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Combinatorial Pharmacogenomic Algorithm is Predictive of Citalopram and Escitalopram Metabolism in Patients with Major Depressive Disorder

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ABSTRACT

Pharmacogenomic tests used to guide clinical treatment for major depressive disorder (MDD) must be thoroughly validated. One important assessment of validity is the ability to predict medication blood levels, which reflect altered metabolism. Historically, the metabolic impact of individual genes has been evaluated; however, we now know that multiple genes are often involved in medication metabolism. Here, we evaluated the ability of individual pharmacokinetic genes (CYP2C19, CYP2D6, CYP3A4) and a combinatorial pharmacogenomic test (GeneSight Psychotropic®; weighted assessment of all three genes) to predict citalopram/escitalopram blood levels in patients with MDD. Patients from the Genomics Used to Improve DEpression Decisions (GUIDED) trial who were taking citalopram/escitalopram at screening and had available blood level data were included (N=191). In multivariate analysis of the individual genes and combinatorial pharmacogenomic test separately (adjusted for age, smoking status), the F statistic for the combinatorial pharmacogenomic test was 1.7 to 2.9-times higher than the individual genes, showing that it explained more variance in citalopram/escitalopram blood levels. In multivariate analysis of the individual genes and combinatorial pharmacogenomic test together, only the combinatorial pharmacogenomic test remained significant. Overall, this demonstrates that the combinatorial pharmacogenomic test was a superior predictor of citalopram/escitalopram blood levels compared to individual genes.

1. Introduction

The clinical use of pharmacogenomics to guide psychotropic medication selection for patients with major depressive disorder (MDD) has increased, which is at least partially attributable to growing concern about the low success rate of standard approaches to treatment selection. Fewer than 40% of patients experience remission after their first medication trial, with rates decreasing further with subsequent medication trials (Rush et al., 2006; Thase et al., 2005). The use of pharmacogenomic testing to identify gene-drug interactions that may contribute to medication failures offers the opportunity to make data-driven decisions to avoid medications that are unlikely to be safe and/
or effective for an individual patient.

The cytochrome P450 2C19 (CYP2C19) gene was among the first included in pharmacogenomic testing based on the demonstrated involvement of the CYP2C19 enzyme in the metabolism of medications across many therapeutic areas (Scott et al., 2012; Whirl-Carrillo et al., 2012). CYP2C19 metabolizes about 30% of the most commonly prescribed psychotropic medications, including more than 60% of antidepressants, making it an important component of pharmacogenomic testing in MDD (Nassan et al., 2016). However, it is now well known that most drugs are impacted by multiple metabolic pathways (McDonnell and Dang, 2013). Thus, it became important to expand pharmacogenomic testing to assess additional genes to more comprehensively and accurately characterize gene-drug interactions for medications that are metabolized by additional enzymes independently or in combination with CYP2C19.

There are ongoing efforts to help “translate” genetic information into clinical recommendations to improve prescribing practices for patients with MDD. However, there is no current global consensus and recommendations vary between professional society guidelines, government agencies, and testing laboratories. This is exemplified by the selective serotonin reuptake inhibitor (SSRI) citalopram, which is often the first line of treatment for depression (Gelenberg et al., 1993 (Revised 2015, Reaffirmed 2015); Rush et al., 2006). Although citalopram is primarily metabolized by CYP2C19, the CYP2D6 and CYP3A4 enzymes are also involved (Sangkuhl et al., 2011). The Clinical Pharmacogenetics Implementation Consortium (CPIC) and FDA make therapeutic recommendations for citalopram based on CYP2C19 metabolizer status. However, CPIC makes clinical recommendations for ultrarapid, rapid, and poor metabolizers (Hicks et al., 2015) while the FDA label only includes a recommended dose change for poor metabolizers (U.S. Food & Drug Administration, 2019; U.S. Food and Drug Administration, 2017). Many clinical testing laboratories make recommendations based on CYP2C19, CYP2D6, and/or CYP3A4; however, some laboratories provide individual phenotype information for each gene (Brennan et al., 2015; Pérez et al., 2017) while others provide a combined phenotype that factors in the combined impact of genetic variations in multiple genes (Hall-Flavin et al., 2012). Phenotype assignments of individual SNPs for all three genes also vary between testing laboratories (Bousman and Dunlop, 2018) and, in some cases, with CPIC guidelines.

