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The 118 A > G polymorphism in the human μ-opioid receptor gene may increase morphine requirements in patients with pain caused by malignant disease

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Background: Dispositions for genes encoding opioid receptors may explain some variability in morphine efficacy. Experimental studies show that morphine and morphine-6-glucuronide are less effective in individuals carrying variant alleles caused by the 118 A > G polymorphism in the μ-opioid receptor gene (*OPRM1*). The purpose of the study was to investigate whether this and other genetic polymorphisms in *OPRM1* influence the efficacy of morphine in cancer pain patients.
Methods: We screened 207 cancer pain patients on oral morphine treatment for four frequent *OPRM1* gene polymorphisms. The polymorphisms were the −172 G > T polymorphism in the 5′untranslated region of exon 1, the 118 A > G polymorphism in exon 1, and the IVS2 + 31 G > A and IVS2 + 691 G > C polymorphisms, both in intron 2. Ninety-nine patients with adequately controlled pain were included in an analysis comparing morphine doses and serum concentrations of morphine and morphine metabolites in the different genotypes for the *OPRM1* polymorphisms.
Results: No differences related to the −172 G > T, the IVS2 + 31 G > A and the IVS2 + 691 G > C polymorphisms were observed. Patients homozygous for the variant G allele of the 118 A > G polymorphism (n = 4) needed more morphine to achieve pain control, compared to heterozygous (n = 17) and homozygous wild-type (n = 78) individuals. This difference was not explained by other factors such as duration of morphine treatment, performance status, time since diagnosis, time until death, or adverse symptoms.
Conclusion: Patients homozygous for the 118 G allele of the μ-opioid receptor need higher morphine doses to achieve pain control. Thus, genetic variation at the gene encoding the μ-opioid receptor contributes to variability in patients' responses to morphine.

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Key words: Cancer; genetic; morphine; opioid receptor; opioid; polymorphism.

THE doses of morphine needed for pain relief vary between individuals. Some of this variation is explained by variable bioavailability and differences in intensities of pain stimuli. However, lack of a direct relationship between serum concentrations of morphine and patient characteristics suggests that inter-individual variability may be related to an interaction between morphine and opioid receptors (1). The morphine metabolite morphine-6-glucuronide (M6G) has high affinity to the μ-opioid receptor, displays higher analgesic potency than morphine in animal models, and contributes to the clinical analgesia produced by morphine (2–5). Animal studies have identified multiple μ-opioid receptor variants that may be responsible for varying analgesic response and adverse effects from morphine and M6G (6). Moreover, experimental studies in animals have shown that antisense targeting of specific exons in the μ-opioid receptor gene (*OPRM1*) has a different influence on antinociceptive effects of various opioids including morphine and M6G (7). Several single nucleotide polymorphisms (SNPs) have been identified in the human *OPRM1* gene (8, 9). Among the most frequent SNPs found are the −172 G > T (in the 5′ untranslated region of exon 1), and the IVS2 + 31 G > A and IVS2 + 691 G > C polymorphisms (both in intron 2). The most common SNP associated with a change in the amino acid sequence is an A to G substitution in exon 1 (118 A > G), causing an exchange of aspargine for aspartate at position 40 (N40D). The frequency of the 118 A > G polymorphism is about 10–14% in Caucasians (9). Lötsch et al. (10) and Skarke et al. (11) showed

in two separate human experimental studies that subjects carrying one or two copies of the variant 118 G allele had decreased pupillary constriction after M6G administration. The response to morphine was unaltered in the study by Lötsch et al. (10) and reduced in the volunteers studied by Skarke et al. (11). Based upon these findings we hypothesized that patients carrying the variant 118 G allele need higher doses of morphine for satisfactory pain relief compared to patients with the wild-type sequence at this position.

Methods

Ethics

The study was carried out in accordance with the principles of the Helsinki declaration. The Regional Committee for Medical Research Ethics, Health Region IV, Norway, approved the study, and all patients gave their oral and written informed consent before entering the study.

