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METHODOLOGY, MECHANISMS & TRANSLATIONAL RESEARCH SECTION

Innate Immune and Neuronal Genetic Markers Are Highly Predictive of Postoperative Pain and Morphine Patient-Controlled Analgesia Requirements in Indian but Not Chinese or Malay Hysterectomy Patients

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Abstract

Objective. Pain severity and opioid requirements in the postoperative period show substantial and clinically significant inter-patient variation due mainly to factors such as age, surgery type, and duration. Genetic factors have not been adequately assessed except for the neuronal *OPRM1* rs1799971 and *COMT* rs4680, whereas the contribution of innate immune signaling pathway genetics has seldom been investigated. **Setting**. Hospital surgical ward. **Subjects**. Women (107 Indian, 184 Malay, and 750 Han Chinese) undergoing total hysterectomy surgery. **Methods**. Morphine consumption, preoperative pain, and postoperative pain were evaluated in relation to genetic variability comprising 19 single-nucleotide polymorphisms (SNPs) in 14 genes involved in glial activation, inflammatory signaling, and neuronal regulation, plus *OPRM1* (1 SNP) and *COMT* (3 SNPs). **Results**. Pre- and postoperative pain and age were associated with increased and decreased morphine consumption, respectively. In Chinese patients, only 8% of the variability in consumption could be explained by these nongenetic and genetic (*BDNF*, *IL1B*, *IL6R*, *CRP, OPRM1*, *COMT*, *MYD88*) factors. However, in Indian patients, 41% of morphine consumption variability could be explained by age (explaining <3%) and variants in *OPRM1* rs1799971, *CRP* rs2794521, *TLR4* rs4986790, *IL2* rs2069762, *COMT* rs4818, *TGFB1* rs1800469, and *IL6R* rs8192284 without controlling for postoperative pain. **Conclusions**. This is the highest known value reported for genetic contributions (38%) to morphine use in the acute postoperative pain setting. Our findings highlight the need to incorporate both genetic and nongenetic factors and consider ethnicity-dependent and nonadditive genotypic models in the assessment of factors that contribute to variability in opioid use.

Key Words: Innate Immune; Neuronal; Genetic Variants; Hysterectomy; Morphine; Postoperative Pain

Introduction

Pain perception from any noxious stimulus, especially surgery, has neuronal, emotional, and humoral immune inputs. Innate immune signaling via microglial and astrocyte activation forms part of the tetrapartite synapse system [1], in which several proinflammatory (e.g., interleukin [IL]-6, IL-1ß) and nociceptive-signaling mediators (tumor necrosis factor [TNF]-α) are strongly implicated. In addition, opioids also activate microglia via an interaction with toll-like receptor 4 (TLR4) [2], leading to a counterintuitive increase in pain behavior in experimental animal models. However, the clinical relevance of these findings remains to be firmly established.

Acute postoperative pain often requires the use of opioids, especially if the pain is moderate to severe in intensity. In the immediate postoperative period, patient-controlled analgesia (PCA) is a commonly used delivery modality for opioids such as morphine [3]. The severity of pain and opioid PCA requirements in the 24- to 48-hour postoperative period show substantial and clinically significant inter-patient variation. Such variability is due to a combination of several nongenetic factors, such as age, surgery, anesthesia type and duration, preoperative pain sensitivity, intraoperative analgesics, and ethnicity [4–8]. Nevertheless, the overall contribution of these factors to variability is small, with much of the remaining variability largely unexplained. Attention has therefore been directed to genetic factors such as the mu opioid receptor gene (*OPRM1*) allelic variant A118G (rs1799971), which causes reduced receptor function, resulting in clinically significantly higher morphine (~40%) doses in Asian patients, including women undergoing total hysterectomy [9]. Catechol-O-methyltransferase affects pain sensitivity as well as opioid receptor density, and three variants of its gene *COMT* have been associated with PCA morphine requirements in Chinese, Malay, and Indian women undergoing hysterectomy [10]. In the same study, a low-pain-sensitivity *COMT* haplotype was also associated with PCA morphine requirements.

We recently showed that in cohorts of Chinese, Malay, and Indian women undergoing elective caesarean section, postoperative morphine PCA requirements were ethnicity and *OPRM1* A118G dependent, as well as *IL1B*, *IL6*, *TLR2*, and *TGFB1* variant dependent, although the pain was mild in most cases and morphine requirements were low (between 4 and 12 mg in the 24-hour postoperative period) [11]. Overall, 10% of the variance in morphine consumption could be accounted for by these genetic variants, ranging from 3% in Chinese to 15% in Indian women.

The aim of the present study was to examine, in a hysterectomy cohort of Chinese, Malay, and Indian women with higher postoperative pain and larger morphine requirements (compared with caesarean), and for whom *OPRM1* [12, 13] and *COMT* [10] variants had already been shown to be important neuronal genetic factors , whether these same or other innate immune signaling variants could be factors contributing to pain and morphine requirements. We also assessed the contribution of nongenetic factors such as age, body weight, preoperative pain tolerance, and surgery duration.

Methods

Study Subjects

A total of 1,046 Chinese, Malay, and Indian (primarily from the Tamil region) women undergoing total hysterectomy surgery were recruited, with clinical details and some results having been previously reported [10, 12]. General anesthesia was induced via body weight–adjusted propofol and fentanyl with atracurium to aid tracheal intubation. Sevoflurane and nitrous oxide were used to maintain anesthesia, supplemented with intravenous (IV) morphine, with ondansetron and dexamethasone administered before surgery completion. All study procedures were approved by the SingHealth Centralized Institutional Review Board (CIRB Ref: 2010/540/A) and the University of Adelaide (H-077-2011). Written informed consent was obtained from the patients after the study was explained to them and before the scheduled procedure.

Data were collected on age, weight, self-reported ethnicity, duration of surgery, morphine and fentanyl use during surgery, postoperative morphine consumption, and pre- and postoperative pain.

Morphine Consumption

On arrival at the postoperative area, all patients received IV PCA set to deliver a 1-mg IV bolus of morphine per demand with a lockout time of 5 minutes, without continuous background infusion. The maximum amount of morphine allowed was 10 mg/hour. The cumulative dose of morphine administered by each patient within every 4-hour period was recorded until 24 hours after surgery. Patients were monitored and could also request additional IV morphine as a 1-mg bolus.

Preoperative Pain Measures

Preoperative pressure pain threshold and tolerance were measured as described previously with a blood pressure cuff on the upper arm, which was inflated; the pressure numbers in kilopascals (kPa) when the patient complained of pain (threshold) and requested cuff deflation (tolerance) were recorded [12]. Preliminary analyses indicated that preoperative pressure pain tolerance (vs threshold) was the better predictor of postoperative pain and morphine PCA use (data not shown). Therefore, only pressure pain tolerance was included in further statistical analyses.

