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Does the A118G Polymorphism at the μ-opioid Receptor Gene Protect against Morphine-6-Glucuronide Toxicity?

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Background: **Some, but not all, patients with renal dysfunction suffer from side effects after morphine administration because of accumulation of the active metabolite morphine-6-glucuronide (M6G). The current study aims to identify genetic causes that put patients at risk for, or protect them from, opioid side effects related to high plasma M6G. Candidate genetic causes are the single nucleotide polymorphism (SNP) A118G of the μ-opioid-receptor gene (OPRM1), which has recently been identified to result in decreased potency of M6G, and mutations in the MDR1-gene coding P-glycoprotein, of which morphine and M6G might be a substrate.**

Methods: **Two men, aged 87 and 65 yr, with renal failure (creatinine clearance of 6 and 9 ml/min) received 30 mg/day oral morphine for pain treatment. Both patients had sufficient analgesia from morphine. However, while one patient tolerated morphine well despite high plasma M6G of 1735 nM, in the patient with M6G plasma concentrations of 941 nM it caused severe sleepiness and drowsiness. Patients were genotyped for known SNPs of the OPRM1 and MDR1 genes.**

Results: **The patient who tolerated morphine well despite high plasma M6G was a homozygous carrier of the mutated G118 allele of the μ-opioid-receptor gene, which has been previously related to decreased M6G potency. In contrast, the patient who suffered from side effects was "wild-type" for this mutation. No other differences were found between the OPRM1 and MDR1 genes.**

Conclusions: **The authors hypothesize that the A118G single nucleotide polymorphism of the μ-opioid-receptor is among the protective factors against M6G-related opioid toxicity. The observation encourages the search for pharmacogenetic reasons that cause interindividual variability of the clinical effects of morphine.**

MORPHINE-6-GLUCURONIDE (M6G) is an active metabolite of morphine. Because it is eliminated *via* the kidney, it accumulates in patients with renal failure. In those patients, M6G rises to more than 4,000 nM (table 1), whereas in healthy volunteers peak M6G concentrations of about 400 nM are reached after oral dosing of 90 mg morphine sulfate.[1] M6G accumulation is a risk factor for opioid toxicity during morphine treatment (table 1). However, this is not seen in every patient with renal insufficiency who receives morphine. The identification of factors that put a specific patient at risk for, or protect a patient from, opioid side effects under high plasma M6G would greatly enhance the individualization, and thus safety, of morphine therapy.

Materials and Methods

Clinical Cases

Patient B was an 87-yr-old man (body weight 66.5 kg, body height 180 cm) suffering from plasmocytoma, osteoporosis, a fracture of the twelfth vertebral body, arterial hypertension, and advanced renal failure (serum creatinine 8.5 mg/dl, calculated creatinine clearance 6 ml/min, blood urea nitrogen 238 mg/dl). The patient was admitted to the hospital for conservative orthopedic treatment. He suffered from severe lower back pain. His mental state was clear and oriented to place, time, and his person. There were no clinical signs for cerebral metastases or increased intracerebral pressure. His pupils were equal in size and reacted to light. Respiratory rate and function was within normal range (10–16/min). The patient had had no treatment with psychopharmacologic drugs or chemotherapy causing sedation. When the pain service was called the pain intensity was 7 on the visual analog scale (VAS; range 0–10). Pain was insufficiently treated with dipyrone (4× 750 mg/day, a nonopioid analgesic without significant antiphlogistic properties) and tramadol (4× 75 mg/day) and subsequently changed to 30 mg/day of oral morphine slow release. One day after morphine administration had been initiated the patient was pain-free (VAS 0–1) but showed sedation level 3 (Sedation score; 0 = no sedation – 6 = not arousable)[2] and drowsiness. He was sleepy but always arousable and answered adequately to questions but initiated no activity on his own. The patient stayed in bed most of the day and had no appetite. Questions concerning orientation to place, time, or to his person were answered slowly. For walking, eating, and drinking he needed assistance. These symptoms developed during the first day of morphine treatment.[3] His relatives were upset by the change in his mental state, but on the other hand, were glad to see him with no or minimal pain. The patient's respiratory rate and function was sufficient, no bradypnea less than 8/min or cyanotic changes were seen. Pupil size was equal in both eyes and pupils were smaller in size than with the treatment before. Reaction to light was slower in both pupils than with tramadol. No other drugs were changed or added. Hemodialysis was not considered necessary. Because of

