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Evaluation of a [¹³C]-Dextromethorphan Breath Test to Assess CYP2D6 Phenotype

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Abstract

A [¹³C]-dextromethorphan ([¹³C]-DM) breath test was evaluated to assess its feasibility as a rapid, phenotyping assay for CYP2D6 activity. [¹³C]-DM (0.5 mg/kg) was administered orally with water or potassium bicarbonate-sodium bicarbonate to 30 adult Caucasian volunteers (n = 1 each): CYP2D6 poor metabolizers (2 null alleles; PM-0) and extensive metabolizers with 1 (EM-1) or 2 functional alleles (EM-2). CYP2D6 phenotype was determined by ¹³CO₂ enrichment measured by infrared spectrometry (delta-over-baseline [DOB] value) in expired breath samples collected before and up to 240 minutes after [¹³C]-DM ingestion and by 4-hour urinary metabolite ratio. The PM-0 group was readily distinguishable from either EM group by both the breath test and urinary metabolite ratio. Using a single point determination of phenotype at 40 minutes and defining PMs as subjects with a DOB < 0.5, the sensitivity of the method was 100%; specificity was 95% with 95% accuracy and resulted in the misclassification of 1 EM-1 individual as a PM. Modification of the initial protocol (timing of potassium bicarbonate-sodium bicarbonate administration relative to dose) yielded comparable results, but there was a tendency toward increased DOB values. Although further development is required, these studies suggest that the [¹³C]-DM breath test offers promise as a rapid, minimally invasive phenotyping assay for CYP2D6 activity.

Keywords

CYP2D6; phenotype; dextromethorphan; breath test

Interindividual variability in the response, intended or unanticipated, to similar doses of a given medication is an inherent characteristic of treated populations. The role of genetic factors in drug disposition and response has been investigated for more than 50 years and has resulted in increasing numbers of examples of how variation in human genes can lead to

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variability in drug responses at the level of the individual patient.¹ Cytochrome P450 2D6 (CYP2D6) is an important example of a clinically relevant drug-metabolizing enzyme for which genotyping and phenotyping information has the potential to improve drug safety and efficacy.²⁻⁴

CYP2D6 is involved in the biotransformation of more than 40 therapeutic entities, including several beta-receptor antagonists, antiarrhythmics, antidepressants, antipsychotics, and morphine derivatives (for an updated list, see <http://medicine.iupui.edu/flockhart/>). Considerable variability in *CYP2D6* gene expression and activity has also been attributed to well-known genetic polymorphisms. To date, more than 60 allelic variants plus additional subvariants of *CYP2D6* have been identified (<http://www.cypalleles.ki.se>). Inheritance of 2 recessive loss-of-function alleles results in a poor-metabolizer (PM) phenotype, whereas combinations of full- or reduced-function alleles result in a wide spectrum of phenotypes, including intermediate (IM), extensive (EM), and ultra-rapid metabolizers (UM). There are considerable differences in allele frequencies among populations, giving rise to variable percentages of UM, EM, IM, and PM subjects within a given population or ethnic group. Prime examples are the nonfunctional *CYP2D6**4 and the reduced-function *CYP2D6**10 and *CYP2D6**17 alleles, which are common in Whites (~20%), Asians (30%-60%), and African Americans (~20%), respectively.⁵

CYP2D6 phenotype may be inferred from genotype data, or it may be determined directly using a pharmacologic phenotyping probe. The latter is accomplished using pharmacologic probes that are primarily dependent on a single metabolic pathway for the formation of the predominant metabolite. The most common procedure is to administer the phenotyping probe orally and collect all urine produced over a specified time interval, usually 4 to 12 hours, although some groups have explored salivary and serum sampling.⁶ Phenotype status is determined through the use of urinary metabolite ratios (MRs) customarily expressed as the molar amount of parent compound present in urine in the numerator and the molar amount of the CYP2D6-dependent metabolite in the denominator. Most CYP2D6 phenotyping studies have been conducted with debrisoquine,⁷ sparteine,^{8,9} metoprolol,¹⁰ or dextromethorphan (DM).¹¹ Population distributions of urinary metabolite ratios in Caucasians are distinctly bimodal,^{7,12} and the antimode is used for phenotype assignment. The use of urinary MRs is somewhat counterintuitive because higher values are indicative of reduced enzyme activity. Using sparteine as a phenotyping probe, PM status is assigned for MRs > 20, whereas the analogous values for debrisoquine and DM are > 12.6 and > 0.3, respectively.⁶