Postoperative Pain Measurement

Immediately after surgery (before morphine PCA) and every 4 hours for 24 hours from the time of arrival at the postoperative recovery area, subjects rated the degree of pain on a visual analog scale (VAS) consisting of a numeric scale ranging from 0 to 10 points, with 0 being "no pain at all" and 10 being "maximum pain." These evaluations were timed with the measurement of vital signs such as blood pressure and pulse rate, with the patient awake.

Genetic Analysis

Before surgery, blood (3 mL) was collected in EDTA tubes and stored frozen. DNA was extracted (Gentra Puregene Blood Kit; Gentra Systems Inc., Minneapolis, MN, USA) and checked for quantity and purity (NanoDrop Spectrophotometer; NanoDrop Technologies, Wilmington, DE, USA). An aliquot was transported to Adelaide, Australia, for multiplex analysis.

Adelaide Sample Analysis

DNA samples were genotyped for immune and opioid signaling SNPs (Supplementary Data Table 1) with the use of a custom-designed multiplex array (Sequenom MassARRAY iPLEX Gold, Sequenom Inc., San Diego, CA, USA) at the Australian Genome Research Facility (Brisbane, Australia). We have reported on this previously [11, 14, 15], together with the rationale for the selection of SNPs in a caesarean section study [11].

Singapore Sample Analysis

Subjects had also been genotyped for *COMT* rs4680, rs4633, and rs4818 SNPs, and haplotypes (low [LPS (rs4633>C + rs4818>G + rs4680>G)], average [APS (rs4633>T + rs4818>C + rs4680>A)], and high [HPS (rs4633>C + rs4818>C + rs4680>G)] pain sensitivity) were inferred , as described previously [10, 16].

Statistical Analysis

Statistical analyses were performed in the R statistical environment [17]. For immune and opioid signaling SNPs included in the Adelaide analysis, chi-squared analysis was used to test for genotype deviations from Hardy-Weinberg equilibrium. Differences in allele frequencies between the three ethnic groups (two-way comparisons) were tested with Fisher's exact test with odds ratio ("stats" package) [17]. The Benjamini-Hochberg step-up procedure [18] for false discovery rate (FDR $\alpha = 0.02$) was used for multiple comparisons adjustment. The "LD" (linkage disequilibrium) function of the "genetics" package [19] was used to assess linkage disequilibrium between SNPs within *IL1B*, *IL10*, *TLR4*, and *CASP1* within each ethnic group. Tightly linked SNPs were combined into haplotypes for analysis.

COMT genotype deviations from Hardy-Weinberg equilibrium and ethnic differences in allele and genotype frequency were determined as previously described [10]. *COMT* haplotypes were analyzed as separate factors of three groups (homozygous carrier, heterozygous carrier, noncarrier). To limit redundant analyses, only *COMT* SNPs and/or haplotypes previously associated with a specific phenotype within an ethnic group [10] were included in the genetic analysis for that corresponding phenotype and ethnic group (as specified below for specific analyses). Where no *COMT* SNPs had previously been identified or investigated by Tan and colleagues [10] for a phenotype, all three *COMT* SNPs were included in the genetic analysis for that corresponding phenotype.

Preoperative pressure pain tolerance was analyzed as a binomial variable, with patients divided into those who could tolerate the maximum 300 kPa ("high pain tolerance") and those who could not ("low pain tolerance"). Major nongenetic variables associated with preoperative pressure pain tolerance (high–low) that needed to be controlled for in subsequent genotype analyses were identified by LASSO regression as described previously [11, 14]. The variables tested were ethnicity (factor), age (in years; $\lambda = -0.3$ Box-Cox–transformed to a Gaussian distribution), and weight (in kilograms; square-root–transformed to a Gaussian distribution). Nongenetic variables selected from LASSO regression were then included in all subsequent analyses involving genetic polymorphisms.

A step-down regression model selection procedure based on cross-validation error (CVE) was used to identify genetic factors (immune and opioid signaling SNPs and all three *COMT* SNPs) associated with preoperative pressure pain tolerance (high–low), fixing major nongenetic predictors as the base model and incorporating first-order genotype interactions with ethnicity (regardless of LASSO regression results), as described previously [11, 14]. The "best" model selected was that with the lowest CVE within the fifth percentile of CVEs from randomized permutation controls (see [11, 14] for full details). No assumptions of dominance were made. However, where the number of homozygous variant individuals within an analysis set was fewer than three, homozygous variant and heterozygous genotypes were combined into a variant carrier group. This procedure was also performed within each ethnic group separately.

The significance of genotype main effects and ethnicity× genotype interactions were determined by likelihood-ratio test ("Anova" function of "car" package [20]), and all genotype multiple comparison (Tukey) post hoc significances within SNPs were determined with the "glht" function of the "multcomp" package [21]. The effects of each predictor (averaging over other terms in the model) in the final selected model were visualized with the "effect" function of the "effects" package [22].

The postoperative pain VAS score immediately after surgery was analyzed as a binomial variable (VAS_0), with patients divided into "low pain" (VAS score <2) and "high pain" (VAS score >2) groups; VAS data could not

be transformed to a normal distribution, and preliminary analyses (not shown) identified this dichotomization as the best reduction to explain most of the relationship with subsequent morphine PCA use and also to distinguish between ethnic groups while enabling binomial generalized linear regression analysis.

Nongenetic variables and genotypes (immune and opioid signaling SNPs and *COMT* rs4818 [10]) associated with VAS_0 were identified via the same procedures as for preoperative pressure pain, with the addition of surgery duration (in minutes; $\log_e$-transformed), amount of morphine used during surgery (in milligrams; square-root–transformed), amount of fentanyl used during surgery (in micrograms; $\lambda = -1$ Box-Cox–transformed), and amount of morphine used in recovery (0 vs >0 mg) as possible nongenetic predictors , with and without controlling for preoperative pressure pain tolerance. The procedures were performed with ethnic groups combined and within each ethnic group separately.

Backward stepwise regressions for postoperative pain did not converge for Indian patients because of small numbers of patients with "low pain" (n = 14). Therefore, chi-squared or Fisher's exact test was used to test for genotype differences (immune and opioid signaling SNPs and all three *COMT* SNPs) in the proportion of patients with "high pain." If SNPs were significantly associated in univariate analysis after correction for multiple testing (FDR = 0.1), they were also combined with major nongenetic predictors in multiple generalized (binomial logistic) regression analysis, with significant genotype differences identified by likelihood-ratio testing with the "Anova" function in the "car" package [20].

