The rare Arg181Cys mutation in the μ opioid receptor can abolish opioid responses

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Conflict of interest

The authors declare that they have no conflict of interest.

Abstract

Background: Genetic variability contributes to variable clinical response to opioids. This study emerged from the observation of three Norwegian patients who showed no or extraordinary poor response to very high doses of opioids. We suspected a genetic defect and applied a “most likely candidate gene” approach to investigate this possibility.

Methods: DNA sequencing was used to search for mutations in coding regions of the *OPRM1* gene, encoding the μ opioid receptor (hMOR), in one patient. The remaining two patients, and two cohorts comprising 2,158 European cancer pain patients and 600 Norwegian healthy volunteers, respectively, were genotyped using a custom-made TaqMan SNP allelic discrimination assay.

Results: DNA sequencing disclosed a homozygous, inactivating Arg181Cys mutation in hMOR in the patient who showed no effects from opioids. The two patients with poor effect from very high doses of opioids were both heterozygous for the mutation. Six heterozygous patients identified among the European cancer patients all used high doses of opioids and/or reported inferior effect on their pain. About one in every 100 Norwegians is heterozygous for the mutation.

Conclusions: The Arg181Cys mutation occurs at clinically relevant frequencies and produces a signaling dead hMOR which may abolish or significantly reduce opioid effects in affected individuals. Anesthesiologists and practitioners in pain medicine should be aware of this mutation as a possible explanation for inefficiency of opioids and consider genotyping in relevant cases. Individuals homozygous for the mutation may need a highly personalized approach to pain therapy.

Introduction

Opioids are the most important analgesics used to treat moderate and severe pain. It is demonstrated in various categories of pain that patients respond differently to opioid treatment, both with regard to dose requirements, degree of pain relief and to the type and severity of adverse effects.[1-4] Individuals may also respond differently to different opioids, which is the rationale for an opioid switch if the first choice opioid does not give adequate pain relief, gives rise to intolerable adverse effects, or both.[5,6] These interindividual differences, differences between ethnic groups, and data from well-designed twin studies strongly suggest that opioid efficacy is influenced by genetic variability.[7,8] Most studies that have addressed the possible impact of genetic variants on the efficacy of opioids have focused on common variants in candidate genes assumed to be important for the pharmacodynamics or pharmacokinetics of opioids.[9-13] With a few exceptions, such as some variants causing inactivity of the CYP2D6 enzyme involved in the metabolism of codeine and tramadol,[14] these variants, including the much studied 118A>G polymorphism (Asn40Asp) in the μ opioid receptor gene (*OPRM1*),[9,10] have shown only minor clinical effects.[15,16] Typically these variants explain only a minor fraction of the total variability of the outcome, and can therefore not be used to predict opioid responses in individual patients. These results suggest that additional genetic variants, perhaps not addressed in previous genetic association studies, or variants that may have escaped identification because they occur at very low frequencies, contribute to the variability of clinical responses to opioids. Effects from rare variants may be difficult to demonstrate in analyses of patient cohorts, especially if multiple rare variants collectively contribute to a larger part of the variability in the population.

The background for the present study was three patients who were reported to our research group by experienced anesthesiologists because of no or extraordinary poor response to high doses of opioids. Because the lack of response in one of the patients was so striking, we

began our studies with the hypothesis that the condition might have a genetic basis. Indeed, DNA sequencing of the coding parts of the *OPRM1* gene, considered to be the most likely candidate because of its essential role for opioid action *in vivo*, disclosed that this patient was homozygous for an inactivating Arg181Cys mutation in hMOR, a rare mutation that had been reported in the literature only once before, and which was shown to produce an inactive receptor *in vitro*.[17] We also present data on the two additional patients, as well as data from genotyping of two larger cohorts.

Methods

Patients and cohorts

Patient 1 was a 52 year old male presenting for inguinal hernia repair ad modum Lichtenstein. The planned anesthetic technique was total intravenous anesthesia (TIVA). During induction with remifentanil infusion the patient showed no signs of opioid effects. The dose of remifentanil was gradually increased to a total dose of 3,000 µg, which is more than 60 times the dose usually needed for surgical anesthesia. Additionally, a bolus dose of 0.3 mg fentanyl was administered, still without any opioid effects. Anesthesia was changed to the use of anesthetic gas, propofol infusion and local anesthetics and the further surgical and anesthetic course was uneventful. The content of the remifentanil syringe was controlled by pharmaceutical analyses in order to exclude syringe swapping or drug theft.

