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Can extremely low or high morphine formation from codeine be predicted prior to therapy initiation?

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ABSTRACT

Activation of codeine by *O*-demethylation into morphine is a prerequisite for its analgesic effects and severe toxicity. Identifying patients in whom morphine is formed either at extremely low or at extremely high amounts may improve efficacy and safety of codeine therapy. To assess how well this identification is possible, we compared the performance of current CYP2D6 phenotype association systems (traditional genotype-based classification, a recently proposed CYP2D6 activity score, and the plasma dextromethorphan metabolic ratio) in 57 healthy Caucasians after oral administration of 30 mg dextromethorphan hydrobromide or 50 mg codeine. Most subjects (87.5%) at the lower 15% of morphine formation from codeine and thus likely to not to respond to codeine therapy were correctly identified by *CYP2D6* genotype- or phenotype-based systems. In contrast, in subjects at the upper 15% of morphine formation being at risk for opioid toxicity, *CYP2D6* genotyping predicted only the 50% who carried gene duplication, whereas dextromethorphan-based phenotyping identified 67.5% of the subjects with high morphine formation. However, satisfactory prediction (87.5%) of high morphine formation was only achieved when combining genotyping with phenotyping. In conclusion, insufficient morphine formation from codeine and thus likely failure of analgesia can currently be well predicted. However, to make codeine therapy safe, extremely high morphine formation has to be predicted as well, which has to be obtained at the effort of combining genotyping with phenotyping.

1. Introduction

Codeine is a WHO step 2 weak opioid analgesic and the narcotic drug most widely used in medical practice around the world [1]. It is extensively metabolized and after a single oral dose of 30 mg, 86.1% was recovered in urine, 59.8% as codeine-6-glucuronide, 7.1% as total morphine, 6.9% as total norcodeine and 11.8% as unchanged codeine [10]. The clinical effects of codeine are mainly attributed to its activation to morphine, which has 200 times higher affinity and 50 times higher intrinsic activity at μ-opioid receptors than codeine itself [34,41]. Morphine is therefore considered to be the active principle of codeine despite some evidence that codeine or codeine-6-glucuronide contribute to the pharmacodynamic effects [14,30,45,46,51].

Since codeine activation is catalyzed by the cytochrome P450 (CYP) 2D6 enzyme [13] that is known to be genetically highly polymorph [9], the effects of codeine are under pharmacogenetic control [16]. Genetically altered codeine effects (Table 1) may occur in subjects with either decreased to absent or highly increased CYP2D6 activity as compared to those occurring in population average. Decreased codeine effects may concern approximately 7–11% of the Caucasian population in whom CYP2D6 is inactive for genetic reasons [4,31,48], with interethnic differences [9], leading to very low or absent morphine formation from codeine. On the other hand, increased codeine effects may concern up to 7% of the Caucasian population in whom CYP2D6 is extremely active [47] leading to very high morphine formation from codeine.

Thus, roughly 1 in 7 of the Caucasians [52] is at risk of either failure or toxicity of codeine therapy due to CYP2D6 genetics-dependent extremely low or high morphine formation, respectively. Identifying these persons at initiation of codeine therapy may improve its efficacy and safety and, considering the wide use of codeine [1], would be a major step toward personalized pain therapy. Recent advances in pharmacogenetics allowing for *CYP2D6* genotype–phenotype association [23] have raised expectations that this identification may have become clinically possible.

To judge to which extent these exceptions can be met, we prospectively compared how current CYP2D6 phenotype association

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Table 1
Evidence for very low or very pronounced effects of codeine in humans that have been related to the *CYP2D6* genotype.