Total PCA morphine use in the first 24 hours after surgery (excluding the recovery period) was square-root–transformed to a normal distribution to facilitate linear regression. Nongenetic variables and genotypes (immune and opioid signaling SNPs and one of the following: *COMT* rs4680 [in near-perfect linkage disequilibrium with rs4633] and APS haplotype within Chinese patients, *COMT* rs4818 within Indian patients, or no *COMT* SNPs within Malay patients [10]) associated with morphine consumption were identified through the use of a procedure similar to that used for postoperative pain, with and without controlling for preoperative pressure pain tolerance and postoperative pain (as a factor consisting of quartiles of VAS score immediately after surgery).

Because the relationship between preoperative pressure pain tolerance and morphine PCA use varied between ethnic groups (see Supplementary Data Figure 1A), analyses were performed within each ethnic group separately. Diagnostic plots (quantile–quantile, Cook's distance) were used to assess the final selected models for normality of residuals and to identify potential outlier cases with high leverage. The relative contributions of each regressor were assessed with the averaging-over-orderings method proposed by Lindeman et al. [23] (implemented with the R package "relaimpo") [24].

Results

Demographics and outcomes for patients with genotype data for all immune and opioid signaling SNPs investigated (excluding *COMT*) are shown in Table 1. In total, 750 Chinese, 184 Malay, and 107 Indian patients were successfully genotyped for all SNPs (including *COMT*). For curation purposes [25], they are categorized as East Asian, East Asian, and Central/South Asian, respectively.

Genetic Variability

Adelaide Sample Analysis

Of 1,046 patients, 1,041 were successfully genotyped for all 21 immune and opioid signaling gene SNPs (n = 5 with no results for any SNP). Variant allele frequencies in each ethnic group are shown in Supplementary Data Table 2. Genotype frequencies in each ethnic group did not deviate significantly from Hardy-Weinberg equilibrium ($P > 0.3$). Significant ethnic differences were found for most of the SNP frequencies (Supplementary Data Table 2).

Linkage disequilibrium between SNPs in *IL1B*, *IL10*, *TLR4*, and *CASP1* within each ethnic group is shown in Supplementary Data Table 3. There was significant (chi-squared $P < 0.01$) linkage disequilibrium between all SNPs within each gene. Near-complete and near-perfect linkage disequilibrium between *IL1B* rs1143627 (T > C) and rs16944 (C > T) SNPs produced three common (T/T-C/C [29%], T/C-C/T [47%], C/C-T/T [22%]) and three rare (T/C-C/C [<1%], C/C-C/T [1%], C/C-C/C [<1%]) diplotypes in all ethnic groups. The three rare *IL1B* diplotypes were combined with the heterozygous diplotype for subsequent analyses. Linkage disequilibrium between the *IL10* rs1800871 and rs1800896 SNPs was near complete but imperfect ($r^2 \ll 1$); therefore, these SNPs were analyzed separately. *CASP1* rs554344 (G > C) and rs580253 (G > A) SNPs were in complete linkage disequilibrium in all ethnic groups, so only the rs554344 SNP was included in further statistical analyses.

COMT Genetic Variability

As reported previously [10], all three *COMT* SNPs were common in all three ethnic groups, with variant allele frequencies above 20% (rs4633 T = 0.30, 0.23, and 0.40; rs4818 G = 0.30, 0.26, and 0.31; and rs4680 A = 0.30, 0.23, and 0.37 in Chinese, Malay, and Indian patients, respectively). *COMT* rs4633 and rs4680 variant alleles were in significant ($P < 3 \times 10^{-16}$) linkage disequilibrium in all three ethnic groups ($r^2 = 0.92$, $D' = 0.95$ in Chinese; $r^2 = 0.88$, $D' = 0.94$ in Malay; $r^2 = 0.91$, $D' = 1.0$ in Indian), whereas the rs4818 variant allele was in significant ($P < 8 \times 10^{-10}$) linkage disequilibrium with wildtype rs4680 and rs4633 alleles in all three ethnic

Table 1. Demographics and outcomes data for Chinese, Malay, and Indian women undergoing total hysterectomy surgery

	Median (1st quartile, 3rd quartile; range) (unless indicated otherwise)		
	Chinese (n = 750)	Malay (n = 184)	Indian (n = 107)
Age, y	48 (45, 51; 16–45)	47 (44, 50: 30–69)	47 (44, 50; 36–67)
Weight, kg	58 (52, 65; 35–92)	68 (57, 70: 40–91)	66 (59, 73; 37–93)
Presurgery pressure pain tolerance, kPa	290 (270, 300; 190–300) (ND = 66)	290 (260, 300; 180–300) (ND = 14)	290 (260, 300; 170–300) (ND = 7)
Surgery duration, min	105 (85, 124; 47–288) (ND = 1)	104 (82, 123; 52–240)	106 (85, 126; 52–240)
Morphine during surgery, mg	9 (8, 10; 4–17)	10 (9, 10; 6–15)	10 (10, 10; 6–15) (ND = 1)
Fentanyl during surgery, μg	110 (100, 130; 70–200)	110 (100, 140; 80–200)	120 (100, 140; 70–190)
Morphine in recovery, mg	1 (0, 3; 0–21) (ND = 1)	1 (0, 3; 0–24)	3 (0, 7; 0–26)
Antiemetic in recovery, n, no:yes	11:739	3:181	2:105
Postsurgery VAS score (premorphine PCA)	2 (0, 3; 0–8)	2 (1, 3; 0–8)	3 (2, 3; 0–10)
"None" (VAS 0), n (%)	194 (26)	38 (21)	10 (9)
"Mild" (VAS = 1–3), n (%)	454 (61)	119 (65)	72 (67)
"Moderate" (VAS = 4–6), n (%)	90 (12)	26 (14)	17 (16)
"Severe" (VAS = 7–10), n (%)	12 (2)	1 (1)	8 (7)
First 24 hours after surgery:			
Total PCA morphine, mg	13 (6, 21; 0–64)	17 (8, 27; 1–71)	25 (17, 37; 1–69)
Average VAS score	1 (0.5, 1.7; 0–5.7)	1 (0.7, 1.7; 0–4.3)	1.7 (1, 2.2; 0–4)
Average nausea score, n, 0:>0 (range)	570:180 (0–1.8)	157:27 (0–0.7)	91:16 (0–1.8)
Vomiting episodes, n, 0:>0 (range)	647:103 (0–4)	174:10 (0–3)	91:12 (0–7)

ND = number of patients with missing data.

groups ($r^2 = 0.10$ to 0.21, $D' = 0.82$ to 0.97). LPS, APS, HPS, and other (rs4633-rs4818-rs4680 C-C-A, T-C-G or T-G-A) haplotype frequencies were 0.30, 0.29, 0.39, and 0.02 in Chinese, 0.28, 0.21, 0.48, and 0.02 in Malay, and 0.30, 0.36, 0.30, and 0.04 in Indian patients, respectively.