Of the 4 prototypic substrates mentioned above, DM, a nonprescription antitussive agent found in many pediatric and adult over-the-counter cough and cold formulations, possesses many qualities of an ideal phenotyping probe.¹³ However, the current protocols involve a urine collection of 4 to 12 hours and are not feasible as a rapid, office-based procedure. Furthermore, apparent phenotype determined from metabolite ratios has been observed to vary up to 20-fold in individuals phenotyped on multiple occasions, with the majority of the variability attributed to variations in urinary pH within the physiologic range.¹⁴ In this report, we describe the initial results from a series of investigations conducted to assess the

feasibility of a [^{13}C]-labeled DM breath test as a rapid assessment of CYP2D6-mediated *O*-demethylation activity.

METHODS

Subjects

The study cohort comprised 30 healthy European American adult volunteers, 15 men and 15 women, between the ages of 19 and 60 years (Table I). The study protocol was approved by the University of Missouri–Kansas City Health Sciences Adult Institutional Review Board, and all volunteers provided written informed consent prior to participation in the study.

None of the study participants had anemia (hematocrit < 34% and/or hemoglobin < 12 g/dL), significant impairment of hepatic (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, or total bilirubin > 2 times the upper limit of normal) or renal function (serum creatinine < 1.8 g/dL), or cardiovascular disease based on history, physical examination, and screening blood tests. Subjects also denied any use of known CYP2D6 inhibitors within 6 months of enrollment. Subjects were selected and assigned to 1 of 3 groups based on *CYP2D6* genotype as follows:

Group PM-0 (n = 10): no functional alleles (genotypes consisting of any 2 loss-of-function alleles: *CYP2D6**3, *4, *5, *6, or *16)

Group EM-1 (n = 10): 1 functional allele (1 functional *CYP2D6**1 or *2 allele and 1 *CYP2D6**3, *4, *5, *6, or *16 loss-of-function allele)

Group EM-2 (n = 10): 2 functional alleles (*CYP2D6**1/*1, *CYP2D6**1/*2, or *CYP2D6**2/*2 genotypes).

All 3 groups were comparable with respect to age, weight, and sex distribution (Table I).

Source of Study Drug and Formulation

(*O*-methyl- ^{13}C)-dextromethorphan hydrobromide ([^{13}C]-DM, CLM-7296-USP) was synthesized by Cambridge Isotope Laboratories (Andover, Massachusetts) and provided as a 99% pure powder meeting USP standards. The oral liquid formulation (15-mg/mL solution) was prepared by Investigational Pharmacy Services at CMH as follows: to 100 mL of Sterile Water for Irrigation was added 16 × 1-g packets of Splenda, 16 drops Bitterness Suppressor (FLAVORx, Inc, Bethesda, Maryland), 8 drops Vitamin/Iron Masking Agent (FLAVORx, Inc), and 2 g [^{13}C]-DM HBr (Cambridge Isotope Laboratories), stirring until the DM had dissolved completely. Water was then added to a total volume of 133 mL, and an aliquot was analyzed by high-performance liquid chromatography (HPLC) to ensure a nominal concentration of 15 mg/mL.

[^{13}C]-DM Breath Test Study Protocol

The protocol consisted of 2 study days separated by at least 1 week for the EM-1 and EM-2 groups and at least 3 weeks for the PM-0 group. On each occasion, subjects received an oral dose of [^{13}C]-DM, 0.5 mg/kg as an oral liquid accompanied by either water or antacid tablets containing 1000 mg anhydrous citric acid, 344 mg potassium bicarbonate, and 1050

mg heat-treated sodium bicarbonate (AlkaSeltzer Gold [ASG]) tablets in a randomized, crossover design as described below (Figure 1A).

