

Analgesia and Opioids: A Pharmacogenetics Shortlist for Implementation in Clinical Practice

Maja Matic,^{1,2} Saskia N. de Wildt,^{2,3} Dick Tibboel,² and Ron H.N. van Schaik^{1*}

BACKGROUND: The use of opioids to alleviate pain is complicated by the risk of severe adverse events and the large variability in dose requirements. Pharmacogenetics (PGx) could possibly be used to tailor pain medication based on an individual's genetic background. Many potential genetic markers have been described, and the importance of genetic predisposition in opioid efficacy and toxicity has been demonstrated in knockout mouse models and human twin studies. Such predictors are especially of value for neonates and young children, in whom the assessment of efficacy or side effects is complicated by the inability of the patient to communicate this properly. The current problem is determining which of the many potential candidates to focus on for clinical implementation.

CONTENT: We systematically searched publications on PGx for opioids in 5 databases, aiming to identify PGx markers with sufficient robust data and high enough occurrence for potential clinical application. The initial search yielded 4257 unique citations, eventually resulting in 852 relevant articles covering 24 genes. From these genes, we evaluated the evidence and selected the most promising 10 markers: cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*), cytochrome P450 family 3 subfamily A member 4 (*CYP3A4*), cytochrome P450 family 3 subfamily A member 5 (*CYP3A5*), UDP glucuronosyltransferase family 2 member B7 (*UGT2B7*), ATP binding cassette subfamily B member 1 (*ABCB1*), ATP binding cassette subfamily C member 3 (*ABCC3*), solute carrier family 22 member 1 (*SLC22A1*), opioid receptor kappa 1 (*OPRM1*), catechol-*O*-methyltransferase (*COMT*), and potassium voltage-gated channel subfamily J member 6 (*KCNJ6*). Treatment guidelines based on genotype are already available only for *CYP2D6*.

SUMMARY: The application of PGx in the management of pain with opioids has the potential to improve therapy. We provide a shortlist of 10 genes that are the most promising markers for clinical use in this context.

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Opioid administration to patients with moderate to severe pain is not without risk. Side effects that may occur with the use of opioids are constipation, nausea, vomiting, sedation, dry mouth, or worse, respiratory depression and delirium. An important obstacle for the use of opioids is the large and unpredictable variability in dose required to reach adequate treatment (1). Opioids are currently dosed based on the patient's clinical presentation, as judged by self-reporting of the patient and/or by nurses using validated pain scales. This is a trial and error approach, starting from a common starting dose. This approach results in an increased risk of side effects in some patients, whereas other patients may be undertreated.

Opioids

In the treatment of moderate acute or chronic pain, a patient will be given opioids when acetaminophen or nonsteroidal antiinflammatory agents do not provide sufficient analgesia. The severity/duration of the pain and the patient's clinical summary (e.g., potential drug–drug interactions, hepatic/renal function) determine the choice of the agent (2). The weak opioids codeine and tramadol are often added to nonopioid compounds to treat pain, while severe pain is mostly treated with morphine. Alternatives for morphine are oxycodone, hydrocodone, and fentanyl, which have different pharmacokinetic (PK)⁴ profiles. Fentanyl, alfentanil, remifentanil, and sufentanil with a rapid C_{max} and short half-life are

¹ Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, the Netherlands; ² Intensive Care and Department of Pediatric Surgery, Erasmus University Medical Center–Sophia Children Hospital, Rotterdam, the Netherlands; ³ Department of Pharmacology and Toxicology, Radboud University Medical Center, Nijmegen, the Netherlands.

* Address correspondence to this author at: Department of Clinical Chemistry, Wytemaweg 80, 3015 CN Rotterdam, the Netherlands. E-mail r.vanschaik@erasmusmc.nl. Received August 22, 2016; accepted December 28, 2016.

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⁴ Nonstandard abbreviations: PK, pharmacokinetic; PGx, pharmacogenetics; MAF, minor allele frequency; UM, ultrarapid metabolizer; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; PD, pharmacodynamic; PCR_RFLP, PCR-restriction fragment length polymorphism; CE-IVD, European Conformity–In Vitro Diagnostic Medical Devices; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; SNP, single nucleotide polymorphism; AUC, area under the concentration curve; MOR, μ -opioid receptor.

selected when rapid onset of action is required, as is the case with acute procedural pain (2). Weak opioids and the more potent compounds appear to carry comparable risk for adverse events, such as respiratory depression. In addition, tramadol has been associated with the development of the serotonin syndrome due to its pharmacological action of serotonin reuptake (3).

