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CYP2D6 Genotype Phenotype Discordance Due to Drug-Drug Interaction

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Abstract

Drug-drug interactions have been demonstrated to alter CYP2D6 enzyme phenotype due to inhibitor ingestion though it is unclear how substrate interactions affect phenotype. This was a pragmatic clinical trial examining the kinetics of a CYP2D6 enzyme probe drug with and without CYP2D6 dependent substrates. Patients were enrolled into an inpatient study unit, orally administered a 2 mg microdose of dextromethorphan to probe enzyme activity with and without CYP2D6 dependent drug-drug interactions. Thirty-nine subjects were enrolled in this trial. Twelve subjects were on no CYP2D6 dependent drugs and 27 were on one or more CYP2D6 dependent drugs. There were 1 poor metabolizer, 5 intermediate metabolizers, 31 normal metabolizers, and 2 ultra-rapid metabolizers. Those with co-ingestion of another CYP2D6 dependent drug were 9.49 (95% CI: 1.54, 186.41; $p = 0.01$) times more likely to have genotype-phenotype discordance based upon the 3 hours DX/DM ratio. CYP2D6 substrate co-ingestions can cause genotype-phenotype discordance.

Keywords

CYP2D6; discordance; enzyme activity; metoprolol; pharmacogenomics; phenotype

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AUTHOR CONTRIBUTIONS

A.M. and K.W. wrote the manuscript; A.M. and P.A. designed the research; A.M., K.M., S.S., H.F., F.A., L.B., and J.S. performed the research; A. M., K.W., D.A., and K.W. analyzed the data; P.A. and L.B. contributed reagents and analytical tools.

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INTRODUCTION

Cytochrome 2D6 (CYP2D6) is one of the most important drug metabolizing enzymes for human elimination of xenobiotics.(1, 2) The enzyme metabolizes approximately 25% of all drugs and genetic polymorphism in the *CYP2D6* gene affects the pharmacokinetics of approximately 50% of these drugs.(3) This polymorphism can lead to drug ineffectiveness, (4–6) adverse drug events (ADEs),(7–9) or supra-effective therapy,(5, 6) even at low doses.

With a few notable exceptions, such as codeine, genotyping of *CYP2D6* has not resulted in better prediction of drug effectiveness or safety commensurate with the number of drugs the enzyme metabolizes.(10–12) A contributing factor to this may be due to discordance of the predicted phenotype from the underlying *CYP2D6* genotype.(12) For instance, the strong CYP2D6 inhibitor, paroxetine, has been shown to alter the pharmacokinetics of metoprolol leading to *CYP2D6* genotype-phenotype discordance.(13) This shift in enzyme phenotype may not be limited to enzyme inhibitors. CYP2D6 substrates may occupy the enzyme's active site thereby interfering with the metabolism of additional drugs, resulting in genotype-phenotype discordance. For instance, the analgesic effectiveness of hydrocodone, which is dependent upon CYP2D6 for conversion to an active metabolite hydromorphone, is better predicted by the absence of a CYP2D6 drug-drug interaction than *CYP2D6* genotype. (11) This phenotypic conversion may be variable between patients with different genotypes.

Polypharmacy is becoming increasingly common, and the effect of drug-drug interactions on drug effectiveness and safety is underappreciated. Forty-eight percent of people in the US take at least one prescription medication and more than 76% of people 60 years or older are on two or more.(14–16) The clinical effects of drug-drug interactions in people with genetic polymorphism adds additional complexity to this problem. Given the frequency of polypharmacy, we must strive to understand these drug-drug and drug-gene interactions in order to better predict the effectiveness and safety of prescribed drugs. Our primary objective was to determine if CYP2D6 drug substrates result in genotype-phenotype discordance of enzyme function and secondarily, whether this translates to discordance of predicted clinical effect.

