Candidate gene analyses for acute pain and morphine analgesia after pediatric day surgery: African American *versus* European Caucasian ancestry and dose prediction limits

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Abstract

Acute pain and opioid analgesia demonstrate inter-individual variability and polygenic influence. In 241 children of African American and 277 of European Caucasian ancestry, we sought to replicate select candidate gene associations with morphine dose and postoperative pain and then to estimate dose prediction limits. Twenty-seven single nucleotide polymorphisms (SNPs) from 9 genes (*ABCB1, ARRB2, COMT, DRD2, KCNJ6, MC1R, OPRD1, OPRM1, UGT2B7*) met selection criteria and were analyzed along with *TAOK3*. Few associations replicated: morphine dose (mcg/kg) in African American children and *ABCB1* rs1045642 (A allele, ß=−9.30, 95% CI −17.25–−1.35, p=0.02) and *OPRM1* rs1799971 (G allele, ß=23.19, 95% CI 3.27–43.11, p=0.02); *KCNJ6* rs2211843 and high pain in African American subjects (T allele, OR 2.08, 95% CI 1.17–3.71, p=0.01) and in congruent European Caucasian pain phenotypes; and *COMT* rs740603 for high pain in European Caucasian subjects (A allele, OR 0.69, 95% CI 0.48–0.99, p=0.046). With age, body mass index, and physical status as covariates, simple top SNP candidate gene models could explain theoretical maximums of 24.2% (European Caucasian) and 14.6% (African American) of morphine dose variances.

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Conflict of interest
None

Introduction

Pain and opioid response are complex, interrelated phenotypes with interacting genetic and environmental factors that contribute to significant inter-individual variability. Although more than 400 genes have been reported to regulate pain pathways[1], smaller subsets may influence specific pain modalities[2]. Few genes (*e.g. OPRM1* and *COMT*) have shown even moderately consistent associations with acute postoperative pain and opioid analgesia and individual single nucleotide polymorphism (SNP) effect size has been uniformly small.[3–8] Adult studies draw principally on European Caucasian and Asian populations and demonstrate significant influence of racial and ethnic backgrounds.[4, 7, 9–11]

Exploratory pediatric studies using small candidate gene panels have shown mixed results for racial/ethnic differences in postoperative pain and opioid response, but also suggest polygenic and racial effects.[12, 13] Maximal contributions of known covariates and candidate genes have not been determined expressly for morphine dosing and no multi-locus candidate gene study has addressed children of African American descent. We previously investigated acute pain and morphine requirement following day surgery tonsillectomy and adenoidectomy in opioid-naïve children of African American or European Caucasian ancestry and, using genome wide association study (GWAS) methodology, identified a novel locus (*TAOK3*) that accounted for 8% of morphine dose variance in European Caucasian subjects.[14] In our retrospective African American and European Caucasian GWAS discovery cohorts, we sought to replicate associations of select candidate genes for morphine dose, high (7/10) pain, and low (3/10) pain, and, using top SNP candidate gene array modelling, estimate the upper limits of predicted race-specific morphine dose variance.

Materials/Subjects and methods

Subjects

This retrospective study was approved by the Children's Hospital of Philadelphia Institutional Review Board with waiver of consent/assent. Final study subjects, genotyped at the Center for Applied Genomics (CAG), had given consent/assent previously and were enrolled in the Institutional Review Board-approved Study of the Genetic Causes of Complex Diseases. All genotyped subjects from the two largest racial cohorts in the CAG database meeting inclusion/exclusion criteria were studied: 241 children of African American ancestry and 277 children of European Caucasian ancestry. (Table 1) Subjects had undergone day surgery tonsillectomy and adenoidectomy, were 4–18 y of age, had no significant obstructive sleep apnea, were managed with morphine as the sole intravenous analgesic, and had documented, serial recovery room pain scores. Full descriptions of these discovery cohorts were reported previously.[14] The primary phenotype was total (intraoperative plus postoperative) morphine in mcg/kg absolute body weight titrated to achieve comfort sufficient to go home. Two pain score-defined phenotypes were used as secondary outcomes: high maximum pain (7/10) for which additional intravenous analgesics were administered in the recovery room, and low maximum pain (3/10) where no further intravenous analgesia was required.

SNP genotyping

All samples were genotyped as a part of our initial GWAS. Briefly, genomic DNA was extracted from blood samples of patients and genotyped on the Illumina Human-Hap550 SNP array (Illumina, San Diego, CA, USA) or the Illumina Human610-Quad version 1 SNP array. Saliva-derived DNA samples from five subjects failed quality control (QC) filtering and were excluded from analyses. For all samples, QC filtering excluded SNPs with call rate <95%, Hardy-Weinberg equilibrium P-value <0.0001, and minor allele frequency <0.01.

Candidate gene selection

Using the Gene Database derived and published by the National Center for Biotechnology Information (NCBI) Reference Sequence and Genome Annotation Groups (https://ncbi.nlm.nih.gov), we conducted a systematic search for genetic loci affecting acute postoperative or experimental pain and morphine response demonstrating both clinical and basic science support. Electronic query included the combined search terms "morphine and pain," yielding 143 genetic loci for which 36 had supporting human clinical data. We excluded 23 loci because their associations were limited to chronic pain phenotypes or they lacked supporting mechanistic study. Only genetic variation due to individual SNPs was considered; copy number variation and variable tandem repeat were excluded. The final set of 9 established candidate genes and their 27 clinically significant variants are listed in Table 2. The *TAOK3* locus was also included for the gene-based analyses and dose prediction models based on its significance in our prior GWAS. Specific candidate gene and SNP features are further described in Supplementary Tables 1 and 2.