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Table 1. Plasma Morphine, M6G and M3G Concentrations, and M3G to M6G Ratios from Reports of Patients with Renal Failure in Whom M6G Had Been Related to Side Effects during Morphine Therapy and the Concentrations Found in the Present Patients

Reference	Study Population	Morphine Dose	Plasma Concentration (nM)			M3G:M6G	Unwanted Clinical Effects
			Morphine	M6G	M3G		
24	3 patients	1: ≈84 mg in 42 h 2: ≈361 in 5 days 3: ≈415 in 3 days	1: < 10 2: < 10 3: up to 39	1: up to 386 2: up to 848 3: up to 2,342	1: 2,040 2: 3,670 3: 10,220	1: 5.3 2: 4.3 3: 4.4	Respiratory depression
25	7 patients with high plasma M6G from a total of 109 patients	90 mg orally or 70–3139 mg intravenously, in 48 h	213–2,390	4,475–38,638	Not given	—	Respiratory depression, moderate to severe cognitive impairment
26	1 patient	30 mg twice daily long-term oral morphine	≈700	≈863	Not given	—	Chronic nausea
20	7 patients	195 mg/d orally or 160 mg/d intravenously	220	1,500	12,000	8.4	Nausea, vomiting, confusion
21	1 patient	30 + 40 mg perioperatively, 36 mg in 18 h postoperatively, 4 mg in the following 13 h	< 81.6	1,483	11,224	7.6	Coma, no respiratory depression
27	1 patient (7 yr old, 27 kg)	115 mg on the first postoperative day	27.4	2,655	13,790	5.2	Respiratory depression
28	1 patient	141 mg/d for 11 d	< 10	4,400	9,200	2.1	Respiratory depression
29	12 patients	2–5 mg/h to 3–15 mg/h for 56–528 h	249	Not given	Not given	—	Depression of consciousness
Current	2 patients ("B" and "S")	30 mg/d orally	B: 35 S: 41.1	B: 941 S: 1,735	B: 3,634 S: 8,251	B: 3.9 S: 4.8	B: Sedation S: None

the severe impairment of the patient's vigilance, morphine was discontinued (total given dose 60 mg) and replaced by 300 mg/day of a slow release tramadol formulation. Forty-eight hours later, the patient's sleepiness had disappeared and normal communication with him was possible. He became more active. In eating, drinking, and walking he was self-dependent again. Tramadol now provided sufficient pain relief. Plasma concentrations of morphine and M6G of 10 and 436 ng/ml, respectively, were measured during the time when the patient had a reduced vigilance.

The second patient, Patient S, was a 65-yr-old man (body weight 61.9 kg, height 168 cm) with advanced renal disease (serum creatinine of 8 mg/dl, calculated creatinine clearance 9 ml/min, blood urea nitrogen 137 mg/dl) caused by glomerulonephritis, failed kidney transplant, peripheral vascular disease with necrotic ulcerations at the right foot, and atrial fibrillation with pacemaker. The patient had increasing pain in his right foot for several days and weeks. Pain-intensity was scored with 9 on the visual analog scale (VAS 0–10). Sleep at night was disturbed because of the pain. His mental state was oriented in place, time, and to his person. Answers to questions came slowly. Respiratory function was normal and pupils reacted equally to light. Oral tilidine/naloxone (300 mg/day, a combination of a weak opioid with an antagonist to eliminate its intravenous abuse potential; tilidine is a prodrug that to become analgesic has to be metabolized to the active nortilidine, which happens mainly during the first-pass effect) and dipyrone (2 g/day) did not sufficiently reduce the patient's pain and were therefore replaced with 30 mg/day morphine plus dipyrone 4 g/day. A tricyclic antidepressant in a low dose of 5 mg doxepin at night was prescribed for sleeping. This regimen adequately relieved the patient's pain and was well tolerated. The pain intensity reduced to VAS 0–1 and sleep at night was possible again. Respiration was normal and pupil size smaller than before. Reaction to light was equal in both eyes. Most importantly, no alterations of the patient's vigilance state were seen. The patient was oriented to place, time, and his person at any time. With the reduced pain he was quicker in his reactions and answers than before. Plasma concentrations of morphine and M6G of 12.3 and 804 ng/ml, respectively, were measured on the eighth day after morphine therapy was begun.