Pharmacogenetics

Many research projects have focused on unraveling the interindividual variability in opioid response to improve pain treatment. Pharmacogenetics (PGx) is one of the most promising disciplines in this respect. It is assumed that specific inherited genetic variations can predict a patient's response to drugs. For example, 12%–60% of the variability in alfentanil response can be explained by genetics, as illustrated in an experimental pain study in twins showing that the strength of the correlation between genetics and thermal pain depended on the pain stimulus applied (4). From the findings of transgenic knockout mouse studies, over 400 genes have been implicated (5). Transgenic knockout mouse studies, candidate gene analyses, and genome-wide association studies in humans all point toward a genetic component in pain and efficacy of pain treatment, thus confirming that PGx may help improve patient care.

Although reviews on this topic have summarized the various scientific findings, they typically have not focused on translation into clinical practice. To answer this gap, we performed a structured literature search on all publications on genes in the pain literature and identified those PGx markers that, in our opinion, may be relevant for further validation for use in clinical practice.

Methods

Using a broad range of search criteria (see Table 1 in the Data Supplement that accompanies the online version of this review at <http://www.clinchem.org/content/vol63/issue7>) we searched the Embase, Medline, Web-of-Science, Cochrane, and Google Scholar databases, which initially yielded 5898 citations. Removal of duplicate citations yielded 4257 unique hits. After exclusion of articles in languages other than English, non-peer reviewed hits (poster presentations), papers focusing on opioid therapy for addiction or the phenotype pain without addressing the effect on opioid therapy, 852 articles remained, covering 24 genes [ATP binding cassette subfamily B member 1 (*ABCB1*),⁵ ATP binding cassette subfamily C member

3 (*ABCC3*), arrestin beta 2 (*ARBB2*), calcium voltage-gated channel subunit alpha1 E (*CACNA1E*), calcium voltage-gated channel auxiliary subunit alpha2delta 2 (*CACNA2D2*), cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*), cytochrome P450 family 3 subfamily A member 4 (*CYP3A4*), cytochrome P450 family 3 subfamily A member 5 (*CYP3A5*), catechol-O-methyltransferase (*COMT*), GTP cyclohydrolase 1 (*GCH1*), 5-hydroxytryptamine receptor 3B (*HTR3B*), interleukin 1 receptor antagonist (*IL1RN*), potassium voltage-gated channel subfamily J member 6 (*KCNJ6*), melanocortin 1 receptor (*MC1R*), opioid receptor mu 1 (*OPRM1*), opioid receptor kappa 1 (*OPRK1*), rhomboid 5 homolog 2 (*RHBDF2*), solute carrier family 22 member 1 (*SLC22A1*), sodium voltage-gated channel alpha subunit 9 (*SCN9A*), signal transducer and activator of transcription 6 (*STAT6*), TAO kinase 3 (*TAOK3*), UDP glucuronosyltransferase family 1 member A1 (*UGT1A1*), UDP glucuronosyltransferase family 1 member A8 (*UGT1A8*), UDP glucuronosyltransferase family 2 member B7 (*UGT2B7*)]. In vitro studies, animal studies, case reports, candidate gene and genome wide approaches were included. Each of these papers was assessed on the following criteria: (a) sufficient evidence to warrant a true effect (several independent studies finding the same effect); (b) minor allele frequency (MAF) of 5% in the white population (high enough to be clinically relevant for screening purposes); (c) the paper included novel findings for which the first criterion obviously could not yet be applied but was interesting because of the potential impact based on a mechanistic point of view. This approach led to a shortlist of 10 genes that we deemed to have the highest potential for application in the clinical field.

PK-Related Candidate Genes

CYP2D6

The most frequently addressed candidate gene in the literature on pain is the highly polymorphic *CYP2D6*. This phase-I liver enzyme is involved in the biological activation of codeine into morphine and tramadol into

gated channel subunit alpha1 E; *CACNA2D2*, calcium voltage-gated channel auxiliary subunit alpha2delta 2; *CYP2D6*, cytochrome P450 family 2 subfamily D member 6; *CYP3A4*, cytochrome P450 family 3 subfamily A member 4; *CYP3A5*, cytochrome P450 family 3 subfamily A member 5; *CYP3A5*, cytochrome P450 family 3 subfamily A member 5; *COMT*, catechol-O-methyltransferase; *GCH1*, GTP cyclohydrolase 1; *HTR3B*, 5-hydroxytryptamine receptor 3B; *IL1RN*, interleukin 1 receptor antagonist; *KCNJ6*, potassium voltage-gated channel subfamily J member 6; *MC1R*, melanocortin 1 receptor; *OPRM1*, opioid receptor mu 1; *OPRK1*, opioid receptor kappa 1; *RHBDF2*, rhomboid 5 homolog 2; *SLC22A1*, solute carrier family 22 member 1; *SCN9A*, sodium voltage-gated channel alpha subunit 9; *STAT6*, signal transducer and activator of transcription 6; *TAOK3*, TAO kinase 3; *UGT1A1*, UDP glucuronosyltransferase family 1 member A1; *UGT1A8*, UDP glucuronosyltransferase family 1 member A8; *UGT2B7*, UDP glucuronosyltransferase family 2 member B7.

