

# Polymorphism of $\mu$ -Opioid Receptor Gene (*OPRM1*:c.118A>G) Does Not Protect Against Opioid-induced Respiratory Depression despite Reduced Analgesic Response

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**Background:** The effect of a single nucleotide polymorphism of the  $\mu$ -opioid receptor at nucleotide position 118 (*OPRM1*:c.118A>G) was investigated on morphine-6-glucuronide (M6G)-induced analgesia and respiratory depression in a group of healthy volunteers.

**Methods:** Sixteen subjects of either sex received 0.4 mg/kg (n = 8) or 0.6 mg/kg M6G (n = 8). At regular time intervals, the isocapnic acute hypoxic ventilatory response, pain tolerance (derived from a transcutaneous electrical acute pain model), and arterial blood samples were obtained. Data acquisition continued for 14 h after drug infusion. Population pharmacokinetic-pharmacodynamic sigmoid Emax models were applied to the respiratory and pain data. All collected data were analyzed using the statistical program NONMEM (San Francisco, CA).

**Results:** Four of the subjects were *OPRM1*:c.118GA heterozygotes, and the remainder of the subjects were *OPRM1*:c.118AA homozygotes. **M6G analgesia:** In contrast to analgesic responses in *OPRM1*:c.118AA homozygotes, responses were small and inconsistent in *OPRM1*:c.118GA heterozygotes and best described by the function  $\text{Effect}(t) = \text{baseline}$  ( $P < 0.01$  vs. *OPRM1*:c.118AA homozygotes). Emax and  $C_{50}$  values in heterozygotes equaled  $0.55 \pm 0.18$  (or a 55% increase in current above baseline) and  $161 \pm 42$  ng/ml, respectively. **M6G-induced respiratory depression:** For the acute hypoxic response, neither Emax nor  $C_{50}$  (value =  $282 \pm 72$  ng/ml) differed between genotypes.

**Conclusions:** The data indicate that the *OPRM1*:c.118A>G polymorphism affects opioid analgesic and respiratory effects differentially. Despite reduced analgesic responses to M6G the *OPRM1*:c.118A>G single-nucleotide polymorphism does not protect against the toxic effects of the tested opioid. However, some caution in the interpretation of the data is needed because of the small sample size. Further studies are needed to explore the link between this polymorphism and respiratory/analgesic responses beyond the small human sample. In *OPRM1*:c.118AA homozygotes, the potency parameters differed by a factor of 2 for analgesic versus respiratory effect. In this respect, M6G differs favorably from morphine.

POTENT opioid analgesics, such as morphine and its metabolite morphine-6-glucuronide (M6G), produce their intended (analgesia) and side effects (such as respi-

ratory depression) by an action at the  $\mu$ -opioid receptor (*OPRM1*).<sup>1-3</sup> Recent studies identified several single-nucleotide polymorphisms (SNPs) of the *OPRM1* gene.<sup>4</sup> The most widespread SNP of the *OPRM1* gene associated with a change in the amino acid sequence of the gene product is the substitution of the nucleotide adenine (A) with guanine (G) in exon 1 at nucleotide position 118 (*OPRM1*:c.118A>G SNP, dbSNP1799971: A>G). The result of this substitution at the receptor level is the exchange of amino acid asparagine (Asn) by aspartate (Asp) at the site of amino acid 40. Various studies have addressed the biologic effect of the *OPRM1*:c.118A>G SNP with respect to (1) opioid affinity to the *OPRM1*,<sup>4,5</sup> (2)  $\mu$ -receptor endocytosis/desensitization,<sup>5</sup> (3) vulnerability to substance abuse (opioid and nonopioid),<sup>6,7</sup> (4) stress response to  $\mu$ -receptor blockade,<sup>8-10</sup> (5) opioid-induced pupil constriction,<sup>11</sup> and (6) opioid-induced analgesia.<sup>12-14</sup> The picture that emerges from *in vitro* studies (1 and 2) is that, in contrast to most opioids (such as morphine and M6G),  $\beta$  endorphin binds three times more tightly to the Asp40 (*OPRM1*:c.118G) variant of the receptor (with three times greater potency) than to the Asn40 (*OPRM1*:c.118A) variant.<sup>4</sup> However, no differences in  $\mu$ -receptor endocytosis or internalization was observed between receptor types, indicating no marked functional differences.<sup>5</sup> Studies in humans<sup>4-6</sup> do point toward differences in opioid response in carriers of the *OPRM1*:c.118G allele compared with homozygous carriers of the *OPRM1*:c.118A receptor form. The cortisol response to opioid receptor blockade with naloxone is greater in carriers of the *OPRM1*:c.118G allele compared with *OPRM1*:c.118AA homozygotes.<sup>8,9</sup> The potency of morphine and its metabolite M6G to constrict the pupil is reduced by a factor of approximately 2 in *OPRM1*:c.118GA heterozygotes and by a factor of 3-4 in *OPRM1*:c.118GG homozygotes compared with *OPRM1*:c.118AA homozygotes.<sup>11</sup> Despite the relatively high frequency of the mutated allele in the population (10-30%),<sup>4,14,15</sup> few studies have addressed the issue of *OPRM1*:c.118A>G SNP and opioid-induced analgesia. Klepstad *et al.*<sup>12</sup> showed that patients with cancer who are homozygous for the *OPRM1*:c.118G allele require twice as much morphine to achieve adequate pain control compared with heterozygous *OPRM1*:c.118G patients and homozygous *OPRM1*:c.118AA patients. In healthy volunteers, we recently observed a threefold

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reduction in M6G analgesic potency in *OPRM1:c.118GA* heterozygotes compared with *OPRM1:c.118AA* homozygotes.<sup>14</sup> Interestingly, the *OPRM1:c.118A>G* polymorphism has not been studied in relation to one of the most important side effect of opioids, respiratory depression. This is of importance because anecdotal data suggest that the *OPRM1:c.118G* allele protects against opioid toxicity.<sup>16,17</sup> In this study, we assessed the analgesic and respiratory effects of M6G in 16 healthy volunteers. We chose to test M6G in this study because we already have evidence for a reduced M6G-induced analgesic response in *OPRM1:c.118GA* heterozygotes.<sup>14</sup>

Note that we use the official SNP notation for the *OPRM1* SNP at nucleotide position 118: *OPRM1:c.118A>G*, with alleles *OPRM1:c.118A* and *OPRM1:c.118G*.<sup>††</sup> Homozygotes are noted as *OPRM1:c.118AA* or *OPRM1:c.118GG*; heterozygotes are noted as *OPRM1:c.118GA*. At the protein level, phenotypes are *OPRM1:p.40Asn* for *OPRM1:c.118AA* homozygotes and *OPRM1:p.40Asp* and *OPRM1:p.40Asn* for *OPRM1:c.118GA* heterozygotes.