Preoperative Pressure Pain Tolerance

Fifty-one percent (348/684) of Chinese, 56% (96/170) of Malay, and 59% (59/100) of Indian patients with preoperative pressure pain tolerance data were classified as having "low pain tolerance." No major nongenetic predictors of "low pain tolerance" were identified by LASSO regression.

In the combined analysis of Chinese, Malay, and Indian patients, *IL10* rs1800871 and *TNFA* rs1800629 variant genotypes were associated with low and high pain tolerance, respectively, while *TLR4* rs4986791 and *IL6R* rs8192284 genotypes were associated with low pain tolerance, depending on ethnicity (Table 2). An alternative model additionally incorporating the *COMT* rs4818 genotype (lower pain tolerance) had the lowest CVE of the models tested but was within the seventh percentile of randomized control CVEs, and thus it is presented in Supplementary Data Table 4.

Within the group of Chinese patients, *IL1B* rs1143634 heterozygosity (no homozygous variants found) and *COMT* rs4818 variant genotypes were associated with high and low pain tolerance, respectively (Table 2). An alternative model additionally incorporating *TNFA* rs1800629, *IL6R* rs8192284, and *CASP1* rs554344 genotypes had a lower CVE but was within the sixth percentile of randomized control CVEs, and thus it is presented in Supplementary Data Table 4. For Malay patients, *IL10* rs1800871, *TLR4* rs4986791, and *IL6R* rs8192284 variant genotypes were associated with low pain tolerance and *OPRM1* rs1799971 variant genotypes with higher pain tolerance (Table 2). Within Indian patients, all tested models were within the 61st percentile or above of randomized controls, and because of the smaller sample size, multiple fitted probabilities were numerically 0 or 1 (perfect separation) and/or confidence intervals could not be estimated (fitted probability near 1).

Postoperative Pain

The proportions of patients with postoperative (before morphine PCA) VAS scores >2 ("high" pain) vs <2 ("low" pain) were 52% in Chinese (n = 284 vs 261, respectively), 57% in Malay (69 vs 52), and 82% in Indian (62 vs 14) patients. Ethnicity and preoperative pressure pain tolerance (less than or greater than 300 kPa) were identified as the major nongenetic predictors of postoperative pain when all ethnicities were combined. With controlling for preoperative pressure pain tolerance, Malay patients had nonsignificantly (adjusted odds ratio [95% confidence interval]= 1.24 [0.82 to 1.89], post hoc $P = 0.5$) and Indian patients significantly (5.11 [2.72 to 10.5], post hoc $P < 0.0001$ [$P = 0.0005$ vs Malay]) greater likelihood of "high pain" than Chinese patients (ethnicity main effect $P = 4 \times 10^{-7}$). With controlling for ethnicity, patients with "low pain tolerance" before surgery were significantly more likely to have "high" pain after surgery (adjusted odds ratio 1.77 [1.30 to 242], $P = 0.0003$).

In combined ethnicity analysis, no model incorporating genetic markers performed adequately compared with randomized data controls, with or without

Table 2. Genetic predictors of low pressure pain tolerance before hysterectomy surgery in Chinese, Malay, and Indian women

Regressor	Adjusted Odds Ratio† (95% CI)		Nested Model Likelihood-Ratio Test *P* Value
Combined ethnicity analysis (Chinese, Malay, and Indian)			
Ethnicity			0.17
Chinese (n = 684)	ref		
Malay (n = 169)	1.19	(0.73 to 1.92)	
Indian (n = 100)	1.71	(0.88 to 3.38)	
IL10 rs1800871			0.009
C/C (n = 114)	ref		
C/T (n = 414)	1.12	(0.73 to 1.73)	
T/T (n = 425)	1.65	(1.07 to 2.56)#	
TNF rs1800629			0.03
G/G (n = 777)	ref		
G/A (n = 166)	1.30	(0.92 to 1.85)	
A/A (n = 10)	0.21	(0.03 to 0.85)#	
Ethnicity × *IL6R* rs8192284‡			0.007
Chinese A/C	1.35	(0.97 to 1.88)	
Chinese C/C	0.91	(0.57 to 1.45)	
Malay A/C	1.15	(0.60 to 2.21)	
Malay C/C	NA		
Indian A/C	1.36	(0.56 to 3.42)	
Indian C/C	0.52	(0.14 to 1.85)	
Ethnicity × *TLR4* rs4986791§			0.04
Chinese C/T	NA		
Chinese T/T	NA		
Malay C/T	7.79	(1.37 to 147)	
Malay T/T	NA		
Indian C/T	1.02	(0.43 to 2.46)	
Indian T/T	NA		
(Intercept)	0.66	(0.42 to 1.03)	
Chinese only			
IL1B rs1143634			0.03
C/C (n = 660)	ref		
C/T (n = 21)	0.36	(0.13 to 0.90)	
T/T (n = 0)	NA		
COMT rs4818			0.007
C/C (n = 333)	ref		
C/G (n = 286)	1.25	(0.91 to 1.72)	
G/G (n = 62)	2.42	(1.37 to 4.38)**	
(Intercept)	0.90	(0.73 to 1.12)	
Malay only			
IL6R rs8192284			0.002
A/A (n = 94)	ref		
A/C (n = 66)	1.25	(0.64 to 2.50)	
C/C (n = 9)	NA		
TLR4 rs4986791			0.008
C/C (n = 160)	ref		
C/T (n = 9)	10.7	(1.72 to 212)	
T/T (n = 0)	NA		
OPRM1 rs1799971			0.008
A/A (n = 57)	ref		
A/G (n = 84)	0.75	(0.35 to 1.55)	
G/G (n = 28)	0.21	(0.07 to 0.58)*#	
IL10 rs1800871			0.09
C/C (n = 21)	ref		

(continued)

Table 2. continued

Regressor	Adjusted Odds Ratio† (95% CI)		Nested Model Likelihood-Ratio Test *P* Value
C/T (n = 78)	1.35	(0.46 to 4.06)	
T/T (n = 70)	2.59	(0.88 to 7.91)	
(Intercept)	0.90	(0.73 to 1.12)	

CI = confidence interval.

