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Novel Associations between *FAAH* Genetic Variants and Postoperative Central Opioid related Adverse Effects

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Abstract

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 in due to opioid induced respiratory depression 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 259 healthy children between 6 and 15 years that 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, respiratory depression (RD), postoperative nausea and vomiting (PONV) and prolonged stay in Post Anesthesia Recovery Room (PACU) due to RD and PONV were analyzed. Five specific *FAAH* SNPs had significant associations with more than

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Conflict of Interests

None of the authors have any conflicts of interest to disclose.

2 fold 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. *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 respiratory depression, and prolonged PACU stay due to opioid adverse effects in white children undergoing tonsillectomy.

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, along with other adverse effects such as Postoperative Nausea and Vomiting (PONV). Genetic factors contribute to significant variability in opioid induced respiratory depression, nausea and analgesia in twin studies.[1, 2] Important genetic risk factors for increased opioid induced postoperative respiratory depression 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 CNS, 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 cannabinoid receptor CB1 are involved in morphine's central nociception and mediate the influence via μ-opioid receptor agonistic action.[17] In addition, anandamide if protected from degradation by FAAH, acts via the CB1 receptor and modulate morphine's analgesia by interactions with kappa opioid receptors (Supplemental Figure 1).[18] In neonatal mice, 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

mortality[20] and morbidity[21, 22] in infants exposed to substance abuse including cannabinoid during the perinatal period and opioid/marijuana abusers.

Opioid and cannabinoid systems reciprocally and synergistically modulate functions at multiple levels. However, 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 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 history of snoring plus respiratory pauses during sleep lasting more than 10 seconds or daytime drowsiness were 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 (PSQ),[23, 24] a validated tool 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 PSQ, the child was considered to have OSA.

Children were excluded if they or their parents were non-English speaking. Children allergic to study medications or who had developmental delay, liver or renal diseases, or preoperative pain requiring analgesics (e.g. chronic tonsillitis) were excluded. Due to limited availability of research coordinators for this study, we were not able to recruit all eligible subjects which resulted in convenience sampling (Figure 1).

Standard Care and Study Procedures

All participants received standard perioperative care, including standardized surgical (electrocautery based) and anesthetic techniques. Anesthesia was induced using sevoflurane followed by a propofol (2 mg/kg) bolus to facilitate endotracheal intubation. Anesthesia was maintained with sevoflurane without the use of neuromuscular blockade. Patients received morphine prior to surgical incision. Children with OSA history received 0.1 mg/kg morphine while those without OSA diagnosis received 0.2 mg/kg. If there were any signs suggestive of pain (clinically significant increase in heart rate and blood pressure) following surgical incision and cauterization, the clinical anesthesia team provided additional morphine at 0.05 mg/kg increments intraoperatively as necessary. All children receive prophylactic ondansetron (0.1 mg/kg) and dexamethasone (0.1 mg/kg) intraoperatively. Significant postoperative pain measured with facial expression; leg movement; activity; cry; and consolability (FLACC) pain score[25] ≥ 4/10 was managed in the postoperative anesthesia care unit (PACU) with rescue doses of morphine (0.05mg/kg increments).

Duration of PACU stay (time to achieve PACU discharge readiness) was defined as the duration in PACU before achieving the following discharge criteria. Level of consciousness: easily arousable or awake, airway: patent with adequate air exchange, core body temperature: ≥ 36·3 degrees Celsius, acceptable pain level (pain score <4), hemodynamically stable, no significant opioid related adverse effects such as PONV and respiratory depression, and surgical site without any bleeding or complications. This is discharge readiness time is different from actual PACU discharge time as we did not want to include delays due to social (non-medical) reasons (e.g. waiting for car ride). If a patient required more than 90 minutes to meet PACU discharge criteria following tonsillectomy, it was defined as a prolonged PACU stay.