RESULTS

Thirty-nine subjects were enrolled in the pragmatic phenotyping analysis. *CYP2D6* genotypes were not known at the time of the kinetic analyses. The demographic distribution was similar between groups (Table 1). Subjects were taking between zero and twenty-four total drugs. Twelve subjects were taking zero CYP2D6 dependent drugs and constituted the no-interaction group. The number of CYP2D6 dependent drugs taken in the interaction group was one (n=16), 2 (n=8), and 3 (n=3). All the co-ingested drugs patients reported taking within 3 half lives were found and confirmed by the qualitative urine drug screen performed by Labcorp.(17) Twenty-five (62.5%) subjects were taking metoprolol succinate for management of their hypertension. As expected, the majority of subjects were normal metabolizers (n=31, 79%). The negative log of the 3 hour DX/DM ratio was predicted by the negative log of DM AUC (R square 0.85, p<0.0001) with this microdose protocol.

Genotype-Phenotype Discordance:

Genotype-phenotype discordance was observed in 14 (35%) of the subjects (Table 2). Those with co-ingestion of another CYP2D6 dependent drug were 9.49 (95% CI: 1.54, 186.41; $p = 0.01$) times more likely to have genotype-phenotype discordance based upon the 3-hour DX/DM ratio. There was one subject that was taking no CYP2D6 dependent co-ingestions that genotyping demonstrated to be a normal metabolizer but phenotyping showed them to be an intermediate metabolizer. Age, ethnicity, and co-ingestion of CYP2D6 dependent drugs were determined to meet covariate selection criteria and were included in the full model. There was collinearity between ethnicity and CYP2D6 co-ingestion, and therefore ethnicity was removed from the model. When included in the full model, age was found to be nonsignificant ($p = 0.21$) in the presence of all other variables. Lastly, genotype was found to be insignificant when stratified by co-ingestion and excluding the person with a PM genotype ($p = 0.26$). See Table 3 for model estimates.

Drug-Drug Interaction Effect on AUC:

The derived drug-drug interaction term (derived K_i term) was insufficient to predict genotype-phenotype discordance in isolation ($p = 0.07$, Table 3). There was still no association when the model controlled for age, CYP2D6 genotype, and CYP2D6 co-ingestion ($p = 0.05$, Table 3). Overall, patients that were taking a CYP2D6 dependent drug had a larger DM AUC (Figure 1) suggesting decreased enzyme metabolic activity. Those who co-ingested CYP2D6 drugs had 21.93 times increase in their DM AUC (CI 95%: 6.72, 71.52). We were unable to stratify this drug-drug interaction effect by the number of co-ingestants beyond the primary covariate of CYP2D6 co-ingestion captured as a binary variable, likely due to smaller numbers in this stratified sample (Table 4). Four patients were taking CYP2D6 inhibitors. Co-ingestion of CYP2D6 dependent drugs was still significant when included in the multivariate model with CYP2D6 inhibitor for DM AUC ($p = 0.002$).

When controlled for metoprolol dose, the median MT/MT-OH ratios at 3 hours significantly increased from phenotypically normal metabolizers (1.21 [IQR: 0.54, 1.73]), to intermediate metabolizers (4.20 [IQR: 3.22, 4.95]), to poor metabolizers (90.30 [IQR: 23.83, 489.09]) ($p = 0.0026$).

Clinical Effects of CYP2D6 Phenotype:

In the subset of subjects taking metoprolol, there was no association between systolic blood pressure decline of 10% from baseline and phenotype ($p = 0.093$, Table 5) or the derived K_i variable ($p = 0.19$, Table 4). Similarly, there was no association between heart rate decline of 10% from baseline and phenotype ($p = 0.22$, Table 5). When the phenotypes were dichotomized into PM/IM and NM/UM groups, blood pressure control was predicted by combined phenotype ($p = 0.03$, Table 5), but heart rate decline was not predicted these combined phenotype groups ($p = 0.08$, Table 5).

The composite secondary outcome of 10% decline in SBP or HR was predicted by the CYP2D6 phenotype. This composite outcome was more likely in patients that were phenotypically intermediate or poor metabolizers ($p = 0.044$, Table 5). The association was most profound in phenotypically intermediate metabolizers ($p = 0.021$); the poor metabolizer

phenotype did not predict the composite outcome, due to small numbers in that subset ($p=0.361$).