Statistical analysis

For the reported candidate gene variants that existed in the post-QC dataset, we extracted their association statistics from our GWAS dataset. For those that did not, we conducted imputation with the Haplotype Reference Consortium (Release 1) as the reference panel on the Sanger Imputation Service (https://imputation.sanger.ac.uk/). All SNP QC steps before and after imputation were carried out following instructions of the Sanger Imputation Service. Association tests were performed on variants with INFO score >0.7, following the same steps as we previously reported for the genotyped SNPs in our GWAS dataset. Linear regressions were carried out to assess association between SNPs and total morphine sulfate dose requirement with age, body mass index (BMI), and American Society of Anesthesiologists' physical status (PS) classification as covariates. Logistic regressions and Fisher's exact tests were performed for high pain and low pain phenotypes.

To understand the overall associations between the candidate genes and the three phenotypes, and to boost power for detecting genetic associations, we conducted gene-set based tests for all 10 candidate genes (including *TAOK3*) using the Versatile Gene-Based Test for Genome-wide Association (VEGAS).[15] This test is based on the sum of association statistics from single SNPs and includes corrections for linkage disequilibrium structure. We defined gene boundaries to include the 50kb regions upstream and downstream of the gene transcript. All SNPs within these boundaries were included in deriving gene-based association statistics.

In addition to testing individual SNP associations within *COMT,* we tested its 4 SNP (rs6269, rs4633, rs4818, rs4680) foundational haplotype linked to pain using linear regression for morphine dose and logistic regression and Fisher's exact tests for pain outcomes as above.

Predictive modeling

Due to the limited size of the study cohorts, contributions from all 27 variant SNPs in the established candidate genes could not be reliably estimated using GCTA software.[16] Furthermore, because of the varied linkage disequilibrium patterns across the many SNPs in each gene and because we sought to define upper limit prediction boundaries, we explored only the simplest model, utilizing the SNP within each gene having the lowest GWAS P-value for total morphine dose. Using software R, (https://r-project.org) we were then able to construct a general linear regression model including this best variant for each of the 10 candidate genes (including *TAOK3*) as well as the covariates of age, BMI, and PS. Lastly, we assessed the relative importance of each variable in the linear model using the software package Relaimpo.[17]

Results

Demographics and phenotypes

Patient demographics and phenotypes by African American and European Caucasian ancestry are summarized in Table 1. Average total morphine dose was greater in the European Caucasian cohort than that in the African American cohort (P<0.001) and was likely need-based, with significantly fewer European Caucasian children having had 3/10 maximum pain, compared to African American subjects (P < 0.005), and slightly more children in the European Caucasian cohort having had 7/10 maximum pain. Regression analyses confirmed age, BMI and PS to be significant covariates for total morphine dose in both cohorts.

Candidate gene SNP variant analyses

The association statistics for 27 SNPs from the select 9 candidate genes are presented in Table 3. We confirmed that the multiple candidate SNPs within *KCNJ6*[18] were not in significant linkage disequilibrium. (Supplementary Figure) Because our first aim was to replicate individual SNP contributions in two new pediatric cohorts, we did not apply multiple testing correction, thus significance threshold was set at P-value<0.05. In the African American cohort, SNPs rs1045642 in *ABCB1* and SNP rs1799971 in *OPRM1* replicated for total morphine dose requirement; for high maximum pain, two SNPs in *KCNJ6* (rs2211843 and rs2835930) reached threshold; and for low pain phenotype two additional SNPs in *KCNJ6* (rs928723 and rs6517442) were significant. In the European Caucasian cohort, for total morphine dose phenotype, no candidate SNP (with the exception of those in *TAOK3*) reached significance; for high maximum pain phenotype, only SNPs rs740603 in *COMT* and rs563649 in *OPRM1* were significant; for low maximum pain phenotype, the *KCNJ6* SNP rs2835925 and *OPRD1* SNP rs569356 reached significance. Because effect directions for rs563649 and rs569356 countered those of reference studies, [19,20] however, these SNP associations failed replication. We noted that *KCNJ6* SNP

rs2211843 was also marginally associated with high maximum pain and low pain maximum phenotype in the European Caucasian cohort (P=0.07 for both phenotypes); the direction of effects was consistent between these two phenotypes (OR > 1 for high maximum pain phenotype and OR < 1 for low maximum pain phenotype in both cohorts), suggesting that carriers of the minor T allele require more morphine than non-carriers. In the African American cohort, *KCNJ6* rs2835930 ORs behaved similarly between high and low pain phenotypes, though the latter association reached only P=0.08.