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 respiratory depression (RD) and refractory Post-Operative Nausea and Vomiting (PONV). Total morphine requirement (mg/kg 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 minutes) secondary to respiratory depression and refractory PONV were assessed consistently by the research coordinator. Total morphine dose was total amount of morphine used (in mg/kg) 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, USA). These included *rs932816, rs4141964, rs3766246, rs324420, rs324419, and rs2295632.* In addition, a genome-wide genotyping was performed on the Illumina Human OMNI-5 genotyping array using the iScan System (Illumina) 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 HWE p-value less than 0.001 were dropped from the study. We identified 39 SNPs in the FAAH gene location and within 5kb 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. Since 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 SVS 7.7.6 (Golden Helix, Bozeman, MT). Up to 10 PCs were also used in the assessment of the potential confounding by population stratification. The genomic inflation factor (λ) was estimated from the median χ^2 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), 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. Hardy Weinberg equilibrium (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, 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 respiratory depression and PONV with 14 FAAH SNPs that had minor allele frequency (MAF) ≥5% in both black and white children. The SNPs were linked with mean D' of 0.967 (whites: 0.976; blacks: 0.9) and mean R^2 of 0.289. Though there was significant correlations between both opioid adverse effects (Spearman correlation), in order to not overestimate associations, we performed a conservative simple Bonferroni correction for multiple comparisons, which

yielded a significance threshold of 0.0018 [p=0.05/(2 outcomes x14 SNPs)]. We also report associations reaching the threshold of 0.05 as nominally associated.

Power analysis—Prior to analysis, we estimated statistical power to detect a genetic effect using Quanto. We varied MAF from 0.2 to 0.5 to capture the expected frequency range of our variants and held α to 0.0006 to account for multiple testing. Assuming a frequency of 17% for PONV and 32% for RD in white, we were 80% powered to detect odds ratios ranging between 3.3 and 3.8 for PONV and 2.6 to 3.1 for RD using our sample of 216 white children. As we were seeking to identify clinically relevant genetic associations, these effect sizes were reasonable.

Results

Demographics

A consort diagram illustrates eligible, approached and enrolled study subjects (Figure 1). Participants were primarily white with slightly more girls. Compared to 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 (MAF) of 5% or more in both white and black children. Tests on Hardy Weinberg equilibrium (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.

Self-reported race and genetic ancestry

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 (PC) 1 and 2 successfully separated white and black 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 to genetic AIMs.

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 2B.

Genetic association with PONV

In white patients, statistically significant association was detected between PONV and five SNPs (*rs4141964, rs3766246, rs324420, 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 and is a subset of children with PONV. When tested genetic association with prolonged stay due to PONV were tested in white children only (since 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), *rs2295633* (p=0.0373), *rs11576941* (p=0.0404), *rs2295632* (p=0.0016), and *kgp12517369* (p=0.0016).

Genetic association with Respiratory Depression

We detected nominal association of respiratory depression with *rs324420, rs4141964, rs2295632, rs3766246, and kgp12517369* in white children (Table 2). No association was detected for any of the SNPs in black children. When genetic association with prolonged stay in PACU due to respiratory depression was tested, no genetic association was detected with prolonged stay in PACU due to respiratory depression in white or black children.

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

Based on single SNP association tests, an interesting region was identified in *FAAH* gene ranging from 46865040 bp 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 (Figure 2A and 2B) with high linkage disequilibrium between these 11 SNPs (Supplemental Figure 2).

Sex specific SNP effect

For significant associations between *rs4141964, rs3766246, rs324420, rs2295632* and *kgp12517369* and PONV, there was 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 PCs, 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, in order 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. *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 folds; 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 folds. Though not statistically significant, *rs324420 AA* genotype children overall stayed in PACU longer [97.9 (84.3 –113.6) minutes] than CC and CA genotype children [83.9 (79.9–88.2 minutes, 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 respiratory depression, 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 *kgp12517369* 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 5 *FAAH* SNPs including a missense polymorphism, *rs324420*, had nominal associations with opioid related respiratory depression 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, *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 Supplemental 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 haploblock of in *FAAH* gene region (Supplemental Figure 2) and is significant associated with both PONV and respiratory depression (Figure 3). Two different central opioid adverse effects, PONV and respiratory depression, are associated with multiple FAAH SNPs with high linkage; 7 SNPs were

associated with PONV and 5 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.[27–32] In the USA, opioid overdose/respiratory depression related deaths are more frequent than motor vehicle related injury deaths.[33] Our finding of association of respiratory depression 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 (e.g. marijuana, synthetic cannabinoids)[22, 34–39] every year, for both the abuse potential and the potential for life-threatening respiratory depression.