⁵ Human genes: *ABCB1*, ATP binding cassette subfamily B member 1; *ABCC3*, ATP binding cassette subfamily C member 3; *ARBB2*, arrestin beta 2; *CACNA1E*, calcium voltage-

O-desmethyltramadol, and also in the conversion of oxycodone to the active metabolite oxymorphone and hydrocodone to hydromorphone (2). Genetic variability in *CYP2D6* can be translated into 4 phenotype groups, namely ultrarapid metabolizer (UM), extensive (or normal) metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM) (6). Over 100 different alleles have been discovered, of which approximately 25% lead to total loss of activity in vivo and 6% result in decreased activity (7). In the white population, 5%–10% of individuals are a PM and 2%–4% a UM. In the Asian population, *CYP2D6* PMs are less common, but IMs are more common, with up to 20% of the Japanese population carrying the *10 decreased activity allele (6). In the African population, the frequently occurring *17 and *29 alleles also lead to an IM phenotype. These alleles are virtually absent in the white population (6).

Several case reports have described severe and even lethal adverse events in individuals with genetically impaired *CYP2D6* metabolism who are treated with codeine and tramadol (8). Most alarmingly, a 13-day-old infant died from morphine toxicity via breastfeeding by the *CYP2D6* genetically impaired mother (9). Another report concerns an adult with a *CYP2D6* UM status who in association with a *CYP3A4* drug–drug interaction and reduced kidney function experienced life-threatening adverse events while treated with codeine (25 mg 3 times daily) (10). Besides codeine, tramadol-related respiratory depression has been reported in a child with obstructive sleep apnea (11) and in an adult with kidney failure (12); both had a *CYP2D6* gene duplication.

Many studies have analyzed associations between *CYP2D6* genotype and codeine PK and pharmacodynamic (PD) outcomes. Platforms used for *CYP2D6* genotyping were PCR–restriction fragment length polymorphism (PCR-RFLP), SNaPshot™, TaqMan®, or the European Conformity–In Vitro Diagnostic Medical Devices (CE-IVD)-approved INFINITI® system (see online Supplemental Table 2). Taking into account the available evidence, the Clinical Pharmacogenetic Implementation Consortium has published clinical recommendations for codeine dosing based on *CYP2D6* genotype (13). Both *CYP2D6* UMs and PMs should avoid codeine: in the former group a rise in morphine formation will increase toxicity risk, while in the latter group no analgesic effect will occur due to reduced morphine formation. Although the evidence is less strong for the other opioids, caution should especially be taken with the use of tramadol, and also oxycodone and hydrocodone, in *CYP2D6* genetically impaired individuals (13). The Clinical Pharmacogenetic Implementation Consortium guideline highlights that the *CYP2D6* genotype information should be considered together with other patient factors in the therapeutic choice for the individual pa-

tient. The safety concerns with regard to genetically impaired *CYP2D6* metabolism are further stressed by inclusion of this information in the drug label of codeine, mentioning that codeine is contraindicated in individuals with known *CYP2D6* UM status (8). Based on the extensive evidence for this gene, the availability of guidelines for healthcare professionals, and the fact that active metabolites of codeine and tramadol are formed by this enzyme, this is a highly suitable biomarker for improving pain therapy in the clinic.

CYP3A4/A5

Two other relevant phase-I enzymes in opioid metabolism are cytochrome P450 family 3 subfamily A members 4 and 5 (*CYP3A4* and *CYP3A5*). These enzymes are especially relevant for inactivation of fentanyl, sufentanil, and alfentanil, where the inactive metabolites are formed via *N*-dealkylation (14). In contrast to *CYP2D6*, *CYP3A4* is involved in the formation of the nonactive metabolites of codeine and tramadol, norcodeine, and *N*-desmethyltramadol, respectively. For oxycodone, 80% of this drug is converted by *CYP3A4* to the nonactive metabolite noroxycodone (2).

