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Genetic variability of the μ -opioid receptor influences intrathecal fentanyl analgesia requirements in laboring women ^{*}

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Abstract

Labor initiates one of the most intensely painful episodes in a woman's life. Opioids are used to provide analgesia with substantial interindividual variability in efficacy. μ -Opioid receptor (μ OR, *OPRM1*) genetic variants may explain differences in response to opioid analgesia. We hypothesized that *OPRM1* 304A/G polymorphism influences the median effective dose (ED₅₀) of intrathecal fentanyl via combined spinal–epidural for labor analgesia. Nulliparous women were prospectively recruited around 35 weeks gestation ($n = 224$), and genotyped for 304A/G polymorphism. Those requesting neuraxial labor analgesia were enrolled in one of the two double-blinded trials: up-down sequential allocation (SA, $n = 50$) and a separate confirmatory random-dose allocation trial (RA, $n = 97$). Effective analgesia from intrathecal fentanyl was defined by ≥ 60 min analgesia with verbal rating score ≤ 1 (scale 0–10) and was compared between μ OR 304A homozygotes (Group A) and women carrying at least one 304G allele (Group G). *OPRM1* 304G allele frequency $f(-)$ was 0.18. Using SA, intrathecal fentanyl ED₅₀ was 26.8 μ g (95% CI 22.7–30.9) in Group A and 17.7 μ g (95% CI 13.4–21.9) in Group G ($p < 0.001$; 304A homozygosity increased the ED₅₀ 1.5-fold). RA confirmed that 304A homozygosity significantly increases intrathecal fentanyl ED₅₀ (27.4 μ g in Group A and 12.8 μ g in Group G [$p < 0.002$; 2.1-fold]). We demonstrate for the first time that the μ OR 304G variant significantly reduces intrathecal fentanyl ED₅₀ for labor analgesia, suggesting women with the G variant may be more responsive to opioids and require less analgesic drugs. These

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Author contributions

Dr. Landau had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design*: Landau, Smiley. *Acquisition of data*: Landau, Kern. *Analysis and interpretation of data*: Landau, Blouin, Smiley, Columb. *Drafting of the manuscript*: Landau, Smiley, Columb. *Critical revision of the manuscript for important intellectual content*: Landau, Kern, Smiley, Columb, Blouin. *Statistical analysis*: Columb. *Obtained funding*: Landau. *Administrative, technical, or material support*: Landau, Kern, Blouin. *Study supervision*: Landau.

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Conflict of interest

None of the authors presents any commercial association or other issues that might pose a conflict of interest.

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findings for intrathecal fentanyl pharmacogenetics may have implications for patients receiving opioids in other settings.

Keywords

OPRM1; 304A/G; A118G; Combined spinal-epidural; Fentanyl; Genetic variability; Human; Labor; Pain; Pharmacogenetics; Polymorphism; Pregnancy; Up-down sequential allocation

1. Introduction

Variability in the experience of labor pain is well known, demonstrated by the ability of some women to undergo full “natural” delivery with minimal analgesic agents whereas others require intense analgesia even in the early stages of labor. In addition to obstetrical conditions, mechanistic explanations for why some women experience more intense labor pain could involve sensitivity to endogenous endorphins and exogenous opioids. Opioids are among the most widely used drugs for the management of acute and chronic pain, despite large interindividual variability in efficacy, side effects, and tolerance profiles. Over the past two decades, epidural and spinal opioids have been increasingly used to provide labor analgesia and treat postoperative pain. The μ -opioid receptor (μ OR), encoded by the gene *OPRM1*, has been the focus of numerous genetic studies because this receptor is the primary site of action for many endogenous opioid peptides, including β -endorphin and enkephalin, as well as being the major target of opioid analgesics [19].

A number of single nucleotide polymorphisms (SNPs) have been described for *OPRM1* [2, 47,48]. An adenine to guanine substitution in gene *OPRM1*₁ that results in an asparagine residue replacing an aspartate² has been reported to occur at a minor allelic frequency of $f(-)$ 0.10–0.30 [4,20,28]. There is major interest in this polymorphism due to its potential pharmacological [4] and physiological consequences [3,9,21,46]. *In vitro*, Bond et al. determined that the presence of at least one 304G (called G118 in that study) allele increases binding affinity and potency of β -endorphin [4]. Thus, hypothetically, individuals carrying the variant receptor gene may show differences in some of the functions mediated by β -endorphin action at the altered μ OR, such as higher thresholds to pain, although this remains to date a controversial concept. Consistent with this early laboratory finding, one *in vivo* study in a human experimental pain model demonstrated that volunteers (women and men) carrying a 304G allele exhibit higher pressure pain thresholds compared with 304A homozygotes [18]. It is to be noted, however, that when heat was the experimental painful modality, lower pain ratings among men but higher pain ratings among women with the variant allele were reported (i.e., a gender/genotype interaction) [18]. Since labor pain is primarily caused by changes of pressure during the contractions transmitted at the level of the lower thoracic and upper lumbar segments, the experimental findings with a pressure stimulus may be more relevant. Finally, the frequency of the 304G variant has been shown to be lower in chronic pain patients as compared to controls [23], suggesting that the variant confers some analgesia or other form of “protection” against pain.

The median effective dose (ED₅₀) of intrathecal fentanyl in women receiving labor analgesia had been previously determined to be 18.2 μ g using the up-down sequential allocation method [33]. We tested the hypothesis that the μ OR 304A/G polymorphism significantly affects the ED₅₀ of intrathecal fentanyl analgesia in laboring women in two prospective studies with distinct methodologies.

²p.N102D previously p.N40D.