Respiratory depression related to opioids is a serious, potentially life threatening, however preventable complication. When we used a clinically relevant, widely accepted definition for respiratory depression, we found associations with *FAAH SNPs* (Table 2). Genetic risk factors (e.g. codeine in ultrarapid metabolizers) can increase the risk of respiratory depression 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.[42] In humans, stress and motion sickness are associated with impaired endocannabinoid activity.[43] Anandamide transport inhibition has been shown to attenuate vomiting in animals.[44] General anesthesia influences anandamide levels in a drug-dependent way, which may explain high incidence of PONV with inhalational anesthetics.[45] Since 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 respiratory depression highlighting higher risk for morphine’s central adverse effects. In our study, we observed a relatively lower incidence of PONV compared to respiratory depression; this could be due to intraoperative prophylactic use of dexamethasone and ondansetron, and possibly due to 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] In rhesus monkeys,

cannabinoid agonists such as tetrahydrocannabinol and anandamide produce antinociception and respiratory depression; 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 respiratory depression that could be reversed by respective antagonists in monkeys[47] and FAAH inhibition can attenuate morphine withdrawal effects in mice,[48] 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 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 requirement. Two small studies that specifically examined sex differences showed greater in morphine-induced respiratory depression in women than men.[49, 50] In our current study, girls had higher incidences of opioid-induced respiratory depression 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 respiratory depression, 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, due 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 respiratory depression. Despite these limitations, the results of the study has 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 respiratory depression 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 respiratory depression 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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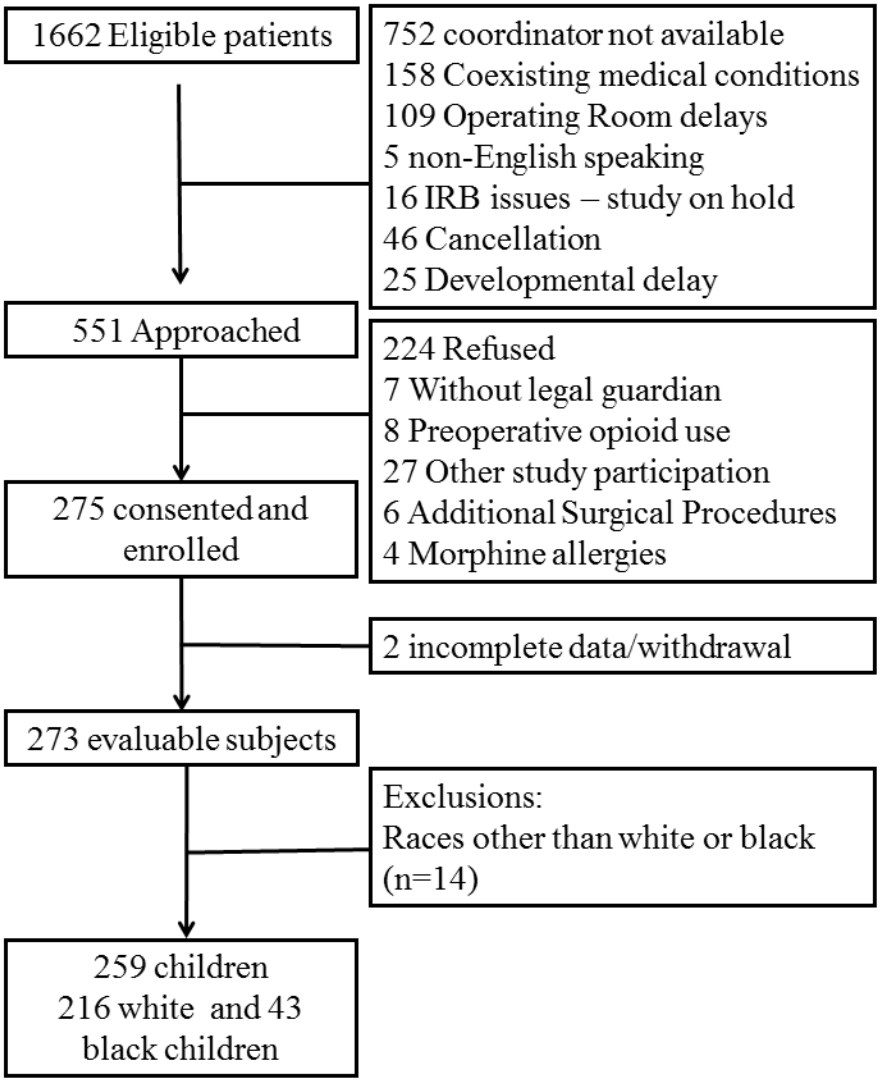

Figure 1. The consort diagram

illustrates the flow of study participants through this clinical trial. Eligible participants, reasons for exclusions, enrolled and analyzed patients are reported. IRB = institutional review board.