Adverse drug events (ADEs):

There were a total of eight reported events captured by the ADE screen. Four of these events were present prior to probe drug administration and thus were not considered associated with the probe drug. One patient described itching at the three-hour time point though no rash was present, and this symptom resolved prior to the final time point. One patient reported a subjective feeling of abdominal pain at the six-hour time point. The patient was observed, and the symptoms resolved after the subject ate some crackers. Two patients reported mild drowsiness at the four-hour time point, and this resolved in one subject, but remained present at discharge in the second subject.

LIMITATIONS

While appropriately powered for our stated primary outcome, the number of patients in this study are small. This means that we can not definitely state how CYP2D6 co-ingestion changes enzyme activity on the population level. The study only included 2 PMs, 4 UMs, and 10 IMs, by genotype, which limits this study's ability to determine the interaction between the underlying genotype and the drug-gene interaction. However, we would not expect PMs or IMs to have increased enzyme activity when faced with an increased substrate load. The clinical effect data associated with metoprolol are also limited by the number of subjects in this subset. We will continue to enroll patients to further explore these trends.

This pragmatic trial allowed patients to take their own medications per their routine. This resulted in variability in timing of their ingestion. We opted for this approach because we desired to examine this interaction in a real world, pragmatic fashion. Examination of drug-drug interactions in this way is inherently less likely to identify clinical effects. Therefore, our study was biased toward the null hypothesis, that substrate ingestion does not cause genotype-phenotype discordance. Thus, our findings are suggestive that genotype-phenotype discordance may be more profound if all interacting drugs are taken at the same time.

The co-ingestion of CYP2D6 inhibitors, while not significant as a co-variate, may have contributed to the inability to stratify the co-ingestion variable by number of CYP2D6 drugs ingestions. There were only 4 subjects that co-ingested an inhibitor; 1 in which the inhibitor was the only CYP2D6 dependent drug taken, 1 was taking 2 CYP2D6 dependent drugs, and 2 were taking 3 CYP2D6 dependent drugs (one of which was the inhibitor). The ingestion of an inhibitor is likely to lead to more profound genotype-phenotype conversion, as demonstrated by Parker, et al.(13) Inclusion of inhibitor co-ingestant as a covariate demonstrated that the ingestion of CYP2D6 dependent substrates remained significant, which argues against inhibitor co-ingestion driving the reported association.

We utilized a microdose of DM as our probe drug. This lower dose was chosen to minimize ADEs and prevent competitive inhibition. The 3 hour DX/DM ratio was used by Frank, et al and this is the first report using the 3 hour/hour DX/DM ratio with microdose DM. We have

provided the analysis that demonstrates the 3 hour microdose ratio predicts the phenotype calculated by the DM AUC, however, this was not the primary aim of this study. The association appears to hold, however a study specifically designed to validate this method may be warranted to confirm this finding. We hypothesized that the derived K_i term would allow for more specific stratification of the substrates taking into account the individual drug dose and the degree of enzyme inhibition. This approach may have been limited by pulling K_i s from 2 separate in-vitro studies rather than a single unified source. K_i s are inherently method dependent and vary between laboratories which may have limited the performance of the derived term. The K_i s we incorporated may themselves not be accurate in individual patients with enzyme polymorphism. The more general binary co-ingestion term was more successful at predicting enzyme phenotype.

DISCUSSION

These data strongly suggest that co-ingestion of CYP2D6 substrates leads to genotype-phenotype discordance. This association with genotype-phenotype discordance and alteration in DM AUC remains present even without inhibitors which confirms an effect of substrates on enzyme phenotype. The one subject not taking any CYP2D6 co-ingestants that was genotyped as a CYP2D6 NM but was phenotyped as an IM was just barely in the IM ratio group. This patient had been taking zolpidem, which we do not consider a CYP2D6 dependent drug because less than 3% of the parent compound is metabolized via this pathway.(18) Zolpidem may have contributed to this phenotype shift or this patient may have a variant in the CYP2D6 gene that we did not capture with our assay. This genotype-phenotype discordance is associated with altered clinical effects in those taking metoprolol, as we've demonstrated with the composite blood pressure and heart rate outcome. Understanding shifts in phenotype due to interaction at the drug metabolizing enzyme level allows a more detailed understanding of the pathophysiology of drug-drug interactions. Other investigators have demonstrated alteration of pharmacokinetics and pharmacodynamics with CYP2D6 inhibitors though this work has never been extended to examine the effects of enzyme substrates on these parameters.(13) While co-ingestion may not always alter the clinical effects, enzyme substrates can have profound effects on drug metabolizing enzyme activity as well as the clinical effects of co-ingested drugs.