On each study day, subjects were admitted to the study unit after an overnight fast. Prior to dosing, the subjects completely emptied their bladders and provided a urine sample to be used as the blank for analysis of DM and metabolites. A baseline breath sample was collected in a 1.2-L aluminum breath bag. Subjects were then administered [^{13}C]-DM, 0.5 mg/kg (to a maximum dose of 60 mg), as an oral liquid solution accompanied by either 230 mL of water or 1 ASG tablet dissolved in 230 mL of water in a randomized manner. Water (230 mL) or 1 ASG tablet in 230 mL water was repeated 15 minutes after dosing. Breath samples were collected at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 180, and 240 minutes after [^{13}C]-DM dosing. The breath samples were collected by momentarily holding the breath for 3 seconds prior to exhaling into the sample collection bag. After collection of the 60-minute breath samples, the subjects were provided with a small meal selected from the standard hospital menu. All urine produced over the 4-hour study period was collected and pooled, the total volume was measured and recorded, and 40-mL aliquots were stored at -20°C until analysis.

A second study was repeated in 15 of the subjects: the 3 PM-0 subjects with the highest values for enrichment of $^{13}\text{CO}_2$ in expired air 60 minutes after the [^{13}C]-DM dose (mean time to peak for enrichment of $^{13}\text{CO}_2$ in expired air in the first study) and the 6 subjects in each of the EM-1 and EM-2 groups with the lowest values for enrichment of $^{13}\text{CO}_2$ in expired air 60 minutes postdose. The study protocol was modified such that the subjects were administered 0.5 mg/kg [^{13}C]-DM with an ASG tablet 15 minutes before dosing and 1 tablet with the [^{13}C]-DM dose. Breath samples were collected immediately before dosing and in duplicate at 5 time points: 30, 40, 50, 60, and 90 minutes after [^{13}C]-DM ingestion (Figure 1B). Urine was collected for the full 4-hour study period. The higher of the 2 DOB values was recorded and compared with the corresponding values obtained for each subject from the previous oral liquid protocol.

Genotype Analysis

CYP2D6 genotype analysis was conducted using long-range polymerase chain reaction (XL-PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP)-based procedures according to standard operating procedures established in the Developmental Pharmacology and Experimental Therapeutics Laboratory and adapted from published methods.^{12,15-17} In brief, genomic DNA was isolated from peripheral mononuclear cells with the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, California). Using 2 oligonucleotide primers designed to differentiate between the *CYP2D6* gene and the inactive *CYP2D7* and *CYP2D8* genes, an initial XL-PCR amplification generated a 6.6-kb-long product comprising the entire *CYP2D6* coding region and flanking regions. A second set of primers within this reaction allowed detection of a diagnostic PCR product that was only produced in the presence of the most common gene duplication events. The 6.6-kb *CYP2D6* XL-PCR product served as a template for subsequent reamplification reactions followed by restriction digestions (PCR-RFLPs) designed to identify key sequence variations associated with the *CYP2D6**2, *3, *4, *6, *7, *8, *9, *10, *17, *29, *36, *40, and *41 alleles. The *CYP2D6**5

(gene deletion) was detected by amplifying a 2.9-kb *CYP2D6**5-derived fragment along a 3.8-kb-long internal control fragment.⁶ When a gene duplication event was detected, additional long-PCR reactions and diagnostic genotyping assays were conducted to determine the nature of the gene that had been duplicated according to the strategy described recently.¹⁶

Analysis of Expired $^{13}\text{CO}_2$

The [^{13}C]-DM breath test is based on the principle that CYP2D6-mediated *O*-demethylation cleaves a $^{13}\text{CH}_3$ group that enters the body's one carbon pool to be ultimately eliminated as $^{13}\text{CO}_2$ in expired air (Figure 2). The concentration of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in the exhaled breath samples was measured by infrared spectrometry using an UBiT-IR₃₀₀ spectrophotometer (Meretek Diagnostics, Lafayette, Colorado). Use of interference filters centered at the distinct $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ absorption features facilitates this measurement. All samples were analyzed within 48 hours of collection. Enrichment of $^{13}\text{CO}_2$ in expired air as a consequence of [^{13}C]-DM is expressed as the increase in the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio relative to predose baseline samples and is termed *DOB*:

$$\text{DOB} = 1000 \times \frac{[(^{13}\text{CO}_2/^{12}\text{CO}_2)_{\text{Postdose}} - (^{13}\text{CO}_2/^{12}\text{CO}_2)_{\text{Baseline}}]}{R_{\text{PDB}}},$$

where DOB is expressed in units of ‰ (per mil (%)), and $R_{\text{PDB}} = 0.0112372$ $^{13}\text{C}/^{12}\text{C}$ in PDB (international standard Pee Dee Belemnite).