## Materials and Methods

### Study Design

Sixteen healthy volunteers (eight men, eight women; aged 18–30 yr) participated in the protocol after approval was obtained from the local human ethics committee (Commissie Medische Ethiek, Leiden, The Netherlands). Oral and written consent was obtained from all volunteers. None of the subjects had a history of illicit drug use; all women were taking oral contraceptives. The subjects were asked to have a normal night of sleep and not to eat or drink for at least 6 h before the study. During the studies, the subjects were allowed to drink water and to eat a light meal.

After arrival in the research unit, electrocardiographic electrodes were placed and an arterial line (for blood sampling) inserted in the left or right radial artery during local anesthesia. In the contralateral arm, an intravenous line was inserted for drug infusion.

### Morphine-6-glucuronide

The subjects were grouped randomly into four groups, receiving (1) 0.4 mg/kg M6G at 09:00 h, (2) 0.6 mg/kg M6G at 09:00 h, (3) 0.4 mg/kg M6G at 18:00 h, and (4) 0.6 mg/kg at 18:00 h. M6G was donated by CeNeS Ltd. (Cambridge, United Kingdom). The local pharmacy performed randomization and prepared the syringes on the day before the experiment. M6G (dissolved in 5 ml normal saline) was infused intravenously over 90 s. Before and after M6G infusion, we obtained analgesic and respiratory responses. These measurements continued

for 14 h after the M6G infusion at regular intervals, with the exception of the sleep period in groups 3 and 4 (from approximately 24:00 to 07:00 h). This design enabled us to obtain measurements evenly spread out over the 14-h time period without the need to wake up subjects during their sleep period.

### Blood Sampling

Blood sampling took place at times  $t = -10, 2, 5, 10, 15, 20, 25, 50, 80, 90, 100, 105, 150, 200, 210$ , and 240 min and every next hour until 12 h after the bolus infusion. In instances where blood sampling coincided with pain assessment, the pain test preceded the sampling. Plasma was separated within 15 min after blood collection and centrifuged for 10 min at  $3,500 \text{ min}^{-1}$ . Plasma samples were immediately stored at  $-25^\circ\text{C}$  until analysis. Plasma M6G concentrations were determined with liquid chromatography tandem mass spectrometry. The lower limits of quantification were set at 2.0 ng/ml. The coefficient of variation varied from 4 to 8% over the calibration range of 2–10,000 ng/ml.

### Acute Pain Model

Acute pain was induced by an electrical current through two surface electrodes (Red Dot; 3M, Neuss, Germany) placed on the skin overlaying the tibial bone (shin bone) of the left leg. The electrodes were attached to a computer-interfaced current stimulator, which was locally designed and constructed. This pain model has been validated previously.<sup>14</sup> The intensity of the noxious stimulation was increased from 0 mA in steps of 0.5 mA/s with a pulse duration of 0.2 ms at 10 Hz (cutoff = 128 mA). The subjects were instructed to press a button on a control box when no further increase in stimulus intensity was acceptable (*i.e.*, pain tolerance). When the subjects pressed the button, the stimulus train ended, and the current was collected and stored on the hard disc of a computer for further analysis. Before drug infusion, the subjects were trained on both sessions for approximately 1 h during which several stimulus trains were applied. These data were discarded. After a subsequent resting period, baseline tolerance was assessed in triplicate. The intensity of antinociceptive measurements was every 10 min during the first 4 h and every 30 min afterward until the 14 h time point was reached. No measurements were made from 23:00 to 07:00 h.

### Respiratory Measurements

End-tidal gas forcing and data acquisition were performed using the dynamic end-tidal forcing technique (see Dahan *et al.*<sup>18</sup> for an explanation of the technique). In brief, a personal computer provided control signals to a set of mass-flow controllers (Bronkhorts, Veenendaal, The Netherlands) so that the composition of the inspired gas mixtures could be adjusted to force end-tidal oxygen and carbon dioxide concentrations to follow a specified

<sup>††</sup> See also Human Genome Variation Society for nomenclature guidelines. Available at: [www.hgvs.org](http://www.hgvs.org). Accessed November 3, 2004.

pattern in time, independent of the ventilatory response. The inspired and expired oxygen and carbon dioxide concentrations and the arterial hemoglobin-oxygen saturation were measured with a Datex Multicap gas monitor (near the mouth) and Datex Satellite Plus pulse oximeter, respectively (Datex-Engstrom, Helsinki, Finland). End-tidal concentrations of oxygen and carbon dioxide, inspired minute ventilation ( $V_i$ ), and oxygen saturation were collected and stored on disc for further analysis.

In this study, we performed steps from normoxia (end-tidal oxygen tension 110 mmHg for 8 min, end-tidal carbon dioxide tension = 50 mmHg) into hypoxia (end-tidal oxygen tension = 45 mmHg—values reached within four to six breaths, duration of hypoxia = 3 min, end-tidal carbon dioxide tension = 50 mmHg). Before drug infusion, control or baseline hypoxic responses were obtained. Next, the drugs were infused. Breathing responses were initially obtained at 30-min intervals (at  $t = 30$  and 60 min after the bolus drug infusion) followed by 60-min intervals until the end of the study (no studies performed from 23:00 to 07:00 h).

The breath-to-breath data of the last 10 breaths of normoxia ( $V_i$ (normoxia)) and the last 10 breaths of hypoxia ( $V_i$ (hypoxia)) were averaged. Because the relation between ventilation and arterial oxygen saturation is linear, we calculated the difference between the hypoxic and normoxic minute ventilation and the oxygen saturation measured by pulse oximetry ( $SpO_2$ ) data points and expressed the acute hypoxic ventilatory response (AHR) or sensitivity as follows:

$$AHR = \frac{V_i(\text{hypoxia}) - V_i(\text{normoxia})}{SpO_2(\text{normoxia}) - SpO_2(\text{hypoxia})} \quad (1)$$

(units L/min per % desaturation).