CYP2D6 genotype	Population	*n*	Oral codeine dose	Clinical effect	References
Only inactive *CYP2D6* alleles	Healthy volunteers	24	75 mg	Decreased analgesia	[42]
	Healthy volunteers	18	170 mg	Decreased analgesia	[14]
	Healthy volunteers	16	120 mg	Decreased respiratory, psychomotor and miotic effects	[8]
CYP2D6 gene amplification	33-year-old dental pain patient	1	60 mg	Euphoria, dizziness, blurred vision, epigastric pain	[12]
	62-year-old pneumonia patient with leukemia history	1	25 mg 3 times a day for 3 days	Arterial P_{O_2} = 56 mm Hg, score of 6 on the Glasgow coma scale (no eye opening, no verbal response, and limb withdrawal after pain stimulation)	[18]
	Breastfed newborn	1	Mother: 30 mg 2 times a day for 2 days, 15 mg 2 times a day subsequently	Child: intermittent periods of difficulty in breastfeeding and lethargy starting on day 7, regained his birth weight on day 11, grey skin and his milk intake fallen on day 12, dead on day 13.	[25,32]
	29-months old child after tonsillectomy	1	1.75 mg/kg	Unresponsiveness, pinpoint pupils, the patient became apneic, complete recovery after naloxone	[50]
	Breastfed newborns	2 of 17	Mother: 120 mg/day	Mother: sedation, nausea, dizziness, weakness Child: extreme drowsiness, poor feeding; complete reversal of the symptoms after switch to formula feeding	[33]

systems [23] predict the clinically most relevant extremes of morphine formation from codeine.

2. Methods

2.1. Subjects and study design

Of 515 healthy Caucasians genotyped for relevant known non-functional *CYP2D6* alleles (∗*3*, ∗*4*, ∗*5*, ∗*6*, ∗*7*, ∗*8* [20]), the reduced function ∗*41* allele [38] and gene amplification [21], 32 men and 25 women (age: 27 ± 5 years, body weight: 70 ± 12 kg) were selected in a manner to obtain a heterogeneous sample of several combinations of *CYP2D6* alleles (Figs. 1 and 2). Very rare alleles, as well as the frequent ∗*2* allele with minor relevance in Caucasians, were excluded [17]. We focused on including subjects at the extremes of expected CYP2D6 phenotypes while reducing the number of subjects with normal CYP2D6 function. Selecting more subjects with only one or zero wildtype ∗*1* alleles deviated the study cohort from a random sample (Fig. 1; χ^2-test of activity score frequencies between the study cohort and a random sample: $p = 0.001$). Subjects with extreme CYP2D6 activity scores [17] were about 2–3 times overrepresented (Fig. 1).

In an open randomized cross-over design, consenting subjects (Ethics guidelines adhered to) received a single oral dose of 30 mg dextromethorphan (DTM) hydrobromide (Hustenstiller-ratiopharm® Kapseln, ratiopharm GmbH, Ulm, Germany) or of 50 mg codeine base (Codein-Tropfen von ct, ct-Arzneimittel GmbH, Berlin, Germany) at a minimum interval of five days. Food was prohibited for 8 h, and all medications including herbals but excluding oral anticonceptionals were not allowed for at least one week prior assessments. A blood sample was drawn at 4 h after drug intake and urine was collected for 8 h.

2.2. Genotyping of CYP2D6

Genomic DNA was extracted from blood using the "blood and body fluid spin protocol" of the EZ1 DNA Blood 200 µl Kit on a BioRobot EZ1 Workstation (Qiagen, Hilden, Germany). Genotyping of *CYP2D6* alleles (∗3, ∗4, ∗5, ∗6, ∗7, ∗8, ∗41 and gene amplification) was performed using validated Pyrosequencing™ assays [15,43] on a PSQ 96MA device (Biotage AB, Uppsala, Sweden). The genetic diagnosis was double checked for all DNA samples with allele ∗*5* or gene amplification and for nine randomly chosen samples in a blinded fashion using PCR-RFLP analysis [39]. Assay results without the selected variant alleles were assigned to the wildtype ∗*1* allele.