Although direct comparison of the plasma samples of the two patients may be flawed by their different timing relative to morphine dosing, the concentrations clearly indicate M6G accumulation whereas morphine concentrations were low. Patient B corresponded to the cases of M6G toxicity listed in table 1[20,21,24–29]. Neither disease state nor concomitant medications (table 2) provided a satisfactory explanation of why patient S showed no such side effects. We therefore screened the patients for genetic polymorphisms in the OPRM1-gene (coding the μ-opioid receptor). The motive to examine polymorphisms of the OPRM1-gene (table 3[8,9,23,30–34]) derives

Table 2. Demographic and Clinical Data for the Two Patients with Impaired Renal Function Who Differed in How They Tolerated Morphine Therapy

	Patient B	Patient S
Demography	Male, 87 yr, 180 cm, 66.5 kg	Male, 65 yr, 168 cm, 61.9 kg
Diagnoses	End-stage renal insufficiency, plasmocytoma, osteoporosis, fracture of the 12th vertebral body, arterial hypertension	End-stage renal insufficiency, peripheral vascular disease with necrotic ulcerations at the right foot, atrial fibrillation
Serum creatinine	8.5 mg/dl	8 mg/dl
Calculated creatinine clearance	6 ml/min	9 ml/min
BUN	238 mg/dl	137 mg/dl
Morphine	30 mg/d sustained release oral formulation	30 mg/d sustained release oral formulation
Concomitant analgesics	—	4 g/d dipyrone
Other concomitant medications (daily doses)	Furosemide (40 mg), amlodipine (5 mg), metoprolol (95 mg), omeprazole (20 mg), calcitriol (0.25 mg), lactulose (10 ml), levofloxacin (250 mg), low molecular weight heparin (0.3 ml)	Calcium carbonate (1.5 g), magnesium, vitamins, iron, folic acid, cyanocobalamine, erythropoietin, low molecular weight heparin (0.3 ml)
Morphine analgesia	Yes	Yes
Morphine side effects	Yes: dizziness, sleepiness, apathy	None

BUN = blood urea nitrogen.

mainly from our recent observation that the potency of M6G is decreased in carriers of the mutated G118 allele of the OPRM1-gene.[4] Specifically, the potency of M6G to produce pupil constriction in a homozygous carrier of the G118 mutated allele was significantly reduced by about four times compared with homozygous carriers of the wild-type allele A118. This makes this single nucleotide polymorphism (SNP) a candidate for explaining why the patient without side effects had sufficient analgesia from morphine while not suffering from central nervous opioid side effects caused by M6G accumulation.

In addition, we screened the patients for genetic polymorphisms in the genes that code for transporters that may play an important role for the CNS concentrations of morphine or M6G. Candidates of such transporters are P-glycoprotein (P-gp) or probenecid sensitive transporters, *e.g.*, multidrug-resistance related proteins (MRP), organic anion transporters (OAT), or organic anion transporter polypeptides (OATP), which are all expressed at the blood brain barrier, and which have glucuronides among their substrates. The rationale for examining genetic polymorphisms of the MDR1 gene (table 3) is based on the evidence for P-gp expression at the blood-brain barrier[5] and on the report that the brain concentrations of M6G[6] and of morphine[7] are significantly increased when P-gp is blocked. In addition, it is known that genetic causes may modify the P-gp functionality. The C3435T SNP in the MDR1-gene is frequent among Caucasians (frequency of the mutated T3435 allele of about 50%.[8,9]) It was shown to result in decreased intestinal P-gp expression with enhanced bioavailability of digoxin.[8] Screening for known polymorphisms of the MRP2 gene was motivated by the knowledge that glucuronides are possible substrates of it,[10] and that coadministration of probenecid, that inhibits MRP2, resulted in enhanced morphine antinociception in rats.[11] For the other candidate, probenecid sensitive transporters that might transport M6G such as OATs and OATPs, there are currently no SNPs known with consequences for the transporter function in general, or for the distribution of a specific pharmaceutical substance. Summaries of the human ABC transporters to which belong MDR1 and MRP can be found in Klein *et al.*,[12] of OATP in Tamai *et al.*,[13] and of OAT in Sweet *et al.* and Sekine *et al.*[14,15] The investigation's protocol had been approved by the Johann Wolfgang Goethe-University of Frankfurt Medical Faculty Ethics Review Board and the patients gave informed consent to enrollment and procedures. The guidelines of the Declaration of Helsinki on biomedical research involving human subjects (Somerset West amendment) were obeyed.