### Genotyping

We used two primers to amplify part of exon 1 of the *OPRM1* gene containing the *OPRM1:c.118A>G* SNP (NM\_000914.1:c.118A>G, dbSNP1799971:A>G): primer Opm1F (5'-GGTCAACTTGTCCCACTTAGATCGC-3') with a single nucleotide substitution (underlined in sequence), which creates a restriction site for the enzyme *Bst*UI when the G118 allele is present and Opm1R (5'-AATCACATACATGACCAGGAAGTTT-3'). Polymerase chain reaction was performed on 100 ng genomic DNA isolated from blood samples in a total volume of 25  $\mu$ l at a final concentration of 10 mM Tris-HCl (pH 8.8), 75 mM KCl, 1.5 mM  $MgCl_2$ , 100  $\mu$ M each dNTP, and 0.025 U/ $\mu$ l *E-Taq* polymerase (Eurogentec, Liège, Belgium) in the presence of 7.5 pmol of primers. Denaturation was 3 min at 94°C, followed by 38 cycles of amplification with denaturation for 30 s at 94°C, annealing for 1 min at 62°C, and extension for 1 min at 72°C, with a final extension for 10 min. A 20- $\mu$ l polymerase chain reaction sample was

### Detection of A118G SNP in $\mu$ -opioid receptor gene (*OPRM1*)

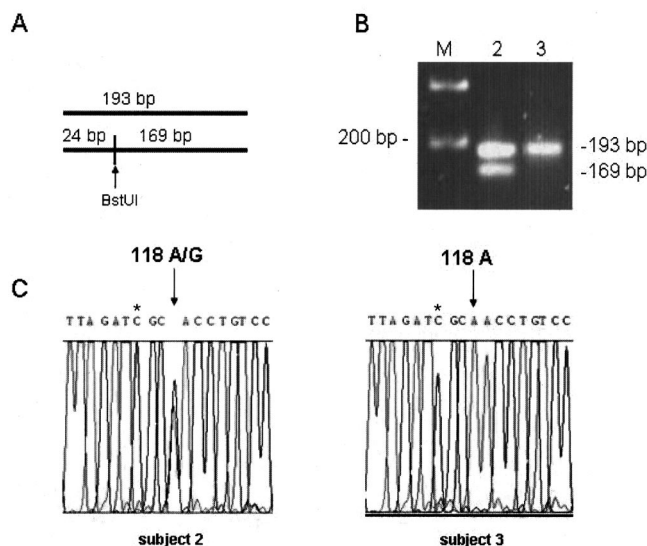


Fig. 1. (A) Detection of the  $\mu$ -opioid receptor gene *OPRM1*: *c.118A>G* single-nucleotide polymorphism (SNP) in two subjects by restriction-length polymorphism analysis. After amplification of part of *OPRM1* exon 1 using modified primer Opm1F, the polymerase chain reaction products were digested with the restriction enzyme *Bst*UI to detect the *OPRM1*: *c.118A>G* SNP. The *OPRM1*: *c.118G>C* substitution generated by primer Opm1F in combination with the *OPRM1*: *c.118A>G* SNP creates an extra *Bst*UI site within the 193-base pair (bp) polymerase chain reaction product, resulting in fragments of 24 and 169 bp after restriction digestion (left). (B) The 193-bp polymerase chain reaction product containing *OPRM1*: *c.118A* is not cut. Subject 2 is an *OPRM1*: *c.118GA* heterozygote as indicated by the presence of 193-bp and 169-bp bands. Subject 3 is an *OPRM1*: *c.118AA* homozygote (193-bp band only, right). M = marker. (C) Confirmation of the presence of the *OPRM1*: *c.118A>G* SNP by direct sequence analysis. Subject 2 is an *OPRM1*: *c.118GA* heterozygote, whereas subject 3 is an *OPRM1*: *c.118AA* homozygote. The position of the G116C substitution generated by primer Opm1F to create the *Bst*UI restriction site on the *OPRM1*: *c.118G* allele is indicated by an asterisk.

analyzed on a 2% agarose gel. Amplified *OPRM1* products were digested with the restriction enzyme *Bst*UI (New England Biolabs, Beverly, MA) according to the recommendation of the manufacturer. Each sample was analyzed on a 2% agarose gel, stained with ethidium bromide, and visualized by an ultraviolet transilluminator. To confirm the results from the restriction fragment length polymorphism analysis, amplified *OPRM1* products were purified by use of the Qiaquick polymerase chain reaction purification kit (Qiagen, Valencia, CA) and sequenced on an ABI 377 sequencer using the same primers and the Big Dye Terminator cycle sequencing kit (Perkin Elmer, Shelton, CT) (see also figure 1).

### Pharmacokinetic-Pharmacodynamic Data Analysis

The pharmacokinetic-pharmacodynamic analysis was performed with NONMEM version V, level 1.1 (a data analysis program for nonlinear mixed effects modeling; San Francisco, CA) using a population approach.<sup>19</sup> First,



a pharmacokinetic analysis was performed. Two- and three-compartment models were fitted to the data. Next, the pharmacodynamic analysis was performed on the analgesic and respiratory data with fixed individual pharmacokinetic parameters.

To eliminate a possible hysteresis between opioid plasma concentrations, as described by the pharmacokinetic model, and analgesic effect, an effect compartment was postulated. This effect compartment equilibrates with the plasma compartment with a time constant  $t_{1/2k_{e0}}$  (blood–effect site equilibration half-life).

### Respiration

Acute hypoxic responses were analyzed using the following inhibitory sigmoid Emax model:

$$\text{AHR}(t) = \text{AHR}_0 \cdot \left[ 1 - \text{Emax} \cdot \frac{(\text{Ce}(t)/C_{50})^\gamma}{1 + (\text{Ce}(t)/C_{50})^\gamma} \right], \quad (2)$$

where  $\text{AHR}(t)$  is AHR at time  $t$ ,  $\text{AHR}_0$  is baseline (= predrug) AHR,  $\text{Ce}(t)$  is the effect site concentration at time  $t$ ,  $C_{50}$  is the effect site or steady state concentration causing a 50% depression of AHR, Emax is the maximum possible effect, and  $\gamma$  is a dimensionless shape parameter.

### Analgesia

We assume that M6G attenuates the response to the applied noxious stimuli by inhibition of signal propagation or central signal processing or both. As a consequence, stronger stimuli are needed before a subject presses the pain tolerance button. The attenuation (A) was described by an inhibitory sigmoid Emax model<sup>20</sup>

$$A(t) = 1 - \text{Emax} \cdot \left[ \frac{(\text{Ce}(t)/AC_{50})^\gamma}{1 + (\text{Ce}(t)/AC_{50})^\gamma} \right], \quad (3)$$

where Emax is the maximum attenuation and  $AC_{50}$  is the effect site concentration causing 50% of the maximal attenuation effect. Because a response of the subject occurs when his or her pain sensation exceeds the response threshold (for pain tolerance), we may rewrite this as

$$\text{Current}(t) = \text{BLN} \cdot \frac{1}{A(t)}, \quad (4)$$

where BLN is baseline (= predrug) current.