†Odds ratio controlling for all other regressors in multiple binomial generalized linear regression, with Chinese (for ethnicity) and homozygous wild-type genotype groups as reference.

‡*IL6R* rs8192284 A/A, A/C, and C/C genotypes: in Chinese, n = 272, 312, and 100; in Malay, n = 94, 66, and 9; and in Indian, n = 51, 34, and 15.

§rs4986791 C/C, C/T, and T/T genotypes: in Chinese, n = 684, 0, and 0; in Malay, n = 160, 9, and 0; and in Indian, n = 64, 33, and 3.

Odds ratio greater than 1 indicates an association with increased likelihood of low pressure pain tolerance (less than 300 kPa), and a ratio of less than 1 indicates an association with high pain tolerance.

Post hoc

*$P < 0.05$ and

**$P < 0.01$ vs homozygous wildtype genotype.

#$P < 0.05$ vs heterozygous genotype.

NA = no such genotype within ethnic group, fitted probabilities numerically 0 or 1 (perfect separation), and/or unable to estimate confidence interval (fitted probability near 1). Indian only model was not significant.

controlling for preoperative pain tolerance (CVEs in >55th and >23rd percentiles of randomized data controls, respectively). However, in both cases, the models with the lowest CVEs relative to randomized data controls identified *TLR4* rs4986790 variant genotypes as associated with "high" postoperative pain (see Figure 1B and Supplementary Data Table 5).

Within Chinese patients, multiple regression identified *IL1B* rs1143634 heterozygosity (no homozygous variants found) as associated with decreased likelihood of "high" postoperative pain when controlling for (adjusted odds ratio [95% confidence interval] = 0.16 [0.02 to 0.58], $P = 0.004$), or not controlling for (0.13 [0.02 to 0.46], $P = 0.0008$) preoperative pressure pain tolerance. Fifty-three percent (279/526) of Chinese *IL1B* rs1143634 C/C genotype patients had "high" postoperative pain, compared with only 13% (2/16) of those with the C/T genotype; median (25th percentile, 75th percentile; range) VAS scores were 2 (0, 3; 0–8) and 1 (0, 2; 0–4) in the C/C and C/T genotype groups, respectively. Within Malay patients, no model incorporating genetics performed adequately compared with randomized data controls, with or without controlling for preoperative pain tolerance (CVEs in >8th and >17th percentiles of randomized data controls, respectively). However, in both cases, the models with the lowest CVEs relative to randomized data controls identified *TLR4* rs4986791, *IL6R* rs8192284, and *TLR2* rs3804100 variant genotypes as associated with "high" postoperative pain (see Figure 1B and Supplementary Data Table 6). Within Indian patients, no genetic factors were significantly associated with postoperative pain by univariate analysis (point-wise $P > 0.1$).

Morphine Consumption

Within the group of Chinese patients, high pre- and postoperative pain was associated with significantly increased morphine PCA use in the 24 hours after surgery (Supplementary Data Table 7 and Supplementary Data Figure 1), and increasing age was associated with significantly decreased morphine PCA use in the 24 hours after surgery (Table 3 and Supplementary Data Figure 1). Accounting for these three nongenetic variables, no model incorporating genetics performed adequately compared with randomized data controls; the model with the lowest CVE relative to randomized data controls (11th percentile) is provided in Supplementary Data Table 7. With controlling for the effect of age only, the *BDNF* rs6265 and *IL1B* rs1143634 variant genotypes were associated with significantly lower morphine PCA use, and the *IL6R* rs8192284, *OPRM1* rs1799971, and *CRP* rs2794521 variant genotypes were associated with significantly higher morphine PCA use (Table 3), with *MYD88* rs6853 and *COMT* rs4680 also retained in the "best" model without being statistically significant factors themselves. In combination, age and genetic factors in Table 3 predicted 8% of the variability in morphine use within Chinese patients. For Malay patients, high preoperative (nonsignificantly) and postoperative (significantly) pain was associated with increased morphine PCA use, and higher age (nonsignificantly) was associated with decreased morphine PCA use (Supplementary Data Table 8 and Supplementary Data Figure 1). Accounting for these three nongenetic variables, or for age only, no model incorporating genotypes performed adequately compared with randomized data controls (>11th percentile); the models with the best CVE relative to randomized data controls are provided in Supplementary Data Table 8.

For Indian patients, high postoperative (but not preoperative) pain was (nonsignificantly) associated with increased morphine PCA use, and higher age was (nonsignificantly) associated with decreased morphine PCA use (Table 4 and Supplementary Data Figure 1).