In the *CYP3A4* gene, over 20 variant alleles have been discovered, most of which are relatively rare with an MAF < 0.01 (10). The major exception is the *22 allele (rs35599367; 15389C>T), with an MAF of 5%–7% in the white population, which encodes decreased *CYP3A4* enzymatic activity (15). Regarding fentanyl, in a European cancer cohort (n = 620) it was shown that genetic variability in both *CYP3A4**22 and *CYP3A5**3 influenced transdermal fentanyl metabolism (16). The time elapsed between patch application and sample collection was not documented, but the length of the interval could have diluted the genetic effect and may have resulted in only a small percentage in fentanyl PK variability explained by *CYP3A4/A5* genetics (16). A recent metaanalysis in the postoperative setting, which for that matter did not include the *CYP3A4**22 allele, concluded that the *CYP3A4**1G allele (rs2242480; 20230G>A) is associated with lower fentanyl consumption in individuals carrying the *1G allele (n = 141) compared to patients carrying the wild-type allele (n = 178) (17). This is in line with a Chinese study on human liver microsomes (n = 88) in which decreased mRNA and the metabolic rate of fentanyl correlated with the *1G allele (18).

Regarding the *CYP3A5* gene, the most frequent genetic variant in white individuals is the inactive *CYP3A5**3 allele (rs776746; 6986A>G). Approximately 80% of white individuals are homozygous carriers of this allele and as a consequence do not express this enzyme (nonexpressors) (19). *CYP3A5* expressors who carry at least one *1 allele are thus expected to have an increased elimination of fentanyl compared to the general popula-

tion and therefore might require higher doses. Although a higher norfentanyl/fentanyl metabolic ratio was observed in carriers of the *CYP3A5*1* allele while on transdermal fentanyl (16), a metaanalysis on postoperative pain nevertheless failed to show an altered fentanyl consumption in carriers of this allele (17). Also, alfentanil PK and PD in healthy individuals (n = 99) were not found to be related with the *CYP3A5*3* allele [see online Supplemental Table 3; *CYP3A5*, reference (1)]. The genotype-phenotype relations between these *CYP3A4* and *CYP3A5* alleles with fentanyl or alfentanil PK and PD have not been addressed in the pediatric population, in which full activity is reached around 1 year of age (20).

Genetic variability in these 2 genes is less extensive compared to that for the *CYP2D6* gene. Most variants in these genes, except the ones mentioned previously, have a low MAF in different ethnic populations and are therefore less relevant for screening purposes in clinical practice. As long as the mentioned *CYP3A4/5* genetic variants are tested concordantly, leaving out the rare alleles is likely not to have affected the outcomes of these studies. In addition, when initial findings in mostly small candidate gene cohorts were inconclusive, the large cancer cohort (n = 620) and the metaanalysis (n = 319) did illustrate an effect on fentanyl PK and PD, respectively, suggesting a study power issue as a cause instead. Because these enzymes are highly relevant for elimination of fentanyl via bioinactivation to norfentanyl, studies are needed that assess if genotype-based dose alterations result in less pain and fewer adverse events.

UGT2B7

The phase-II enzyme UGT2B7 is involved in the conversion of morphine into morphine-3-glucuronide (M3G) and the active metabolite morphine-6-glucuronide (M6G), with a formation ratio of 9:1. While UGT2B7 is the primary enzyme involved in M3G formation, UGT2B7, UGT1A1, and UGT1A8 are most likely involved in the conversion from morphine to M6G (21).

The most described *UGT2B7* single nucleotide polymorphism (SNP) 802C>T (rs7439366) leads to a 2-fold decrease in transcriptional activity in hepatoma and colon cell lines (22). This SNP is in complete reverse linkage disequilibrium with 6 promoter situated SNPs (-1306G>A; -1299C>T; 1112C>T; -900A>G; -327G>A; -161C>T; *2 haplotype, MAF 50%). In this *2 haplotype, SNP -900G>A [also known as -842G>A (rs7438135)] is believed to be the causative variation (22). In agreement with the in vitro data, the metabolite/morphine metabolic ratio was lower in (n = 20) -842G allele carriers with sickle cell disease (23). This effect was confirmed for morphine PK in a pilot study (n = 15) with preterm newborns (24), but in a larger cohort of older children (n = 146) this variant was not found to be related to morphine clearance (25).

Adult Japanese patients (n = 32) carrying the *UGT2B7*2* allele reported less nausea while on oral morphine therapy for cancer pain than did patients carrying the wild-type *UGT2B7* allele (26). However, not all clinical studies found an effect of these *UGT2B7* polymorphisms on morphine PK or PD [see online Supplemental Table 3; UGT2B7, references (1-4)].