Likelihood ratio tests were performed to determine whether  $\gamma$  and Emax equaled 1. The interindividual variability of each model parameter was assumed to be log-normally distributed and was characterized by percent coefficient of variation. The improvement of the model fit by inclusion of covariates (time of infusion, sex, and genotype) was tested using the likelihood ratio criterion. Separate analyses were performed on pain tolerance,  $\text{Vi}(\text{normoxia})$ , and AHR.  $P$  values less than 0.01 were considered significant (e.g., a decrease of >

6.63 in the NONMEM objective function). Values are reported as population value (median)  $\pm$  SE.

## Results

The mean age of the subjects was 21.5 yr (range, 19–23 yr), and the mean weight was 71.1 kg (range, 55–91 kg). The sexes were evenly spread over the two M6G doses. All 16 subjects completed the protocol without major side effects. Nausea or vomiting did not occur.

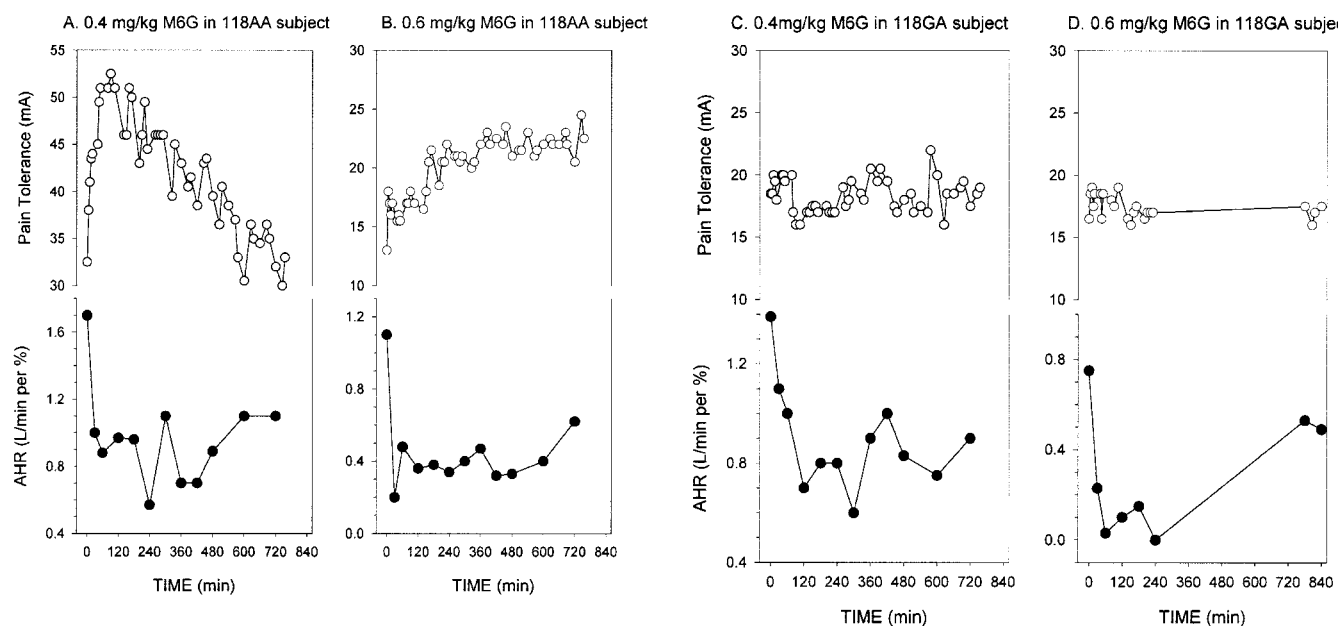
We observed the *OPRM1:c.118G* allele with a frequency of 12.5%. Four of the subjects were heterozygous for the *OPRM1:c.118G* allele (genotype: *OPRM1:c.118GA*; on protein level: *OPRM1:p.40Asp + OPRM1:p.40Asn*), the remaining subjects were all homozygous for the *OPRM1:c.118A* allele (genotype: *OPRM1:c.118AA*; on protein level: *OPRM1:p.40Asn*). Examples of analgesic and hypoxic responses of homozygous *OPRM1:c.118AA* and heterozygous *OPRM1:c.118GA* individuals are given in figure 2. It illustrates the general observation that M6G produces consistent analgesic and respiratory responses in homozygous *OPRM1:c.118AA* individuals. *OPRM1:c.118GA* subjects showed M6G-induced respiratory depression, just like the *OPRM1:c.118AA* subjects, however, coinciding with small and inconsistent analgesic responses.

### M6G-induced Analgesia in *OPRM1:c.118GA* versus *OPRM1:c.118AA* Subjects

Analysis of the data of the 16 subjects indicated an improvement in data fits (at the  $P < 0.01$  level) when genotype (but not time of infusion or sex) was included as a covariate. However, we were unable to obtain a reliable estimate of the potency of M6G in the four *OPRM1:c.118GA* subjects (because of their small and inconsistent responses). The *OPRM1:c.118GA* analgesic data were best described by the function  $\text{Effect}(t) = \text{baseline}$ . In contrast, *OPRM1:c.118AA* subjects increased their current tolerance by 55% above baseline. The parameter estimates of the NONMEM analysis are given in table 1. In figure 3, some examples of the data fits in *OPRM1:c.118AA* and *OPRM1:c.118GA* subjects are given.

### M6G-induced Respiratory Depression in *OPRM1:c.118GA* versus *OPRM1:c.118AA* Subjects

All subjects showed respiratory depression in response to M6G infusion. To get an indication of the response of *OPRM1:c.118GA* subjects relative to the *OPRM1:c.118AA* subjects, we plotted the individual responses of the *OPRM1:c.118GA* subjects against the mean *OPRM1:c.118AA* responses  $\pm$  95% confidence intervals for the 0.4 and 0.6 mg/kg M6G groups in figure 4. Apart from showing a dose-dependent effect of M6G on the AHR, it shows that M6G produces respiratory depression in both



**Fig. 2.** Analgesic versus respiratory responses. Influence of morphine-6-glucuronide (M6G) on pain tolerance and the ventilatory response to acute hypoxia (AHR) in two *OPRM1:c.118AA* homozygotes (118AA; A and B) and *OPRM1:c.118GA* heterozygotes (118GA; C and D). In contrast to the respiratory responses, analgesic responses differed between genotypes, with small and inconsistent analgesic response in carriers of the *OPRM1:c.118GA* allele.

genotypes, with little difference in time course or magnitude of effect. The results of the population pharmacodynamic analysis indicate that inclusion of covariates time of infusion, sex, and genotype did not improve the model fits. The pharmacodynamic parameter values are given in table 1.  $E_{max}$  did not differ from 1, indicating that  $AHR = 0$  was the maximum effect. In figure 5, we

give some examples of the data fits in *OPRM1:c.118AA* and *OPRM1:c.118GA* subjects.