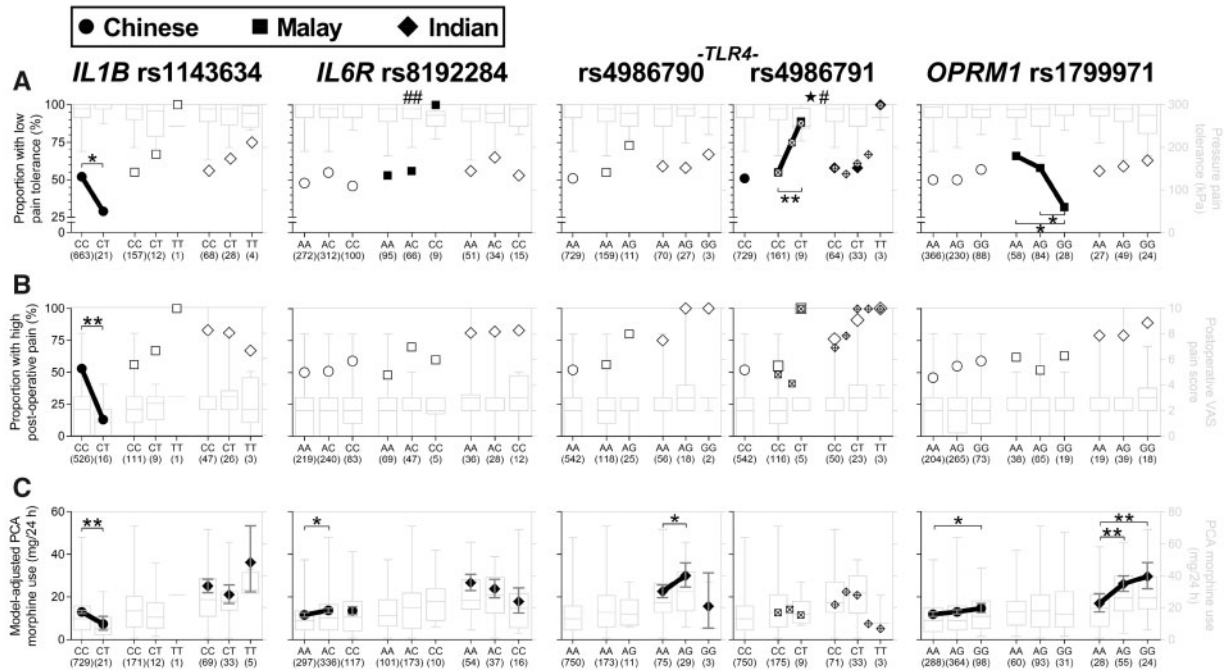

Figure 1. Major genetic influences on **(A)** preoperative pressure pain tolerance, **(B)** postoperative pain, and **(C)** postoperative morphine PCA use in Chinese (circle), Malay (square), and Indian (diamond) women undergoing total hysterectomy. Only polymorphisms of genes identified in three or more models of pain or morphine use are shown. "Low pain tolerance"= pressure pain tolerance less than 300 kPa (maximum). "High postoperative pain"= postsurgical VAS scores (before morphine PCA) >2. Letters are genotypes, and numbers in brackets are genotype N. **(A–B)** Points represent unadjusted proportions plotted on the left y-axis. **(C)** Filled points and error bars are back-transformed mean ± 95% confidence interval of morphine PCA use, with other model main effects held to typical values (mean or proportional distribution) for multiple regression models in Chinese patients {$\sqrt{}$(morphine PCA use) ~ age [Box-Cox ($\lambda = -0.3$) transformed] + rs1143634 + rs6265 + rs8192284 + rs2794521 + rs1799971 + rs4680 + rs6853} and Indian patients {$\sqrt{}$(morphine PCA use) ~ age [Box-Cox ($\lambda = -0.3$) transformed] + VAS pain score immediately after surgery (VAS quartiles, unordered factor) + rs1143634 + rs1799971 + rs2069762 + rs2794521 + rs4986790 + rs8192284}. Filled points highlight genotypes selected as predictors in multiple regression models for the respective responses **(A–C)**, with significant post hoc differences connected by solid lines. Crossed squares and diamonds represent combined *TLR4* rs4986790 and rs4986791 genotypes, with intermediate points representing mixed heterozygous–homozygous genotypes. All box-plots are unadjusted medians, 25th–75th percentiles, and range plotted on the right y-axis. Type II likelihood-ratio chi-squared *$P < 0.05$ genotype main effect, or #$P < 0.05$, ##$P < 0.01$ genotype × ethnicity interaction, for multiple binomial generalized linear regression model: Pain tolerance (low vs high) ~ ethnicity + rs1800871 + rs1800629 + rs4986791 + rs8192284 + ethnicity*rs4986791 + ethnicity*rs8192284. Tukey post hoc *$P < 0.05$ and **$P < 0.01$ (within-ethnicity multiple regression models).

With controlling for postoperative pain and age, the *IL1B* rs1143634 heterozygote and *CRP* rs2794521 and *IL6R* rs8192284 homozygous variant genotypes were associated with lower morphine PCA use, and the *TLR4* rs4986790 heterozygote, *IL1B* rs1143634 homozygous variant, and *OPRM1* rs1799971 and *IL2* rs2069762 variant genotypes were associated with increased morphine PCA use (Table 4). With controlling for the effect of age only, *CRP* rs2794521, *IL6R* rs8192284, *TLR4* rs4986790, *OPRM1* rs1799971, and *IL2* rs2069762 (but not *IL1B* rs1143634) were similarly associated with morphine PCA use (as when controlling for postoperative pain), with *COMT* rs4818 and *TGFB1* rs1800469 additionally associated with higher and lower morphine use, respectively (Table 4). In combination, these variables predicted 41% of the variability in morphine use within Indian patients.

Diagnostic plots indicated acceptable normality of residuals, with no high leverage outliers for all models described here and in the Supplementary Data.

For individual or linked SNPs identified in three or more models of pre- or postoperative pain or morphine PCA use, an additional visual summary of their effect patterns across each of the facets of pain and analgesia investigated, as well as similarities or differences in effects across ethnicities, is provided in Figure 1.

Discussion

We aimed to investigate innate immune signaling pathway genetic contributions to variability in preoperative pain tolerance, postoperative pain, and morphine PCA requirements in Chinese, Malay, and Indian women undergoing hysterectomy surgery. We previously showed immunogenetic associations with morphine use after caesarean surgery [11], but it was unclear whether this was due to the underlying pain phenotype or to morphine response per se. In the present study, our patients had a higher incidence of moderate–severe pain and required higher postoperative morphine, enabling further

Table 3. Predictors of morphine PCA use in the first 24 hours after surgery in 750 Chinese women undergoing total hysterectomy, without controlling for preoperative pain tolerance or acute postoperative pain

Coefficients	Estimate (95% CI)		ANOVA *P* Value	Contribution‡ to Model ($R^2 \times 10^{-2}$)
Age†	–5.56	(–8.4 to –2.7)	0.0002	1.8
IL1B rs1143634			0.004	1.1
C/C (n = 729)	ref			
C/T (n = 21)	–0.89	(–1.49 to –0.29)		
BDNF rs6265			0.007	1.2
G/G (n = 194)	ref			
G/A (n = 372)	–0.38	(–0.61 to –0.14)**		
A/A (n = 184)	–0.17	(–0.45 to 0.10)		
IL6R rs8192284			0.02	1.1
A/A (n = 297)	ref			
A/C (n = 336)	0.29	(0.08 to 0.51)*		
C/C (n = 117)	0.27	(–0.02 to 0.56)		
CRP rs2794521			0.03	0.9
T/T (n = 494)	ref			
T/C (n = 239)	0.27	(0.05 to 0.48)*		
C/C (n = 17)	–0.25	(–0.92 to 0.41)		
OPRM1 rs1799971			0.05	0.8
A/A (n = 288)	ref			
A/G (n = 364)	0.15	(–0.07 to 0.36)		
G/G (n = 98)	0.38	(0.07 to 0.70)*		
COMT rs4680			0.05	0.8
G/G (n = 373)	ref			
G/A (n = 305)	–0.16	(–0.37 to 0.04)		
A/A (n = 72)	–0.39	(–0.73 to –0.04)		
MYD88 rs6853			0.14	0.2
A/A (n = 701)	ref			
A/G (n = 48) or G/G (n = 1)	–0.30	(–0.70 to 0.10)		
(Intercept)	16.3	(9.7 to 22.9)		
Model $R^2 = 0.08$				

Coefficient estimates are for the square-root transformation of total morphine consumption (milligrams in 24 hours after surgery); positive coefficient estimates indicate an association with increased morphine use (homozygous wildtype genotype groups as reference).