Gene-set based analyses

The VEGAS association tests are shown in Table 4. The majority of candidate genes contain SNPs with nominal significance (P < 0.05) for each of the three outcomes, but the tests for most of the selected candidate genes did not reach significance. In the African American cohort, *ABCB1* reached significance for both morphine requirement and high pain phenotypes (P=0.030) and *KCNJ6* was marginally associated with low pain (P=0.081). As expected from our prior GWAS findings, *TAOK3* was associated with high pain in African American subjects (P=0.049). In the European Caucasian group, we also reconfirmed significant associations between *TAOK3* and total morphine sulfate dose (P=6 ×10^{-5}), as well as with high maximum pain phenotype (P=0.0015). For the remainder of candidate genes studied in European Caucasian subjects, we only observed significant association for *ARRB2* with low maximum pain phenotype (P=0.026) and marginal significance of *OPRM1* (P=0.079) and *COMT* (P=0.057) with total morphine sulfate dose.

Association of *COMT* foundational haplotype with morphine requirement and pain phenotypes

In European Caucasian children, we confirmed the three major *COMT* haplotypes, with ATCA being most prevalent (48%) and ACCG being the least common (8.1%). (Table 5) The latter haplotype was associated with reduced total morphine dose requirement (P=0.023), a finding principally driven by male subjects. (Supplementary Table 3) The African American cohort exhibited greater heterogeneity in *COMT* haplotype combinations. (Table 5) The three most frequent were ACCG (28%), ATCA (26%) and GCCG (20%). Interestingly, a less frequent haplotype (ATCG, 4.5%) displayed consistent associations with all three phenotypes and was not significantly influenced by gender. (Supplementary Table 4)

Statistical modeling for total morphine dose requirements and prediction limits

Results of the top SNP array and covariate linear regression models for African American and European Caucasian subjects are shown in Table 6. In African American subjects, only two variants reached relative importance > 0.02: rs6957599 in *ABCB1* and rs858008 in *KCNJ6*. Top variants in *TAOK3*, *OPRM1* and *COMT* all were of relative importance < 0.01. In the European Caucasian cohort, the relative importance of *TAOK3* SNP rs795484 was 0.0642. Other variants of relative importance > 0.02 include rs3778153 in *OPRM1* and rs3788317 in *COMT*. The genetic factors and covariates together contributed to 24.2% variance in total morphine dose requirement in the European Caucasian cohort and 14.6% in the African American cohort.

Discussion

This candidate gene replication study lends further support to several loci previously associated with acute postoperative pain and morphine analgesia (*ABCB1, ARRB2, COMT, KCNJ6, OPRM1*) and to *TAOK3*, but highlights the limits of and inconsistencies within the current opioid pharmacogenetics literature. As other investigators were unable demonstrate associations between 22 candidate genes and opioid analgesia in adult oncologic pain,[21] we too were unable to replicate the majority of prior acute pain/opioid analgesia genetic association findings in children. Establishing the upper bounds of race-specific prediction models using the most significant SNPs within 10 candidate genes and 3 demographic covariates, we could not explain more than 25% of morphine dose variability.

In the largest genotyped African American cohort with detailed analgesia/pain phenotype data to date, we validated associations between morphine requirement and postoperative pain and SNPs within *ABCB1* and *OPRM1*. Using gene-based testing, *ABCB1* was further associated with both morphine requirement and high pain. The minor allele A at rs1045642 within *ABCB1* was associated with decreased morphine requirement consistent with that in postnephrectomy adults[22] and following abdominal hysterectomy.[23] In postoperative children treated with comparable morphine dose across rs1045642 genotype, episodes of severe pain were fewer in minor variant subjects.[13] Furthermore, fentanyl requirements in intensive care[24] and postoperative pain[25] were reduced for those with the minor allele. Small, morphine-specific studies in predominantly European Caucasian subjects have not shown analgesic dose effects for this SNP, however.[12,26–28] Differences in allelic frequencies of *ABCB1* variants by race/ethnicity have been reported (T allele, 56.1% for European Caucasian subjects; 20.2% for African American.)[29] With varied linkage disequilibrium patterns about rs1045642 and racial/ethnic frequency differences for distinct *ABCB1* haplotypes,[29] consistent association for this locus cannot be expected across race.

The minor allele (G) at rs1799971 in *OPRM1* was strongly associated with increased morphine requirement (ß= 23.2 mcg/kg, P=0.02) in children of African American descent, but this finding did not extend to European Caucasian subjects, where the *OPRM1* locus overall only reached gene-based marginal significance. The non-synonymous rs1799971 SNP in *OPRM1* is the most extensively studied in opioid pharmacogenetics and has been shown to alter receptor expression and second messenger coupling.[30,31] A comprehensive review and meta-analysis of this SNP and adult postoperative opioid requirements shows heterogeneity of effect, but robust association for G-allele carriers and higher opioid dose in Asians, morphine users, and patients recovering from surgery on a viscus.[7] That the effect was stronger in morphine (*versus* fentanyl) analgesia-based studies,[11,26,32–36] supports ligand-specific, variant-mediated pharmacodynamic differences at *OPRM1*.[37] None of these studies included subjects of African American ancestry; however, in a study of adult experimental pain, the 7.4% of African American subjects with the minor allele exhibited no sensitivity differences.[38] We hypothesize that our primary rs1799971 association finding for morphine dose, rather than pain, best supports *OPRM1* as a pharmacogene, much as we believe *TAOK3* to be.[39] Our cohorts show that allele rarity (G minor allele frequencies of 0.13, European Caucasian; 0.034, African American does not, in this instance, explain association disparities across races/ethnicities as has been proposed previously.[7, 40]