This trial attempted to account for variable enzyme effects caused by different drug substrates utilizing a derived term that accounted for all CYP2D6 dependent drugs and their doses. Other probe drugs, as well as other substrates, may yield different shifts in the enzyme phenotype. These shifts are ultimately dependent upon how tightly the enzyme binds each individual drug, and at what dose. For instance, a low dose drug that binds very tightly may still be out-competed by a drug at high dose even if the enzyme has lower affinity for the drug. It is likely that the resulting enzyme activity is altered differentially depending upon the specific substrate; the substrate affinity for the enzyme and the dose may be important factors that affect enzyme activity. We chose DM because of the high enzyme affinity for this drug but different probe drugs are likely to give different results. Whilst our pre-specified analysis did not identify a unifying term, we did observe a trend that suggests the need to enroll more subjects for future analyses. Ultimately, with no prior data to guide us, it was unclear how many subjects we would need to examine this derived

term. Additionally, these data are likely stratified by underlying enzyme function and, perhaps, allelic variation. Thus, a larger study focused on this question is warranted.

We found no association with drug-drug interactions and ADEs. This was expected since the most common adverse drug events are off-target effects, such as nausea. ADEs that are exaggerations of the intended clinical pharmacology may be more common, may be predicted, and subsequently prevented by starting at lower doses in those with at risk genotypes. In this example, poor metabolizers were expected to tolerate lower doses of metoprolol, as demonstrated by other investigators.(19) Clinically, patients that have lower enzyme activity don't tolerate significant up-titration of an enzyme dependent drug which minimizes the associations of ADEs in ecologic studies.(20, 21) Starting at a low dose and titrating upward is the safest method when no genotype information is available. It may be possible to start patients on higher doses in those with normal enzyme function and no drug-drug interaction or utilize a different drug entirely in patients not likely to meet therapeutic levels, such as those with ultra-rapid enzyme activity.

We were not able to predict phenotypes stratified by the number of CYP2D6 co-ingestions. This is likely due to the lower number of subjects ingesting more than two CYP2D6 dependent drugs and the presence of CYP2D6 inhibitors interspersed throughout the groups. Our results demonstrate that the composite clinical phenotype of BP and HR decline is affected by CYP2D6 dependent drug-drug interactions. A large trial that includes genotyping and drug-drug interactions must be undertaken to definitely determine the utility of genotyping in metoprolol treatment.

In conclusion, CYP2D6 substrate co-ingestion leads to genotype-phenotype discordance. Enzyme phenotype is associated with clinical effect in those taking metoprolol succinate. Larger studies that phenotype patients with broader *CYP2D6* genotype representation and increased stratification of co-ingested CYP2D6 dependent drugs may allow for predictive modeling of clinical drug effects.

METHODS

Study Design/setting:

This was a prospective pharmacokinetic study in the University of Colorado Denver (UCD) Colorado Clinical Translational Science Institute (CCTSI) Clinical Translational Research Center (CTRC). The CTRC inpatient facilities include nursing staff, bionutritional support, and phlebotomy for pharmacokinetic analyses. The study was approved by the local institutional review board.

Patients:

A subset of patients from a parent clinical trial (NCT02293096) with uncontrolled HTN between 30 and 80 years of age were enrolled. Exclusion criteria included end-stage liver disease, glomerular filtration rate < 60 ml/min/1.73 m², pregnancy, American Association of Anesthesiologists (ASA) classification of > 3 , prisoners or wards of the state, decisionally challenged individuals, heart rate < 60 beats per minute, AV block > 240 msec, active

reactive airway disease, illicit drug use in the preceding 30 days (excluding marijuana), allergy to metoprolol succinate, or severe peripheral arterial circulatory disorders.