†Age (years) data are transformed (Box-Cox $\lambda = -0.3$) for regression analysis.

‡Averaging-over-orderings method. Post hoc

*$P < 0.05$ and

**$P < 0.01$ vs homozygous wildtype genotype.

identification of a role for innate immune genetics in contributing to morphine requirements. We were able to investigate and adjust for underlying variability in preoperative pain sensitivity and postoperative pain, in addition to consequent morphine requirements.

No demographic factors including ethnicity significantly affected pain tolerance preoperatively. When all patients were combined, the *IL10* rs1800871 T/T genotype was associated with low and the *TNFA* rs1800629 A/A genotype with high pressure pain tolerance. The proportion of patients with low pain tolerance was higher (significantly or nonsignificantly [data not shown]) in *IL10* rs1800871 T/T than in C carriers in Chinese (54% vs 47%), Malay (64% vs 52%), and Indian (74% vs 56%) patients. *IL10* haplotypes homozygous for the linked rs1800871 C and rs1800896 G alleles are associated with higher IL-10 expression, driven mainly by the rs1800896 G/G genotype [26, 27]. As IL-10 is antiinflammatory, a contribution of rs1800871 T/T directly to reduced IL-10 (or linkage to other *IL10* SNPs that reduce IL-10) might swing the phenotype toward a proinflammatory high pain state, thus supporting an association with low pain tolerance. The *IL10* rs1800896 genotype was not associated with pain tolerance, likely because of the low frequency of the G/G genotype. Conversely, TNF-α is proinflammatory, and the rs1800629 A allele causes increased TNF-α expression in vitro [28, 29] and increased proinflammatory cytokine release after surgery [30], but the clinical pain consequences are complex. The *TNFA* rs1800629 G/A and A/A genotypes are associated with increased risk of chronic pain conditions [31–33] and postoperative pain severity [34]. Our findings align with those of Reyes-Gibby et al. [34] with regard to lower pressure pain sensitivity for the A/A genotype (including no clear allele–dose relationship), but there was no similar association of the *TNFA* rs1800629 genotype with postoperative pain or morphine requirements.

Postoperative 24-hour morphine consumption substantially differed between ethnicities, ranging from 13 mg (median) in Chinese to 25 mg in Indian patients, but with large variability within each ethnic group. As

Table 4. Predictors of morphine PCA use in the first 24 hours after surgery in 107 Indian women undergoing total hysterectomy

	With Control for Postoperative Pain				Without Control for Postoperative Pain			
Coefficients	Estimate (95% CI)		ANOVA *P* Value	Contribution-to Model ($R^2 \times 10^{-2}$)	Estimate (95% CI)		ANOVA *P* Value	Contribution-to Model ($R^2 \times 10^{-2}$)
Postoperative VAS			0.6	1.7				
VAS <2 (n = 14)	ref							
VAS = 2 (n = 31)	0.04	(–0.86 to 0.95)						
VAS = 3 (n = 37)	0.43	(–0.46 to 1.3)						
VAS ≥4 (n = 25)	0.12	(–0.80 to 1.0)						
Age†	–7.9	(–16 to 0.23)	0.06	2.9	–7.6	(–15 to 0.34)	0.06	2.7
CRP rs2794521			0.001	10.4			0.005	9.3
T/T (n = 43)	ref				ref			
T/C (n = 48)	0.52	(–0.05 to 1.1)			0.43	(–0.14 to 1.0)		
C/C (n = 16)	–0.98	(–1.8 to –0.18)*###			–0.86	(–1.6 to –0.08)##		
OPRM1 rs1799971			0.001	9.0			8.0×10^{-5}	10.4
A/A (n = 28)	ref				ref			
A/G (n = 55)	1.0	(0.36 to 1.6)**			1.0	(0.40 to 1.6)**		
G/G (n = 24)	1.3	(0.55 to 2.1)**			1.6	(0.88 to 2.4)***		
TLR4 rs4986790			0.03	4.7			0.02	5.0
A/A (n = 75)	ref				ref			
A/G (n = 29)	0.73	(0.11 to 1.3)*			0.83	(0.24 to 1.4)*		
G/G (n = 3)	–0.79	(–2.4 to 0.86)			–0.30	(–2.0 to 1.4)		
IL2 rs2069762			0.03	4.4			0.07	3.8
T/T (n = 24)	ref				ref			
T/G (n = 53)	0.46	(–0.21 to 1.1)			0.45	(–0.21 to 1.1)		
G/G (n = 30)	1.0	(0.27 to 1.8)*			0.87	(0.14 to 1.6)		
IL6R rs8192284			0.06	3.2			0.1	2.8
A/A (n = 54)	ref				ref			
A/C (n = 37)	–0.29	(–0.87 to 0.29)			–0.20	(–0.75 to 0.36)		
C/C (n = 16)	–0.94	(–1.7 to –0.15)			–0.83	(–1.6 to –0.05)		
IL1B rs1143634			0.09	2.8				
C/C (n = 69)	ref							
C/T (n = 33)	–0.42	(–1.0 to 0.16)						
T/T (n = 5)	1.0	(–0.30 to 2.3)						
COMT rs4818							0.1	3.7
C/C (n = 53)					ref			
C/G (n = 41)					0.38	(–0.17 to 0.94)		
G/G (n = 13)					0.77	(–0.06 to 1.6)		
TGFB1 rs1800469							0.16	3.1
C/C (n = 33)					ref			
C/T (n = 60)					–0.05	(–0.67 to 0.56)		
T/T (n = 14)					–0.81	(–1.7 to 0.08)		
(Intercept)	21	(2.7 to 40)			21	(2.2 to 39)		
		Model $R^2 = 0.39$				**Model $R^2 = 0.41$**		

Coefficient estimates are for the square-root transformation of total morphine consumption (milligrams in 24 hours after surgery); positive coefficient estimates indicate an association with increased morphine use (postoperative VAS ≤1, or homozygous wildtype genotype groups, as reference).

†Age (years) data are transformed (Box-Cox $\lambda = -0.3$) for regression analysis.

‡Averaging-over-orderings method. Post hoc

*$P < 0.05$ and

**$P < 0.01$ vs homozygous wildtype genotype. Post hoc

##$P < 0.01$ and

###$P < 0.001$ vs heterozygous genotype.

expected, postoperative VAS scores before initiation of morphine PCA were positively correlated with subsequent morphine use. Lower preoperative pressure pain was also correlated with morphine PCA use in Chinese and Malay, but not Indian, patients.