2. Methods

2.1. Patient recruitment

After Institutional Review Board approval and informed written consent, 224 nulliparous women with uncomplicated singleton vertex pregnancies scheduled to deliver at term at the Geneva University Hospital (HUG) were recruited between 33 and 37 weeks gestation at the prenatal clinic. Each mother was informed about the analgesic procedure and told that the decision to deliver with or without neuraxial analgesia, as well as the timing of analgesia, would be left to her discretion. Blood samples (10 ml, EDTA tubes) were obtained for immediate genotyping (<72 h) from all subjects. Non-inclusion criteria at the time of recruitment were cardiovascular disease, history of drug abuse or use of chronic pain medication. Only mothers who elected neuraxial analgesia during labor were enrolled in the actual drug studies during labor. Women who were of a gestational age <36 weeks or who had a diagnosis of pre-eclampsia at the time of analgesia request, and all women with an obstetrical indication for a cesarean delivery without a trial of labor, were excluded from further participation in the study. In addition, because intrathecal fentanyl alone does not provide sufficient analgesia during the second stage of labor, only women requesting analgesia early in labor (cervical dilatation less than 6 cm) were studied; of these, only those continuing in labor for at least one hour were included in the final analysis.

2.2. Overview of two studies performed

Two distinct prospective, double-blinded studies were performed. The first study estimated ED₅₀ using a previously validated up-down sequential allocation (SA) design [12], whereas the second study estimated the ED₅₀ using a random-dose allocation (RA) method. After recruitment in the prenatal clinic, women were assigned to two groups according to genotype. Group A consisted of wild-type homozygotes (304A) while Group G included heterozygotes and homozygotes carrying the 304G allele. At the time of labor, women in spontaneous labor (SL) who requested neuraxial analgesia were assigned to one of the two up-down sequences according to their genotype (SA–SL). Once the SA sequences were completed, subsequent women in spontaneous labor were enrolled in the random-dose allocation (RA–SL) study. In order to determine if findings in spontaneous labor are generalizable to women undergoing labor induction, recruited women undergoing induction of labor with prostaglandin (misoprostol) were enrolled in a separate random-dose allocation (RA–IL) protocol. Each protocol is described in more detail below.

2.3. General clinical methods

Subjects and the anesthesiologist performing combined spinal–epidural analgesia (CSE) were blinded at all times in both studies to genotype and fentanyl dose. Fentanyl doses were prepared in a total volume of 2 ml by the HUG pharmacy in sterile pre-filled syringes. The syringes were placed in sealed envelopes and stored by a member of the team who was not involved in the anesthetic care of any of the subjects enrolled in the study and were always available for recruited subjects who requested neuraxial analgesia.

CSE analgesia was initiated with subjects in the sitting position. Using the loss-of-resistance to saline technique, an 18G Tuohy needle was introduced into the epidural space at the L2–3, L3–4, or L4–5 interspace. A 27G Whitacre needle was passed through the Tuohy needle into the subarachnoid space. After return of cerebrospinal fluid, women received a single intrathecal injection of fentanyl as determined by the dose allocation. After injection of the study solution, the Whitacre needle was withdrawn and an epidural catheter was threaded 3–5 cm into the epidural space. The Tuohy needle was withdrawn and the catheter secured before the patient was repositioned supine with left uterine displacement. The catheter remained unused until the parturient requested additional analgesia or until the predetermined time of 60 min. No

medication was injected through the epidural catheter for at least 20 min after the intrathecal injection. Women were asked to rate their pain according to an 11-point verbal rating score (VRS) for pain intensity in which 0 represented no pain and 10 the worst pain imaginable before CSE and at 5, 10, 15, 20, 30, 45 and 60 min after spinal injection. Maternal blood pressure and fetal heart rate were also recorded at the same time points. Cervical dilatation was checked within the hour preceding the CSE procedure and thereafter for obstetric indications. Side effects, such as maternal hypotension, pruritus (on a 4 point scale: 0 = none; 1 = mild; 2 = moderate; 3 = severe requiring nalbuphine), new nonreassuring fetal heart rate (FHR) abnormalities, or uterine hyperactivity, were recorded. FHR tracings were assessed by the attending obstetrician (fetal bradycardia was defined as FHR <100 bpm for more than 90 s). Uterine activity was assessed using external tocography and clinical evaluation by the attending obstetrician; the presence of uterine hyperactivity was defined as ≥ 2 contractions lasting ≥ 60 s. Cardiotocographic recordings were continuously performed for 15 min before and 60 min after the CSE.

Hypotension was treated with intravenous (i.v.) ephedrine 5–15 mg according to our usual clinical practice. No other medications were administered within 60 min of the intrathecal injection. Additional medication such as nalbuphine for pruritus or oxytocin for augmentation of labor was given only after the completion of the study period (≥ 60 min after the intrathecal dose).

2.4. Initial sequential allocation study

For the up-down sequential allocation study (SA–SL), the initial dose of fentanyl was 18 μg for women in both genotypic groups and was based on a previous estimate of ED₅₀ [33]. The up-down testing interval between subjects was 2 μg . Individual patient outcomes were categorized as success, failure, or rejection, similar to previous minimal local analgesic concentration (MLAC) [12] and minimal analgesic dose (MAD) [33] methodologies. The MLAC methodology has been extensively used in obstetric anesthesia investigations to assess differences in potencies and analgesic responses both to opioids and local anesthetics [1,6, 12,13,31,36–38,39,40,41]. We used the same criteria to define successful analgesia or failure as those used in previous studies assessing median effective doses of opioids or local anesthetics, as indicated below:

Success, defined as analgesia achieved within 20 min (VRS for pain intensity $\leq 1/10$) and lasting at least 60 min, resulted in a dose reduction of 2 μg for the next woman in that genotype-grouped sequence. At 60 min, study participation ended and women received analgesia via the indwelling epidural catheter according to usual practice.

Failure, defined as analgesia not achieved within 20 min (VRS for pain intensity $> 1/10$), or VAPS $\leq 1/10$ within 20 min but not lasting at least 60 min, resulted in a dose increase of 2 μg for the next subject in that sequence. If a woman was not comfortable within 20 min after the intrathecal injection (VRS for pain intensity $> 1/10$ or request for further analgesia), additional anesthetics were administered via the epidural catheter per standard practice.