CYP2D6 Genotyping and Phenotype Determination

Genomic DNA was extracted from whole blood via the Puregene® Blood Core kit B (Qiagen) according to the manufacturer's instructions. *CYP2D6* and *ADRB1* were genotyped using the Multiplex SNaPshot technique previously described.(22) This assay detects 20 *CYP2D6* clinically significant variants and identifies copy number variants. Predicted phenotypes were determined utilizing CYP2D6 activity score, as described by Gaedigk *et al.*(23) The CYP2D6 enzyme phenotype was determined based upon the 3 hour DM/DX ratio with ranges validated by Jurica *et al.*(24) Subjects were determined to have genotype-phenotype discordance if the phenotype predicted by the underlying genotype was different than the measured phenotype, determined by probe drug metabolism.

CYP2D6 probe and kinetic time points

An oral 2 mg microdose of dextromethorphan was administered as the phenotyping probe drug. Microdosing (2 mg versus the typical therapeutic dose of 20 mg) of dextromethorphan allows for subtherapeutic dosing, thus minimizing the risk of probe-drug interaction and adverse drug events(25). Measurement of serum dextromethorphan (DM) and its metabolite, dextrophan (DX), is considered the most reliable CYP2D6 phenotyping probe.(24, 26)

The parent compound and the metabolite were detected in EDTA plasma samples obtained at time 0, 1, 4, and 6 hours post dose. A reversed-phase ultra-performance liquid chromatographic tandem mass spectrometry (UPLC-MS/MS) assay for the determination of DM, its metabolite DX, metoprolol (MT), and its metabolite alphahydroxy metoprolol (MT-OH) in human EDTA plasma was validated.(24)

This method utilized stable labeled deuterated internal standards (DM-IS, DX-IS, MT-IS, MT-OH IS). The samples were treated with β -glucuronidase solution to convert dextrophan-*o*-glucuronide to dextrophan prior to extraction. A solid phase extraction procedure utilizing Strata-WCX cartridges was used to prepare the samples for analysis. The chromatographic separation was performed on a Waters 1.8 μ m HSS T3, 100 \times 2.1mm reverse-phase analytical column. Two different mobile phases were utilized for a gradient elution for the compounds. Mobile phase A consisted of 5:95 (acetonitrile:ultrapure water) containing 0.1 % formic acid (v:v). Mobile phase B consisted of 90:10 (acetonitrile:ultrapure water) containing 0.1% formic acid (v:v). The gradient separation changed linearly from 90% mobile phase A to 50% mobile phase A over 3 minutes at a flow rate of 0.50mL/min. The detection of DM, DX, MT, MT-OH, and their respective internal standards were achieved by protonated electrospray ionization on a TSQ Vantage detector. Precursor/product SRM transitions (m/z) in the positive ion mode were 284.4/116.2 and 289.4/121.2 for MT-OH and MT-OH IS, respectively, and 268.4/116.2 and 275.4/123.2 for MT and MT-IS, respectively. Precursor/product transitions (m/z) for positive ion mode were 258.4/157.2 and 261.4/157.2 for DX and DX-IS, respectively, 272.4/171.2 for DM and DM-IS, respectively, 434.402/258.402 for dextrophan-*o*-glucuronide was also evaluated for completeness of enzymatic treatment. MT-OH, DM, MT utilize a linear (1/x weighted)

regression while DX utilized a quadratic (1/x weighted) regression. MT, and MT-OH had a quantifiable range of 0.25ng/mL to 250ng/mL with a lower limit of quantitation (LLOQ) of 0.25ng/mL. DM and DX had a quantifiable range of 0.025ng/mL to 25.0ng/mL with a LLOQ of 0.025ng/ml. The assay used 0.2mL of human plasma.