In Chinese patients, *IL1B* rs1143634 heterozygotes (C/T) had lower morphine requirements, with likely contributions from significantly higher preoperative pain tolerance (~1/3 the odds of low pain tolerance) and lower postoperative pain (~1/7 the odds of high postoperative pain). With nongenetic factors incorporated, *IL1B* rs1143634 C/T genotypes had nonsignificantly lower morphine requirements (data not shown) and were not retained in the "best" model for predicting morphine requirements. An association between the *IL1B* rs1143634 C/T genotype and reduced pain and morphine

requirements is consistent with decreased serum IL-1b (proinflammatory) [35, 36]. The effect of the *IL1B* rs1143634 genotype in Chinese was not reflected in Indian patients, for whom we observed higher morphine requirements in heterozygotes after caesarean surgery. Therefore, although our findings indicate an important role for this gene variant in contributing to postoperative analgesia, genotype effects appear both ethnicity and context dependent, possibly confounded by significant genotype–by–psychological factor interactions [37, 38].

Though not always statistically significant, the linked *TLR4* variants demonstrated a pattern of lower pain tolerance (significantly overall and within Malay patients), higher postoperative pain (nonsignificant), and higher morphine requirements (significantly in Indian patients). This is likely due to its frequency being 0% in the Chinese, 3% in the Malay, and 16–18% in the Indian cohort. The effect on morphine use in Indians persisted after adjustment for postoperative pain and appears to affect underlying pain and morphine analgesia. The rs4986790 missense variant causes reduced TLR4 signaling in vitro, ex vivo, and in vivo [39–41]. A priori we would predict this to translate to an "anti-inflammatory" phenotype and associate with lower pain and morphine use. One explanation is that this variant causes a dysregulated (i.e., not simply anti-inflammatory) immune phenotype, which may also help to explain the association of these *TLR4* variants with increased risk of endometriosis [42].

The *IL6R* rs8192284 variant demonstrated ethnicity- and phenotype-dependent effects on pain tolerance and morphine use, and not always in a classical additive or allele-dominant manner. It was associated with decreased morphine use in the Indian cohort and increased use in the Chinese cohort, even though the variant frequency was similar. IL-6 signaling is complex, involving pro- and anti-inflammatory pathways, together with two types (membrane-bound and soluble) of IL-6R signaling [43]. The variant is associated with increased and decreased risk of different inflammatory component diseases [44–46]; as such, our findings are not inconsistent but may reflect a complex ethnicity-dependent role in postoperative morphine use.

The *CRP* rs2794521 genotype significantly influenced morphine requirements in Chinese (T/C significantly higher, C/C nonsignificantly lower) and Indian patients (T/C nonsignificantly higher, C/C significantly lower), and for the latter it explained 10% of variability, the highest of all the genetic contributors, and without any significant effect on underlying pre- or postoperative pain. The mechanism behind the apparent nonadditive nature of the genotype's effect on morphine use is unknown, but it may relate to the presence of and linkage disequilibrium with other more functionally relevant *CRP* SNPs [47] not included in the genotyping array.

In the Indian cohort only, *IL2* rs2069762 significantly contributed 4% to increased morphine use with controlling for age and postoperative pain. It is associated with increased IL-2 secretion [26] and would cause a proinflammatory effect consistent with increased postoperative pain and increased morphine use. *TGFB* rs1800469 was retained in the best model of morphine use in Indian patients without adjustment for underlying pain but was not significantly associated with either pre- or postoperative pain. Though not a significant predictor of morphine use, lower morphine use in rs1800469 variant genotypes reflects their association with decreased morphine use in Indian caesarean patients [11]. This is consistent with *TGFB1* rs1800469 increasing TGF-b1 (anti-inflammatory) expression [48, 49], which is expected to have an anti-inflammatory effect with less postoperative pain and lower morphine use. *OPRM1* rs1799971 associations with increased morphine use in the Chinese and Indian cohorts were expected and reported previously [9] in Asians, who have the highest variant frequency (37% in our Chinese, 49% in our Indian cohorts). As expected [10], the *COMT* rs4680 A/A genotype was associated with (borderline $P = 0.05$) lower morphine use among Chinese patients; however, this explained less variability than any of the polymorphisms discussed above. Our results with *OPRM1* and *COMT* are also consistent with the recent Clinical Pharmacogenetics Implementation Consortium guidelines review [50]. In contrast to Tan et al. [10], in the Indian cohort, the COMT rs4818 variant was associated with higher morphine use, contributing almost 10% to relative morphine dose requirements. Although *TLR2* rs3804100 was previously [11] associated with decreased morphine use in Chinese caesarean patients, it was not associated with morphine use in the present hysterectomy study.

We highlight key points pertaining to genetic studies addressing postoperative pain severity and analgesic use. First, known or likely nongenetic factors need to be accounted for in the assessment of genetic factors. Increasing age associates with reduced postoperative morphine [3, 8], which was found in the largest cohort, Chinese patients, even though the highest age was 45 years. It is unsurprising that pre- and postoperative pain influences postoperative morphine use, but it is a key consideration when genetic factors can influence analgesia either directly or indirectly via effects on underlying pain. Second, ethnicity per se is important in the reporting of pain severity. Our findings support those of Chan et al. (2011) showing that Singaporean Indians report greater pain severity than Singaporean Chinese [51]. Third, genetic findings in one population may not be found in others (e.g., *TLR4* rs498670 in Indian but not Chinese patients), which is partly due to inter-ethnic differences in polymorphism frequencies and may contribute to inter-ethnic differences in pain and morphine requirements. Fourth, even in a small number of patients, genetic association signals can be uncovered (e.g., within the 107 Indian patients). Finally, a "proinflammatory"

genotype (e.g., based on in vitro function) does not always translate to increased pain or opioid requirements, and life history, ethnicity, and clinical context may shape associations between polymorphisms and postoperative pain and analgesic efficacy.

Conclusions

Although the genotyping array of this study interrogated only a small fraction of the genetic variability in the innate immune signaling pathway that might contribute to pain and opioid response, we were able to explain 40% of morphine requirements in the Indian cohort. The combined genetic component (38%) is the highest value reported for genetic contributors to morphine use in the acute postoperative pain setting. Innate immune SNPs were identified alongside the known pain/opioid response genes *OPRM1* and *COMT*. Our findings highlight the need to incorporate both genetic and nongenetic factors and consider ethnicity-dependent and nonadditive genotypic models in the assessment of factors that contribute to variability in opioid use.

Supplementary Data

Supplementary Data may be found online at http://painmedicine.oxfordjournals.org.

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