2.3. Determination of codeine, dextromethorphan and their relevant metabolites

Codeine, codeine-6-glucuronide (C6G), morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) were ana-

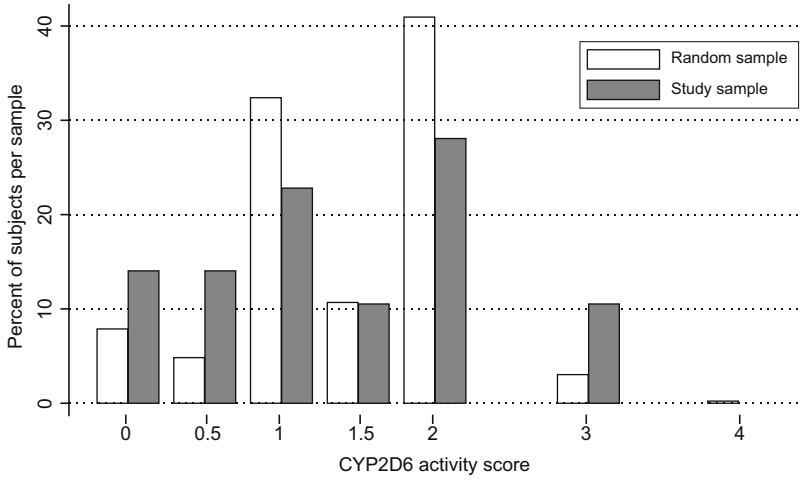

Fig. 1. Distribution of CYP2D6 activity scores [17] in the present study sample and comparison to a random sample of Caucasian subjects from which the actual cohort was obtained.

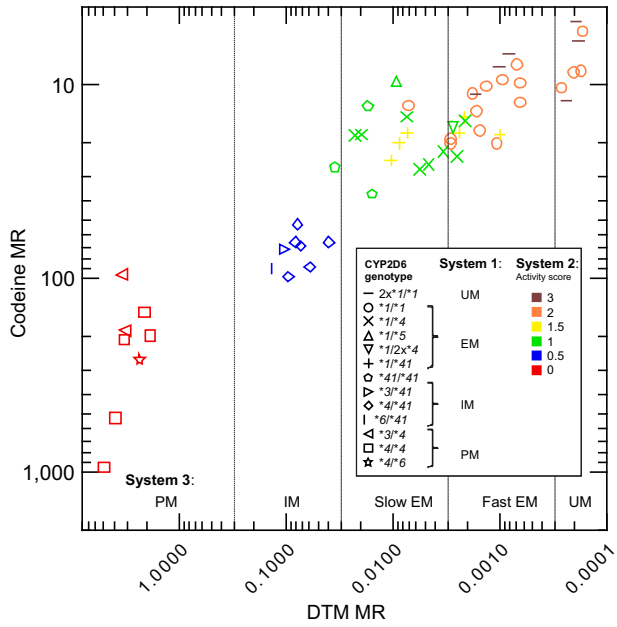

Fig. 2. Original values of codeine MRs ($n = 57$), relative to the dextromethorphan MRs and symbol coded for *CYP2D6* genotypes. The subjects' *CYP2D6* genotypes and their classification by phenotype prediction systems 1–3 are indicated. Please note the reversed axes scaling which provided placement of higher values associated with CYP2D6 activity toward the upper and the right sides.

lyzed after solid phase extraction of plasma samples. Dextromethorphan (DTM), dextrorphan (DT), dextrorphan glucuronide (DTG) and dextrorphan sulphate (DTS) were analyzed without prior extraction after 1:100–1:10000 dilutions of the samples, except for ultra rapid metabolizers with expected very low parent compound concentrations. Deuterized substances were used as internal standards. HPLC analysis was done under gradient conditions using a Synergi Hydro-RP and a Synergi Polar-RP column (Phenomenex, Aschaffenburg, Germany) for codeine plus metabolites and DTM plus metabolites, respectively. Mass spectrometry (MS) and tandem MS analyses were performed on a 4000 Q TRAP triple quadrupole mass spectrometer with a Turbo V source (Applied Biosystems, Darmstadt, Germany) in the positive ion mode. The following precursor-to-product ion transitions of m/z were used for the multiple reaction monitoring: codeine: 300→152, C6G: 476→300, morphine: 286→152, M3G and M6G: 462→286, codeine-d_3: 303→152, C6G-d_3: 479→303, morphine-d_3: 289→152, M3G-d_3 and M6G-d_3: 465→289 (dwell time 50 ms), DTM: 272→215, DT: 258→157, DTG: 434→258, DTS: 338→258 (dwell time 150 ms). Concentrations of the calibration standards, quality controls and unknowns were evaluated by Analyst software (version 1.4; Applied Biosystems, Darmstadt, Germany). Variations in accuracy and intra-day and inter-day precision were <15% over the range of calibration of 0.05 or 0.1–1250 ng/ml for codeine or metabolites, respectively, and of 0.1–1250 ng/ml for DTM and DT. The lower limit of quantification (LOQ) was 0.1 ng/ml for morphine and codeine, 0.05 ng/ml for their glucuronides, and 0.1 ng/ml for DTM and DT. Concentrations below LOQ were set at ½ LOQ. In addition, dextromethorphan and relevant metabolites were analyzed in urine.