The DM/DX 3-hour ratios were calculated to determine the CYP2D6 phenotype of each patient (based on cutoff values determined by Jurica J, *et al*(24)). Three of the DM 3-hour concentrations were below the limit of quantitation (BLQ), therefore half the LLOQ was imputed for these BLQ values based on the presence of DX . The MT:MT-OH 3-hour ratios were also calculated. One MT 3-hour concentration and one MT-OH 3-hour concentration were BLQ and half of the LLOQ was imputed for these values based on the presence of MT-OH and MT, respectively.

Drug Co-ingestion

A structured drug co-ingestion tool was utilized to captured prescriptions, over-the-counter medications, vitamins, and supplements. This tool captured the doses and times of the last dose of co-ingestants. Any drug taken within 3 of its half-lives was considered to have a potential CYP2D6 drug-drug interaction. We confirmed that stated drugs were taken using a comprehensive qualitative urine drug screen. This method did not confirm stated timing of ingestion, but demonstrated the patients were taking the drugs they claimed and no others. Subjects took all medications per their routine, with the exception of the dextromethorphan probe drug and the metoprolol succinate (when part of the subject's medication list), which was taken after obtaining the baseline kinetic sample. When metoprolol was taken, it was scheduled by protocol to be at least 20 hours after the patient's prior dose.

Area Under the Curve modeling

The area under the curve (AUC) was determined for DM, DX, MT, and MT-OH utilizing the trapezoidal rule,

$$AUC_{0-\infty} = \sum [(C_n + C_{n-1})/2] \times (t_n - t_{n-1}) \dots + C_{last}/ke = \frac{c_1}{\Delta t} \ln \frac{c_2}{c_1}$$

The tail (+ C_{last}/ke) was not included in the AUC calculation for MT and MT-OH as these were at steady state concentrations. The DM/DX and MT/MT-OH AUC ratios were calculated.

Drug-drug interaction modeling

A unique CYP2D6 drug-drug interaction variable was derived in an attempt account for interaction as a single term. This term was derived from the sum of the doses of CYP2D6 dependent drugs divided by sum of each CYP2D6 substrate inhibition constant (K_i) for each drug, as determined by Shen *et al*, Vandenbrink *et al* Zhou *et al*, and Kerry *et al*.(2, 27–29) The K_i values are provided in Appendix 1.

$$[\text{Dose}_1 + \text{Dose}_2 + \text{Dose}_3 + \text{Dose}_n \dots] / [K_{i-1} + K_{i-2} + K_{i-3} + K_{i-n} \dots]$$

Adverse drug events:

All adverse drug events during the trial were captured using a structured form. Abdominal pain, nausea, vomiting, diarrhea, shortness of breath, bradycardia, ventricular dysrhythmias, lightheadedness, syncope, rash/itching, and other adverse drug events, reported by the patient but not listed above, prior to probe administration and at every kinetic analysis time point were captured.

Metoprolol Clinical Effectiveness Outcomes:

A clinical effectiveness analysis was performed in the subset of subjects taking metoprolol. The primary outcome for this analysis was systolic blood pressure (SBP) decline of 10% from baseline, captured in the parent clinical trial over 4 weeks of therapy. Secondary outcomes were heart rate (HR) decline of 10% from baseline, and a composite of SBP and/or HR decline of 10%. Heart and blood pressure changes were not expected and were not observed during this trial since metoprolol succinate was at steady state following 4 weeks of therapy (data not shown).

Statistical Analysis

Logistic regression was used to predict genotype-phenotype discordance using co-ingestion of CYP2D6 drugs, age, ethnicity, and underlying *CYP2D6* genotype as potential covariates. Each potential covariate was assessed univariately as a predictor for genotype-phenotype discordance. Any potential covariate that was found to meet the significance criteria of $p < 0.20$ was included in the full model to determine potential confounding. Any variable that was found to be insignificant ($p > 0.05$) using Chi-square likelihood ratio tests was excluded from the final model. This analysis was repeated by combining the genotypes into two groups "Poor/Intermediate" and "Normal/Ultra". Finally, genotype was used, excluding the lone poor metabolizer genotype subject, to stratify the results based on CYP2D6 co-ingestion.