Failure to find the effect of this genetic variant could be due to confounding by other genetic variants. A study that used human livers from white individuals (n = 54) identified a particular haplotype associated with UGT2B7 activity (27). This haplotype, with an MAF of 12%, included the intronic variant IVS1 + 985A>G (rs62298861), which was consistently correlated with increased mRNA expression and increased glucuronidation. More research is needed to address the relative importance of this variant in addition to the previously mentioned *UGT2B7*2* allele. Also, although the negative studies [see online Supplemental Table 3; *UGT2B7*, references (1-4)] were usually larger (n = 70-175) and assessed multiple *UGT2B7* SNPs by means of sequencing, probably even larger cohorts would be required to provide enough power to detect a significant hit among these variants. Moreover, genetic variability in other UGT enzymes has been associated with morphine PK. Therefore UGT2B7 variants should be considered together with UGT1A1 and UGT1A8 genetic variability.

ABCB1

The ATP binding cassette subfamily B member 1 (ABCB1), also referred to as P-glycoprotein or Multidrug Resistance Protein 1, is known from its role as an efflux pump in the intestine and at the blood-brain barrier. In vitro and in vivo studies have shown that morphine, fentanyl, and oxycodone are substrates for the ABCB1 transporter (28). Although for the *ABCB1* gene over 8000 SNPs are reported, only 4% of those in fact have a MAF above 5%.

The SNP 3435C>T (rs1045642) has been studied most. Healthy individuals genotyped as 3435TT showed a 2-fold decrease in duodenal expression (29). Assuming in addition that there is lower expression at the blood-brain barrier in 3435TT genotyped individuals, this finding fits with the observed higher morphine concentrations found in the cerebrospinal fluid after intravenous injection (30). Consequently, lower opioid doses might be needed in these patients since they would risk side effects on normal dosages. While lower opioid consumption has indeed been found in the postoperative setting (n = 152) in 3435TT genotyped individuals (31), this is not a uniform finding [see online Supplemental Table 3; ABCB1, references (1-5)]. Sample sizes in these mostly postoperative studies that failed to find a significant effect of the 3435C>T variant were comparable with that of the previous positive study (32). Therefore, this result

could have been a false-positive finding. Additionally, the metaanalysis on postoperative pain (including 7 clinical studies with 1632 unique patients) was also unable to illustrate an effect of SNP 3435C>T and 2677G>T/A on opioid requirement (17). An increased risk for morphine-induced nausea and vomiting was reported in whites with the 3435TT genotype undergoing colorectal surgery (32). A higher frequency of opioid-related adverse events such as sweating and sedation was reported in 3435TT genotyped individuals receiving remifentanyl for spinal fusion surgery (33). Other SNPs in *ABCB1*, 1236C>T (rs1128503) and 2677G>T/A (rs2032582), have also been found to be related to adverse events (26, 32, 34, 35). Other studies, including one in a pediatric population, could not confirm this increased risk for adverse events in carriers of the 3435T allele [see online Supplemental Table 3; *ABCB1*, references (5–8)].

The 3435C>T, 2677G>T/A, and 1236C>T SNPs are in linkage disequilibrium and are thus often found in the same patient. Overall, studies performed on these 3 *ABCB1* SNPs have demonstrated inconsistent findings, implicating some effect but also illustrating that other genetic or nongenetic factors play a role. Recently, several new polymorphisms in this transporter gene have been assessed in relation with opioid-induced respiratory depression, in which the SNP rs9282564, with a frequency of 11% in white individuals, was associated with an increased risk for developing this adverse event and correlated with a prolonged hospital stay in a study on 263 children (36).

ABCC3

The *ABCC3* gene encodes the ATP binding cassette subfamily C member 3 transporter, also known as Multidrug Resistance Protein 3. A study in mice genetically lacking *ABCC3* revealed that this transporter is involved in the efflux of M3G and M6G from the liver to the bloodstream (37). In a study with 103 adult white individuals, 51 genetic variations were discovered, but only the promoter SNP –211C>T (rs4793665) was associated with significantly decreased *ABCC3* mRNA expression and alterations in the binding of transcription factors (38). A study with 105 children undergoing adenotonsillectomy indicated that the –211C>T polymorphism was associated with lower M3G and M6G plasma concentrations (39). All 12 patients with undetectable M6G concentrations were carriers of the T-allele, which is in line with *in vitro* data from Lang et al. (38). Findings of studies on the developmental pattern of the *ABCC3* transporter during childhood are inconsistent. Based on the proteomic study that did illustrate a “low to high” developmental pattern, adult protein expression values are reached within the first 5 postnatal weeks (40). There-

fore, it would be worthwhile to assess if the reported effect of the –211C>T variant on morphine PK could also be found in the neonatal population. More studies are needed to confirm this effect on the metabolism of morphine. Additionally, the *ABCC3* gene requires further screening for relevant genetic variants in other, non-Caucasian populations.