Association was validated in European Caucasian children with *ARRB2* and low pain phenotype using gene-based testing. In mice, this locus has been shown to enhance morphine analgesia in a knockout model[41] and also following antigene RNA administration that selectively targets *ARRB2* transcription start sites, downregulating expression.[42] *ARRB2* variants can alter morphine analgesic response in adult European Caucasian oncology patients[43] and acute nociception response variability under general anesthesia.[44] While rs7223183 represented our best SNP association, functional studies are limited and it remains unclear which SNP(s) is(are) most relevant. Haplotype effects are likely as has been shown for methadone responsiveness.[45]

Although several SNPs in *COMT*, both single variants and SNPs in combination haplotype have been associated with pain and opioid analgesia, only rs740603 replicated. In our European Caucasian subjects the A minor allele was associated with reduced odds of high pain, consistent with European Caucasian adult minor allele homozygotes who reported decreased pain levels following third molar extraction.[46] It is unclear why this 2kB upstream intron 1 variant replicated, while the more established functional variant, rs4680, did not. Most studies showing decreased morphine requirements associated with the rs4680 minor allele (A) have been in adults of European Caucasian or Asian descent.[3,8,47,48] Small, mixed-race pediatric studies have not consistently supported rs4680 effect direction: the A allele may be associated with increased pain on mobilization following surgery[13] and with decreased postoperative analgesic administration.[49] For children of African American descent rs4633 was of marginal significance with the T allele conferring reduced odds of having high pain. This is consistent with adult female T carriers of Asian descent requiring less postoperative morphine[8] and, in a primarily European Caucasian pediatric population, TT homozygotes having lower maximum postoperative FLACC scores.[49]

The *COMT* locus may be better linked to functional outcomes through haplotype [6,50] and multigene epistatic analyses.[51,52] *COMT* haplotype was shown to predict *in vitro* COMT activity and correlate with chronic temporomandibular joint pain development in European Caucasian adults.[50] Although gene-based analysis showed *COMT* to be marginally associated with morphine requirement in European Caucasian subjects, foundational haplotypes failed replication. In fact, the high pain sensitivity haplotype (ACCG) was associated with decreased morphine requirement, an effect driven by males. Our effect direction also contrasts with a recent report of increased postoperative fentanyl requirements in Asian subjects with ACCG haplotype.[53] However, *in vivo* differences in COMT activity and pain associated with the ACCG haplotype may result from epistatic interactions with other genes,[52] and along with ligand-specificity, may explain result discrepancies. The less common ATCG haplotype, previously described in 1% of European Caucasian and African American subjects,[49] was more likely in an African American child with low pain and had a consistent marginal correlation with lower morphine dose requirement. This haplotype deserves further investigation regarding COMT enzyme activity and opioid analgesic associations. Because of complex global differences in genetic variation and linkage disequilibrium at *COMT*,[54] race-stratified studies are essential.

With significant and marginally significant associations representing multiple relational nodes across phenotype and race, our composite data suggest an important role for *KCNJ6*

in postoperative pain managed with morphine. Early studies showed gene-based differences in both nociception and opioid analgesia,[55,56] and recent work has shown the gene product GIRK2 to be required for opioid-mediated peripheral analgesia.[57] In European Caucasian adults having had total knee arthroplasty, Bruehl *et al* found 8 SNPs within *KCNJ6* associated with analgesic order phenotype.[18] Although our replication findings for several of these SNPs centered on pain phenotype, each association was in the same effect direction: for subjects of African American ancestry, significance was replicated at rs2211843 for high pain (marginal in the European Caucasian cohort for both pain phenotypes); rs2835930 for high pain (marginal for low); and rs928723 for low pain. Children of African American ancestry demonstrated significant association at rs6517442 for low pain and marginal significance for morphine dose, also consistent with Elens *et al.*[58] In subjects of European Caucasian ancestry, an additional SNP identified by Bruehl, rs2835925, was associated with low pain in a direction consistent with the initial discovery cohort. Despite many phenotype and racial consistencies, gene-based analyses for *KCNJ6* showed only marginal significance for low pain. Few SNPs have shown functional significance *in vitro*, although rs2835930 may influence *KCNJ6* expression in the brain.[59]

With these replication results and reconfirmation of *TAOK3* significance at a gene-based level, we were encouraged to model best SNP arrays for all studied candidate genes to estimate their maximum genetic contribution to morphine dose variability. Others have shown that SNP combinations across *ABCB1, COMT, ESR1, OPRM1,* and *UGT2B6* better predict morphine requirement and pain phenotype than isolated SNP or single gene variants alone.[5,23,51,60] While heritability may reach 60% for experimental pain and opioid analgesia phenotypes,[9] recent work on *COMT, ESR1,* and *OPRM1* suggests that an array of 3 – 9 SNPs explain only 5–10.7% of variance in adult postoperative morphine consumption.[5] Our results, which include 10 SNPs/genes and 3 demographic covariates, vary by race and confirm the limited potentials of current candidate gene arrays to predict morphine requirements. Compared to well-characterized disease states such as pediatric onset autoimmune disorders where GWAS-significant SNP contribution to phenotypic variance ranges from 16 – 85%,[61] clinical opioid response in children is less well defined and more variable, significantly reducing potential SNP-explained phenotypic variance.