Patient 2 was a 52 year old female presenting with a right-sided non-small cell lung cancer, brainstem metastases, and metastases to the left femur, sacrum and spinal column. She was treated with radiation therapy to most lesions. Late in the trajectory she was admitted to a palliative care unit with severe pain (7-10 on a 0-10 numerical rating scale; NRS-11) in the lumbar dorsal region and the left hip, increasing when weight bearing. Her analgesic regimen on admission consisted of dexamethasone 4 mg x 3 po, phenytoin 150 mg x 2 po, paracetamol 1 g x 4 po, transdermal fentanyl 375 µg x h^{-1} and morphine sulphate mixture 120 mg prn. Despite the use of multiple prn-doses she reported only moderate effect on the pain. Nor did she report typical opioid adverse effects.

Patient 3 was a 47 year old female with a pancreatic carcinoma. She had extensive metastatic liver disease that caused pain with an intensity of 8-10 (NRS-11). She had previously received pregabalin and glucocorticosteroids, both stopped due to adverse effects. Treatment was rapidly escalated to dipyrone 1 g x 4 and oxycodone controlled-release 360 mg x 2 and

additional nasal fentanyl 200 µg and oxycodone immediate–release 60 mg for breakthrough pain 6 times daily but without satisfactory pain control. Other drug regimens were initiated, and prior to her death she received a subcutaneous infusion of hydromorphone 120 mg x $24h^{-1}$, ketamine 75 mg x $24h^{-1}$, clonidine 600 µg x $24h^{-1}$, and midazolam 5 mg x $24h^{-1}$ plus oral methadone 70 mg x 3, dipyrone 1 g x 4 and dexamethasone 4 mg x 2. Despite the very high opioid doses she was not pain relieved before clonidine was introduced. She did not experience any opioid related adverse effects.

Cohort 1 consisted of 600 Norwegian individuals included in a local biobank of healthy volunteers (blood donors).

Cohort 2 consisted of 2,158 cancer patients included in a biobank developed for the research on opioids, the European Pharmacogenetic Opioid Study (EPOS).[15] All patients had advanced cancer disease and were treated with opioids for cancer pain.

Isolation of DNA

Genomic DNA was extracted from EDTA whole blood using the Gentra Puregene blood kit (QIAGEN Science, Germantown, MD, USA).

PCR amplification and DNA sequencing

DNA sequencing was carried out for exons 1 through 4 in the *OPRM1* gene; methodological details are only given for the relevant exon 2. Exon 2 of the *OPRM1* gene was amplified from genomic DNA using AmpliTaq Gold® polymerase (Applied Biosystems), and primers 5'-ACTCAACAAAGCAGCATCG-3' (forward) and 5'-CTAAGACAATGGGGCACTCC-3' (reverse). Resulting PCR products were recovered from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). Isolated DNA was sequenced using the same forward and

reverse primers and the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer). Sequences were resolved on an Applied Biosystems 3730 DNA sequencer. The validity of the sequencing result was checked by sequencing of DNA obtained from a second blood sample from the same patient.

Targeted genotyping

Targeted genotyping of the Arg181Cys mutation (NCBI dbSNP rs79910351) in the 600 Norwegian healthy volunteers was performed at the Centre for Integrative Genetics (CIGENE), University of Life Sciences, Oslo, using a Sequenom MassARRAY® system. Heterozygous samples were then re-genotyped, together with the samples from 2,158 European cancer patients and the two female patients (Patient 2 and Patient 3), at the HUNT Biobank (Levanger, Norway) using a custom-made TaqMan SNP allelic discrimination assay on an ABI 7900HT Fast Real-Time PCR instrument (Applied Biosystems, Foster City, CA). There was 100% concordance between genotypes obtained by the two genotyping methods.

Ethics approval

All patients consented to participate in the study and to have their case history published. The studies including the two cohorts were both approved by The Regional Committee for Medical Research Ethics, Central Norway. "Arg181Cys mutation in the μ opioid receptor – clinical implications" (Approval document REC Central: 2014/350, 4th of April 2014, Chairman: Sven Erik Gisvold); "Biobank for pharmacogenetic and physiologic studies in volunteers" (Approval document REC Central: 4.2007.2232, 7th of November 2007, Chairman: Arne Sandvik); "European Pharmacogenetic Opioid Study (EPOS)" (Approval document REC Central: 119-03, 27th of September 2003, Chairman: Arne Sandvik)

Results

Patient 1 was a 52 year old male who showed no effects from very high doses of opioids. Sequencing of exons 1 through 4 in the *OPRM1* gene, encoding the classical μ opioid receptor (hMOR-1), disclosed a homozygous C to T missense mutation in exon 2 (c.541C>T), predicting a change from arginine (Arg) to cysteine (Cys) at amino acid position 181 in hMOR-1 (p.Arg181Cys) (Fig. 1).

Patients 2 and 3 were both females who were treated with opioids for cancer pain. Both patients showed extraordinary poor response to high opioid doses. Genotyping using a custom-made TaqMan SNP allelic discrimination assay revealed that they both were heterozygous for the Arg181Cys mutation.