Rejection, i.e., elimination or censoring of the subject's data from final analysis, occurred in presence of analgesic *failure* and cervical dilatation > 8 cm within the 60 min study period (indicating rapid progression of labor and lack of the table 60 min study period necessary for study completion). Cervical dilatation was checked if women complained of severe pain or urge to push during the first hour, and outcome was defined as *rejection* due to rapid progress of labor. Rejections were not included in the up-down sequence response analysis and resulted in a repeated dose for the next subject in that sequence.

2.5. Subsequent random-dose allocation study

For the random-dose allocation study in spontaneously laboring subjects and subjects undergoing induction of labor (RA–SL and RA–IL), fentanyl doses between 2.5 and 35 μg (27 evenly spaced log intervals with 2 doses per level) were randomly allocated. Definitions of success, failure and rejection were the same as those described for the SA study.

2.6. DNA collection and purification

Blood was obtained from subjects when they were initially recruited into the study in the prenatal clinic. Genomic DNA was isolated the same day from whole venous blood by a non-phenolic method using Puregene Blood Extraction Kit (Gentra, Minneapolis, MN) and tested for DNA integrity by gel electrophoresis and purity/quality by optical densitometry (260/280 nm).

2.7. SNP genotyping

All genotyping was performed at the Geneva University Hospital. Sixty nanograms of DNA from subjects was used to amplify DNA encoding the μOR via polymerase chain reaction (PCR) under standard conditions using the annealing primers forward (5' end-labelled with biotin) 5'-CCGGC CGTCAGTACCAT-3' and reverse 5'-GTAGAGGGCCATG ATCGTGAT-3' http://www.ensembl.org/Homo_sapiens/gene_view?gene=ENSG00000112038#ENST00000330432> ENST00000330432. Polymorphism 304A/G (GATGGCRA CCTGT) was then genotyped on the 246 bp PCR fragment by a minisequencing method (Pyrosequencing; Uppsala, Sweden; <http://www.pyrosequencing.com>) using sequencing (reverse) primer 5'-TGGGTCCGACAGGT-3' as follows. PCR products were immobilized with Dynabeads (Dyna, Oslo, Norway) by a 15 min, 65 °C incubation in a buffer containing 10 mM Tris–HCl, 2 M NaCl, 1 mM EDTA and 0.1% Tween 20. These were then removed from solution using magnetic separation, denatured with 0.5 M NaOH and washed with 200 mM Tris–acetate, 50 mM MgAc₂. The remaining single stranded DNA was then hybridised with the internal 'sequencing' primer, by heating the mix to 80 °C and slowly cooling it to room temperature. The remaining steps were processed using the Pyrosequencing automated station (model PSQ96MA), i.e., enzyme and substrate mixes were added to each well, and the reactions processed at 28 °C by the sequential addition of single nucleotides with a predetermined order. Luciferase peak heights were proportional to the number of nucleotide incorporations, which have been shown to have a high degree of correspondence (5% error rate) in a number of experimental settings as well as in our previous work [28].

2.8. Statistical analysis

Demographic and clinical data were collected and are presented as mean (SD), median [interquartiles] and counts as appropriate. Means were analyzed using unpaired Student's *t*- or Welch's *t*-tests for differing variances, medians [interquartiles] by Mann–Whitney *U*-test, and counts or proportions by Fisher's exact test. Time-based data were analyzed using log rank tests. The median effective dose (ED₅₀) was estimated with 95% confidence interval (CI) from each up-down sequence using the method of Dixon and Massey [17] and analyzed with probit regression analyses as back-up or sensitivity tests. The effect size was assessed by estimating the ratio with 95% CI of the ED₅₀ estimations. Logistic regression and analysis of covariance (ANCOVA) were used to examine for potential confounding variables. Analyses were carried out using the following software: Excel 2000 (Microsoft Inc., Redmond, WA), Number Cruncher Statistical Systems 2004 (NCSS Inc., Kaysville, UT), Minitab 14.20 (Minitab Inc., State College, PA), and Prism 4.03 (GraphPad Software Inc., San Diego, CA). Statistical significance was defined as $p < 0.05$ (two-sided).

Sample size estimations were based on data from a previously published study with intrathecal fentanyl [33]. Assuming a coefficient of variation of 0.18, a minimum of 18 subjects per group would be required to detect a 30% difference in the ED₅₀ between genotype groups with α error <0.05 and β error <0.20 with an up-down sequential study design.

3. Results

3.1. Patient recruitment and enrollment in studies

The disposition of all enrolled subjects is shown in the flow diagram (Fig. 1). Two hundred and twenty-four ($n = 224$) women were recruited and genotyped between December 2003 and October 2006; the minor allele frequency, $f(-)$, was 0.18. Technical problems prevented genotyping in one woman, who was excluded from the study. Self-reported ethnic background and genotype frequencies for the remaining recruited women ($n = 223$) are presented in Table 1. Of these, 158 women ($n = 110$ and $n = 48$ for Groups A and G, respectively) received CSE analgesia under the study protocol (Fig. 1). The distribution of genotypes was not different in women excluded from the study because of advanced cervical dilatation (>6 cm) or lack of request for neuraxial analgesia (Fig. 1).

3.2. Demographics and obstetrical data

Demographic data and obstetric outcomes were similar in women enrolled in the SA and RA parts of the study (data not presented). Tables 2 and 3 present these same results in terms of genotype groups. Demographic data are similar between Groups A and G. The study groups were also similar with respect to incidences and treatment of side effects such as pruritus, hypotension and fetal bradycardia (Table 3).