Of the associations we investigated, many were established for somewhat different phenotypes and each could have risen to inclusion through positive publication bias. Importantly, all were derived in European Caucasian or Asian populations; relevant, but unexamined loci with stronger genetic effects in African American subjects are possible. European Caucasian-derived, African American-replicated associations may reflect particularly robust associations, as shown for asthma.[62] Our small cohorts do not allow for comprehensive analysis of multiple SNPs each expected to make modest contributions to phenotype. Candidate gene variant odds ratios are also small, making them more difficult to replicate. Individual SNP associations are not necessarily responsible for phenotype; causal SNPs could be in linkage disequilibrium with those studied. Finally, we expect that rare variants and additional GWAS-identified loci, such as *TAOK3*, now showing increased importance across other clinical pain and analgesia phenotypes,[63] will become essential components of larger and more precise genetic testing arrays for morphine analgesia and acute postoperative pain.

Summary

This candidate gene replication study in pediatric postoperative pain and opioid analgesia lends additional support to SNPs in *ABCB1* (rs1045642) and *OPRM1* (rs1799971) for morphine dose phenotype in African American subjects; *COMT* (rs740603) for high pain in European Caucasian subjects; and *KCNJ6* (rs928723, rs2211843, rs2835925, rs2835930, rs6517442) for interrelated pain phenotypes across both races. *ABCB1* (African American) and *ARRB2* (European Caucasian) show gene level significance. *COMT* foundational haplotypes failed replication. Our prediction models explain between 14.6% (African American) and 24.2% (European Caucasian) of morphine dose variability. *TAOK3* (rs795484) remains a principal contributor to morphine dose in European Caucasian subjects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1.

Subject Demographics and Phenotypes

Characteristics	European Caucasian (n=277)	African American (n=241)
Age (mo)	100.9 (45.2)	102.6 (40.2)
Sex M/F (%)	49.1/50.9	45.6/54.4
Weight (kg)	33.7 (18.9)	40.1 (23.3)
Height (cm)	129.6 (20.7)	134.1 (19.4)
BMI (kg/m^2)	18.6 (4.5)^a	20.6 (6.5)^a p<0.001
Physical status (%) 1/2/3	20.9/75.1/4.0	15.8/81.3/2.9
Morphine (mcg/kg)	132.4 (40.9)^b	118.6 (39.8)^b p<0.001
Pain ≥ 3 [*] (%)	15.7^c	26.7^c p<0.01
Pain ≥ 7 [*] (%)	49.3^d	46.6^d p=0.596

Summary demographics including age, weight, height, and BMI and the total morphine dose phenotype are reported as mean followed by SD in parentheses. Specific statistical comparisons are indicated with lettered superscripts.

[a,b] based on t-test for equal means

[c,d] based on Fisher's exact test

[*] Maximum pain scores (normalized to a 0 – 10 scale) were not consistently reported in 10.4% of subjects and could not be included. BMI = body mass index, SD = standard deviation.

Table 2.

Candidate genes and variants associated with acute postoperative or experimental pain and morphine analgesia

Gene	SNP	Functional Consequence	Supporting Clinical Studies	Supporting Basic Science
ABCB1	rs1045642	SC 3435T>C	13,22	29,64–68
ARRB2	rs1045280	SC, IV, NcTV Ser280	44	41,42,69,70
COMT	rs4680	MS, UV Val158Met	3,8,13,47,49–52,71–74	50
	rs4818	SC, UV Leu136 408C>G/T	3,49,50,72,73,75	50
	rs6269	IV, UV, UtrV5' Promoter region for S-COMT	49,50,72,75	50
	rs4633	SC, UV His62 186C>T	8,49,50,72	50
	rs740603	IV, UV 3545A>G	46	
DRD2	rs6277	SC 957C>T	76	77,78
KCNJ6	rs2835859 rs1543754 rs858035 rs9981629 rs928723 rs2835925 rs2211843 rs1787337 rs2835930 rs6517442	IV, UV	18,58,79,80	55–57,81–83
MC1R	rs1805007 rs1805008 rs1805009	MS, UV Arg151Cys Arg160Trp Asp294His	84	84–86
OPRD1	rs1042114 rs2234918 rs569356	MS, SC, UV Phe27Cys Gly307	19,48	87–91
OPRM1	rs1799971 rs563649	MS, IV, NcTV,UtrV5' A118G	7,11,20,23,32,33,36,38,40,51,92–94	95–103
UGT2B7	rs7439366	MS Tyr268His 802C>T	23,104	105

Candidate genes from the National Center for Biotechnology Information Gene Database meeting selection criteria. IV=intron variant, MS=missense, NcTV=non-coding transcript variant, SC=synonymous codon, UtrV5=untranslated region variant 5 prime, UV=upstream variant (2kB)

Table 3.