This example of citalopram highlights three key considerations for the clinical utilization of pharmacogenomic testing: 1) what genes should be tested, 2) what genotypes and phenotypes should be used for each gene, and 3) how to interpret multiple genetic variations across multiple genes that are relevant for a single medication. The last point is particularly important for medications like citalopram that have complex metabolic pathways (Sangkuhl et al., 2011). Alteration in one metabolic pathway could be compensated for or amplified by an alteration in another pathway. In addition, secondary metabolic pathways are often less efficient, and the combined effect of multiple genetic variations may not be equal. Therefore, the interpretation of multiple genetic variations and genes that impact a single medication must account for the combined, weighted impact of all relevant phenotypes.

Given the number of critical factors that must be considered in pharmacogenomic testing, each test should be thoroughly validated to be appropriate for clinical use. An important assessment of the validity for any pharmacogenomic test is the ability to accurately predict medication blood levels, which reflects the ability to predict changes in metabolism based on the tested genes and assigned phenotypes (individual and combined). In this study, we assessed the ability to predict medication blood levels using individual genes compared to a combinatorial pharmacogenomic test (GeneSight® Psychotropic) in patients with MDD taking citalopram or escitalopram as part of the Genomics Used to Improve DEpression Decisions (GUIDED) trial (Greden et al., 2019). This included an evaluation of individual gene phenotype calls as made by the testing laboratory and by CPIC recommendations as well as the combined phenotype from the combinatorial pharmacogenomic test.

2. Methods

2.1. Cohort

GUIDED was a large, patient- and rater-blinded, randomized, controlled trial comparing the impact of treatment selection guided by combinatorial pharmacogenomic testing (guided-care) versus treatment as usual on outcomes of patients with MDD. Patients were included if they had a diagnosis of MDD, a score of ≥11 on the self-rated and clinician-rated 16-item Quick Inventory of Depressive Symptomatology Scale, and at least one failed psychotropic medication trial within the current depressive episode (defined as inadequate efficacy after 6 weeks of treatment, discontinuation due to adverse events, or intolerability). A detailed description of the study design has been previously published (Greden et al., 2019). The trial was approved by the Copernicus Group independent review board (INC1-14-012) and all patients provided written informed consent.

We evaluated all patients who met eligibility criteria at the screening visit, were identified as taking citalopram or escitalopram at the time of the screening visit (either in their medical history at screening or by indicating that citalopram or escitalopram were being dropped at the baseline visit), specified their citalopram or escitalopram dose, and had provided a blood sample. Patients were excluded from analysis if their citalopram or escitalopram blood levels were below the lower level of quantification. Citalopram and escitalopram were selected for this analysis because, combined, they were the most common antidepressants being taken at baseline for which blood levels were obtainable in the GUIDED trial.

To examine the generalizability of the phenotype combinations identified in the GUIDED trial sample, we examined the phenotypes in a large clinical population. This sample consisted of a sequential series of patients who underwent combinatorial pharmacogenomic testing ordered by their treating clinician in the course of their clinical care. This included patients who received clinical testing between April 2014 to October 2019. Only patients who provided consent for their information to be used for research purposes were included.

2.2. Combinatorial Pharmacogenomic Testing


For each medication, a combined phenotype was assigned based on a weighed algorithmic assessment of individual phenotypes in genes involved in the pharmacokinetics (metabolism) or pharmacodynamics (mechanism of action) of that medication. The relative contribution of each enzyme involved in the metabolism of the medication was weighted. These pharmacokinetic weights were combined with pharmacodynamic weights in an algorithm that describes the additive or offsetting effects that each enzyme has on medication metabolism, as well as the impact of genetic variation on efficacy or side effects.

The resultant combined phenotype for each medication was used to categorize the medication according to the predicted level of gene-drug interactions in three categories: ‘use as directed’ (no gene-drug interactions), ‘use with caution’ (moderate gene-drug interactions), or ‘use
with increased caution and with more frequent monitoring (significant gene-drug interactions). The test report included recommendations or highlighted relevant considerations for each medication based on the type and severity of predicted gene-drug interactions. This may have included recommendations to increase dose, decrease dose, consideration of reduced efficacy or increased side-effect risk, or that the medication is contraindicated. For example, citalopram would be in the “significant gene-drug interaction” report category with a recommendation to decrease the dose for an individual who is a CYP2C19 intermediate metabolizer, CYP2D6 poor metabolizer, and CYP3A4 normal metabolizer due to the combined weights of the pharmacokinetic genes. In comparison, the phenotype based on CYP2C19 alone would not indicate a recommendation to change the starting dose (Hicks et al., 2015).