Group G requested analgesia at a later point in labor (greater cervical dilation) compared with Group A (2 cm versus 0 cm, medians; $p = 0.0067$). This finding is internally consistent with a tendency to shorter time to delivery after CSE injection in Group G (Table 2). In addition, Group G subjects had more rapid progress of labor within the first hour of study, resulting in a greater proportion of rejections for cervical dilation ≥ 8 cm (8/110 rejections in Group A versus 9/48 in Group G, $p = 0.048$) (Table 3). Logistic regression and ANCOVA were used to adjust for variability due to cervical dilatation for all subjects ($N = 158$, *treatment-received analysis*) and differences in cervical dilatation did not adversely confound the effect size.

3.3. Results from specific study protocols

Table 4 summarizes fentanyl ED₅₀ doses for the sequential allocation study and the random allocation study. In the SA–SL study ($n = 50$), 26 women who were homozygous for the wild-type 304A allele (Group A) and 24 women carrying the mutant 304G allele (Group G) were studied. One rejection in Group A and 5 in Group G resulted in $n = 25$ and $n = 19$ in the respective groups for the *per-protocol* ($n = 44$) analysis of analgesic outcomes. All 50 subjects were included in the *treatment-received* analysis. In the RA study ($n = 108$), 84 women were 304A homozygous (Group A); of these 47 were in spontaneous labor (RA–SL) and 37 women underwent induction of labor (RA–IL). Twenty-four women were carrying at least one 304G allele (Group G); of these 11 were in spontaneous labor and 13 underwent induction of labor. Seven rejections in Group A and 4 in Group G resulted in $n = 77$ and $n = 20$ in the respective groups for the *per-protocol* ($n = 97$) analysis. All 108 subjects were included in the *treatment-received* analysis.

3.4. Median effective fentanyl dose and ratios

In the SA study, *per-protocol* analysis showed that the ED₅₀ was significantly ($p = 0.0091$) greater in women in Group A (26.8 μg , 95% CI 22.7–30.9) compared to women in Group G

(17.7 µg, 95% CI 13.4–21.9), with a 95% CI for the difference between groups of 2.6 and 15.7 µg (Fig. 2). The ED₅₀ was higher by a factor of 1.51 (95% CI 1.18–2.01) for the 304A homozygous genotype. Results of the back-up probit regression analysis are shown in Table 4A. The dose–response relationships, as assessed using probit regression, confirmed the significant effects of dose ($p = 0.008$) and genotype variant ($p = 0.009$).

The RA study demonstrated no significant ($p = 0.23$) effect of mode of initiation of labor (spontaneous versus induced labor) on ED₅₀ of fentanyl, so these groups were combined for analysis (Table 4B). Using probit analysis the ED₅₀ estimate was significantly ($p = 0.002$) greater in Group A (27.4 µg, 95% CI 22.5–32.2) compared to Group G (12.8 µg, 95% CI 5.5–20.0) (Fig. 3). The *treatment-received* analysis confirmed the significant effects of dose ($p < 0.001$) and genotype variant ($p = 0.007$). The effect size, as assessed by the ED₅₀ ratio was 2.14 (95% CI 1.30–5.07) by the *treatmentreceived* analysis, was still confirmatory.

4. Discussion

We demonstrate a significant increase in sensitivity to the analgesic effect of intrathecal fentanyl in laboring women carrying the 304G allele of *OPRM1*, confirmed by two different methodological approaches to estimate the ED₅₀ in two separate cohorts. The converse is that women 304A homozygous require significantly more fentanyl for labor analgesia. This is the first published clinical trial demonstrating that genetic variants of µOR affect the response to neuraxial fentanyl or any other neuraxial opioid. In our study, women were genotyped well before their request for labor analgesia, and the dose of fentanyl, the opioid usually used to provide labor analgesia, was allocated according to genotype in a double-blind manner. With early genotyping, we were able to use the up-down sequential methodology with separate sequences for the two genotypic groups to determine intrathecal fentanyl ED₅₀ (the dose providing total analgesia for 60 min in 50% of women) per genotype. We then replicated our findings in a separate study using random-dose allocation. A pharmacogenetic effect of µOR on the efficacy or dose–response to intrathecal fentanyl has never been described, although the 304A/G polymorphism has been studied in different clinical settings with different opioids given systemically.

Our finding of a 1.5- to 2-fold difference in ED₅₀ according to genotype is clinically relevant, because provision of optimal labor analgesia remains an ongoing challenge. A major goal of labor analgesia is minimal motor impairment, emphasizing the need to minimize local anesthetic use. Similarly, there is a need to reduce opioid doses in order to minimize opioid-related side effects such as pruritus and fetal bradycardia [44]. Due to our study design, a large proportion of 304A homozygotes ($n = 35$) received doses of fentanyl larger than commonly given clinically (25 µg or more) and in women with this genotype, the occurrence of pruritus or fetal bradycardia appeared to be unrelated to the dose. We cannot draw the same conclusions for women carrying the 2 other genotypes (304A/G or 304G homozygous), because only one woman in that group received a dose above 25 µg (27 µg). Therefore, according to our findings, genotyping may well help improve the delivery of labor analgesia because a 1.5- to 2-fold increase in fentanyl dose is not trivial.

While maternal demographics and neonatal data were similar between groups, the median cervical dilatation at the time of analgesia request was significantly less in 304A homozygotes than that in 304A/G or 304G homozygotes. This is of interest since women received CSE analgesia by their own request at the time they experienced painful contractions. It has been demonstrated that epidural analgesic requirements increase with the progress of labor and cervical dilatation [6,45]. The expectation would be that women carrying the mutant G allele should have had increased analgesic requirements due to the greater cervical dilatation at which

they requested analgesia; our finding that these women require *less* fentanyl may actually underestimate the true effect of genotype.

There are several possible explanations for such a difference in cervical dilatation at the time of analgesia request. Women with the G variant may have higher pain tolerance and therefore request epidural analgesia at a later point in labor, which would be consistent with the *in vitro* finding of enhanced β -endorphins binding [4]. This genetic variant may have an effect on the progress of labor, resulting in more rapid dilatation in the variant genotypes, or may be associated with other genetic factors controlling the rate of cervical dilation and progression of labor. Although there is no clinical evidence for such an effect, the 304G variant alters activation of the hypothalamic–pituitary–adrenal (HPA) axis stress-induced production of cortisol [9,21,46]; it is possible that this or other hormonal-related effects could impact on the course of labor. This would be consistent with our finding of nine rejections for rapid progress of labor in women Group A (19%) versus only eight in Group G (7%) and a tendency to a shorter duration of labor from the time of intrathecal injection with that genotype.