Patients

Two hundred and seven patients receiving chronic morphine treatment for cancer pain were screened for *OPRM1* polymorphisms. All patients were in-patients at the 900-bed tertiary St. Olavs. University Hospital, Trondheim, Norway. A subgroup of 99 patients was selected for an analysis searching for the relation between genotypes and efficacy of morphine. One global item of the Brief Pain Inventory (BPI) was used for this selection (12, 13). Patients reporting four or less on the 11-point numeric rate BPI scale were considered to be adequately treated and included (14). This selection was performed to compare variability in the pharmacological observations related to genetic dispositions and not those caused by inadequate morphine doses.

The following information was collected from the hospital records for each patient: Age, gender, ethnicity, cancer diagnosis, time since diagnosis, presence of metastases and time since start of morphine. The daily morphine doses were collected from the patients' ward charts. Survival times from the time of inclusion were obtained from the death records of Norway.

Assessments

Pain was measured using the item of 'average pain' during the last 24 h in the BPI. The patients rated pain on an 11-point numeric scale, where 0 represents 'no pain' and 10 represents 'pain as bad as you can imagine'. The BPI is developed for the use in cancer pain patients, is validated in Norwegian, and is recommended by the European Association of Palliative Care for use in clinical studies (12, 13, 15).

The European Organization for Research and Treatment of Cancer core quality-of-life questionnaire (EORTC QLQ-C30) version 3.0 was used to assess the patients' nausea/vomiting, constipation, fatigue and tiredness (16). Cognitive function was assessed by the Mini Mental State (MMS) examination. The MMS score ranges from 0 to 30; higher scores meaning better cognitive function (17). The patients' functional status was assessed by the Karnofsky performance status (18).

Blood samples and analyses

All blood samples for determination of serum concentrations of morphine, M6G and M3G were obtained at the same time of the day during the routine morning round for collecting blood samples. The blood samples were separated by centrifugation (3000 r.p.m., 10 min) and stored at −85°C. All samples were analyzed for serum concentrations of morphine; M6G and M3G applying liquid chromatography mass spectrometry (19). The limits of quantification were 0.35 nmol l^{-1} for morphine and 2.2 nmol l^{-1} for M6G and M3G. The analytical coefficients of variation were 3.0% for morphine, 5.5% for M6G and 7.0% for M3G.

Serum concentrations of creatinine (reference interval; male<120, female<100 μmol l^{-1}) and albumin (reference interval; 37–48 g l^{-1}) were determined using standard analytical methods.

Pharmacogenetic analyses

Genomic DNA was isolated from 50 μl to 200 μl EDTA blood on a MagNA Pure LC (Roche Diagnostics Scandinavia AB, Bromma, Sweden) using the MagNA Pure LC DNA Isolation *Kit*I, applying the manufacturer's high-performance protocol. Purified genomic DNA was eluted in 100 μl elution buffer and stored at −20°C. Polymorphisms in the *OPRM1* gene were detected using the LightCycler (Roche Diagnostics AB, Bromma, Sweden) fluorescence resonance energy transfer method (20). PCR amplifications were performed in 20-μl reactions on a LightCycler System (Roche), using 2 μl purified genomic DNA and the LightCycler-FastStart DNA Master Hybridization Probes kit (Roche). PCR primers (Eurogentec, Seraing, Belgium) and fluorescence-labeled probes (TIBMOLBIOL, Berlin, Germany) are shown in Table 1. Conditions for PCR and melting curve analyses are shown in Table 2.

Polymorphisms were verified by DNA sequencing. PCR products were purified using the CONCERT™ Rapid PCR Purification System (GibcoBRL, Gaithersburg, MD) and eluted in 50 μl nuclease-free

Table 1

Hybridization probes and primers used for *OPRM1* genotyping.