2.4. System comparison to predict CYP2D6-mediated morphine formation from codeine

CYP2D6 activity expressed as morphine formation from codeine was quantified by calculating the metabolic ratio (MR) as $Codeine\ MR = \left(\frac{C_{codeine}+C_{C6G}}{C_{morphine}+C_{M3G}+C_{M6G}}\right)$. Three systems were used to predict it. System 1 was the traditional classification defining a poor metabolizer (PM) as non-carrier of a functional *CYP2D6* allele, an intermediate metabolizer (IM) as a carrier of a non-functional and a reduced functional allele, an extensive metabolizer (EM) as a carrier of two copies of functional alleles, and an ultra rapid metabolizer (UM) as a carrier of more than two copies of functional alleles. System 2 was the recently proposed CYP2D6 activity score [17] consisting of the sum of one point assigned to each full-function allele and ½ point assigned to each reduced function allele. System 3 was the classification system based on the plasma DTM MR [6] calculated as $Dextromethorphan\ MR = \left(\frac{C_{dextromethorphan}}{C_{dextrorphan}+C_{dextrophan\ \ glucuronide}+C_{dextrorphan\ \ sulphate}}\right)$ [40]. DTM assignment was: PM: DTM MR ⩾ 0.3, IM: 0.03 < DTM MR ⩽ 0.3, EM: 0.0003 ⩽ DTM MR < 0.03 (exploratory with sub-classification into slow EM: 0.003 ⩽ DTM MR < 0.03 and fast EM: 0.0003 ⩽ DTM MR < 0.003), and UM: DTM MR < 0.0003 [17]. However, since the DTM MR phenotype normative values originate from urine data [17], the agreement between plasma and urine-based DTM MRs was verified by identifying a high Pearson correlation [37] of $r^2 = 0.8736$, $p < 0.0001$, a low bias of 0.023 for the difference between plasma and urine MRs, and a narrow limit of agreement [5] between MRs of −0.87 and 0.91, which was maintained when leaving out the extreme phenotypes.

We let the systems predict the codeine MR by employing leave-one-out cross-validation [24]. This iteratively calculated the codeine MR of each subject from the data of the other 56 subjects according to the particular system's rules. That is, each class was assigned a value of the codeine MR that is the arithmetic mean of the codeine MRs of the remaining members of the respective class, excluding the value of the subject actually to be predicted. The value of the class to which the subject to be predicted belonged was then assigned as the codeine MR for the subject actually to be predicted. Subsequently, codeine MR values were used to identify subjects at the lower or upper 15% of the codeine MR distribution ($n = 8$ each) thus targeting subjects at risk for codeine side effects or ineffectiveness, respectively. The 15th percentiles were chosen as the double of the 7th percentiles at which the limits are found in the average Caucasian population to account for the fact that subjects with a low CYP2D6 activity score [17] were about 2–3 times overrepresented in the study sample (Fig. 1). The success to correctly assign a subject to very low or very high morphine formation was compared between systems by submitting the observed and predicted assignments to Cochran's Q tests [11]. In addition, we checked whether or not assignment was improved when combining genotype-based systems 1 and 2 with the phenotype-based system 3.

3. Results

Codeine was well tolerated in all subjects with occasional reports of feeling slightly tired. CYP2D6 phenotype assignment systems performed identically at the upper 15% of the codeine MR distribution and correctly predicted 7/8 of the subjects with very low morphine formation (Table 2).