We also determined that induction of labor with misoprostol, at least in our obstetric practice, did not influence spinal fentanyl ED₅₀ in comparison to women undergoing spontaneous labor. The ED₅₀ of early labor epidural sufentanil using the up-down methodology has been shown to be increased by a 1.3 factor in women undergoing induction of labor versus spontaneous labor [7]. Other studies have shown that women with induced labors requested neuraxial analgesia at less cervical dilatation. Indications for misoprostol inductions may have changed in recent years, and the low cesarean section rate in our IL group (similar to the SL group) suggests that most labors were probably not dystocia. Dystocia has been shown to increase both analgesia requirement and the cesarean delivery rate [22].

Our results are not consistent with findings of a handful of studies assessing the clinical efficacy and toxicity of morphine-6-glucuronide compared to morphine in subjects with 304A/G polymorphism [29,30,42], and several trials examining chronic morphine requirements in cancer patients [5,23,25] or the use of postoperative i.v. morphine via patient-controlled analgesia [10,11,14,23]. In these various settings, patients with at least one 304G allele exhibited a trend towards *increased* systemic morphine requirements, findings which are in the opposite direction to our results with intrathecal fentanyl. Although our study was not designed to investigate the molecular pathways by which laboring women carrying the 304G allele exhibit a lower intrathecal fentanyl ED₅₀ or why our results differ from those reported in clinical studies of systemic opioids, it is of interest to consider potential mechanisms.

One explanation for increased analgesia in 304G carriers would be that the variant μ OR demonstrates increased binding specifically in response to intrathecal fentanyl. While an increased binding affinity for β -endorphins was reported by Bond et al. [4], this was not the case in their study for smaller endogenous opioid peptides or μ -preferring opioid agonists, or for the opioid antagonist, naloxone, nor was this finding replicated by others [2,3] or by the same group recently [26]. In fact, taken together, *in vitro* studies tend to suggest that the 304A/G polymorphism is more likely to affect μ OR function via alterations in expression, transduction systems or receptor trafficking rather than via altered receptor binding affinity [32]. Nevertheless, whether these *in vitro* findings can be extrapolated to the phenotype of the human variant μ OR in spinal cord neurons remains to be determined.

Spinal and systemic opioid pharmacokinetics and pharmacodynamics may be quite different; enhanced analgesia in response to intrathecal fentanyl in the presence of the 304G allele may not exist in response to intravenous fentanyl or other opioids via the intravenous route. One could speculate that human spinal cord receptor function and signal transduction is selectively more altered by the 304G variant than supraspinal receptors.

The different nature of the nociceptive stimulus in labor versus that perceived in experimental models using psychophysical testing, or in acute postoperative and chronic settings as well as the modalities used to describe pain perception and analgesic responses could also contribute to inconsistent findings. Indeed, similar disparate results in human genetic studies of pain sensitivity have been shown to occur with the common polymorphism of the catechol-*O*-methyltransferase gene (Val158Met) [34]; while one study found a significant genetic association [49], another did not [24], a third described a significant effect but not for that polymorphism [16], and a fourth reported that findings depended on stimulus modality [15].

Our clinical trial in laboring women bears the obvious limitation that we only studied pregnant women, and therefore gender specific as well as pregnancy-related differences could exist. Recent studies have demonstrated pregnancy-induced antinociception [8,35] indicating that pregnancy, via hormonal changes or other mechanisms modifying the opioid system, may produce a pattern of results that may not be applicable to non-pregnant women and men. One must use caution in extrapolating between different studies, drugs, and clinical settings. Our current findings are based on a sample of healthy nulliparous young women in labor, and it is not known whether the association between the μ OR 304A/G polymorphism and clinical response would exist with other opioids, given via alternate routes in patients suffering different pain syndromes.

In this study, we studied only one polymorphism of the *OPRM1* gene. Since numerous functional SNPs have been reported on this gene [43] and many other candidate genes have been suggested for the study of pain and analgesic responses [27], we cannot exclude linkage disequilibrium between the 304G allele and another functional variant or report on the possible polygenic nature of this pharmacogenetic effect.

Despite these clinical limitations, our dose–response data, gathered by different methodologies in two separate cohorts, strongly suggest that this common SNP of *OPRM1* significantly affects the response to intrathecal fentanyl administered for labor analgesia. Future work should be aimed at determining whether this study in pregnant women has implications for patients receiving opioids in other settings. If confirmed in other clinical settings and with other opioids, use of *OPRM1* 304A/G genotyping may improve the provision of analgesia in the not-too-distant future.