Polymorphism	Position of polymorphism	Probe sequence (sensor/anchor)	PCR primers (forward/reverse)
−172 G > T	Exon 1, 172 base pairs upstream of start A_{+1}TG	5'-AGAATGTCAGATGCTCAT**T**CTCGG-Fl-3'/ 5' LC Red 640-CCCCTCCGCCTGACGCTCCTCTC-3'	5'-CACAGAAGAGTGCCCAGTGAA-3'/ 5'-AGCCAGGAGCACCGAGACT-3'
118 A > G	Exon 1, 118 base pairs downstream from start A_{+1}TG	5' LC Red640-CTTAGATGGC**A**ACCTGTCCGACC-3'/ 5'-CCCGGTTCCTGGGTCAACTTGTCC-Fl-3'	5'-GCTTGGAACCCGAAAAGTCT-3'/ 5'-GTAGAGGGCCATGATCGTGAT-3'
IVS2+31 G > A	Intron 2, nucleotide position 31	5'-CATATCAGGCTGTGAACC**C**TCCT-Fl -3'/ 5' LC Red640-CCCTCAGGCTGGTAA**C**ATCACTCACC-3'	5'-TTAATAGGCCTAAAATTATGAG-3' 5'-TTGGTCTTCCTGTAATGTTC-3'/
IVS2+691 G > C	Intron 2, nucleotide position 691	5'-ATTTTGCTCATTGATTTTTA**G**CC-Fl-3'/ 5' LC Red640-TGACCAGAGCTAACTTAAGGCTATAAAGAA-3'	5'-CTTAATGTGATCGAAGTGGACT-3'/ 5'-GTAATGATGAGCACTGGCAT-3'

LC Red640 = Light Cycler Red640; Fl = fluorescein.

H_2O. DNA cycle-sequencing was carried out in 20 µl reactions on a Perkin Elmer GenAmp PCR system 9700 (Perkin Elmer, CA), using 6 µl of purified PCR product, 3.2 pmol primer and an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer, Foster City, CA) as described by the manufacturer. The forward and reverse sequencing primers were the same as those used in the PCR amplification. Conditions for the sequencing of PCR products were 25 cycles with 30 s at 96°C, 15 s at 50°C, and 4 min at 60°C. Residual dideoxy terminators were removed by ethanol precipitation and sequences analyzed on an Applied Biosystems 377 DNA sequencer (Foster City, CA).

Statistics

Descriptive data are given as mean ± SD. Comparisons between the different genotype groups for each of the *OPRM1* polymorphisms were performed with one-way ANOVA tests. For polymorphisms where no (IVS2 + 31 G > A) or only one (−172 G > T) homozygous variant patient was detected, a Student's *t*-test was applied when comparing the two remaining genotypes. A *P*-value less than 0.01 was considered statistical significant in order to give some protection against multiple tests. Because this report was obtained from data generated from a patient population included into a study originally designed for assessing the relations between patients' characteristics and serum concentrations of morphine and metabolites (1, 21), a prestudy formal sample size calculation was not performed. The statistical software SPSS for Windows vs. 10.07 was used for all statistical analyses (SPSS, Chicago, IL).

Results

Patients

Two hundred and seven consecutively referred patients using morphine for pain caused by a malignant disease were genotyped for polymorphisms in the *OPRM1* gene. After excluding patients who did not complete the BPI questionnaire (n = 47) and those with uncontrolled pain (BPI average pain score above four) (n = 61), 99 patients remained for analysis of the relations between genotypes and pharmacological observations. All patients were Caucasians.

−172 G > T, IVS2 + 31 G > A and IVS2 + 691 G > C polymorphisms

The frequency of each polymorphism and the genotype distributions among the 207 patients are given in

Table 2

Amplification and melting curve conditions for *OPRM1* polymorphisms.

Polymorphism	PCR cycling conditions				Melting curve conditions	
	Denaturation	Annealing	Extension	Cycles	Stepwise temperature decrease	Temperature increase
−172 G > T	95°C, 15 s	60°C, 7 s	72°C, 14 s	40	95°C for 15 s, 66°C, 58°C, 55°C and 40°C for 20 s each	40°C → 95°C at 0.4°C s^{-1}
118 A > G	95°C, 15 s	60°C, 7 s	72°C, 12 s	40	95°C for 15 s, 73°C, 71°C, 68°C and 40°C for 20 s each	40°C→85°C at 0.4°C s^{-1}
IVS2+31 G > A	95°C, 15 s	56°C, 5 s	72°C, 10 s	40	95°C for 15 s, 70°C, 65°C, 60°C and 40°C for 20 s each	40°C→85°C at 0.4°C s^{-1}
IVS2+691 G > C	95°C, 15 s	57°C, 7 s	72°C, 14 s	40	95°C for 15 s, 60°C, 55°C, 52°C and 40°C for 20 s each	40°C→95°C at 0.4°C s^{-1}

Table 3. No significant differences in morphine doses or opioid serum concentrations between the wild-type patients and patients heterozygous for the −172 G > T polymorphism were evident for the 99 patients who had well-controlled pain (Table 4). Only one of these patients was homozygous for the −172 T allele. Thus, we were not able to conclude on the clinical characteristics related to the individuals' homozygous variant for the −172 G > T polymorphism. We observed no statistical significant differences of morphine doses or serum concentrations related to the two intron polymorphisms, IVS2 + 31 G > A and IV2 + 691 G > C (Table 4).