To assess the frequency of the Arg181Cys mutation, we genotyped two cohorts: 600 Norwegian healthy volunteers (Cohort 1), and 2,158 European cancer pain patients receiving an opioid (Cohort 2). Among the 600 healthy volunteers, seven individuals were found to be heterozygous, whereas none were homozygous for the mutation. Among the European cancer patients, six patients were found to be heterozygous for the mutation (Norway: 3/513; Switzerland: 1/108; Great Britain: 1/261; Iceland: 1/143; Italy: 0/451; Germany: 0/439; Lithuania: 0/54; Finland: 0/30; Sweden: 0/125; Denmark: 0/28; Greece: 0/6). None of the patients were homozygous for the mutation. Of the six heterozygous patients, three used high opioid doses ((morphine equivalent oral daily doses (MEDD) > 400 mg)), one used MEDD 162 mg but had severe pain and was still under opioid titration, one used 60 mg MEDD but with the dose limited because of adverse effects. This patient was not able to report his pain intensity and died six days later. Finally, one patient used only MEDD 50 mg but had high pain intensity (Table 1).

Discussion

The present study highlights the importance of considering less frequent genetic variants as possible contributors to interindividual variation in the clinical response to opioids. To the best of our knowledge, this is the first report ever of a patient who is most likely clinically insensitive to opioids due to a homozygous missense mutation in hMOR. This mutation was first reported in 2009 by Ravindranathan *et al*., who identified the mutation after screening 550 subjects of the San Diego Sibling Pair study cohort.[17] However, the clinical implications of the mutation have never before been explored. The Arg181Cys mutation is located in the 2nd intracellular loop of hMOR, a region shown to be of key importance for signaling through intracellular effector proteins.[18] When the mutant receptor was functionally characterized *in vitro*, it failed to internalize in response to 10μM of the potent opioid peptide DAMGO ([D-Ala2,*N*-MePhe4,Gly-ol5]enkephalin), and was demonstrated by an intracellular Ca^{2+}-release assay to be signaling dead.[17] These data support the homozygous Arg181Cys mutation as the direct cause for opioid insensitivity in Patient 1. Importantly, the mutation may be critical to the signaling properties of all known alternatively spliced forms of hMOR.[19,20] All these variants, with one exception, hMOR-1S, a short variant exclusively encoded by exon 1 and exon 4, and with unknown function, contain the same exon 2 where the mutation is located. Given that the mutant receptors are unable to signal to all opioids, this will be equivalent to a “knock-out” of the *OPRM1* gene in individuals homozygous for the Arg181Cys mutation.

Although the implications of being heterozygous for the Arg181Cys mutation are less conclusive, our data are in favor of reduced opioid effects in heterozygotes. Firstly – based on genotyping of the two cohorts, the estimated frequency of heterozygotes among Norwegians is approximately 1% (10/1,113; both cohorts merged); thus the likelihood of reporting two heterozygous patients (patients 2 and 3) “by chance” (i.e. if the heterozygous state does not alter opioid efficacy) is only 1 in 10,000. This strongly indicates that heterozygotes can be

identified on the basis of their poor response to opioids, without *a priory* knowledge of their genotype. Secondly - individuals heterozygous for the Arg181Cys mutation will have, in theory, only half the functional receptors present in a normal individual.[17] Previous studies in mice have demonstrated a 60% decrease in MOR binding,[21] and reduced sensitivity to morphine in heterozygous MOR deficient mice.[22,23] This is in accordance with impaired, but not totally abolished, opioid efficacy in the two heterozygous female patients. Finally - the six heterozygous patients identified among the 2,158 European cancer pain patients all used high doses of opioids and/or reported inferior effect on their pain. It should be noted, however, that high opioid doses are not unusual among patients with advanced cancer disease. On the other hand, not all patients with advanced cancer disease are in need of high opioid doses, which may also be true for some patients who are heterozygous for the mutation.

Our data indicate that the frequency of heterozygotes may vary between different European populations - e.g. the frequency may be lower in Germans (0/439) and Italians (0/451) - although genotyping in larger cohorts is needed to obtain a more reliable estimate of the frequency in these populations. Data deposited in NCBI dnSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=79910351), based on genotyping of 60,553 individuals (different populations and ethnic groups merged), suggest that the overall frequency of heterozygotes may be somewhat lower than observed among Norwegians. Opioids are used at essentially every hospital worldwide, on a daily basis, to control moderate and severe pain. This highlights the importance of exploring in more detail the clinical implications of being heterozygous for the Arg181Cys mutation.