2.3. Individual Gene Phenotype Assignments

CPIC guidelines include recommendations for assigning CYP2C19 (Hicks et al., 2015) and CYP2D6 (Caudle et al., 2019) phenotypes for SSRIs. This includes assignments as ultrarapid, rapid (CYP2C19 only), normal (extensive), intermediate, or poor metabolizers based on the number of increased, normal, decreased, or no function alleles (Supplemental Table 1) (Caudle et al., 2019; Hicks et al., 2015). In brief, individuals with increased function alleles (alone or in combination with normal function alleles) or duplications of functional alleles were defined as ultrarapid metabolizers. Individuals with only normal function alleles were defined as poor metabolizers. Individuals were defined as normal, intermediate, or rapid metabolizers based on a combination of normal function, decreased function, increased function, and/or no function alleles. Of note is that CPIC guidelines provide recommendations for CYP2D6 phenotypes for SSRIs but do not include dosing recommendations for citalopram (Hicks et al., 2015).

As part of the combinatorial pharmacogenomic test performed in the GUIDED trial, phenotypes were assigned for each individual gene. For CYP2C19 and CYP2D6, these phenotypes differed from CPIC guidelines for SSRIs for some allele interpretations (Supplemental Table 1). Specifically, CPIC classified individuals with CYP2C19 *2/*17 as intermediate metabolizers and individuals with CYP2C19 *1/*17 as rapid metabolizers (Hicks et al., 2015). The combinatorial pharmacogenomic test classified individuals with both of these genotypes as normal metabolizers. In addition, the pharmacogenomic test did not include a rapid metabolizer phenotype. For CYP2D6, the combinatorial pharmacogenomic test and CPIC differed in the definitions of poor and ultrarapid metabolizers. Patients with two alleles that have at least moderately decreased activity were called poor metabolizers for the combinatorial pharmacogenomic test. This increased the number of poor metabolizers compared to CPIC guidelines, which only included carriers of two non-functional alleles (Caudle et al., 2019). In addition, the combinatorial pharmacogenomic test included the CYP2D6*2A allele as an increased function allele, which increased the number of ultrarapid metabolizers beyond those with gene duplications.

2.4. Blood Concentrations

Citalopram (R/S-citalopram) and escitalopram (S-citalopram only) blood concentrations were quantified using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The methodologies utilized here could not distinguish between citalopram enantiomers. However, the S-citalopram enantiomer is the active ingredient in both citalopram and escitalopram and the concentration/dose ratio accounts for dosing differences in citalopram and escitalopram treatment. As such, it is unnecessary to distinguish enantiomers for this analysis.

Venous blood samples (3mL) were stored in EDTA vials (BD Vacutainer® EDTA tubes, BD Diagnostics, Franklin Lakes, NJ, USA) and were kept at -80 degrees Celsius until analysis. The LC-MS/MS system utilized Sciex Triple Quadrupole 6500PLUS instrument (AB Sciex LP, Ontario, Canada) coupled with two Shimadzu Nexera X2 LC-30AD pumps (Shimadzu Scientific Instruments, Columbia, MD, USA), and a CTC PAL-xt autosampler (CTC Analytics AG, Zwingen, Switzerland). The drug concentrations from 10μL of lyed whole blood were determined by Precera Bioscience, Inc. (Franklin, TN, USA). The lower limit of quantification was 5ng/mL and the upper limit of quantification was 1000ng/mL. Detection was performed by multiple reaction monitoring (MRM) in the positive mode with the following m/z transition: 325.1 → 262.151. The time since last dose and concomitant medications were uncontrolled.