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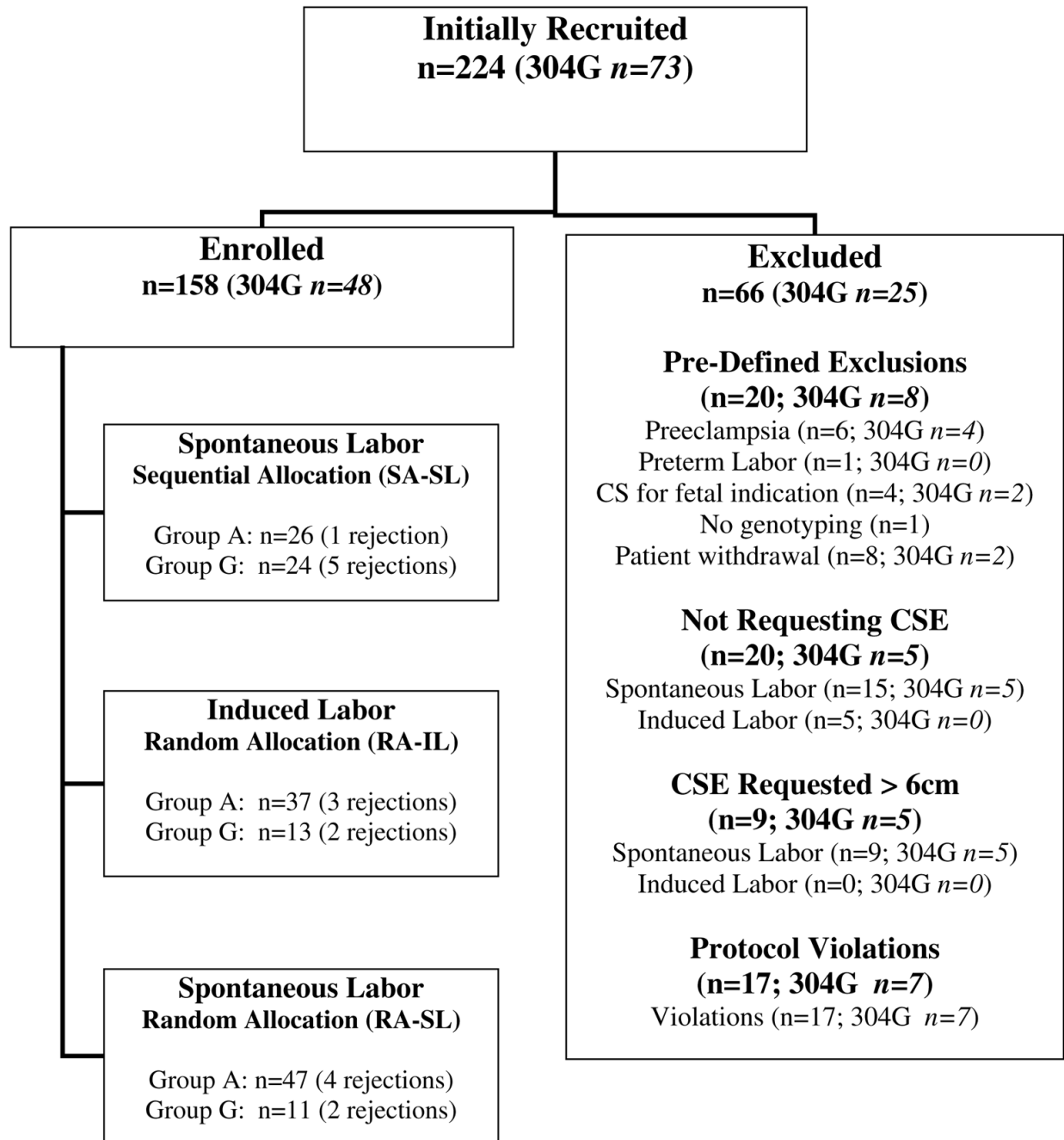
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**Fig. 1.**

Flow diagram of all recruited subjects. Total number of subjects (n); women in Group G (carrying one or more 304G allele is represented as an italic n). Women with no CSE ($n = 20$) are women who did not request neuraxial analgesia during labor and delivery. Patient withdrawal ($n = 8$) occurred when women had agreed in the antenatal clinic to have blood drawn for genotyping and subsequently did not want to be included in the study when requesting neuraxial analgesia. Protocol violations ($n = 17$) included women receiving intramuscular pethidine prior to CSE ($n = 4$), or anesthesiologist not available to perform the study at the time of CSE request ($n = 13$).