SLC22A1

The Organic Cation Transporter 1, encoded by the *SLC22A1* gene, is primarily present on the sinusoidal membrane of hepatocytes. It is responsible for the uptake of positively charged compounds at physiological pH, such as morphine and the active metabolite of tramadol, *O*-desmethyltramadol (41). The role of *SLC22A1* loss-of-function polymorphisms (*2, *3, *4, *5, *6) on tramadol PK and PD was emphasized by the finding of increased *O*-desmethyltramadol plasma concentrations and prolonged contraction of the eye pupils (PD marker for opioid effect) in healthy volunteers (42). Another study in 205 adult postoperative patients on patient-controlled analgesia found that those with 2 inactive *SLC22A1* alleles had higher *O*-desmethyltramadol area under the concentration curve (AUC) and lower tramadol consumption during the first 24 h after surgery (43). In line with these observations, *O*-desmethyltramadol/tramadol metabolic ratios in newborn infants carrying the *SLC22A1* low activity genotype were higher than those in infants carrying 2 *SLC22A1* wild-type alleles (44). Organic Cation OCT1 is expressed from the first postnatal day and increases quickly in the following months (45, 46), which fits with the found genotype–phenotype correlation.

In healthy volunteers with the previously mentioned deficient *SLC22A1* alleles, an increase of 56% in morphine AUC after codeine administration was found (47). The association of these deficient *SLC22A1* alleles with morphine was confirmed in a pediatric cohort with 220 children aged 6–15 years. The children with 2 loss-of-function alleles treated with intravenous morphine for postoperative pain had significantly lower clearance compared with patients with wild-type alleles (39). The authors proposed that these genetic variants could explain why children with a white background experienced more adverse events than African American children, in whom the variant alleles are less frequent. *SLC22A1* deficient alleles may be especially relevant for patients carrying the *CYP2D6* UM status: both genetic variants lead to an increase in morphine and *O*-desmethyltramadol plasma concentrations and subsequently increase toxicity risk. The list of SNPs addressed in studies should be extended based on Seitz et al. (48) to also cover genetic variability in other ethnic populations than the Caucasian population.

PD-Related Candidate Genes

OPRM1

Opioids such as morphine and fentanyl exert their analgesic effect primarily via the μ -opioid receptor (MOR), encoded by the *OPRM1* gene. Despite the large amount of genetic variability in *OPRM1* (>700 SNPs with MAF >5%), only a handful of variants have been addressed in clinical studies. With a relatively high MAF among whites (15%) and Asians (40%), 118A>G (Asn40Asp, rs1799971) seems the most prominent variant (49). In vivo studies on 118A>G have illustrated reduced signal transduction for the receptor carrying this variant, reduced *OPRM1* expression, and reduced binding affinity of morphine and its active metabolite M6G. On the other hand, higher binding affinity for endogenous opioids has been found, as discussed in (49). More recently, 2 independent metaanalyses on postoperative pain in adults, sample size 4607 (49) and 3144 (18) respectively, showed that carriers of the 118A>G variant have indeed higher opioid requirements (17, 50) and a lower risk for adverse events (17).

Like morphine, alfentanil and oxycodone display lower affinity/potency for the MOR in individuals having the 118G allele [see online Supplemental Table 3; *OPRM1*, references (1–3)]. A metaanalysis on the use of fentanyl for labor pain found a lower requirement in carriers of the minor 118G allele [see online Supplemental Table 3; *OPRM1*, reference (4)]. The effect of this genetic variant seems inconclusive for codeine, tramadol, and sufentanil [see online Supplemental Table 3; *OPRM1*, references (5–12)]. Children who were born with the neonatal abstinence syndrome due to the in utero exposure of opioids and who carried the 118G allele had milder symptoms, illustrated by a shorter hospital stay and lower risk for any treatment of neonatal abstinence syndrome (51). Our study in newborn infants illustrated an increased risk for rescue analgesia with morphine while being on the ventilator in carriers of the *OPRM1* 118G allele in combination with the *COMT* 472GG genotype (52). A Japanese cohort with adult patients undergoing major abdominal surgery assessed 5 tag SNPs (118A>G, IVS2 + 691G>C; rs2075572, IVS3 + 5953G>A; rs599548, IVS3 + 8449A>G, TAA+2109A>G), of which only 118A>G was significantly associated with opioid consumption (53). In summary, based on the extensive literature, the 118A>G variant seems to be a potential biomarker for use in clinical practice. However, we need to elucidate the magnitude of its effect on opioid response compared to other genetic variants (e.g., *CYP2D6*, *SLC22A1*). Also, the seemingly different effect per type of opioid requires more research.