Candidate SNP associations with morphine dose and maximum postoperative pain in children of European Caucasian and African American ancestries

European Caucasians				Morphine sulfate dose (mcg/kg)			High pain (7/10)			Low pain (3/10)		
Gene	SNP	A1	MAF	Beta	CI95	P	OR	CI95	P	OR	CI95	P
ABCB1	rs1045642	A	0.48	−4.34	−2.63,12.29	0.20	0.89	0.624,1.27	0.53	0.91	0.57,1.46	0.72
ARRB2	rs1045280	C	0.29	2.01	−5.46,9.48	0.60	0.90	0.611,1.33	0.59	0.75	0.44,1.29	0.36
COMT	rs4680	A	0.48	4.22	−2.36,10.81	0.21	0.86	0.60,1.22	0.39	1.13	0.71,1.81	0.63
	rs4818	G	0.43	0.71	−6.02,7.43	0.84	1.15	0.81,1.64	0.44	0.76	0.47,1.23	0.28
	rs6269	G	0.44	0.09	−6.61,6.78	0.98	1.14	0.80,1.62	0.48	0.82	0.51,1.32	0.47
	rs4633	T	0.48	4.22	−2.36,10.81	0.21	0.86	0.60,1.22	0.39	1.13	0.71,1.81	0.63
	rs740603	A	0.46	2.96	−3.89,9.82	0.40	0.69	0.48,0.99	**0.046**	1.32	0.83,2.11	0.28
DRD2	rs6277	G	0.46	−4.22	−10.62,2.18	0.20	0.75	0.53,1.05	0.09	1.25	0.78,2.00	0.40
KCNJ6	rs2835859	C	0.081	−1.15	−12.57,10.27	0.84	1.28	0.68,2.39	0.44	0.42	0.13,1.41	0.18
	rs1543754	C	0.5	−2.85	−9.35,3.66	0.39	1.15	0.81,1.63	0.43	1.05	0.66,1.67	0.91
	rs858035	G	0.29	0.39	−6.86,7.63	0.92	1.05	0.72,1.54	0.80	1.10	0.66,1.82	0.70
	rs9981629	C	0.42	−1.65	−8.52,5.21	0.64	0.84	0.59,1.21	0.35	1.11	0.69,1.78	0.72
	rs928723	C	0.49	1.28	−5.60,8.16	0.72	1.05	0.74,1.51	0.77	1.24	0.78,1.98	0.41
	rs2835925	G	0.19	1.70	−6.67,10.06	0.69	0.87	0.56,1.36	0.54	1.84	1.08,3.13	**0.03**
	rs2211843	T	0.24	4.83	−2.63,12.29	0.21	1.44	0.97,2.13	0.07	0.56	0.30,1.03	0.07
	rs1787337	A	0.39	0.13	−6.34,6.59	0.97	1.12	0.79,1.57	0.53	0.96	0.59,1.55	0.90
	rs2835930	A	0.23	2.67	−5.33,10.67	0.51	1.19	0.78,1.80	0.43	0.99	0.57,1.73	1.00
	rs6517442	C	0.36	−1.58	−9.07,5.91	0.68	1.23	0.83,1.81	0.31	0.85	0.52,1.39	0.54
MC1R	rs1805007	T	0.071	0.35	−12.68,13.39	0.96	0.60	0.29,1.22	0.16	1.52	0.67,3.45	0.35
	rs1805008	T	0.071	2.49	−10.89,15.88	0.72	1.00	0.50,2.01	1.00	0.80	0.30,2.10	0.82
	rs1805009	C	0.022	−9.65	−31.47,12.18	0.39	1.50	0.40,5.54	0.55	0.00	IND	0.38
OPRD1	rs569356	G	0.12	−3.02	−13.3,7.26	0.57	0.70	0.40,1.22	0.21	1.92	1.03,3.56	**0.05**
	rs1042114	G	0.12	−2.15	−12.4,8.10	0.68	0.72	0.42,1.25	0.24	1.87	1.01,3.48	0.069
	rs2234918	C	0.43	−2.86	−9.48,3.76	0.40	1.25	0.88,1.76	0.22	0.86	0.53,1.38	0.55
OPRM1	rs1799971	G	0.13	−0.72	−10.4,8.96	0.88	1.30	0.78,2.16	0.32	0.96	0.48,1.90	1.00
	rs563649	T	0.090	−5.96	−17.29,5.38	0.30	0.54	0.29,0.99	**0.046**	0.74	0.31,1.81	0.68
UGT2B7	rs7439366	C	0.46	−3.47	−9.99,3.06	0.30	0.79	0.56,1.13	0.20	1.00	0.63,1.60	1.00
African Americans				Morphine sulfate dose (mcg/kg)			High pain (7/10)			Low pain (3/10)		
Gene	SNP	A1	MAF	Beta	CI95	P	OR	CI95	P	OR	CI95	P
ABCB1	rs1045642	A	0.21	−9.30	−17.25,−1.35	**0.02**	1.12	0.71,1.76	0.64	1.15	0.69,1.90	0.60
ARRB2	rs1045280	T	0.43	2.65	−3.91,9.22	0.43	0.95	0.65,1.39	0.78	0.94	0.61,1.45	0.78
COMT	rs4680	A	0.28	1.26	−6.32,8.85	0.74	0.91	0.59,1.38	0.64	0.69	0.42,1.14	0.15
	rs4818	G	0.16	2.56	−5.46,10.58	0.53	1.40	0.87,2.27	0.17	0.67	0.38,1.21	0.18