One should also be aware of other high effect-size mutations in the *OPRM1* gene that may have similar effects. Large scale sequencing has revealed variants in the *OPRM1* gene that occur at low frequencies but reside in regions that may be critical for receptor function, such as the Ser268Pro variant which was shown to be a loss-of-function mutation for the hMOR.[24]

Additional rare variants in the *OPRM1* gene are continuously being uncovered and entered into databases as a result of ongoing large scale sequencing efforts. Although the clinical implications of these mutations are yet to be explored, at least some of them are likely to contribute to the number of individuals who may not respond normally to opioid therapy. Even though the number of individuals carrying one particular rare variant allele of the *OPRM1* gene may be low, the total number carrying some rare *OPRM1* allele, or mutation in some other gene important for opioid effects, may collectively constitute a clinically relevant fraction of the population. Such mutations may therefore explain a relevant fraction of the minority of patients who do not respond normally to best-practice opioid therapy.

There are some limitations to our study. The number of patients included is small. Studying a larger number of patients would strengthen the validity of the results – especially with regard to the effect in heterozygotes. Also, although our patients did receive several different opioids, it remains to be established whether the mutant receptor is unable to signal to all opioids used clinically.

For patients homozygous for the Arg181Cys mutation the information about an expected abolished opioid effect is vital. Although the frequency of homozygotes is low (approximately 1 in 40,000), it still represents about 20 individuals living within our hospital catchment population of 800,000. These patients may need a highly personalized approach to pain therapy, e.g. in relation to cancer disease or after surgery. It should also be kept in mind that close relatives of these patients (parents, children, or siblings) are likely to be heterozygous for the mutation. Anesthesiologists and practitioners in pain medicine should be aware of this mutation as a possible explanation for inefficiency of opioids, and consider genotyping in relevant cases - including in patients reporting a family history of poor pain relief from opioids.

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Figure 1

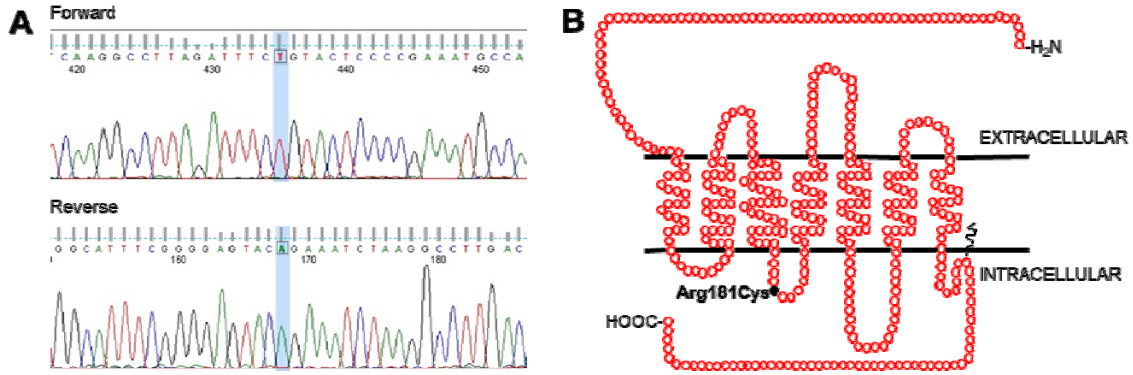
A
Forward
Reverse
B
H_2N
EXTRACELLULAR
INTRACELLULAR
Arg181Cys
HOOC

Figure legends

Figure 1: The mutated region of *OPRM1* exon 2 in the homozygous patient, and position of the mutation in hMOR-1. A. Electropherograms showing the forward and reverse sequences. The homozygous C>T mutation is indicated by the blue box. **B.** Schematic sketch of the μ opioid receptor (hMOR-1) showing the position of the Arg181Cys mutation in the intracellular loop between the 3rd and 4th transmembrane regions (filled circle).

Table 1. Demographics of heterozygous subjects identified among 2,158 European cancer pain patients

Subject	Gender	Age	Karnofsky*	Cancer	EORTC Pain score**	BTP ***	MEDD (mg x $24h^{-1}$) ****	Other analgesics
1	Male	43	80	Prostate	100	Yes	162; still under titration	Dexamethasone Paracetamol NSAID
2	Male	62	60	Myelomatosis	67	No	50	
3	Female	64	50	Gynecological	33	No	540	Dexamethasone Antidepressant
4	Male	77	30	Prostate	Not able to give self-reports, died 6 days later	No	60; limited because of adverse effects	Dexamethasone Paracetamol
5	Female	24	60	Sarcoma	100	Yes	440	Paracetamol
6	Male	82	60	Prostate	67	Yes	550	Methylprednisolone

* Karnofsky status scale; performance score ranging from 100 - no signs of disease, to 10 - death immediately expected. [25]

** EORTC QLQ-C30; pain score scale ranging from 0 – no pain, to 100 - maximal pain. [26]

*** BTP; Break through pain

**** MEDD; Morphine Equivalent Oral Daily Dose