COMT

Next to *OPRM1* and *CYP2D6*, the *COMT* gene is one of the most frequently analyzed candidate genes in the pain field. This gene codes for the catechol-*O*-methyltransferase (COMT) enzyme, which by regulation of MOR expression is involved in numerous physiological functions including pain perception. This relationship between COMT activity and MOR protein expression levels has been illustrated with the *COMT* VAL158MET (472G>A, rs4680) variation in post-mortem human brains. Individuals genotyped with MET158MET (rs4680) had higher MOR protein expression and lower met-enkephalin concentrations (54). In line with these findings, MET158MET-genotyped patients required less opioid for cancer pain (55–58) and postoperative pain (59–62) or showed fewer side effects (63). In line with this protective effect of the rs4680 polymorphism, preterm infants with the MET158 allele sooner reached a pain-free state after remifentanyl or morphine premedication for endotracheal intubation (64). In an additional study, ventilated newborns who carried the MET158 allele with the *OPRM1* 118AA genotype were less likely to require rescue morphine (52). Other studies, however, did not find an association with pain and/or opioid requirement [see online Supplemental Table 3; *COMT*, references (1–3)].

Furthermore, a haplotype approach including the 472G>A variation has been applied. Based on SNPs rs6269, rs4633, rs4818, and rs4680, 3 functional haplotypes have been defined, namely low pain sensitivity (GCGG), average pain sensitivity (ATCA), and high pain sensitivity (ACCG) (65). More recently, the low pain sensitivity haplotype has been associated with the highest pain score and opioid consumption in the postoperative pain setting (60). In another postoperative cohort, the average pain sensitivity haplotype was found to be related to the lowest opioid requirement (62). While extensive data has been generated on *COMT* genetic variability and opioid therapy, more research is needed due to the controversial findings.

KCNJ6

The G protein-activated inwardly rectifying potassium 2 (GIRK2 or Kir3.2) channel, encoded by the *KCNJ6* gene, is one of the downstream signaling effectors of the MOR. The effect of -1250G>A (rs6517442) and 1032A>G (rs2070995) on postoperative analgesia was assessed in adult patients undergoing gastrectomy and colectomy for gastric and colorectal cancer (66). Homozygous 1032A allele patients and patients carrying the haplotype -1250G/1032A more often required rescue analgesia. Lower *KCNJ6* mRNA expression levels were found in 1032AA individuals, while the -1250G>A SNP did not alter this expression (66). Another study on 1032A>G with 352 chronic pain patients showed a

Table 1. Shortlist of candidate genes for PGx opioids.^a

Gene ^b	Chromosome	SNP	rs number	Frequency (%)	Frequency (%)	Frequency (%)	Opioid associated with genetic variant
				Africans	Asians	Whites	
CYP2D6	22	PM	–	1	1	10	Codeine, hydrocodone, oxycodone, tramadol
		UM	–	20	2	2	Codeine, hydrocodone, oxycodone, tramadol
OPRM1	6	118A>G	rs1799971	(G) ^c 1	(G) 39	(G) 16	Morphine, fentanyl, oxycodone, alfentanil, opioids
COMT	22	472G>A	rs4680	(A) 28	(A) 28	(A) 50	Morphine, remifentanyl, opioids
		408C>G	rs4818	(G) 17	(G) 34	(G) 40	Morphine, remifentanyl, opioids
		186C>T	rs4633	(T) 29	(T) 27	(T) 50	Morphine, remifentanyl, opioids
SLC22A1	6	(*2) Met420del	rs72552763				Codeine, tramadol, morphine
		(*3) 181C>T	rs12208357	(T) 0.4	(T) 0	(T) 6	Codeine, tramadol, morphine
		(*4) 1201G>A	rs34130495	(T) 0.3	(T) 0	(T) 2	Codeine, tramadol, morphine
		(*5) 1393G>A	rs34059508	(A) 0	(A) 0	(A) 2	Codeine, tramadol, morphine
		(*6) 262T>C	rs55918055	(C) 0	(C) 0	(C) 0.3	Codeine, tramadol, morphine
ABCC3	17	–211C>T	rs4793665	(C) 41	(C) 14	(C) 44	Morphine
CYP3A4	7	(*1G) 20230G>A	rs2242480	(A) 85	(A) 27	(A) 8	Fentanyl
		(*22) 15389C>T	rs35599367	(T) 0.08	(T) 0	(T) 5	Fentanyl
CYP3A5	7	(*3) 6986A>G	rs776746	(A) 82	(A) 29	(A) 6	Fentanyl
UGT2B7	4	–900G>A	rs7438135	(G) 23	(G) 28	(G) 49	Morphine
		IVS1 + 985A>G	rs62298861	(G) 27	(G) 4	(G) 16	Morphine
ABCC1	7	1236C>T	rs1128503	(T) 14	(T) 63	(T) 42	Fentanyl, morphine, oxycodone
		2677G>T/A	rs2032582	(G) 0	(G) 38	(G) 53	Fentanyl, morphine, oxycodone
		3435C>T	rs1045642	(T) 15	(T) 40	(T) 52	Fentanyl, morphine, oxycodone
KCNJ6	21	–1250G>A	rs6517442	(G) 6	(G) 31	(G) 32	Morphine, remifentanyl, opioids
		1032A>G	rs2070995	(A) 0.6	(A) 36	(A) 20	Morphine, opioids