2.5. Statistical Analysis

Log-transformed concentration/dose ratios based on the dose at the screening visit were used to account for the impact of dosing differences between patients. The concentration/dose ratio also accounts for dosing differences in citalopram and escitalopram treatment. As such, all analyses were performed for the combined cohort of patients taking citalopram or escitalopram (referred to as citalopram throughout). Percent changes in the concentration/dose ratio were reported on a non-log scale. The ability to predict citalopram concentration/dose ratios was assessed for the combinatorial pharmacogenomic test according to the level of gene-drug interactions and metabolic impact based on only the relevant pharmacokinetic genes (CYP2C19, CYP2D6, CYP3A4): significant gene-drug interaction with decreased metabolism, moderate gene-drug interaction with decreased metabolism, no gene-drug interaction, moderate gene-drug interaction with unknown metabolic impact, moderate gene-drug interaction with increased metabolism, significant gene-drug interaction with increased metabolism.

CYP2C19, CYP2D6, and CYP3A4 were also evaluated individually based on phenotype: ultrarapid metabolizer, intermediate metabolizer, normal metabolizer, poor metabolizer. Individual gene assessments for CYP2C19 and CYP2D6 were performed separately using CPIC recommended phenotypes (Caudle et al., 2019; Hicks et al., 2015) and phenotypes assigned during combinatorial pharmacogenomic testing. Individual CYP2C19 genotypes were also evaluated when they differed between CPIC recommendations and the combinatorial pharmacogenomic test.

To test the association between genetic factors and drug metabolism, ANCOVA tests with categorical genetic variables were used to assess the relationship between log-transformed concentration/dose ratios and genotypes, phenotypes, and gene-drug interactions from the combinatorial pharmacogenomic test. To test the linear relationship between recommendations from the pharmacogenomic test and from CPIC with drug metabolism, ANCOVA tests with numerically transformed phenotypes and gene-drug interactions were used to compare the variability explained by the recommendations from CPIC and from the combinatorial pharmacogenomic test as described in the Supplemental Methods.

The relationship between citalopram log-transformed concentration/dose ratios and each of the single gene numeric scores were assessed individually. Then they were assessed in a single ANCOVA with the numeric score for the combinatorial pharmacogenomic test to show the amount of unique variance explained by each variable; that is, accounting for an overlap in recommendations for some patients. Age and smoking status were included as covariates in all analyses. Pairwise comparisons were not conducted for samples sizes of less than 5. Statistical analyses were performed using R, version 3.4.4.

3. Results

3.1. Cohort

In the GUIDED trial, blood level and dose information were available for 96 patients taking citalopram and 95 patients taking escitalopram. Although blood sample collections were not controlled for time
of drug intake, there was a significant relationship between log-transformed concentration and dose ($r^2 = 0.49$, $p < 0.0001$; Table 1). In the combined cohort ($N = 191$), the mean age was 49.7 years, 72% of patients were female, and 95% of patients were non-Hispanic/Latino (Table 1). Overall, 13% of patients had a history of smoking. Smokers and non-smokers had significantly different citalopram blood levels, with a 31% reduction in non-transformed blood levels in smokers ($p = 0.008$). There were no substantial differences in demographics between patients taking citalopram or escitalopram (Table 1).

### 3.2. Validity of Individual Genes and Phenotypes

Fig. 1 shows the concentration/dose ratios for citalopram according to phenotype calls for CYP2C19 made according to the combinatorial pharmacogenomic test and according to CPIC guidelines. Log concentration/dose ratios for citalopram were significantly different across all CYP2C19 phenotype classifications as defined by the pharmacogenomic test ($p = 0.0007$; Fig. 1A). Compared to CYP2C19 normal metabolizers, CYP2C19 ultrarapid metabolizers had a 42% decrease in concentration/dose ratios ($p = 0.04$) and CYP2C19 intermediate metabolizers had a 40% increase in concentration/dose ratios ($p = 0.001$) when the combinatorial pharmacogenomic test phenotype assignments were used.

Log concentration/dose ratios for citalopram also were significantly different across all CYP2C19 phenotype classifications as defined by CPIC ($p = 0.004$; Fig. 1B). CPIC-classified intermediate metabolizers had a 29% increase in concentration/dose compared to CPIC CYP2C19 normal metabolizers ($p = 0.03$). Patients with the CYP2C19*2/*17 genotype were classified as intermediate metabolizers based on CPIC guidelines but were classified as normal metabolizers by the combinatorial pharmacogenomic test. Concentration/dose ratios for these patients did not significantly differ from patients with the normal metabolizer CYP2C19*1/*1 genotype ($p = 0.86$; Supplemental Figure 2). Additionally, there was no difference between concentration/dose ratios for CPIC-classified rapid and normal metabolizers ($p = 0.34$). This is due to the fact that patients with the CYP2C19*1/*17 genotype were considered rapid metabolizers according to CPIC guidelines and there was no significant difference in concentration/dose ratios between CYP2C19*1/*17 and CYP2C19 *1/*1 ($p = 0.33$; Supplemental Figure 2).