Screening for Single Nucleotide Polymorphisms of the Genes Coding μ-Opioid Receptors or ABC Transporters

Genomic DNA was prepared from a blood sample using standard techniques. SNPs were detected by sequence analysis, using products of the polymerase chain reaction (PCR) from LightCycler (Roche, Mannheim, Germany) reactions that had been performed as a screening test before sequencing. After purification with the QIAquick PCR purification kit (QIAGEN, Hilden, Germany) each sample was resuspended in 20 μl distilled water. Approximately 50 ng PCR product was analyzed using an ABI Prism BigDye Terminator Cycle Sequencing Kit (Perkin-Elmer/Applied Biosystems, Weiterstadt, Germany). For primers (TIB MOLBIOL, Berlin, Germany) used for sequencing see table 3. The PCR sequencing reaction was performed in a total volume of 10 μl containing 2 μl premix (AmpliTaq DNA polymerase, BigDye terminators (A-dR6G; C-dROX; G-dR110; T-dTAMRA), desoxynucleoside triphosphates, Mg^{2+}, Tris-HCl pH

Table 3. Known Single Nucleotide Polymorphisms of the Human *OPRM1* (Chromosome 6q24–q25), *MDR1*, and *MRP2* Genes (Chromosomes 7q21 and 10q24, Respectively) that Result in an Amino Acid Exchange or Have Been Shown to Have Functional Consequences and Genotypes of the Two Patients at the Respective Gene Positions; Primers Used for Sequencing Are Also Given

Gene	Exon	Nucleotide Position	Amino Acid Exchange	Frequency of Mutated Allele (%)	Frequency of Heterozygous Subjects (%)	Reference	Patient B	Patient S	Primers
OPRM1	1	C12G	Ser4Arg	?	?	30	C/C	C/C	
	1	C17T	Ala6Val	1.9	3.8	23	C/C	C/C	Forward: 5′-GCT TGG AAC CCG AAA AGT CT-3′
				10		31			
	1	G24A	Silent	2	?	23	G/G	G/G	Reverse: 5′-ACT TGA GTA CGC CAA GGC ATC-3′
	1	A118G	Asn40Asp	11.5	19.2	23	A/A	G/G	Forward: 5′-GTC AGT ACC ATG GAC AGC AG-3′
									Reverse: 5′-GTA GAG GGC CAT GAT CGT GAT-3′
	2	C440G	Ser147Cys	0.3	0.6	32	C/C	C/C	Forward: 5′-CCA TTT GGA ACC ATC CTT TG-3′
	2	A454G	Asn152Asp	?	?	33	A/A	A/A	Reverse: 5′-GAC CAA TGG CTG AAG AGA GG-3′
	3	G779A	Arg260His	< 1	0.7	23	G/G	G/G	Forward: 5′-CTG GGA AAA CCT GCT GAA GA-3′
	3	G794A	Arg265His	?	?	33	G/G	G/G	Reverse: 5′-CCA GCA GAC GAT GAA CAC AG-3′
	3	T802C	Ser268Pro	?	?	33	T/T	T/T	
MDR1	2	A61G	Asn21Asp	11.2	20.6	9	A/G	A/G	Forward: 5′-AGG AGC AAA GAA GAA GAA CTT TTT TAA ACT GAT C-3′
				9.3	17.6	8			Reverse: 5′-GAT TCC AAA GGC TAG CTT GC-3′
	5	T307C	Phe103Leu	0.6	1.2	9	T/T	T/T	Forward: 5′-GTG GTT GCA CAC AGT CAG CA-3′
									Reverse: 5′-GGA GGA TGT CTA ATT ACC TGG TCA-3′
	11	G1199A	Ser400Asn	5.5	11.1	9	G/G	G/G	Forward: 5′-CAG CTA TTC GAA GAG TGG GC-3′
				6.5	12.9	8			Reverse: 5′-CCG TGA GAA AAA AAC TTC AAG G-3′
	21	G2677T	Ala893Ser	41.6	49.2	9	T/T	T/T	Forward: 5′-TGC AGG CTA TAG GTT CCA GG-3′
				63.9	43.4	8			Reverse: 5′-GTT TGA CTC ACC TTC CCA G-3′
	21	G2677A	Ala893Thr	0.9	2	9	NA	NA	Forward: 5′-TGC AGG CTA TAG GTT CCA GG-3′
									Reverse: 5′-TTT AGT TTG ACT CAC CTT CCC G-3′
	26	A3320C	Gln1107Pro	0.2	0.4	9	A/A	A/A	
	26	C3396T	Ala1132Ala	0.3	0.5	8	C/C	C/C	Forward: 5′-ATC TGT GAA CTC TTG TTT TCA GC-3′
	26	C3435T	Ile1145Ile	50.3	47.7	8	T/T	T/T	Reverse: 5′-TCG ATG AAG GCA TGT ATG TTG-3′
				53.9	50.5	9	—	—	
MRP2	10	G1249A	Val417Ile	12.5	20.8	34	G/G	G/G	Forward: 5′-GGG TCC TAA TTT CAA TCC TTA-3′
									Reverse: 5′-TAT TCT TCT GGG TGA CTT TTT-3′
	18	C2302T	Arg768Trp	1	2.1	34	C/C	C/C	Forward: 5′-GGA GTA GTG CTT AAT ATG AAT-3′
	18	C2366T	Ser789Phe	1	2.1	34	C/C	C/C	Reverse: 5′-CCC ACC CCA CCT TTA TAT CTT-3′
	28	C3972T	Ile132Ile	21.9	35.4	34	C/T	C/T	Forward: 5′-TGC TAC CCT TCT CCT GTT CTA-3′
									Reverse: 5′-ATC CAG GCC TTC CTT CAC TCC-3′
	31	G4348A	Ala1450Thr	1	2.1	34	G/G	G/G	Forward: 5′-AGG AGC TAA CAC ATG GTT GCT-3′
									Reverse: 5′-GGG TTA AGC CAT CCG TGT CAA-3′