118 A > G polymorphism

The frequency of the 118 A > G polymorphism and the genotype distribution among the 207 patients is given in Table 3. Of the patients who reported adequate pain control, 78 patients were homozygous wild-type, 17 patients were heterozygous for the 118 A > G polymorphism and four patients were homozygous for the variant 118 G allele. All patients homozygous for the 118 G were males. The different genotype groups (homozygous wild-type, heterozygous, and homozygous variant) were compared with respect to possible confounding factors. No differences between the various genotypes were detected for key variables such as age, primary tumor locations, time since diagnosis, time from start of morphine, time from inclusion until death, and Karnofsky performance status (Table 5). There were no differences in serum creatinine concentrations or serum albumin concentrations between the three cohorts (Table 5). Pain intensity was significantly different between groups, with the patients heterozygous for the 118 A > G polymorphism having more pain (Table 6). The intensities of other symptoms such as fatigue, nausea and vomiting, dyspnea, sleep disturbance, loss of appetite and constipation were similar between the three cohorts (Table 6). The patients' cognitive function measured by the MMS sum score was similar between the groups (Table 6).

The patients homozygous for the 118 G allele received significantly higher daily morphine doses compared to the wild-type patients (Table 7). The serum concentrations of morphine, M6G and M3G showed significantly higher clinical results in patients homozygous for the 118 G allele with statistical outcomes on the borderline of the defined *P*-value in the statistical tests (Table 7).

All patients detected as homozygous variant for the 118 A > G polymorphism were males; therefore, we also compared the morphine doses in an analysis excluding females. Of 61 males 45 were homozygous wild-type, 12 were heterozygous for the 118 A > G polymorphism and four were homozygous for the 118 G allele. The morphine doses in these male genotype groups were 96 ± 92, 78 ± 54 and 225 ± 143 mg $24\,h^{-1}$, respectively ($P = 0.02$ for differences

Table 3

Frequencies of screened polymorphisms in the *OPRM1* gene.

Polymorphism	Affected codon	Wild-type	Heterozygous	Variant	Frequency of occurrence* (variant)
−172 G > T		185	20	1	5.3%
118 A > G	(N40D)	167	35	4	10.4%
IVS2+31 G > A		166	40		9.7%
IVS2+691 G > C		30	100	76	61%

*Frequency of occurrence = (detected variant alleles/total of individuals × 2) × 100%.

Table 4

Pharmacological observations related to the −172 G > T, IVS2+31 G > A and IV2+691 G > C polymorphisms.				
		Wild-type (n = 90)	Heterozygous (n = 8)	Variant (n = 1)
−172 G > T	Morphine dose (mg 24 h^{-1})	96 ± 92	108 ± 73	30
	Morphine serum conc. (nmol l^{-1})	71 ± 67	55 ± 46	34
	M6G serum conc. (nmol l^{-1})	387 ± 430	479 ± 421	168
	M3G serum conc. (nmol l^{-1})	2180 ± 1985	3196 ± 3221	1017
IVS2+31 G > A		(n = 83)	(n = 16)	(n = 0)
	Morphine dose (mg 24 h^{-1})	94 ± 87	111 ± 107	–
	Morphine serum conc. (nmol l^{-1})	69 ± 63	73 ± 78	–
	M6G serum conc. (nmol l^{-1})	397 ± 458	371 ± 214	–
	M3G serum conc. (nmol l^{-1})	2262 ± 2246	2196 ± 1174	–
IV2+691 G > C		(n = 14)	(n = 46)	(n = 39)
	Morphine dose (mg 24 h^{-1})	99 ± 133	87 ± 68	108 ± 96
	Morphine serum conc. (nmol l^{-1})	57 ± 70	67 ± 60	77 ± 69
	M6G serum conc. (nmol l^{-1})	407 ± 495	319 ± 273	471 ± 528
	M3G serum conc. (nmol l^{-1})	1780 ± 1578	2109 ± 1823	2580 ± 2518