Gene	SNP		MAF		CI95	P	OR	CI95	P	OR	CI95	P
	rs6269	G	0.38	3.51	−3.32,10.34	0.32	1.33	0.90,1.96	0.15	0.79	0.51,1.23	0.30
	rs4633	T	0.31	−2.69	−9.97,4.59	0.47	0.70	0.47,1.06	0.09	0.90	0.57,1.43	0.65
	rs740603	G	0.43	−0.95	−7.68,5.79	0.78	1.01	0.69,1.49	0.94	0.92	0.60,1.42	0.71
DRD2	rs6277	A	0.14	0.80	−8.35,9.94	0.86	0.75	0.44,1.28	0.30	1.24	0.70,2.20	0.46
KCNJ6	rs2835859	C	0.35	−5.34	−12.33,1.65	0.14	1.17	0.79,1.74	0.42	0.71	0.45,1.12	0.14
	rs1543754	G	0.43	2.73	−3.89,9.35	0.42	0.79	0.54,1.15	0.21	0.99	0.64,1.52	0.95
	rs858035	G	0.26	−4.83	−12.37,2.72	0.21	0.94	0.61,1.44	0.77	1.24	0.77,2.00	0.37
	rs9981629	C	0.37	−5.13	−11.77,1.52	0.13	0.74	0.50,1.09	0.13	0.88	0.56,1.36	0.56
	rs928723	C	0.37	−4.83	−11.52,1.86	0.16	0.83	0.56,1.23	0.35	1.58	1.03,2.44	**0.04**
	rs2835925	G	0.042	−0.65	−17.93,16.63	0.94	0.56	0.20,1.51	0.24	1.80	0.68,4.77	0.23
	rs2211843	T	0.13	6.79	−3.12,16.71	0.18	2.08	1.17,3.71	**0.01**	0.72	0.37,1.42	0.35
	rs1787337	G	0.18	−0.01	−9.29,9.28	1.00	0.75	0.46,1.24	0.26	1.38	0.81,2.36	0.23
	rs2835930	A	0.29	1.03	−6.39,8.45	0.78	1.68	1.11,2.54	**0.01**	0.65	0.40,1.06	0.08
	rs6517442	C	0.091	−9.52	−20.47,1.44	0.09	0.64	0.32,1.27	0.20	2.17	1.10,4.29	**0.024**
MC1R	rs1805007	T	0.022	18.97	−3.46,41.39	0.10	2.71	0.69,10.63	0.14	0.30	0.04,2.40	0.23
	rs1805008	T	0.016	−8.90	−35.12,17.32	0.51	0.22	0.03,1.93	0.14	2.81	0.56,14.1	0.19
	rs1805009	C	0.0060	4.00	−38.32,46.32	0.85	IND	IND	0.13	0.00	IND	0.39
OPRD1	rs569356	G	0.032	−7.23	−27.47,13	0.48	0.70	0.23,2.19	0.54	0.82	0.22,3.04	0.77
	rs1042114	G	0.034	−4.29	−24.11,15.53	0.67	0.85	0.29,2.49	0.76	0.75	0.20,2.72	0.66
	rs2234918	T	0.36	−0.60	−8.05,6.86	0.88	0.70	0.47,1.04	0.078	1.13	0.73,1.75	0.58
OPRM1	rs1799971	G	0.034	23.19	3.27,43.11	**0.02**	1.48	0.54,4.06	0.44	0.92	0.29,2.90	0.88
	rs563649	T	0.095	4.65	−6.80,16.1	0.43	1.09	0.56,2.10	0.80	1.25	0.61,2.56	0.54
UGT2B	rs7439366	T	0.29	−0.19	−7.90,7.51	0.96	0.83	0.54,1.26	0.38	1.22	0.77,1.95	0.39

SNP= single nucleotide polymorphism; MAF=minor allele frequency; CI95=95% confidence interval; P=P-value; OR=odds ratio.

IND=indeterminant, based on minor allele frequency of 0 for either the phenotype of interest or its comparator. Significant p-values are indicated in bold.

Table 4.

Candidate gene-based associations with morphine dose and maximum postoperative pain in children of European Caucasian and African American ancestries

European Caucasians								
Gene	Morphine sulfate dose			High pain (7/10)			Low pain (3/10)		
	Best SNP[a]	SNP Pval	Gene Pval	Best SNP[a]	SNP Pval	Gene Pval	Best SNP[a]	SNP Pval	Gene Pval
ABCB1	rs4148732	0.047	0.652	rs7793196	0.0341	0.346	rs12720066	0.101	0.911
ARRB2	rs4346260	0.0765	0.374	rs11869640	0.00368	0.121	rs7223183	0.0076	**0.0256**
COMT	rs3788317	0.00225	0.057	rs5993875	0.0325	0.233	rs3804047	0.0473	0.586
DRD2	rs4274224	0.0277	0.199	rs6589382	0.00534	0.227	rs4938025	0.102	0.81
KCNJ6	rs2836035	0.0174	0.542	rs11910276	0.0204	0.581	rs2836014	0.0181	0.871
MC1R	rs885479	0.0618	0.138	rs3803688	0.0236	0.429	rs2302898	0.152	0.734
OPRD1	rs1338062	0.0716	0.338	rs157198	0.144	0.551	rs499062	0.0342	0.201
OPRM1	rs3778153	0.00749	0.0792	rs1319339	0.00926	0.572	rs7738859	0.0183	0.406
TAOK3	rs795484	1.01E-06	**6.00E-05**	rs795484	4.10E-05	**0.00152**	rs9943819	0.071	0.387
UGT2B7	rs7662632	0.107	0.354	rs7662632	0.0943	0.301	rs4348160	0.324	0.794