† Sequence is not translated.

9,0), 1 μl PCR primer (10 μM), 3 μl purified PCR product from the LightCycler, and additional distilled water. After initial denaturation at 95°C for 3 min PCR was carried out with 25 cycles of denaturation (95°C, 10 s), annealing (55°C, 5 s), and extension (60°C, 90 s). To purify the sequenced PCR product the method of ethanol precipitation was applied. After drying the pellet for 5 min at 45°C in a vacuum centrifuge, it was resuspended in 25 μl Template Suppression Reagent (TSR, PE/Applied Biosystems, Weiterstadt, Germany). Analysis was carried out at an ABI PRISM 310 Genetic Analyzer (PE/Applied Biosystems, Weiterstadt, Germany).

Results

Patient S, who tolerated morphine treatment despite high plasma M6G, was homozygous for the mutated G118 allele of the OPRM1 gene, whereas patient B, whose vigilance was severely impaired, was a homozygous carrier of the wild-type allele A118 (Fig. 1). In the other SNPs searched for in the OPRM1, MDR1, and MRP2 genes, the patients did not differ (table 3).

Discussion

Considering our recent report of a decreased potency of M6G in carriers of the mutated G118 allele of the OPRM1 gene,[4] we would like to hypothesize that the A118G SNP could be a reason why one patient tolerated very high M6G plasma concentrations whereas the other patient showed typical opioid CNS side effects. The specific relation of the vigilance effects to morphine/M6G is emphasized by their disappearance after morphine had been replaced by tramadol. The latter also makes a pharmacodynamic interaction between coadministered quinolones or β-blockers as the cause for the