#### M6G Pharmacokinetics

The observed differences in analgesic effect between genotypes were unrelated to differences in the pharmacokinetics of M6G. Plasma concentrations did not differ

**Table 1.** Population Analysis of the Influence of Morphine-6-glucuronide on Analgesia and AHR in Homozygous Carriers of the *OPRM1:c.118A* Allele and Heterozygous Carriers of the *OPRM1:c.118G* Allele

	Analgesia		AHR	
	<i>OPRM1:c.118AA</i>	<i>OPRM1:c.118GA</i>	<i>OPRM1:c.118AA</i>	<i>OPRM1:c.118GA</i>
Baseline				
Value	14.5 mA		$1.13 \text{ l} \cdot \text{min}^{-1} \cdot \%^{-1}$	
SE	1.7		0.12	
%CV	38		36	
$AC_{50}$ or $C_{50}$ , ng/ml				
Value	161	—	282	—
SE	42	—	75	—
%CV	*	—	48	—
$E_{max}^{\dagger}$				
Value	0.55	—	1	—
SE	0.18	—	—	—
%CV	101	—	—	—
$t_{1/2}k_{e0}$ , h				
Value	7.8	—	5.1	—
SE	11.9	—	1.3	—
%CV	204	—	*	—
$\gamma$				
Value	1	—	1	—
SE	—	—	—	—
%CV	—	—	—	—

$AC_{50}$  and  $C_{50}$  = potency parameters; AHR = acute hypoxic response; %CV = percent coefficient of variation or between-subject variability;  $E_{max}$  = maximal effect;  $\gamma$  = a dimensionless shape parameter;  $t_{1/2}k_{e0}$  = blood-effect site equilibration half-life.

\* Not included in statistical model.  $\dagger$  Proportion of baseline.

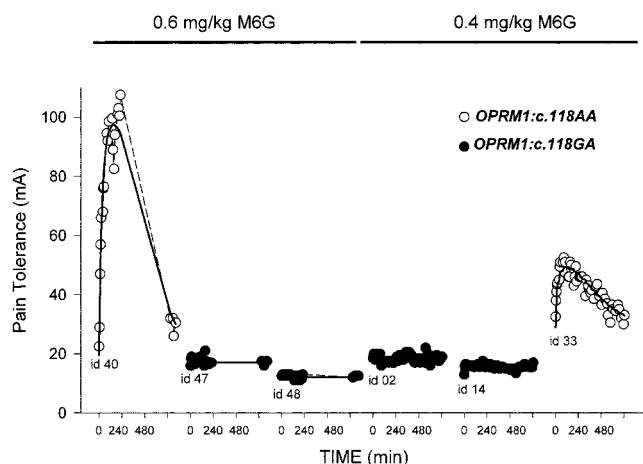


Fig. 3. Examples of model fits of the analgesic data from *OPRM1:c.118AA* (white circles) and model fits of all of the *OPRM1:c.118GA* subjects (black circles). The data presented with white and black symbols are the measured data from the current study. Continuous lines are the predicted responses. Note that for *OPRM1:c.118GA* subjects, the predicted responses are equal to baseline (Effect(t) = baseline). M6G = morphine-6-glucuronide.

between genotypes. The population pharmacokinetic analysis indicated that the pharmacokinetic data were best described by a three-compartment model, with parameter values very similar to those observed previously (data not shown).<sup>14</sup>

## Discussion

A population pharmacokinetic–pharmacodynamic model was developed to assess the effect of the *OPRM1:c.118A>G* SNP on M6G-induced analgesic and respiratory responses. We observed that the *OPRM1:c.118G* allele had no effect on M6G-induced respiratory responses, whereas it caused a severe reduction in the analgesic efficacy of M6G.

In contrast to previous studies, we analyzed the respiratory data using an inhibitory sigmoid Emax model. We

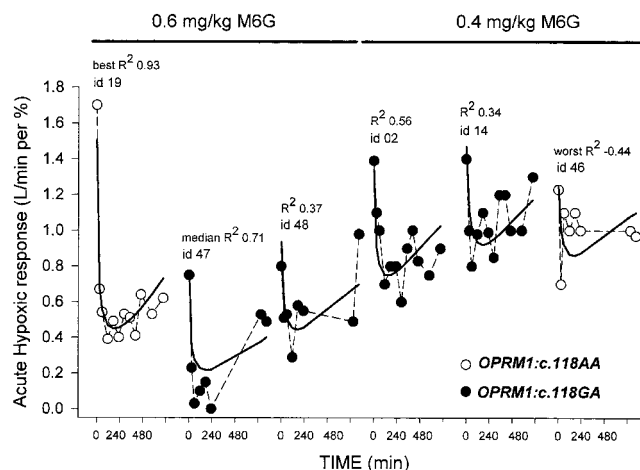


Fig. 5. Model fits of the respiratory data from *OPRM1:c.118AA* subjects (118AA) and *OPRM1:c.118GA* subjects (118GA). Measured data in *OPRM1:c.118AA* (○) and *OPRM1:c.118GA* (●) subjects, respectively. Continuous lines = predicted responses. Best, median, and worst fits are shown plus all four *OPRM1:c.118GA* fits. M6G = morphine-6-glucuronide.

previously used a power model to analyze the respiratory effect of M6G. Because the power model does have our preference considering the flexibility of the model (see Dahan *et al.*<sup>21</sup> for a discussion on the model), we also analyzed the current data set with the power model. Because the analysis with the sigmoid Emax model resulted in significantly better data fits (NONMEM objective function differed by 17 points), we present the analysis of the sigmoid model.