African Americans								
Gene	Morphine sulfate dose (mcg/kg)			High pain (7/10)			Low pain (3/10)		
	Best SNP[a]	SNP Pval	Gene Pval	Best SNP[a]	SNP Pval	Gene Pval	Best SNP[a]	SNP Pval	Gene Pval
ABCB1	rs6957599	0.0137	**0.0297**	rs1922240	0.0192	**0.0297**	rs12720067	0.0427	0.578
ARRB2	rs754814	0.0678	0.727	rs9890937	0.0396	0.755	rs4790230	0.03302	0.256
COMT	rs6518591	0.0331	0.5	rs737866	0.0717	0.426	rs7289747	0.00938	0.274
DRD2	rs4438071	0.0366	0.341	rs2587550	0.0279	0.446	rs4438071	0.0207	0.802
KCNJ6	rs858008	0.00294	0.563	rs2835931	0.0169	0.44	rs2835822	0.00196	0.0806
MC1R	rs2302898	0.195	0.67	rs7205500	0.323	0.851	rs3803688	0.183	0.709
OPRD1	rs2236857	0.0632	0.309	rs150093	0.0053	0.119	rs150093	0.116	0.849
OPRM1	rs1294092	0.0221	0.164	rs6923231	0.00858	0.344	rs613355	0.0107	0.287
TAOK3	rs7307953	0.0958	0.504	rs428073	0.00964	**0.0491**	rs7299040	0.0822	0.727
UGT2B7	rs11931604	0.149	0.413	rs4587017	0.0319	0.112	rs6850028	0.00234	0.21

[a] =Best SNP=SNP of the lowest P-value in each gene. Significant p-values are indicated in bold.

Table 5.

Association of *COMT* foundational haplotype (rs6269, rs4633, rs4818, rs4680) with morphine dose and maximum postoperative pain in children of European Caucasian and African American ancestries

European Caucasians									
	Morphine sulfate dose (mcg/kg)			High pain (7/10)			Low pain (3/10)		
Haplotype	F	Beta	P	F+	F−	P	F+	F−	P
ATCA	0.48	4.22	0.21	0.45	0.50	0.386	0.51	0.47	0.502
GCGG	0.43	0.706	0.837	0.46	0.42	0.44	0.39	0.45	0.321
ACCG	0.081	−13.6	**0.0227**	0.085	0.078	0.784	0.098	0.079	0.565
African Americans									
	Morphine sulfate dose (mcg/kg)			High pain (7/10)			Low pain (3/10)		
Haplotype	F	Beta	P	F+	F−	P	F+	F−	P
ATCA	0.26	0.496	0.901	0.24	0.27	0.437	0.21	0.28	0.146
GCCA	0.021	8.63	0.499	0.026	0.01	0.386	0.018	0.020	0.877
GCGG	0.16	4.05	0.377	0.18	0.12	0.0852	0.12	0.16	0.204
ACGG	0.033	−8.01	0.463	0.031	0.034	0.848	0.031	0.034	0.870
ATCG	0.045	−14.1	**0.0579**	0.028	0.067	**0.0558**	0.085	0.035	**0.0326**
GCCG	0.20	0.731	0.861	0.21	0.21	0.892	0.20	0.21	0.911
ACCG	0.28	−0.393	0.921	0.29	0.28	0.815	0.34	0.26	0.108

F=frequency in all subjects; F+=frequency in subjects with pain phenotype; F−=frequency in subjects without.

Significant p-values are indicated in bold.

Table 6.

Linear regression model components for morphine dose by race: top SNPs by candidate gene and covariates

European Caucasians				African Americans			
SNP variant	Gene	Estimate (mcg/kg)	Relative importance	SNP variant	Gene	Estimate (mcg/kg)	Relative importance
rs1338062_T	OPRD1	−7.75	0.0128	rs2236857_G	OPRD1	−9.61	0.0168
rs7662632_C	UGT2B7	−5.84	0.0118	rs11931604_C	UGT2B7	−12.48	0.0041
rs3778153_A	OPRM1	9.53	0.0249	rs1294092_C	OPRM1	−1.95	0.0005
rs4148732_G	ABCB1	9.44	0.0152	rs6957599_A	ABCB1	19.21	0.0263
rs4274224_G	DRD2	9.96	0.0190	rs4438071_T	DRD2	−6.32	0.0087
rs795484_A	TAOK3	15.66	0.0642	rs7307953_C	TAOK3	8.13	0.0078
rs885479_A	MC1R	19.88	0.0112	rs2302898_T	MC1R	−3.36	0.0019
rs4346260_A	ARRB2	3.89	0.0041	rs754814_G	ARRB2	−4.73	0.0065
rs2836035_C	KCNJ6	−13.57	0.0143	rs858008_T	KCNJ6	10.52	0.0280
rs3788317_T	COMT	−10.48	0.0260	rs6518591_G	COMT	6.11	0.0093
Age_(mo)		0.010	0.0099	Age_(mo)		−0.25	0.0223
BMI (kg/m^2)		−3.13	0.0728	BMI (kg/m^2)		0.85	0.0105
PS (1,2,3)		−8.05	0.0139	PS (1,2,3)		7.62	0.0339

Linear regression model components for total morphine dose requirement in children of European Caucasian or African American descent. BMI= body mass index, PS= American Society of Anesthesiologists' physical status classification, SNP= single nucleotide polymorphism.