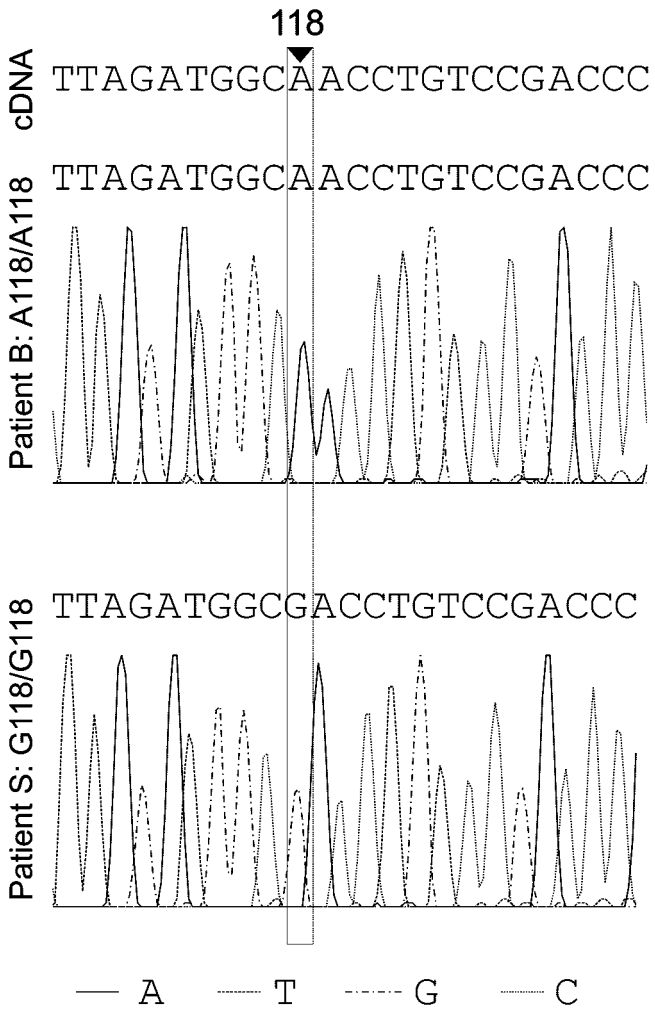

Fig. 1. Results of the sequence analysis of μ-opioid-receptor gene. Only the fragment of the sequence spanning the gene sector of the single nucleotide polymorphism at position 118 is shown (position 118 marked with a rectangle). The sequence was compared with the OPRM1 cDNA (gene data base access number L25119; first line in the figure, "cDNA").

CNS side effects unlikely. Such an assumption would require an explanation of why the interaction happened with morphine only, but not with tramadol, which is partly a μ-opioid agonist. Another drug interaction with morphine, M6G, or both may be caused by omeprazol, which has recently been reported to block P-gp.[16] By this mechanism, and assuming that M6G is a substrate of P-gp, the M6G concentrations in the CNS could have been increased in patient B. However, it is reported that omeprazol is likely to reach plasma concentrations high enough to block P-gp only in cytochrome P450 (CYP) 2C19 poor-metabolizers, who eliminate omeprazol slowly. Patient B, however, showed an extensive metabolizer phenotype predicted on the basis of genotyping for the most relevant defective alleles *CYP2C19 *2* and **3*.[17,18] By this, together with the identical genotype of the patients with respect to *MDR1*, P-gp blockade mediated M6G[6] or morphine[7] accumulation in the CNS are not more likely to have occurred in patient B than in patient S.

Because morphine-3-glucuronide (M3G) has been hypothesized to be a functional antagonist of morphine and M6G,[19] the side effects of M6G in patient S could have been antagonized by his higher plasma M3G compared to patient B. However, the elimination of M6G and M3G is equally affected in patients with renal failure, as indicated by an almost straight-line correlation between M3G and M6G plasma concentrations over a wide range of serum creatinine values.[20] The assumption of such protection raises the questions why patients ever develop M6G toxicity and are not always protected from it by M3G. The M3G:M6G concentration ratios of 3.9 and 4.8 for patient B and patient S, respectively, are both within the range of those calculated from the reported concentrations values of M3G and M6G from patients with renal failure and M6G-caused side effects during morphine therapy (2.1 to 7.6, table 1). Even a ratio of 7.6 or 8.4 did not protect other patients from long-lasting M6G induced coma[21] or other opioid side effects,[20] respectively. Furthermore, the hypothesis of M3G as an antagonist of the depressing actions of morphine and M6G has been repeatedly contradicted.[22]

Age might explain why patient B, aged 87, was more susceptible to the central depressing effects of M6G than 67-yr-old patient S. Another factor that might have contributed to the difference in M6G-tolerability between the patients might be the development of opioid tolerance in patient S. He was treated with morphine for a longer period of time than patient B, and one could speculate that the intolerance of morphine treatment of patient B would have disappeared later because of tolerance development. However, in contrast to patient B, patient S tolerated morphine from the beginning of the therapy.

The mutated G118 allele has a frequency among Caucasians of about 11.5%.[23] The expected frequency of homozygous and heterozygous patients is approximately 2 and 20%, respectively. Clinical experience suggests that more than one-fifth of the subjects receiving morphine despite renal dysfunction tolerate that treatment without developing severe side effects. Thus, the G118 may be one but not the only factor that protects a patient with renal failure from M6G-related opioid toxicity. On the other hand, there might be additional risk factors for M6G toxicity such as age, drug interactions, or disease state. To enlarge the support of our hypothesis of a protective effect of the G118 allele against M6G toxicity, and to identify other factors that play a role in the development of side effects, more patients need to be studied. Nonetheless, the evidence presented here encourages the hope that pharmacogenetics will provide a finer tool to individualize therapy than blunt contraindication of morphine for patients with renal failure.

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