All data are given as mean ± SD. No statistical differences were observed between the genotypes [one-way ANOVA (IV2+691 G > C) and Student's *t*-test (−172 G > T, IVS2+31 G > A)].

between groups). The corresponding serum concentrations of morphine were 63 ± 68, 59 ± 44 and 117 ± 92 nmol l^{-1}.

Discussion

This is the first study to document a clinically significant opioid-receptor polymorphism in cancer pain patients. We have verified our primary hypothesis that cancer patients homozygous for the 118 G allele need higher morphine doses to achieve adequate pain control. The influence from the 118 A > G polymorphism on morphine efficacy has been investigated in two human experimental models with contradictory results (10, 11). One study observed no significant influence from 118 A > G polymorphism on morphine-induced pupil constriction (10), while the

Table 5

Patient demographics for 118 A > G genotype groups.			
	Wild-type (AA) (n = 78)	Heterozygous (AG) (n = 17)	Variant (GG) (n = 4)
Gender male: female	46:32	12:5	4:0
Age	63 ± 14	64 ± 13	66 ± 13
Tumor diagnosis			
Urologic	25	6	2
Lung	14	4	2
Breast	13	3	0
Gastrointestinal	12	1	0
Others	14	3	0
Karnofsky performance status	69 ± 12	67 ± 12	60 ± 22
Creatinine serum conc. (μmol l^{-1})	82 ± 20	96 ± 42	88 ± 18
Albumin serum conc. (g l^{-1})	34 ± 5	32 ± 6	31 ± 3
Time since diagnosis (months)	37 ± 46	55 ± 62	34 ± 52
Time since start morphine (months)	2.8 ± 4.8	2.0 ± 2.2	2.5 ± 3.0
Survival time after study (months)[a]	3.9 ± 3.4	5.4± 5.2	3.9 ± 3.7

All numbers are absolute numbers or mean ± standard deviation. No statistical significant differences were observed between groups (one-way ANOVA).
[a]Two wild-type and two heterozygous patients were lost to follow-up.

Table 6

Patient symptoms for 118 A > G genotype groups.

	Wild-type (AA) (n = 78)	Heterozygous (AG) (n = 17)	Variant (GG) (n = 4)
BPI average pain	1.9 ± 1.5	3.1 ± 1.1*	2.0 ± 1.2
Fatigue EORTC score	64 ± 24	61 ± 26	75 ± 23
Nausea and vomiting EORTC score	27 ± 27	22 ± 23	25 ± 40
Dyspnea EORTC score	36 ± 33	33 ± 33	42 ± 32
Sleep EORTC score	29 ± 32	27 ± 38	17 ± 19
Appetite EORTC score	53 ± 38	43 ± 37	50 ± 43
Constipation EORTC score	47 ± 38	57 ± 35	58 ± 50
Mini Mental Examination sum score	26 ± 4	26 ± 3	28 ± 2

All numbers are mean ± standard deviation.
All scales and single items of the EORTC QLQ-C30 questionnaire are linearly transformed, giving a score range from 0 to 100; higher scores represent higher levels of symptoms.
*$P = 0.01$ for differences between BPI average pain scores (one-way ANOVA). No statistical significant differences were observed between groups for the other symptoms.

other study reported decreased pupillary responses to morphine in patients carrying the variant 118 G allele (11). The morphine metabolite M6G contributes to the opioid analgesia produced by morphine (3–5). M6G displays decreased opioid efficacy, assessed by pupil constriction, in individuals heterozygous or homozygous variant for the 118 A > G *OPRM1* gene polymorphism (11, 12). Our speculation is that morphine has inferior efficacy in patients with the 118 G allele due to loss of the analgesic contribution from M6G and perhaps also due to the decreased analgesic efficacy of morphine. We observed no evidence indicating that any of the other polymorphisms screened in this study, −172 G > T, IVS2+31 G > A or IVS2+691 G > C, influence the clinical efficacy of morphine.