Analytical Methods for DM and Metabolites in Urine

Urinary concentrations of DM and its metabolites DX, 3-MM, and 3-HM were quantified by HPLC with fluorescence detection according to methods established in the laboratory.¹⁸ Pooled urine samples were adjusted to a pH between 4 and 5 with sodium acetate (50 mM, pH 3) and subjected to deconjugation by the addition of 25 μL of beta-glucuronidase (~100 000 U/mL; Sigma, St Louis, Missouri) to each milliliter of urine and incubation overnight at 37°C. Levallorphan tartrate (10 μL , 50 $\mu\text{g}/\text{mL}$) was added to each 1-mL aliquot of deconjugated sample and served as an internal standard. Samples were subsequently filtered to remove unwanted particulates with a 0.22- μm nylon centrifuge filter (Spin-X, Costar, Cambridge, Massachusetts), and aliquots of the filtrate (20-50 μL) were analyzed by HPLC. DM and its metabolites were resolved on a reversed-phase Nova-Pak Phenyl column (3.9 $\times$ 150 mm, 4 μm particle size; Waters, Milford, Massachusetts) using a Hewlett Packard HP 1100 series HPLC system equipped with HP1100 de-gasser, quaternary pump, autosampler, column heater, and fluorescence detector. The mobile phase consisted of 10 mM sodium acetate, pH 4 (mobile phase A), and acetonitrile (mobile phase B) and was delivered at a constant flow of 1 mL/min. The solvent program was as follows: 0 to 13.5 minutes, 14% B; 13.5 to 13.6 minutes, a linear gradient from 14% to 28% B; 13.6 to 33 minutes, 28% B; 33 to 33.1 minutes, a linear gradient from 28% to 14% B; and 33.1 to 37 minutes, 14% B. The column temperature was maintained at 45°C, and the column effluent was monitored by fluorescence detection ($\lambda_{\text{ex}} = 235$; $\lambda_{\text{em}} = 310$). Analytes were quantified by peak height measurements using a 7-point standard curve prepared daily in drug-free urine. Standard

curves for all analytes were linear over a range of 0.1 to 10 nmol/mL ($r^2 > 0.99$). The coefficient of variation for all standards was <10% at concentrations spanning the linear range.

Statistical Analysis

The maximum DOB value ($C_{\max}$) and the time to maximum DOB value ($t_{\max}$) were determined by visual inspection of the data. To identify DOB values relevant to making the DM breath test a onetime point breath collection test, we selected time points bracketing the average $t_{\max}$. All parameters were summarized by descriptive statistics and were compared between the CYP2D6 genotype groups by analysis of variance (ANOVA) with post hoc analysis using Tukey's Honestly Significant Different (HSD) test. Student t test for paired data was used for comparisons between the 2 ASG administration protocols. All statistical analyses were conducted using JMP6 (SAS Institute, Cary, North Carolina).

RESULTS

Initial [^{13}C]-DM Breath Test Study Protocol

The average time course of $^{13}\text{CO}_2$ enrichment in expired air (as measured by the DOB value) for each group is presented in Figure 3. On average, the CYP2D6 PM-0 group could be readily distinguished from either EM group (EM-1 and EM-2). The major effect of ASG administration was a tendency to shorten the time to peak $^{13}\text{CO}_2$ enrichment in expired air (not statistically significant), whereas no demonstrable effect on the peak DOB value achieved was observed (Table II). However, ASG administration was associated with better discrimination of PMs from EMs compared with water alone at time points between 30 minutes and 120 minutes after dosing (Figure 4A-F), an effect that was particularly pronounced between 40 minutes and 60 minutes after dosing. In this study, maximum performance was achieved with a single-point determination of phenotype at 40 minutes and defining the PM phenotype as DOB ≥ 0.5 . Using these criteria, the sensitivity was 100%, and specificity was 95% with 95% accuracy, resulting in misclassification of 1 EM-1 subject as a PM using either genotype or urinary DM/DX ratio as the "gold standard"; no PM-0 or EM-2 subjects were misclassified (Table III, Figure 5).

