important in minimizing the negative impact of central opioid adverse effects.

Another central opioid adverse effect following surgery is PONV, often referred to as ‘the big little problem’ after anesthesia.28 In humans, stress and motion sickness are associated with impaired

**Figure 3.** Missense FAAH SNP, rs324420, and risk of respiratory depression and PONV. Compared with CA genotype of FAAH polymorphism, rs324420, children with AA genotype had higher risk of PONV (odds ratio of 2.73 (1.58–4.73), P = 0.0003) and RD (odds ratio of 1.61 (1.01, 2.59), P = 0.0473); on the other hand, relatively children with CC genotype had less risk of PONV and RD than children with CA and AA genotypes. FAAH, fatty acid amide hydrolase; PONV, postoperative nausea and vomiting.
endocannabinoid activity. 43 Anandamide transport inhibition has been shown to attenuate vomiting in animals. 54 General anesthesia influences anandamide levels in a drug-dependent way, which may explain high incidence of PONV with inhalational anesthetics. 45 As PONV remains as a big problem despite prophylactic anti-emetics and is often associated with opioids, we associated FAAH genetic variants with refractory PONV and prolonged PACU stay due to PONV. In white children, statistically significant higher risk for refractory PONV was detected with FAAH SNPs (Table 2). Though protection against nausea is anticipated with expected higher levels of endocannabinoid with these genotypes, the emetogenic effect of morphine was more pronounced than endocannabinoid effects in these patients. Same polymorphisms were also independently associated with RD, highlighting higher risk for morphine’s central adverse effects. In our study, we observed a relatively lower incidence of PONV compared with RD; this could be due to intraoperative prophylactic use of dexamethasone and ondansetron, and possibly due to the antiemetic properties of expected high endocannabinoid levels (with low expected FAAH function) with associated FAAH SNPs.

Though the exact molecular mechanisms behind central opioid adverse effects and genetic variations in endocannabinoid system are not well known, we found significant associations. Opioid and cannabinoid systems reciprocally and synergistically modulate functions at multiple levels including co-localization of opioid and cannabinoid receptors, direct receptor associations, altered release of endogenous peptide, shared signal transduction pathways, mutual potentiation, receptor cross-talk and cross-tolerance. 46, 47 In rhesus monkeys, cannabinoid agonists such as tetrahydrocannabinol and anandamide produce antinociception and RD; these effects were reversed with a specific cannabinoid receptor antagonist but not the opioid antagonist, revealing a cannabinoid mechanism. 47 Though opioids and cannabinoids can independently cause analgesia and RD that could be reversed by respective antagonists in monkeys 48 and FAAH inhibition can attenuate morphine withdrawal effects in mice, 49 in humans, synergism between opioid and endocannabinoid systems are not well studied. Our study provides early evidence of synergistic postoperative effects between opioids and endocannabinoid system. Our earlier study demonstrated that white children had higher incidence of opioid-related adverse effects than black children following surgery. 26 Though black children required higher total morphine dose than white children in this current study, they tended to have lower incidence of refractory PONV (7 versus 17%, P = 0.159). In this study, we have found that some of the allelic frequencies of FAAH polymorphisms associated with opioid-related PONV and RD are significantly different between Caucasian and African-American children (Table 2) consistent with previous human studies 15 and could potentially explain and contribute to racial differences in clinical outcomes.

Though an adult volunteer study found associations between cold pain sensitivity and variations in FAAH in a gender-dependent manner, 15 in our pediatric study, we did not find any association between FAAH SNPs and morphine dependence. Two small studies that specifically examined sex differences showed greater morphine-induced RD in women than men. 49, 50 In our current study, girls had higher incidences of opioid-induced RD and PONV with higher doses of morphine than boys (data not shown). However, frequencies of FAAH variants in boys and girls did not explain the sex differences in postoperative RD and PONV in our study; furthermore, no sex-specific FAAH associations with clinical outcomes were observed.

There are a few limitations in our study. Although our study found an association between FAAH genetic variants and postoperative opioid-induced RD, it is not possible to say whether these differences are related to specific FAAH genetic variants per se or to some unknown or not measurable variable that are highly linked to FAAH polymorphisms studied; sequencing of the entire FAAH gene might provide additional information. Secondly, owing to local demographics, we enrolled mainly African-American and Caucasian children. Our current study does not address other races. We did not explore interactions between FAAH and other genes in study (which is relatively small for testing multiple gene-gene interactions) that might affect the incidence of RD. Despite these limitations, the results of the study have novel, clinically and economically important findings as it demonstrates that FAAH variants are associated with central opioid adverse effects and prolonged stays in PACU in children, which were reproduced in an extended cohort.

In conclusion, we found novel associations between FAAH polymorphisms and postoperative outcomes, PONV and RD in children undergoing tonsillectomy. Though our study demonstrates clinically and economically important associations between genetic variants of FAAH and central opioid-related adverse effects and there are biological functional evidences to support our associations, causality for these adverse effects needs to be further studied. In future, when managing pain with possible proactive genotyping to personalize care, potentially higher incidences of opioid-induced RD and PONV in children with certain FAAH genetic variants need to be anticipated. To advance personalized pain management, more studies are needed to validate our findings in diverse population and understand the mechanistic pathways behind the genetic associations with opioid-related adverse effects.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Trial Registration. This clinical trial reflects a portion of a larger study, Personalizing Perioperative Morphine Analgesia in Children, NCT01140724, registered at clinicaltrials.gov.

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Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)