During treatment of cancer pain morphine is titrated to the dose that gives adequate pain relief. Thus, in this study altered properties of the μ-opioid receptor caused by genetic polymorphism are not reflected by differences in symptoms intensities, but by differences in morphine doses. Based upon this assumption we have shown a clinical relevance of a specific polymorphism in the gene encoding the μ-opioid receptor in cancer patients.

As stated above the 118 A > G polymorphism displayed contradictory findings in human experimental studies on opioid efficacy following intravenous administration of morphine (10, 11). These findings may question the clinical significance of the 118 A > G polymorphism. However, because of an extensive morphine first-pass metabolism, M6G is found in higher serum concentrations during oral compared to intravenous morphine administration (22). Moreover, experimental studies in man have shown that M6G is transported slowly trough the blood–brain barrier and therefore has little effect after short-term administrations (23, 24). Consequently, genetic variability in M6G efficacy is expected to influence morphine efficacy only during chronic morphine administration, as found in this study.

We identified four patients homozygous for the 118 G allele. We recognize that a larger number of patients homozygous for the 118 G allele would have improved the statistical power of this study. We screened 207 patients in our study to identify four

Table 7

Pharmacological observations for 118 A > G genotype groups.

	Wild-type (AA) (n = 78)	Heterozygous (AG) (n = 17)	Variant (GG) (n = 4)
Morphine dose (mg 24 h^{-1})	97 ± 89	66 ± 50	225 ± 143*
Morphine serum conc. (nmol l^{-1})	71 ± 67	52 ± 46	117 ± 92
M6G serum conc. (nmol l^{-1})	404 ± 449	267 ± 237	711 ± 517
M3G serum conc. (nmol l^{-1})	2300 ± 2166	1666 ± 1462	3815 ± 2729

All numbers are mean ± standard deviation.
*$P = 0.006$ for differences in morphine doses between groups (one-way ANOVA). No statistical significant differences were observed for the other observations.

homozygous variant patients. To assess a high number of homozygous patients would require the inclusion of a very large number of patients. This difficulty is also reflected in the three previous papers on the effects of the 118 A > G polymorphism on morphine efficacy in humans, which include one or two homozygous individuals for the 118 G allele (11, 12, 25). With a small number who are at risk, a skewed distribution by chance of clinical factors may have contributed to a difference in morphine consumption. In particular, a skewed distribution of the severity of the cancer disease could confound our primary outcome. It is not possible to objectively quantify the individual cancer patients' nociceptive stimuli. However, when analyzing for surrogate end-points associated with the severity of the malignant disease, such as time since diagnosis, time from start of morphine, performance status and time from the study until death, we observed similar results in the four patients homozygous for the 118 G allele as in the rest of the study population. Additionally, patients' characteristics including age, renal function and liver function did not differ between genotypes. One striking observation was that all patients homozygous for the 118 G allele were male. Consequently, the morphine doses were also compared between genotypes in an analysis excluding females. This analysis gave similar results as found in the main cohort.

To prove causality between morphine doses and genetic variability it is necessary to titrate the morphine dose for each individual to the same clinical end-point. According to this ideal standard we observed a weakness in our study, namely a significantly higher pain score among patients heterozygous for the 118 A > G polymorphism, suggesting that these patients were not adequately titrated. This may explain the lack of a clear gene-dose effect; that is, the morphine dose of heterozygous carriers was not in-between the morphine doses of the homozygous variant and the wild-type carriers. The intensities of adverse effects were similar between the cohorts. Consequently, there are no indications that the morphine doses were different between groups due to dose-limiting adverse effects.

Genetic variability at the *OPRM1* gene and at other genes encoding functions related to opioid pharmacology may explain interindividual variability in opioid efficacy. Such variability may contribute to the variable analgesic effect and tolerance for opioids in individuals (26, 27), and the large interindividual variations in equianalgesic doses between opioids (27). We report that the 118 A > G polymorphism at the *OPRM1* gene influences the clinical efficacy of morphine when administered to patients with pain caused by malignant disease. This knowledge is a step towards a better understanding of the complexity of biological factors modifying clinical analgesic responses to opioids.

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