Logistic regression was used to determine associations between binary variables and potential confounders. Chi-square likelihood ratio tests were used to determine overall significance for predictors. Simple linear regression and multivariate linear regression was used to test associations between continuous outcomes and potential covariates. Negative log transformations of variables were performed for continuous variables whose distributions were non-normally distributed. Variable selection for covariates was carried out as in the primary analysis.

In order to have 80% power to detect a 0.6 difference in the AUC ratio of metabolites with 0.4 standard deviation at the 0.05 alpha level(30), 10 patients in each group were needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STUDY HIGHLIGHTS**What is the current knowledge on the topic?**

CYP2D6 enzyme activity can be decreased by co-ingestion of inhibitors.

What question did this study address?

Does co-ingestion of CYP2D6 enzyme substrates alter enzyme activity or drug effectiveness.

What does this study add to our knowledge?

Co-ingestion of CYP2D6 dependent drugs alters CYP enzyme phenotype from what is predicted by underlying *CYP2D6* genotype. This CYP2D6 drug-drug interaction alters metoprolol effectiveness.

How might this change clinical pharmacology or translational science?

Clinicians should consider CYP2D6 drug-drug interaction when prescribing drugs dependent upon this enzyme. Substrate dependent drug-drug interaction at the drug metabolizing enzyme can lead to altered drug effectiveness.

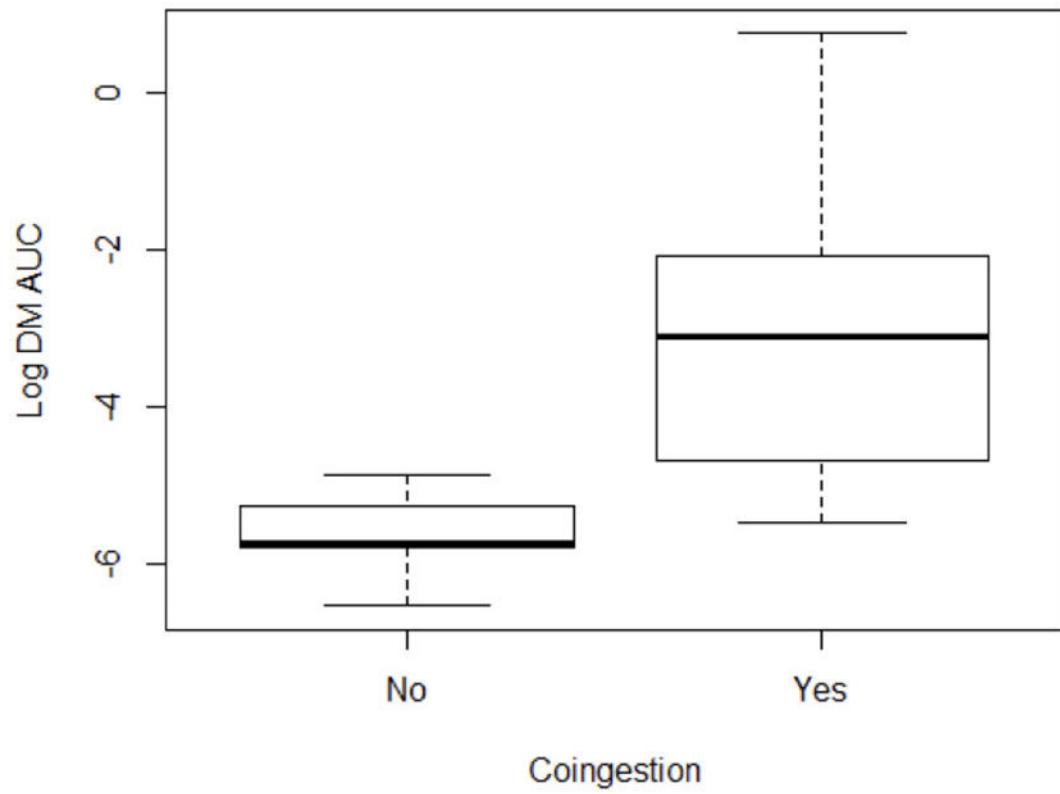


Figure 1:
Distribution of the Log DM AUC values given whether or not the subject was co-ingesting any CYP2D6 dependent drugs

Table 1.