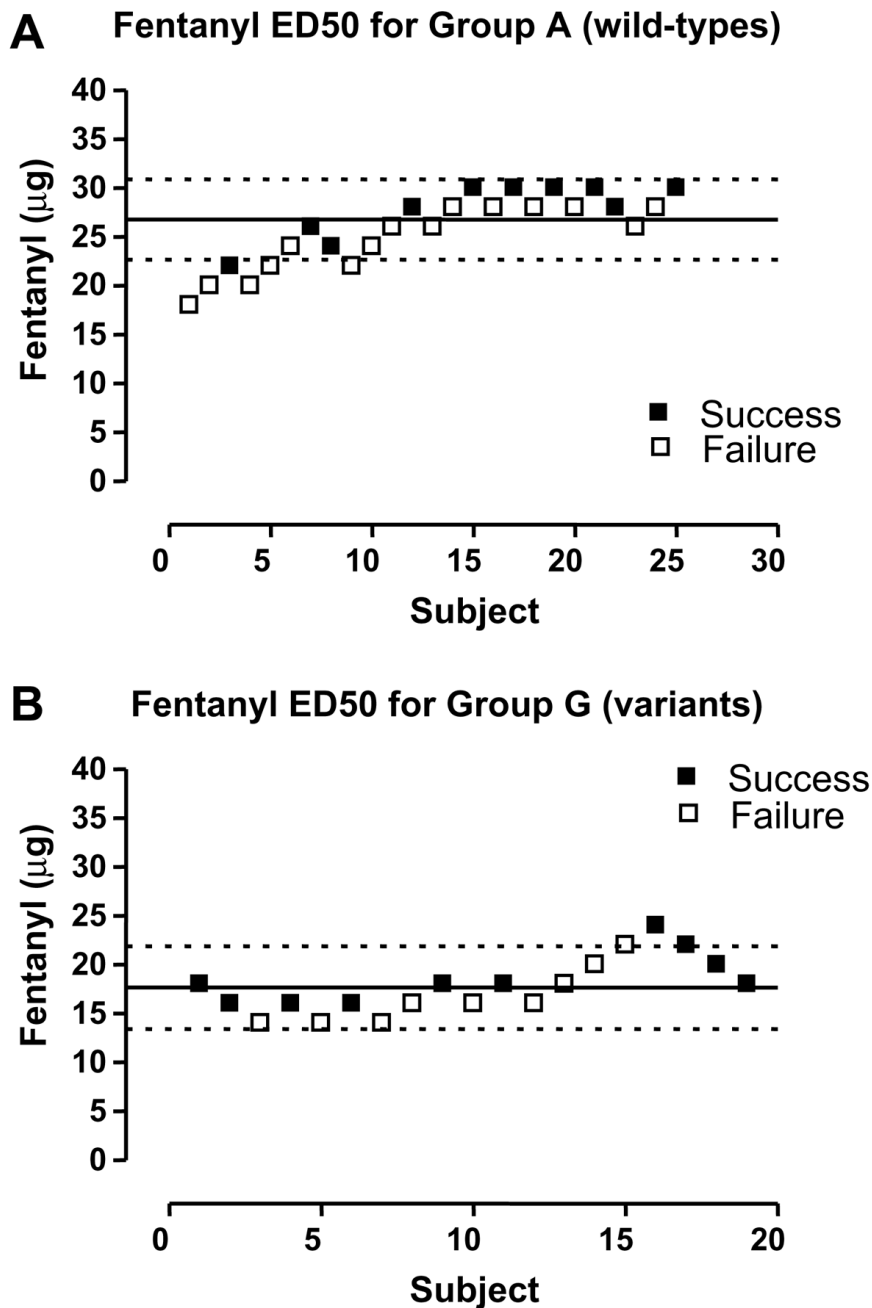


Fig. 2. Median effective doses (ED₅₀) of intrathecal fentanyl according to genotype using sequential allocation (SA). Data show effective (success) and ineffective (failure) analgesic outcomes for wild-types (Group A) and women carrying one or more 304G allele (Group G) with solid bars representing ED₅₀ and dotted bars representing 95% confidence intervals (95% CI). (A) Using up-down analysis, ED₅₀ was 26.8 µg (95% CI 22.7–30.9) in women 304A homozygotes (per-protocol analysis, $n = 25$). (B) Using up-down analysis, ED₅₀ was 17.7 µg (95% CI 13.4–21.9) in women carrying at least one 304G allele (per-protocol analysis, $n = 19$), ED₅₀ ratio (A:G) for genotype is 1.51 (95% CI 1.18–2.01), $p = 0.009$.

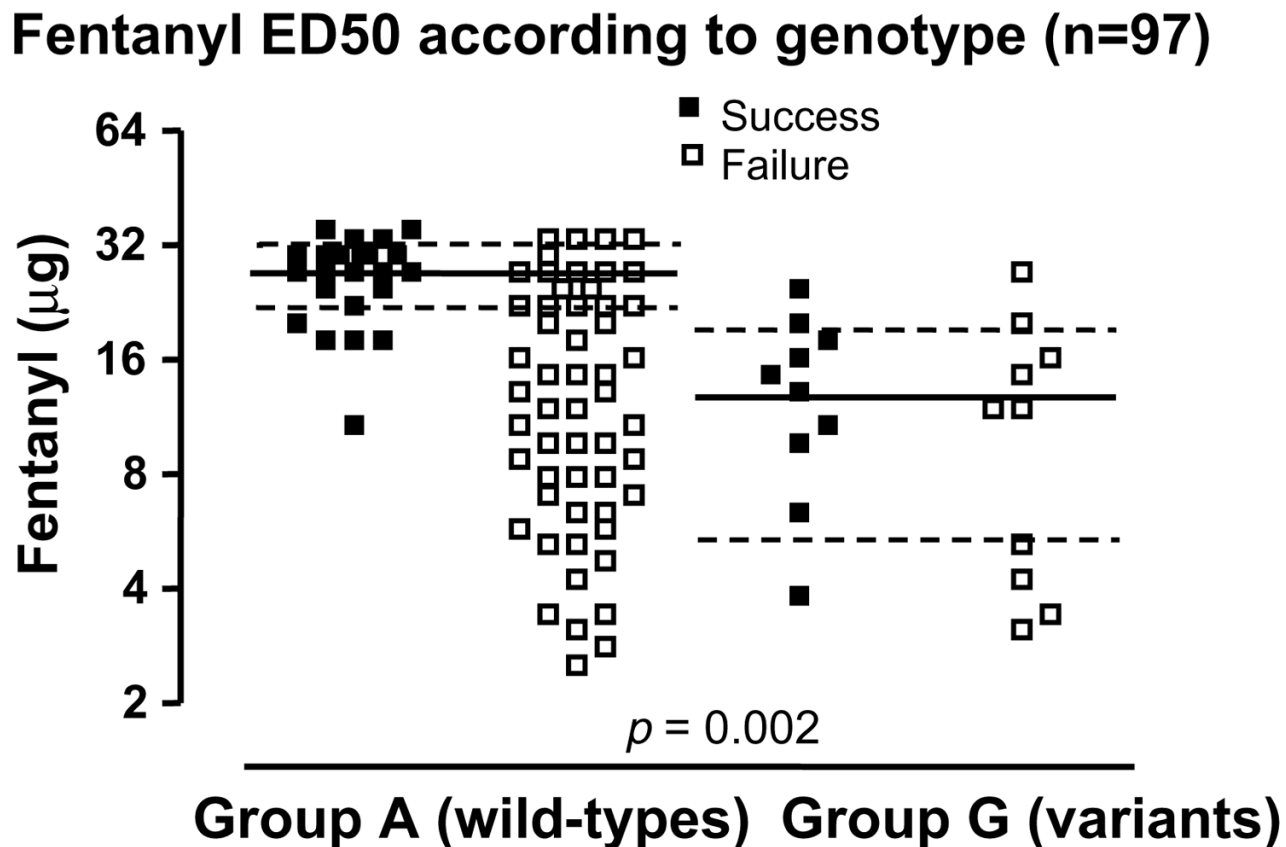


Fig. 3. Median effective dose (ED₅₀) of intrathecal fentanyl using random allocation (RA). Data show effective (success) and ineffective (failure) analgesic outcomes for wild-types (Group A) and women carrying one or more 304G allele (Group G) with solid bars representing ED₅₀ and dotted bars representing 95% confidence intervals (95% CI). ED₅₀ was 27.4 µg (95% CI 22.5–32.2) in women 304A homozygotes ($n = 77$) and 12.8 µg (95% CI 5.5–20.0) in women carrying at least one 304G allele ($n = 20$) using per-protocol analysis ($N = 97$). ED₅₀ ratio (A:G) for genotype is 2.14 (95% CI 1.30–5.17), $p = 0.002$.