A potential drawback of our study is the small number of *OPRM1:c.118GA* subjects in our sample. The small sample size may have caused the overestimation and/or underestimation of the genotype effect for analgesia and respiration, respectively. To increase the power of our study, we performed a *post hoc* analysis on an extended data set. We increased the number of *OPRM1:c.118GA* subjects in the analysis of the analgesic data to 10 by adding data from a previous study from our laboratory on M6G analgesia.<sup>14</sup> In that study, subjects received 0.3

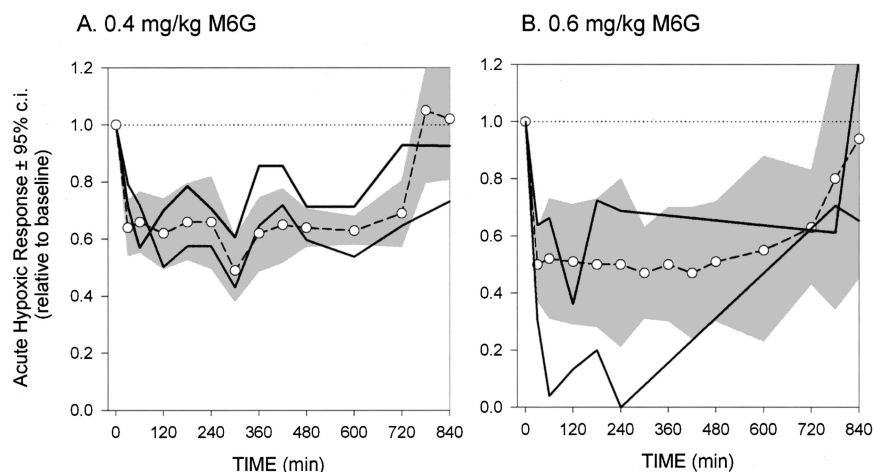


Fig. 4. Ensemble average (○) ± 95% confidence intervals (c.i.; gray area) of the respiratory data of *OPRM1:c.118AA* subjects and individual responses of the four *OPRM1:c.118GA* subjects (continuous black lines). (A) Responses after 0.4 mg/kg morphine-6-glucuronide (M6G); (B) responses after 0.6 mg/kg M6G. All data are relative to baseline (baseline = 1).

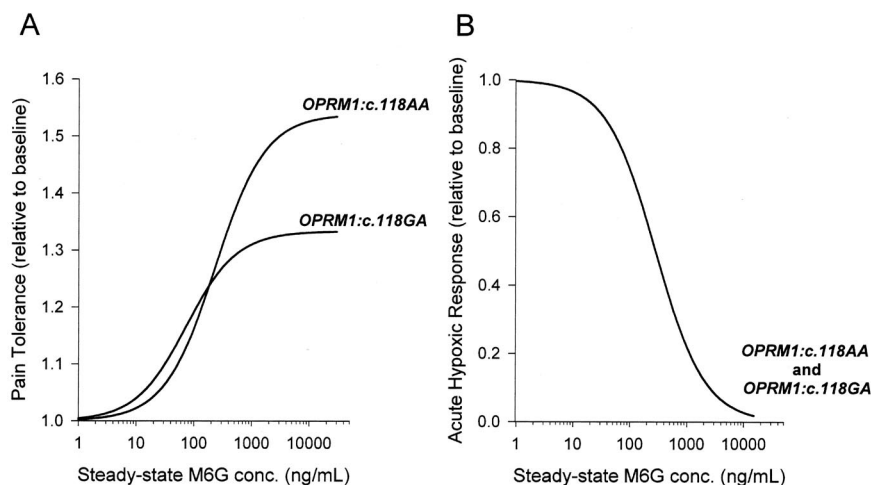


Fig. 6. Steady state response of the morphine-6-glucuronide (M6G) concentration versus pain tolerance (A) and the acute hypoxic ventilatory response (B). For pain tolerance but not for respiration, a significant difference was observed between the *OPRM1:c.118AA* and *OPRM1:c.118GA* genotype with lesser M6G efficacy in *OPRM1:c.118GA* heterozygotes. For pain tolerance (but not for respiration) we performed a *post hoc* analysis including six *OPRM1:c.118GA* heterozygotes from a previous study (Romberg et al.<sup>14</sup>).

mg/kg intravenous M6G (two thirds given as a bolus over 90 s, the remainder given as a continuous infusion over 1 h). Analgesic measurements, identical to those of the current study, were obtained for 7 h. Reanalysis of the total data set yielded the following population parameter estimates for the *OPRM1:c.118GA* subjects:  $E_{\max} = 0.30 \pm 0.07$ ,  $AC_{50} = 59 \pm 12$  ng/ml,  $t_{1/2k_{e0}} = 2.0 \pm 0.8$  h, and a value of  $\gamma$  not different from 1 (see also fig. 6 for the steady state dose-response relation of the 10 *OPRM1:c.118GA* and 12 *OPRM1:c.118AA* subjects). This indicates a sharp reduction in opioid efficacy in heterozygous *OPRM1:c.118GA* subjects with only a small analgesic effect, which occurred rapidly after M6G was infused. The magnitude and rapid onset of effect suggests that the analgesic response in the 10 heterozygous 118A>G subjects bears characteristics of a placebo response rather than a pure M6G-induced analgesic effect. The placebo response (related to phenomena such as anticipation, memory and suggestion) may be apparent because of the lack of significant M6G effect in this group. Studies on the development of placebo analgesia in *OPRM1:c.118GA* versus *OPRM1:c.118AA* subjects are needed to increase our insight in this matter.

For the respiratory data we were unable to extend our data set. Despite the fact that all four *OPRM1:c.118GA* subjects displayed overt respiratory depression (fig. 4), which contrasted sharply with their small and inconsistent analgesic responses (fig. 3), we believe that our respiratory data must be interpreted with care. Although our data clearly indicate that the *OPRM1:c.118G* allele is linked to a significant decrease in M6G analgesic efficacy, the absence of such an association with respect to M6G-induced respiratory effect should be considered preliminary. It may be that the effect of the *OPRM1:c.118G* allele is much smaller than its effect on antinociception and hence could not be unearthed from the small sample size. However, we believe that our results are more than coincidental (fig. 4) and suggest that the *OPRM1:c.118G* allele will not protect for M6G (or any other opioid)-induced respiratory depression. We there-

fore hypothesize at this point that the *OPRM1:c.118G* allele affects opioid analgesic and respiratory effect differentially.