Demographics of study participants.

Characteristic	All N=39	Not Taking a CYP2D6 Dependent Drug n=12	Taking CYP2D6 Dependent Drug n=27
Age, median (IQR)	58 (53, 64)	60 (58, 65)	57 (52, 63)
Male Gender, n (%)	22 (56%)	7 (58%)	15 (56%)
Race, n (%)	17 (44%)	2 (17%)	15 (56%)
Black or African American	22 (56%)	10 (83%)	12 (44%)
White			
Hispanic Ethnicity, n (%)	4 (10%)	2 (17%)	2 (7%)
Hispanic or Latino			
CYP2D6 Genotype, n (%)	1 (3%)	0 (0%)	1 (4%)
Poor	5 (13%)	3 (25%)	2 (7%)
Intermediate	31 (79%)	9 (75%)	22 (81%)
Normal	2 (5%)	0 (0%)	2 (7%)
Ultra rapid			
Number of CYP2D6 Dependent Drugs, including metoprolol succinate	12 (31%) 16 (41%) 8 (21%)	12 (100%) NA NA	NA 16 (59%) 8 (30%)
0	3 (8%)	NA	3 (11%)
1			
2			
3			
Taking a CYP2D6 inhibitor, n	4	NA	4
DM/DX Ratio, median (IQR)	0.01 (0.01- 0.11)	0.003 (0.003–0.005)	0.045 (0.009–0.123)
MT/MT OH Ratio, median (IQR)	1.80 (1.12- 4.51)	NA	1.80 (1.12–4.51)

Table 2.

Subjects with genotype-phenotype discordance stratified by the number of CYP2D6 dependent drugs co-ingested.

Number of CYP2D6 dependent drugs	0 n=12	1 n=16	2 n=8	3 n=3
Number (%) of subjects with discordance based on the 3-hour DX/DM ratio n=14 subjects	1 (8%)	7 (43%)	4 (50%)	2 (67%)

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Table 3.

Chi-Square of Model Variables Predicting Genotype-Phenotype Discordance, univariate and multivariate analyses.

Univariate Analyses			
Variable	Chi-Square	DF	<i>p</i> -value
Age	2.94	1	0.09
Race	0.36	1	0.64
Ethnicity	2.98	1	0.08
Gender	0.49	1	0.48
CYP2D6 Co-ingestion	6.24	1	0.01
<i>CYP2D6</i> Genotype	1.16	3	0.13
Combined Genotype	1.16	1	0.28
Derived Ki term	3.33	1	0.07
Multivariate Analyses			
Age	1.59	1	0.21
<i>CYP2D6</i> Genotype	1.013	3	0.26
CYP2D6 Co-ingestion	3.933	1	0.047
Ranked Dose over Ki	3.99	1	0.05

Table 4:

Univariate model predicting log DM AUC stratified by number of co-ingested CYP2D6 dependent drugs.

Number of CYP2D6 drugs Co-ingested	Estimate	Standard Error	P-value
Co-ingestion (yes/No)			0.004*
Zero vs Two or more	2.272	0.897	0.02*
Zero vs One	2.963	0.795	0.001*
One vs Two or More	0.691	0.833	0.417

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Table 5.

Clinical effects in those taking metoprolol predicted by phenotype, the Ki formula, and combined phenotype (PM/IM or NM/UM). Adjusted for metoprolol does.

Univariate Analyses				
Outcome	Predictor	LR Chisq	Df	Pr(>Chisq)
10 % Blood pressure decline				
	Phenotype	4.754	2	0.090
	Ki	1.699	1	0.190
	Combined Phenotype	4.702	1	0.029*
10% Heart rate decline				
	Phenotype	3.016	2	0.175
	Ki	0.577	1	0.257
	Combined Phenotype	2.964	1	0.069
Composite of either 10% Blood pressure or HR decline				
	Phenotype	6.289	2	0.029*
	Ki	0.790	1	0.273
	Combined Phenotype	5.190	1	0.020*