Modified [^{13}C]-DM Breath Test Study Protocol

Although, on average, the EM-2 and EM-1 groups could be easily distinguished from the PM-0 group, low DOB values at specific points in time (ie, 60 minutes; Figure 4D) and variability in the EM-1 group at almost all time points remained a concern. We considered the possibility that the bicarbonate load from ASG administration could affect the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio and also that by having subjects blow into just 1 bag, there could be dilution of $^{13}\text{CO}_2$ by dead space air collected in that single sample. Therefore, the study protocol was modified such that an ASG tablet was administered 15 minutes before dosing and 1 tablet with the [^{13}C]-DM dose, and the study was repeated in the 6 EM-2 and 6 EM-1 subjects with the lowest DOB measurements at 60 minutes and 3 PM-0 subjects with the highest DOB values at 60 minutes. All subjects were administered 0.5 mg/kg [^{13}C]-DM. Furthermore, breath samples were collected immediately before dosing and in duplicate at 5 time points: 30, 40, 50, 60, and 90 minutes post [^{13}C]-DM ingestion, and the higher of the 2

DOB values was recorded and compared with the corresponding values obtained for each subject from the previous oral liquid protocol (Figure 6). Although there appeared to be good agreement between the 2 study days ($r^2 = 0.537$; solid line in Figure 6A), there was a tendency toward higher DOB values when ASG was given 15 minutes before and with [^{13}C]-DM as the majority of points fell above the line of unity (dashed line in Figure 6A). The differences between the 2 study days for each subject at various time points are presented in Figure 6B. Between 40 and 60 minutes after dosing, only 1 subject (PM-0) was misclassified with the modified protocol on a single (50-minute) occasion.

A comparison of the mean ($\pm$ SD) DOB values at the 40-, 50-, and 60-minute time points for the 15 subjects who participated in all 3 protocols can be found in Table IV. No statistically significant effect of treatment protocol was observed for the PM-0 and EM-1 groups. For the EM-2 group, the protocol in which ASG was administered 15 minutes before and with [^{13}C]-DM resulted in significantly greater mean DOB values at 40, 50, and 60 minutes after dosing ($P < .05$; Table IV).

DISCUSSION

The first step in pharmacogenetic strategies to improve drug dosing (or, more broadly, to implement personalized medicine) is to acquire specific genetic information from individual patients—their genotype—for the pathway(s) involved in all components of drug disposition. The next step is to interpret that genetic information such that the most likely outcome of drug administration can be anticipated. In the context of drug biotransformation, this process involves predicting the effect of allelic variation on drug biotransformation, the consequent change in drug clearance, and, by extension, altered dose requirements.

Predicting phenotype from *CYP2D6* genotype can be challenging given the number of *CYP2D6* allelic variants and the resulting complexity of allele combinations, especially those encoding proteins of variably reduced function (ie, *CYP2D6*10*, *CYP2D6*17*, and *CYP2D6*29*). To simplify this process, we have developed the concept of “activity score”¹⁷ and have explored its application in adults¹⁹ and children.^{20,21}

Phenotype assessment will have greatest practical clinical importance if it can be implemented in such a manner as to provide “real-time” information to aid in drug dosage selection. In this article, we have presented data demonstrating preliminary feasibility for the development of a rapid breath test to assess *CYP2D6* phenotype. The advantages that such a test would offer over other *CYP2D6* phenotyping measures include that it is minimally invasive in nature and that it has the potential to be performed in a physician’s office, with results available in approximately 1 hour.