In contrast, CYP2D6 phenotype assignment systems performed differently at the lower 15% of the codeine MR distribution indicating very high morphine formation (Table 2). While genotype-based systems (1 and 2) correctly identified subjects with gene duplication to be at the lower edge of codeine MR distribution (Fig. 2), only ½ of these subjects were correctly assigned to high enzyme activity by the phenotype-based system 3. In contrast, subjects without gene duplication but with high morphine formation were only identified by phenotyping (Cochran's $Q = 6$, $p = 0.05$).

The reciprocally good performance of genotype- and phenotype-based systems to identify carriers and non-carriers of CYP2D6

Table 2
Correct ("1") and incorrect ("0") identification of subjects at the upper or lower 15% of codeine MRs of a cohort of $n = 57$, i.e., with very low or very high morphine formation, respectively, by three different systems for CYP2D6 phenotype assessment. System 1: classical genotype-based classification, System 2: CYP2D6 activity score [17], System 3: dextromethorphan MR-based classification [40]. The phenotype or genotype information used by the particular systems is indicated at the top. The right part of the table shows that combination of system 1 or 2 with system 3 increases detection of subjects with extremely high morphine formation.

Subject	Genotype	Dextromethorphan MR	Correct prediction				
			System 1 *Genotyping*	System 2 *Genotyping*	System 3 *Phenotyping*	System 1+ System 3 *Genotyping + Phenotyping*	System 2 + System 3
High codeine MR (low morphine formation)							
n^{th} Highest codeine MR[†]							
1	∗4/∗4	4.99	1	1	1	1	1
2	∗4/∗4	3.91	1	1	1	1	1
6	∗3/∗4	3.17	1	1	1	1	1
3	∗4/∗6	2.32	1	1	1	1	1
8	∗4/∗41	0.09	0	0	0	0	0
4	∗4/∗4	3.23	1	1	1	1	1
5	∗4/∗4	1.84	1	1	1	1	1
7	∗4/∗4	2.10	1	1	1	1	1
		% correct	87.5	87.5	87.5	87.5	87.5
Low codeine MR (high morphine formation)							
n^{th} lowest codeine MR[†]							
2	∗1/∗1	0.0002	0	0	1	1	1
5	∗1/∗1	0.0007	0	0	0	0	0
7	∗1/∗1	0.0002	0	0	1	1	1
8	∗1/∗1	0.0002	0	0	1	1	1
1	2x∗1/∗1	0.0002	1	1	1	1	1
3	2x∗1/∗1	0.0002	1	1	1	1	1
4	2x∗1/∗1	0.0008	1	1	0	1	1
6	2x∗1/∗1	0.0010	1	1	0	1	1
		% correct	50	50	62.5	87.5	87.5

[†] Rank order of lowest and highest codeine MRs.

amplification, respectively, to be at the upper edge of CYP2D6 function distribution, suggested combining systems to improve prediction of very high enzyme activity. Indeed, the greatest fraction of subjects was correctly assigned to the upper 15% of morphine formation by (i) more than two copies of allele *∗1* or a DTM MR < 0.0003 [17] (systems 1 and 3), or (ii) from a CYP2D6 activity score >2 or a DTM MR < 0.0003 [17] (systems 2 and 3).

4. Discussion

Extremely low morphine formation from codeine was predicted at the same hit rate by any major contemporary CYP2D6 phenotype assignment systems based on the subject's genotype, phenotype or both. Detection that a person carries only non-functional alleles or a high DTM MR suffices to identify probable codeine ineffectiveness due to lack of formation of relevant amounts of morphine [8,19,42]. However, not all subjects with very low codeine *O*-demethylation to morphine were detected, probably because of environmental factors [2] influencing the CYP2D6 phenotype. In a clinical setting, drug interactions modulating CYP2D6 activity may be added to the latter [26,44,49,54].