Although our study was not intended to explain the putative mechanism of the differential effect of the *OPRM1:c.118G* allele on analgesia and respiration, some speculation in this respect is of interest. The *OPRM1:c.118A>G* substitution occurs within exon 1 of the *OPRM1* gene and is expressed in the N-terminal region of the seven-transmembrane extracellular structure of the  $\mu$ -opioid receptor.<sup>4</sup> The resultant exchange of amino acid asparagine by aspartate results in the loss of a putative site for N-glycosylation.<sup>4</sup> *In vitro* studies did not show a difference in M6G binding between *OPRM1:p.Asp40* and *OPRM1:p.Asn40* receptors.<sup>5</sup> However, some SNPs of the *OPRM1* gene (other than *OPRM1:c.118A>G*) have been associated with alteration in G-protein coupling,<sup>22</sup> suggesting an important change in the functionality of  $\mu$ -opioid receptor variants. *In vivo* differences in  $\mu$ -opioid receptor functionality induced by the *OPRM1:c.118A>G* SNP remain unknown. Animal studies using antisense directed at exon 1 of *Oprm1* indicate that M6G (but not morphine) antinociceptive effects remain unaltered when blocking exon 1. For example, in rats, treatment with antisense probes targeting exon 1 did not block M6G analgesia but significantly reduced morphine analgesia, whereas probes targeting exons 2 or 3 decreased M6G but not morphine analgesia.<sup>23</sup> Similarly, *Oprm* exon 1 knockout mice displayed analgesic responses to M6G (but not to morphine),<sup>24</sup> whereas exon 2 knockout mice displayed no analgesia in response to morphine but small (non-opioid-related) hyperalgesic responses to M6G.<sup>3</sup> The data from exon 1 knockout mice must be interpreted with care because it is unknown whether the observed analgesic responses were blocked by  $\mu$ -opioid receptor antagonists. Our current findings are partially in contrast with the animal work. Our data suggest that exon 1 of the human *OPRM1* gene is an essential requirement for at least part of M6G-induced analgesia (in contrast to the data in exon



1 knockout mice) but not for M6G respiratory effect. Our data then suggest the existence of distinct  $\mu$ -opioid receptor variants (e.g., splice variants) expressed in neurons involved in pain processing and those involved in the control of breathing. The receptors involved in pain processing are critically dependent on the functionality of exon 1 of the receptor gene, whereas receptors expressed in respiratory pathways are not. Another possibility is that the variation at nucleotide 118 of exon 1 of the *OPRM1* gene (which alters a glycosylation site in the receptor) impacts on receptor targeting to the cell surface and that in some neurons (nociceptive but not respiratory) receptors are not properly located on the neuron surface to be fully functional. Evidently, further investigations aimed at studying the molecular effects of the *OPRM1:c.118A>G* variation in respiratory and nonrespiratory neurons are needed to clarify our insight in the above.

The clinical implications of our data are that the *OPRM1:c.118G* allele does not protect for the toxic effects of M6G, which may occur after morphine or M6G administration in patients with renal impairment. This contrasts a hypothesis of Lötsch *et al.*<sup>25</sup> that the *OPRM1:c.118G* allele is among the protective factors against M6G-related toxicity such as severe sleepiness and drowsiness in renal patients. However, before definite conclusions from our study can be drawn regarding any association between the *OPRM1:c.118G* allele and M6G respiratory responses, studies with larger sample sizes are required. Our data are in close accord with a recent clinical study.<sup>12</sup> Patients with cancer who were heterozygous for the *OPRM1:c.118A>G* polymorphism had significantly more pain at equal steady state morphine and M6G concentrations. Furthermore, patients who were homozygous for the *OPRM1:c.118A>G* polymorphism required significantly more morphine for adequate pain relief (homozygous *OPRM1:c.118AA* and *OPRM1:c.118GG* subjects required 97 mg/24 h and 225 mg/24 h, respectively) with corresponding greater morphine and M6G steady state plasma concentrations. Interestingly, side effects such as fatigue, nausea and vomiting, dyspnea, and constipation did not differ among the three genotypes.<sup>12</sup>

Finally, some comments on the pharmacodynamic parameter values are needed. (1) Although the pharmacodynamic parameter values (and their variability) are in accord with previous findings on the analgesic properties of M6G for both genotypes,<sup>14</sup> the onset/offset times and potency of M6G respiratory effect are different from those reported previously. A value for  $t_{1/2k_{e0}}$  of 1 h and a relatively low potency ( $C_{50} > 450$  ng/ml) for the acute hypoxic ventilatory response after 0.2 mg/kg M6G was reported by us.<sup>26</sup> We relate the longer onset/offset half-life and increased potency in the current study to the greater M6G doses given (0.4 and 0.6 mg/kg), which caused greater concentrations of M6G in the brain. M6G is known to pass the blood-brain barrier slowly and is a

substrate of P-glycoprotein,<sup>27</sup> an adenosine triphosphate-dependent drug efflux pump expressed in brain capillary endothelial cells. Saturation of the P-glycoprotein efflux pump occurring at high M6G brain concentrations may have caused trapping of the M6G molecule within the brain compartment causing prolonged central effects at a relatively low potency compared with effects observed at much lower M6G brain concentrations. (2) In homozygous *OPRM1:c.118AA* subjects, we observed greater M6G plasma concentrations needed for 50% respiratory effect relative to 50% analgesic effect. This indicates that less M6G is needed for its intended effect than for its unintended effect (potency ratio  $AC_{50}:C_{50} \sim 1:2$ ). In this respect, M6G differs favorably from morphine, which has an  $AC_{50}:C_{50}$  ratio of 1:1 (i.e., similar morphine concentrations are needed to cause 50% respiratory and analgesic effects).<sup>28</sup> Our observations (this study and the data from Dahan *et al.*<sup>28</sup>) are in agreement with earlier statements and strengthen our belief that M6G has an increased margin of safety relative to that of morphine.<sup>26,28,29</sup>