A critical question worth addressing is that of the general value of CYP phenotype measures relative to genotyping. There are now a number of well-developed and highly reliable assays available for determining CYP genotype. There is also a Food and Drug Administration (FDA)–approved PCR-based genotype test, “Amplichip,” from Roche/Affymetrix. Whereas attempts have been made to simplify the assignment of *CYP2D6* phenotype from genotype data,⁶ considerable variability in phenotype measures, such as urinary metabolite ratios,

exists for a given phenotype. Furthermore, genotype information is of limited utility in situations where a particular allelic variant has differential effects on the intrinsic clearance of CYP2D6 substrates or when the phenotype changes, as has been reported during pregnancy^{22,23} or with drug-drug interactions (such as occur with coadministration of CYP2D6 inhibitors). A phenotyping test may also add value by helping to resolve the significant intersubject variability of active metabolite concentrations that has been observed within genotype classes subsequent to administration of certain CYP2D6-dependent drugs (even when accounting for medication history). A good example of this occurrence is the significant variability in endoxifen concentrations that has been observed within genotype classes during tamoxifen treatments.^{24,25}

It is interesting to speculate on the enhanced separation of PM-0 and EM-2 breath phenotypes we have observed with coadministration of ASG with DM. It is unlikely that the effect is a function of reduced gastrointestinal acidity as DM has a pKa of 8.3,²⁶ and an increase in gastric pH to a value of 4²⁷ would be unlikely to result in a substantial proportion of drug being present as the nonionized form. An alternative possibility is that ASG enhances gastric motility,²⁸ thereby enhancing the delivery of DM to the duodenum and thus its availability for absorption and subsequent biotransformation in the liver by CYP2D6. Assuming this latter mechanism is operative and that intestinal absorption of DM is a rate-limiting step for first-pass metabolism of DM, it would then not be surprising that the more rapid metabolizers (EM-2 group or subjects with 2 fully functional alleles) would exhibit the greatest “benefit” from accelerating DM bioavailability. However, it is also possible that other factors influencing gastric motility, such as food or concomitant administration of drugs such as metoclopramide, may affect performance of the test in a clinical setting.

The considerable intersubject variability we see in breath test–derived phenotype within the EM-1 group is another issue that merits discussion. One reason to believe this intersubject variability may be real rather than a measurement artifact is that significant inter-subject variability in phenotype among EM-1 subjects is also observed in urine-based CYP2D6 phenotyping tests using DM as a pharmacologic probe. Similarly, these urine-based assays show significant heterogeneity among EM-1 subjects and significant overlap with phenotype results for EM-2 subjects. In fact, the limitations of the DM urinary metabolite ratio, especially the influence of variation in urinary pH on the measured ratios,^{14,29} are well known¹³ and likely contribute to the poor correlation between the urinary DM/DX ratio and dextromethorphan oral clearance.³⁰

On the other hand, 2 specific characteristics of the breath test may produce added measurement variability. First, a rapid breath test that seeks to classify phenotype in under an hour may be particularly sensitive to intersubject variations in absorption and first-pass drug biotransformation via the gastrointestinal tract. Second, the DOB levels that are measured in EM-1 subjects are typically only 3-fold to 10-fold above the measurement resolution and repeatability of the instrumentation used to make the measurements. However, if our observed variability in the EM-1 group were primarily due to measurement errors, one would expect that variance to be essentially the same for the EM-1 and EM-2 groups. This is not the case as the coefficients of variation for the DOB values at 40 minutes for the 2 different ASG protocols were 50.0% and 57.1% for the EM-1 group compared with

11.8% and 16.7% for the EM-2 group (Table IV). Thus, we believe that the wide variance in EM-1 responses more likely reflects “real” variability rather than simply a consequence of measurement error. Nevertheless, to help resolve these issues, future studies will focus on assessing the *intrasubject* reproducibility of breath test phenotype—particularly among EM-1 subjects and subjects with intermediate metabolizer phenotypes (IMs).

In summary, the [^{13}C]-DM breath test we have developed offers promise as a rapid, office-based, and minimally invasive phenotyping assay for CYP2D6 activity. However, its value as a clinically useful phenotyping assay will depend strongly on the accuracy with which it can ultimately identify, in larger, more diverse population studies, true CYP2D6 PMs and distinguish the lowest activity IMs and EM-1s from their higher activity counterparts. An ability of the breath test to accurately and quantitatively discriminate higher activity UMs from EMs would also add to its value, as will more information on the precision and the stability of the measure (intraindividual variability) over time. These are the performance metrics by which the value of the [^{13}C]-DM phenotyping breath test will ultimately be judged in relation to alternative, established phenotyping and genotyping protocols. Further studies are clearly needed to properly answer these questions.