Extremely high morphine formation was comprehensively quantified neither by genotype-based nor by phenotype-based systems alone. Adding phenotyping to genotyping was most useful for detecting extremely high morphine formation. This fits to the fact that the UM phenotype has a prevalence of up to 7% [47,52] although only 1–3% of Middle Europeans carry *CYP2D6* gene duplications [3,9,39,48]. Again, the discrepancy may owe to concurrent non-genetic and environmental influences on CYP2D6 activity [2]. Thus, codeine safety can be obtained at the temporal and material costs of *CYP2D6* genotyping plus phenotyping. Presently, genotyping required 17 h for DNA extraction, PCR and sequencing including unavoidable waiting times for assay completion, costs with the Pyrosequencing™ technique €12.60 per subject but can be performed once and at any time. DTM-based phenotyping took 7.5 h and might be faster when drawing an earlier blood sample, costs €3.50 per plasma sample but requires the logistics of probe drug administration, sampling time and sophisticated analytics. In addition, it should be performed close to therapy to keep changing environmental or co-therapy effects at a minimum to make full use of this particular advantage of phenotyping over genotyping. An alternative to DTM-based phenotyping would be measuring parent and metabolite concentrations after the first codeine dose and subsequently making therapeutic decisions in terms of alternative drugs or dose adaptation. However, this requires various analytical assays and abandons the DTM-based normative values convenient for phenotype assignment [17]. Replacing codeine with non-CYPD6 dependent analgesics might be a reasonable alternative.

Since current *CYP2D6* genotype–phenotype assignment systems are pharmacokinetically focused, we analyzed plasma concentrations rather than pharmacodynamic effects. This is a limitation because the whole variability of codeine or morphine pharmacodynamics has been neglected. Indeed, codeine ineffectiveness is probably insufficiently explained by low morphine formation. The fraction of 7–11% CYP2D6 PMs among Caucasians [4,31,48] is too low to explain the high numbers needed to treat 50 or 9 to achieve at least 50% relief of dental or postsurgical pain, respectively, by administration of 60 mg codeine [35]. Non-genetic factors such as pain characteristics or simply the low morphine dose resulting from codeine probably play a role. In addition, codeine analgesia is probably modulated by additional genetic factors affecting the effects of morphine such as variants altering drug transport [7], opioid receptor expression [53] or signaling [36], nociception or pain (for review, see [22,29]) and genetic variants in other enzymes such as CYP3A [27] or UGT2B7 [33] accounting for approximately 70% of the metabolism of codeine [10]. Similarly, high morphine formation is probably a prerequisite but not a sufficient single reason to cause severe codeine side effects. Codeine toxicity is rarer than expected from up to 7% Caucasian phenotypic UMs and documented only in a few case reports [12,18,25,32,33,50], and even rarer than 1–3% of carriers of *CYP2D6*

amplification. This is supported by the absence of considerable codeine side effects in the present subjects at the upper 15% of morphine formation.

The preference for subjects with non-*∗1* alleles to be included in the cohort probably affected the estimates of the diagnostic performance of the genotype–phenotype association systems [28]. A random sample would have included comparatively more EMs, of whom some might have had metabolic ratios within those observed in subjects with gene amplification, which might have further decreased the genotype-based systems' diagnostic performance. For this reason and because of the relatively small sample size, sensitivity and specificity calculations were not performed because the results would not be representative for the average population. However, the subjects with 2nd and 5th highest morphine formation from codeine (see column "*n*th lowest codeine MR" of Table 2) did not carry *CYP2D6* gene amplification but had codeine MRs well within the range of subjects with gene duplication. This emphasizes that pre-selection of the subjects and setting a limit at the lower 15th percentile of the MR distribution had not invalidated the present conclusions.

In conclusion, highly insufficient formation of the active metabolite morphine being most relevant for the clinical ineffectiveness of codeine can be predicted by each of the current CYP2D6 phenotype assignment systems, i.e., by *CYP2D6* genotyping or by phenotyping using the probe drug dextromethorphan. This is probably able to identify a part of the subjects who do not benefit from codeine therapy. However, to make codeine therapy safe, extremely high morphine formation has to be predicted as well. This can currently be only obtained at the effort of combining genotyping with phenotyping.

Conflicts of interest

The authors have no conflicts of interest.

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