^a VAF = minor allele frequency derived from dbSNP (NCBI).

^b Genes are sorted based on relative importance and evidence for application in clinical practice.

^c Capital letters in parentheses indicate variant.

trend in the same direction (67). In contrast to the adult postoperative study with the combined –1250G/1032A haplotype (66), preterm infants with the –1250AA genotype who received either morphine or remifentanyl needed more time to reach a pain-free state after endotracheal intubation (64). In an explorative analysis in 311

adult white patients undergoing total knee arthroplasty, both –1250G>A and 1032A>G were not associated with opioid dose (68). Of the 69 SNPs identified and analyzed in this study, significant hits were found with 8 other polymorphisms (rs1543754, rs858035, rs9981629, rs928723, rs2835925, rs2211843, rs1787337, rs2835930). These

SNPs were combined in a genetic risk score and validated in a cohort with healthy individuals and patients with low back pain. Several SNPs in the *KCNJ6* gene have been reported to be related to opioid response but negative findings have been published as well. Therefore, the functional consequences of the mentioned variants and their relative effects on either mRNA or protein expression need to be further studied.

Conclusions

Our extensive literature search resulted in a shortlist of 10 genes (Table 1) that merit further study in a clinical setting. The most solid evidence of a clinically relevant PGx effect on the analgesic treatment with opioids is available for genetic variation in *CYP2D6*, *COMT*, *SLC22A1*, and the genetic variant *OPRM1* 118A>G. As clinical guidelines for codeine and *CYP2D6* genotyping have been formulated (13) and *CYP2D6* genotyping has been successfully implemented in pediatric clinical practice (69), PGx seems promising to personalize opioid therapy. *CYP2D6* genotyping is complicated when genome-wide association study arrays are used. However, the studies addressing *CYP2D6* genotype and codeine therapy did not use genome-wide array-based platforms (see online Supplemental Table 2). Mostly, PCR-RFLP, SNaPshot, TaqMan, or the CE-IVD INFINITI array were used. For the other 6 genes, more evidence is needed. Prospective studies comparing current treatment strategies with a genotype-based strategy as well as cost-effectiveness studies are required before preemptive testing of these genes in clinical practice is undertaken.

Less evidence is available for the role of PGx in analgesic treatment with opioids in the pediatric population, with the exception of the *CYP2D6* gene. Only a handful of papers have focused on other PGx markers of opioids in this population (24, 39, 51, 52, 64, 70–73). In fact, PGx is age independent as far as the effect on enzymatic activity is concerned, yet if a gene is not expressed in childhood a genetic polymorphism will have

no clinical impact. Thus, we emphasize that next to PGx, ontogeny is also of importance in this age group. Newborns and infants cannot self-report pain and are more prone to opioid-induced severe adverse events such as respiratory depression (74), which stresses the need for a more personalized approach. The neonatal period until infancy is the most interesting in view of the developmental changes that occur in this period. At birth and shortly thereafter major changes are seen in the activity of liver enzymes (75) and transporters (76). For example, in the second postpartum week undetectable *CYP2D6* expression was observed in 13% of liver samples (77). This correlates with the expected 10% of *CYP2D6* PMs in the adult population (77). In addition, based on adult data the *OPRM1* 118A>G variant seems promising for application in clinical practice. Studies assessing whether these findings apply to pain phenotypes in different pediatric age ranges are needed, as well as studies on the clinical consequence of *SLC22A1* genetic variability in the pediatric population. Sufficient evidence on tramadol and morphine PK with *SLC22A1* genetics has been found in adult and pediatric populations, with all studies replicating and confirming previous findings (39, 42, 44, 47).

The application of PGx in the management of pain with opioids certainly has the potential to improve therapy. There is a shortlist of candidate genes that are relevant in the pain treatment with opioids, but additional studies are needed that focus on clinical use and on young children.

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