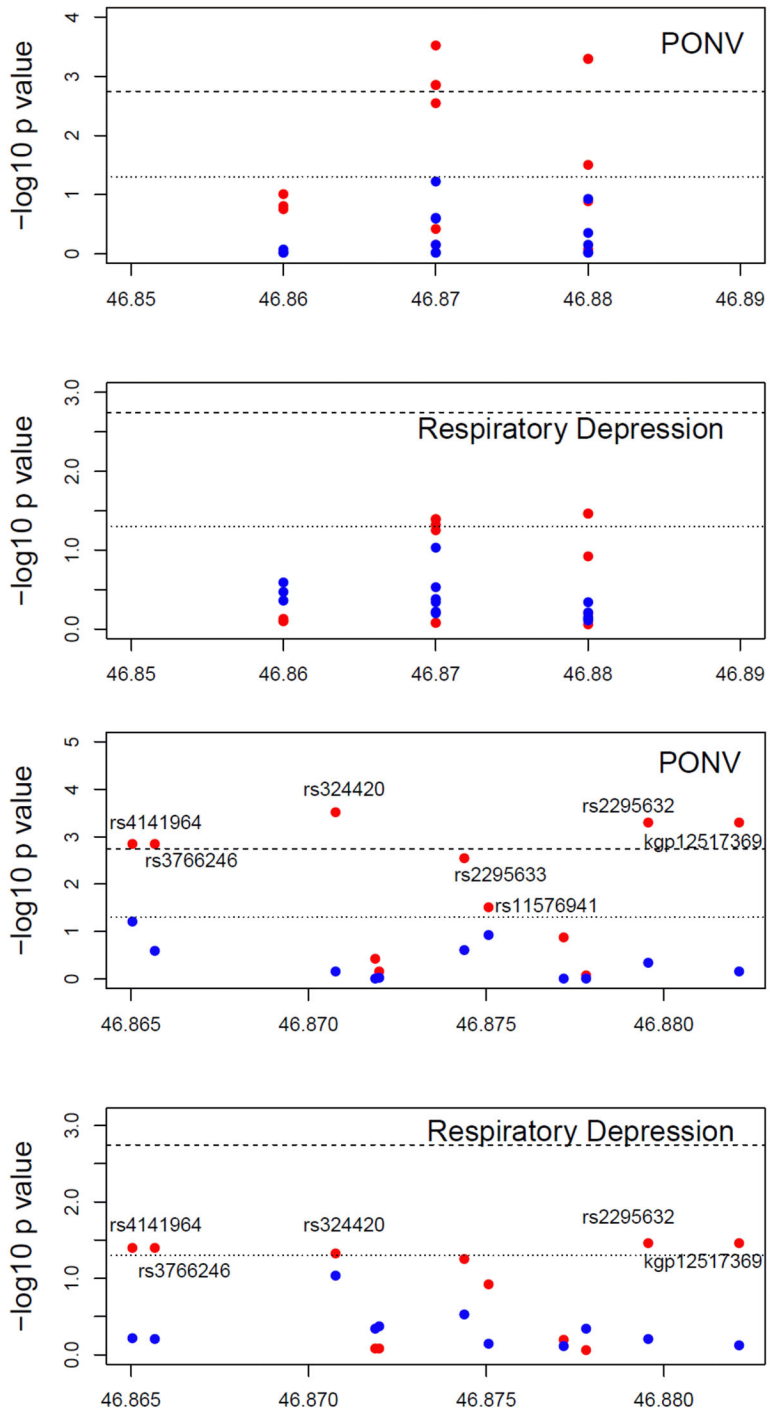

Figure 2. FAAH genotypes associated with Respiratory Depression, PONV and Morphine requirement

Figure 2a. They axis shows the $-\log_{10}$ 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. PACU = Post Anesthesia Care Unit; RD = respiratory

depression; PONV = Postoperative Nausea and Vomiting; FAAH = Fatty Acid Amide Hydrolase.