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Figure 1.

Pictorial representation of the study protocols used in the development of the $[^{13}\text{C}]$ -dextromethorphan ($[^{13}\text{C}]$ -DM) breath test. In the first study (A), subjects were randomized to receive water or an effervescent antacid tablet in water (1000 mg anhydrous citric acid, 344 mg potassium bicarbonate, and 1050 mg heat-treated sodium bicarbonate; ASG, in a randomized, crossover design as described in the text. The timing of ASG administration differed between the 2 protocols, with ASG (or water) being administered with and 15 minutes after $[^{13}\text{C}]$ -DM administration in the first study (A) compared to 15 minutes before and with $[^{13}\text{C}]$ -DM administration in the second study (B). In the second study, breath samples were obtained in duplicate at each of the designated times.

Figure 2.

Biotransformation of [^{13}C]-dextromethorphan ([^{13}C]-DM). CYP2D6 catalyzes the O-demethylation of DM to form dextrorphan (DX) with the cleaved ^{13}C -methyl group entering the 1-carbon pool and appearing as $^{13}\text{CO}_2$ in expired air. O-Demethylation of 3-methoxymorphinan (3-MM) to form 3-hydroxymorphinan (3-HM) is favored over N-demethylation of DX given the relative K_m values for the reactions and the ease with which DX is glucuronidated in vivo. K_m values have been derived from Kerry NL, Somogyi AA, Bochner F, Mikus G. The role of CYP2D6 in primary and secondary oxidative metabolism of dextromethorphan: in vitro studies using human liver micro-somes. *Br J Clin Pharmacol.* 1994;38:243-248.

Figure 3.

Mean delta-over-baseline (DOB)-time profiles for subjects with 2 (EM-2; squares), 1 (EM-1; inverted triangles), or no (PM-0; circles) functional CYP2D6 alleles when [^{13}C]-dextromethorphan ([^{13}C]-DM) was administered with water (control; white symbols) or 2 effervescent antacid tablets (ASG; black symbols). EM, extensive metabolizer; PM, poor metabolizer.

Figure 4.

Distribution of individual data points according to genotype at (A) 30 minutes, (B) 40 minutes, (C) 50 minutes, (D) 60 minutes, (E) 90 minutes, and (F) 120 minutes after dosing with [^{13}C]-dextromethorphan ([^{13}C]-DM). At each time point, delta-over-baseline (DOB) values obtained after water administration are represented by gray circles, and the solid black circles represent DOB values associated with ASG administration.

Figure 5.

Relationship between CYP2D6 phenotype measured by the breath test results (delta over baseline [DOB]) relative to CYP2D6 phenotype assigned by the urinary [^{13}C]-dextromethorphan/dextrorphan (DM/DX) ratio. A poor metabolizer phenotype is assigned for DOB values < 0.5 and a DM/DX ratio > 0.3. EM, extensive metabolizer; PM, poor metabolizer.

Figure 6.

Comparison between delta-over-baseline (DOB) values obtained when ASG is given 15 minutes before and with dex-tromethorphan (DM) relative to when it is given with and 15 minutes after $[^{13}\text{C}]$ -DM (A). The dotted line represents the line of unity between the 2 study protocols, whereas the solid line is the line of best fit. Although there is good agreement between the 2 study days ($r^2 = 0.537$), the DOB values show a trend toward higher values when ASG is given 15 minutes before and with $[^{13}\text{C}]$ -DM (majority of points fell above the line of unity). (B) Changes in individual DOB values at 40, 50, and 60 minutes after DM dose. Between 40 and 60 minutes after dosing, only 1 subject (PM-0) was misclassified on a single occasion (50 minutes, but not 40 or 60 minutes) with the modified protocol. PM, poor metabolizer; EM, extensive metabolizer. PM-0, open circles; EM-1, gray circles; EM-2, solid black circles.