Table 1

Genotype and allele frequencies and ethnic distribution of the 304A/G polymorphism for all recruited women ($n = 223$)

	Genotype frequency, n (%)			Minor allele frequency, $f(-)$
	A/A	A/G	G/G	G
All cases, $n = 223$	150 (67.3)	62 (27.8)	11 (4.9)	0.188
Caucasian, $n = 213$	147 (69.0)	56 (26.3)	10 (4.7)	0.178
Asian, $n = 10$	3 (30.0)	6 (60.0)	1 (10.0)	0.400

Caucasian and Asian populations are in Hardy–Weinberg equilibrium.

Genotype frequencies for this SNP were different between Caucasians and Asians ($p < 0.05$).

Table 2Demographics and clinical obstetrical variables for all all enrolled women (*treatment-received*, $n = 158$)

	Group A ($n = 110$) [*]	Group G ($n = 48$) ^{**}	<i>p</i> -Value
Maternal age (year)	29.7 (5.3)	28.7 (5.1)	>0.20
Maternal weight (kg)	76.9 (13.8)	78.0 (19.2)	>0.20
Maternal height (cm)	163.1 (10.6)	164.8 (8.1)	>0.20
Gestational age (weeks)	40.0 (1.1)	39.8 (1.1)	>0.20
Cervical dilatation at CSE placement (cm)	0 [0,2]	2 [0,3]	0.0067
Time from CSE placement to delivery (min)	415 [270,600]	345 [225,518]	0.071
Induced labor (IL) (%)	34%	27%	>0.20
Neonatal weight (kg)	3.280 (0.455)	3.255 (0.350)	>0.20
Type of delivery			0.11
Spontaneous vaginal delivery (n)	54	29	
Instrumental vaginal delivery (n)	35	16	
Cesarean delivery (n)	21	3	

Data are presented as mean (SD), median [interquartiles] and count.

Group A: 304A homozygotes;

* including 8 rejected cases.

Group G: 304A/G heterozygotes ($n = 40$) and 304G homozygotes ($n = 8$);

** including 9 rejected cases.

Table 3CSE, analgesia and side effects for all enrolled women (*treatment-received*, $n = 158$)

Variable	Group A (N = 110)	Group G (N = 48)	p-Value
Rejected (>8 cm within 1 h of CSE)	8	9	0.048
VRS at CSE placement (0–10 cm)	9 [8,10]	9 [8,10]	0.15
Pruritus (0–3; 4 point scale)	1 [0,1]	0 [0,1]	>0.20
Nalbuphine (given after 60 min for pruritus) (<i>n</i>)	4	1	>0.20
Ephedrine use (<i>n</i>)	6	4	>0.20
Fetal bradycardia with uterine hypertonus (<i>n</i>)	3*	1**	>0.20

Data are presented as count and median [interquartiles].

Group A: 304A homozygotes.

Group G: 304A/G heterozygotes ($n = 40$) and 304G homozygotes ($n = 8$).

VRS: visual rating scale for pain intensity (0 = no pain, 10 = worst pain imaginable).

Pruritus scale: 0 = none; 1 = mild; 2 = moderate; 3 = severe requiring nalbuphine.

Fetal bradycardia (FHR <100 bpm for more than 90 s) with uterine hypertonus (2 contractions lasting ≥ 60 s) occurring within 15 min after spinal injection

* doses: 22, 27 and 30 μg in Group A;

** dose 14.5 μg in Group G; all 4 cases in spontaneous labor.

Table 4**Table 4A. Median effective dose (ED₅₀) for the sequential allocation (SA) study**

Analysis	Group A (<i>n</i> = 25)	Group G (<i>n</i> = 19)	ED ₅₀ ratio (A:G)	<i>p</i> -Value
Up-down analysis	26.8 (22.7–30.9)	17.7 (13.4–21.9)	1.51 (1.18–2.01)	0.009
Probit regression	27.5 (24.7–30.3)	17.3 (14.3–20.3)	1.59 (1.30–1.98)	0.009

Table 4B. Median effective dose (ED₅₀) for random allocation (RA) study using probit analysis

ED ₅₀ (95% CI) µg	Group A (<i>n</i> = 77)	Group G (<i>n</i> = 20)	All subjects (<i>n</i> = 97)
Spontaneous labor (<i>n</i> = 52)	29.1 (22.3–35.9)	15.8 (4.7–26.9)	27.4 (20.7–34.2)
Induced labor (<i>n</i> = 45)	24.3 (17.8–30.9)	10.4 (1.7–19.2)	21.8 (14.8–28.8)
All subjects	27.4 (22.5–32.2)	12.8 (5.5–20.0)	25.4 (19.6–31.1)

Results are ED₅₀ (µg) or ratio with 95% confidence interval.

Group A: 304A homozygotes.

Group G: 304A/G heterozygotes (*n* = 17) and 304G homozygotes (*n* = 2).

ED₅₀ ratio (A:G) for genotype is 2.14 (95% CI 1.305.17), *p* = 0.002.

ED₅₀ ratio (spontaneous:induced) for labor onset is 1.26 (95% CI 0.841.97), *p* = 0.23.

Tests for goodness of fit and parallelism were *p* = 0.53 and *p* = 0.16, respectively.

Group A: 304A homozygotes.

Group G: 304A/G heterozygotes (*n* = 16) and 304G homozygotes (*n* = 4).