Figure 2b. The y axis shows the $-\log_{10}$ P values and the x axis shows the chromosomal positions of the of the 11 FAAH SNPs between 46.86 to 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. PACU = Post Anesthesia Care Unit; RD = respiratory depression; PONV = Postoperative Nausea and Vomiting; FAAH = Fatty Acid Amide Hydrolase.

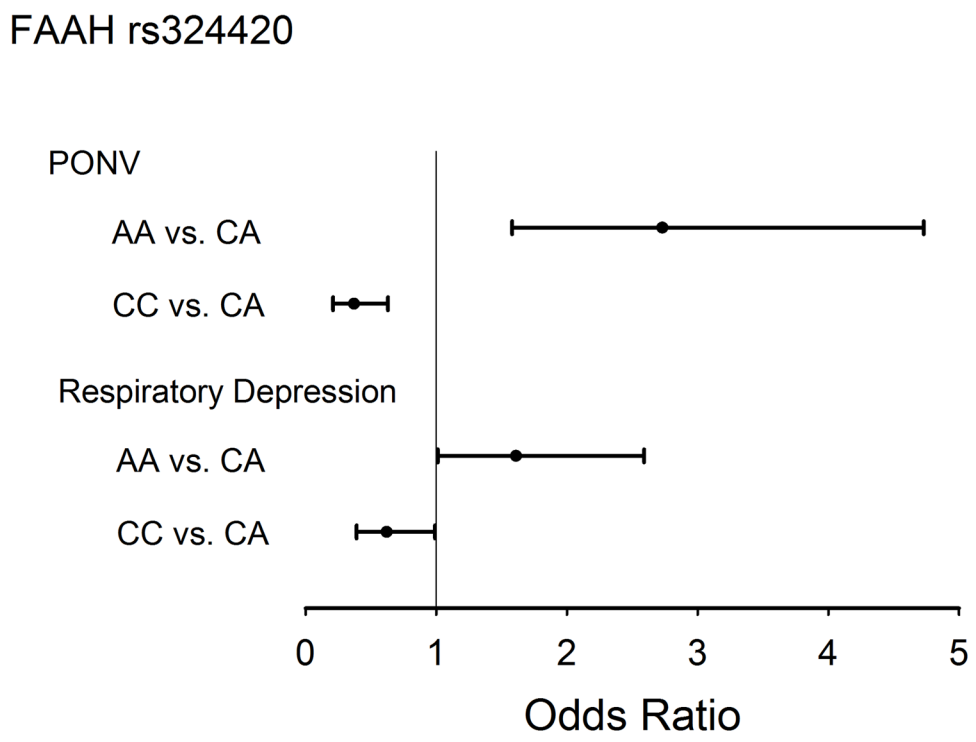

Figure 3. Missense *FAAH SNP, rs324420* and risk of Respiratory Depression and PONV

Compared to 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. PONV = Postoperative Nausea and Vomiting; FAAH = Fatty Acid Amide Hydrolase.

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) (median (IQR))	0.19 (0.17–0.21)	0.20 (0.16–0.20)
Sex (N, %)		
Male	105 (49%)	19 (44%)
OSA (N, %)		
Yes	93 (43%)	29 (67%)
PONV (N, %)		
Yes	36 (17%)	3 (7%)
Respiratory Depression (N, %)		
Yes	68 (32%)	17 (40%)
Total morphine requirement (mg/kg)	0.25 (0.08)	0.30 (0.09)

Age, weight, BMI z score and intra-operative morphine requirement are shown as median and inter-quartile range (IQR); total morphine requirement is shown as mean (standard deviation); sex and Obstructive Sleep Apnea (OSA) are shown as frequencies and proportions. BMI z scores were calculated using CDC growth charts.

Table 2

Single FAAH SNP associations with PONV and Respiratory Depression

			white				black				
outcome	**SNP**	**location**	**minor allele (%)**	**p HWE**	**p association**	**OR (95% CI) Beta ± SE**	**minor allele (%)**	**p HWE**	**p association**	**OR (95% CI) Beta ± SE**	**Putative 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

Note: effects were shown as odds ratio (OR) and 95% CI for Postoperative Nausea and Vomiting (PONV) and respiratory depression (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.