Table IDemographic Characteristics of the *CYP2D6* Genotype Groups

Characteristic	Genotype Group			P Value
	PM-0	EM-1	EM-2	
Sex	4F; 6M	5F; 5M	6F; 4M	.669
Age, mean $\pm$ SD (range), y	42.6 $\pm$ 9.2 (29.6–55.5)	40.1 $\pm$ 9.2 (27.1–57.6)	35.8 $\pm$ 9.5 (23.9–54.5)	.228
Weight, mean $\pm$ SD (range), kg	81.5 $\pm$ 19.3 (53.8–120.4)	83.5 $\pm$ 20.7 (54.0–111.4)	74.4 $\pm$ 13.8 (52.3–100.0)	.508

EM, extensive metabolizer; PM, poor metabolizer.

Table II

Mean $\pm$ SD Breath Test Parameters Following Administration of 0.5 mg/kg [^{13}C]-Dextromethorphan as an Oral Liquid Formulation

Group	Treatment	Peak DOB	$t_{\max}$, min
EM-2	Water	2.1 ± 0.6	84 ± 62
EM-1	ASG	2.4 ± 0.5	56 ± 13
	Water	2.2 ± 0.6	142 ± 82
PM-0	ASG	2.0 ± 0.7	84 ± 55
	Water	0.9 ± 0.5	141 ± 94
	ASG	0.6 ± 0.6	74 ± 102

EM, extensive metabolizer; PM, poor metabolizer; DOB, delta over baseline.

Table III
Sensitivity, Specificity, PPV, NPV, and Accuracy of the [¹³C]-Dextromethorphan Breath Test

DOB	40 min			50 min			60 min		
	PM-0	EM-1	EM-2	PM-0	EM-1	EM-2	PM-0	EM-1	EM-2
0.5	10	1	0	10	2	0	9	3	0
> 0.5	0	9	10	0	8	10	1	7	10
Sensitivity, %			100			100			90
Specificity, %			95			90			85
PPV, %			91			83			75
NPV, %			100			100			94
Accuracy, %			97			93			87

PPV, positive predictive value; NPV, negative predictive value; DOB, delta over baseline; PM, poor metabolizer; EM, extensive metabolizer.

Table IV

Comparison of Mean ($\pm$ SD) DOB Values for Each Treatment Protocol at 40, 50, and 60 Minutes After Administration of [^{13}C]-Dextromethorphan

Protocol	40 min			50 min			60 min		
	PM-0	EM-1	EM-2	PM-0	EM-1	EM-2	PM-0	EM-1	EM-2
Water	-0.1 $\pm$ 0.6	1.2 $\pm$ 0.6	1.2 $\pm$ 0.6 ^a	0.0 $\pm$ 0.2	1.3 $\pm$ 0.4	1.3 $\pm$ 0.3 ^a	0.3 $\pm$ 0.4	1.3 $\pm$ 0.5	1.5 $\pm$ 0.5 ^a
ASG* t ₀ and t ₊₁₅	0.3 $\pm$ 0.1	1.2 $\pm$ 0.6	1.7 $\pm$ 0.2 ^{a,b}	0.1 $\pm$ 0.3	1.0 $\pm$ 0.6	2.0 $\pm$ 0.2 ^b	0.1 $\pm$ 0.4	1.0 $\pm$ 0.8	1.8 $\pm$ 0.3 ^{a,b}
ASG [†] t ₋₁₅ and t ₀	0.0 $\pm$ 0.1	1.4 $\pm$ 0.8	1.8 $\pm$ 0.3 ^b	0.3 $\pm$ 0.3	1.6 $\pm$ 1.2	2.1 $\pm$ 0.3 ^b	0.1 $\pm$ 0.2	1.7 $\pm$ 1.0	2.2 $\pm$ 0.2 ^b

PM-0, n = 3; EM-1, n = 6; EM-2, n = 6. Superscript letters (a, b) indicate homogeneous subsets of treatment protocols using Tukey's Honestly Significant Different (HSD) test at a given time point. DOB, delta over baseline; EM, extensive metabolizer; PM, poor metabolizer.

* ASG, 1 tablet with the dose of [^{13}C]-dextromethorphan ([^{13}C]-DM) and 1 tablet 15 minutes after dosing.

[†] ASG, 1 tablet 15 minutes before the dose of [^{13}C]-DM and 1 tablet with the dose; higher of 2 breath samples obtained at each time point used.