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## References

- Matthes HWD, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, LeMeur M, Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL: Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the  $\mu$ -opioid-receptor gene. *Nature* 1996; 383:819–23
- Dahan A, Sartori E, Teppema L, Olivieri C, Nieuwenhuijs D, Matthes HWD, Kieffer BL: Anesthetic potency and influence of morphine and sevoflurane on respiration in  $\mu$ -opioid receptor knockout mice. *ANESTHESIOLOGY* 2001; 94:824–32
- Romberg R, Sartori E, Teppema L, Matthes MWD, Kieffer BL, Dahan A: Comparison of morphine-6-glucuronide and morphine on respiratory depressant and antinociceptive responses in wild type and  $\mu$ -opioid receptor deficient mice. *Br J Anaesth* 2003; 91:862–70
- Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schlager J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L: Single nucleotide polymorphism in the human mu opioid receptor gene alters  $\beta$ -endorphin binding and activity: Possible implications for opiate addiction. *Proc Natl Acad Sci U S A* 1998; 95:9608–13
- Beyer A, Koch T, Schröder H, Schulz S, Hölt V: Effect of A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human mu-opioid receptor. *J Neurochem* 2004; 89:553–60
- Franke P, Wang T, Nöthen MM, Knapp M, Neidt H, Albrecht S, Jahnke E, Propping P, Maier W: Nonreplication of association between  $\mu$ -opioid-receptor gene (*Oprm1*) A118G polymorphism and substance dependence. *Am J Med Gen* 2001; 105:114–9
- Compton P, Geschwind DH, Alarcón M: Association between human  $\mu$ -opioid receptor gene polymorphism, pain tolerance and opioid addiction. *Am J Med Gen B* 2003; 121B:76–82
- Wand GS, McCaul M, Yang X, Reynolds J, Gotjen D, Lee S, Ali A: The mu-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade. *Neuropsychopharmacology* 2002; 26:106–14
- Hernandez-Avila CA, Wand G, Luo X, Gelernter J, Kranzler HR: Association between the cortisol response to opioid blockade and the Asn40Asp polymorphism at the  $\mu$ -opioid receptor locus (*Oprm1*). *Am J Med Gen B* 2003; 118B:60–5
- Miller GM, Bender J, Tiefenbacher S, Yang H, Novak MA, Madras BK: A mu-opioid receptor single nucleotide polymorphism in rhesus monkey: Association with stress response and aggression. *Mol Psychiatry* 2004; 9:99–108
- Lötsch J, Skarke C, Grösch S, Darimont J, Schmidt H, Geisslinger: The polymorphism A118G of the human mu-opioid receptor gene decreases the pupil



constrictory effect of morphine-6-glucuronide but not that of morphine. *Pharmacogenetics* 2002; 12:3-9

12. Klepstad P, Rakvåg TT, Kaasa S, Holthe M, Dale O, Borchgrevink PC, Baar C, Vikan T, Krokan HE, Skorpen P: The 118A>G polymorphism in the human  $\mu$ -opioid receptor gene may increase morphine requirements in patients with pain caused by malignant disease. *Acta Anaesthesiol Scand* 2004; 48:1232-39

13. Skarke C, Darimont J, Schmidt H, Geisslinger G, Lötsch J: Analgesic effects of morphine and morphine-6-glucuronide in a transcutaneous pain model in healthy volunteers. *Clin Pharmacol Ther* 2003; 73:107-21

14. Romberg R, Olofsen E, Sarton E, denHartigh J, Taschner PEM, Dahan A: Pharmacokinetic-pharmacodynamic modeling of morphine-6-glucuronide induced analgesia in healthy volunteers. *ANESTHESIOLOGY* 2004; 100:120-33

15. Landau R, Cahana A, Smiley RM, Antonarakis SE, Blouin JL: Genetic variability of  $\mu$ -opioid receptor in an obstetric population. *ANESTHESIOLOGY* 2004; 100:1030-3

16. Lötsch J, Zimmerman M, Darimont J, Marx C, Dudziak R, Skarke C, Geisslinger G: Does the A118G polymorphism at the  $\mu$ -opioid receptor gene protect against morphine-6-glucuronide? *ANESTHESIOLOGY* 2002; 97:814-9

17. Hirota T, Ieiri I, Takane H, Sano H, Kawamoto K, Aono H, Yamasaki A, Takeuchi H, Masada M, Shimizu E, Higuchi S, Otsubo K: Sequence variability and candidate gene analysis in two cancer patients with complex clinical outcomes during morphine therapy. *Drug Metab Disp* 2003; 31:677-80

18. Dahan A, deGoede J, Berkenbosch A, Olivier ICW: The influence of oxygen on the ventilatory response to carbon dioxide in man. *J Physiol (Lond)* 1990; 428:485-99

19. Beal SL, Sheiner LB: *NONMEM User's Guide*. San Francisco, University of California, San Francisco, 1999

20. Sarton E, Olofsen E, Romberg R, den Hartigh J, Kest B, Nieuwenhuijs D, Burm A, Teppema L, Dahan A: Sex differences in morphine analgesia. *ANESTHESIOLOGY* 2000; 93:1245-54

21. Dahan A, Nieuwenhuijs D, Olofsen E, Sarton E, Romberg R, Teppema L: Response surface modeling of alfentanil-sevoflurane interaction on cardiorespiratory control and Bispectral Index. *ANESTHESIOLOGY* 2001; 94: 982-91

22. Wang D, Quiallan JM, Winans K, Lucas JL, Sadée W: Single nucleotide polymorphisms in the human  $\mu$  opioid receptor gene alter basal G protein coupling and calmodulin binding. *J Biol Chem* 2001; 276:34624-30

23. Rossi GC, Leventhal L, Pan YX, Cole J, Su W, Bodnar RJ, Pasternak GW: Antisense mapping of MOR-1 in rats: Distinguishing between morphine and morphine-6 $\beta$ -glucuronide antinociception. *J Pharmacol Exp Ther* 1997; 281: 109-14

24. Schuller AGP, King MA, Zhang J, Bolan E, Pan YX, Morgan DJ, Chang A, Czick ME, Unterwald EM, Pasternak GW, Pintar JE: Retention of heroin and morphine-6 $\beta$ -glucuronide analgesia in a new line of mice lacking exon 1 of MOR-1. *Nat Neurosci* 1999; 2:151-6

25. Lötsch J, Zimmerman M, Darimont J, Dudziak R, Skarke C, Geisslinger G: Does the A118G polymorphism at the  $\mu$  opioid receptor gene protect against morphine-6-glucuronide toxicity? *ANESTHESIOLOGY* 2002; 97:814-9

26. Romberg R, Olofsen E, Sarton E, Teppema L, Dahan A: The pharmacodynamic effect of morphine-6-glucuronide *versus* morphine on hypoxic and hypercapnic breathing in healthy volunteers. *ANESTHESIOLOGY* 2003; 99:788-98

27. Huwyler J, Drewe J, Kuseman C, Fricker G: Evidence for P-glycoprotein-modulated penetration of morphine-6glucuronide into brain capillary endothelium. *Br J Pharmacol* 1996; 118:1879-85

28. Dahan A, Romberg R, Teppema L, Sarton E, Bijl H, Olofsen E: Simultaneous measurement and integrated analysis of analgesia and respiration after an intravenous morphine infusion. *ANESTHESIOLOGY* 2004; 101:1201-9

29. Romberg R, Olofsen E, Sarton E, Teppema L, Dahan A: Increased margin of safety of morphine-6-glucuronide relative to morphine (letter). *ANESTHESIOLOGY* 2004; 100:1622