CYP2D6 was significant when the phenotypes assigned by the combinatorial pharmacogenomic test were used ($p = 0.01$; Fig. 1C). Similarly, CYP2D6 was significant when the phenotypes were assigned using CPIC guidelines ($p = 0.002$; Fig. 1D). This analysis utilized the recently released updates to CPIC recommendations for CYP2D6 phenotyping (Caudle et al., 2019). In comparison, CYP2D6 was not significant when the previous CPIC recommendations for CYP2D6 phenotypes were utilized ($p = 0.13$; Supplemental Figure 3) (Hicks et al., 2015). There was no difference between CYP3A4 normal and intermediate metabolizers ($p = 0.51$; Fig. 1E). There were no patients identified as CYP3A4 poor metabolizers.

### 3.3. Validity of Combinatorial Pharmacogenomic Test Compared to Individual Genes

There were 17 unique combinations of CYP2C19, CYP2D6, and CYP3A4 phenotypes in this cohort (Table 2). The most common combination was normal metabolizer in all three genes (76/191, 39.8%). A total of 94 (49.2%) patients were poor, intermediate, or ultrarapid metabolizers in CYP2D6 and/or CYP3A4. The individual gene phenotypes for CYP2C19, CYP2D6, and CYP3A4 were factored into the combined phenotypes that informed the final report category and dose recommendations. This is demonstrated in Fig. 2A, where the final combinatorial pharmacogenomic report category is broken down by the contributing phenotypes. Solid color bars in Fig. 2A indicate that the final report category was based on an individual phenotype, where blue and purple bars correspond to genes other than CYP2C19. Overall, 44.5% (85/191) of patients had a variation in only one gene, 75.3% (64/85) of whom had a variation in a gene other than CYP2C19. For example, all patients for whom citalopram was in the “moderate gene-drug interaction with increased metabolism” report category were CYP2D6 ultrarapid metabolizers. Bars with multiple colors in Fig. 2A indicate that the final report category was informed by variations in more than one gene. Overall, 15.7% (30/191) of patients had an alteration in multiple genes. For example, 56.2% (9/16) of patients for whom citalopram was in the “significant gene-drug interaction with decreased metabolism” report category were CYP2C19 intermediate metabolizers and CYP2D6 poor metabolizers.

Citalopram log concentration/dose ratios were significantly different between report categories from the combinatorial pharmacogenomic test ($p = 0.003$; Fig. 2B). Citalopram was categorized as having significant gene-drug interactions with increased metabolism using the combinatorial pharmacogenomic test for 6 patients (3.1%). The concentration/dose ratios were 47% lower in these patients compared to when citalopram had no gene-drug interaction ($p = 0.03$). These patients were all CYP2C19 ultrarapid metabolizers. Citalopram was categorized as having significant gene-drug interactions with decreased metabolism using the combinatorial pharmacogenomic test for 16 (8.4%) patients. The concentration/dose ratios were 54% higher in these patients compared to when citalopram was subject to no gene-drug interactions ($p = 0.005$; Fig. 2B). However, only 3 (19%) of these patients were CYP2C19 poor metabolizers (Fig. 2A). The remaining 13 patients would not have been candidates for dose modification based on CYP2C19 alone but were identified as having significant gene-drug interactions with decreased metabolism based on the combination of CYP2C19, CYP2D6, and CYP3A4 phenotypes (Supplemental Table 2).

Further evaluation of the individual phenotypes that contributed to the final test report category in Fig. 2A demonstrates the frequency with which the addition of CYP2D6 and CYP3A4 resulted in a different combined phenotype compared to CYP2C19 alone. For example, 35 patients were CYP2C19 intermediate metabolizers, which is not associated with a CPIC recommended dose modification (Hicks et al., 2015); however, 11 of these patients (31.4%) were categorized as having significant gene-drug interactions with decreased metabolism according to the pharmacogenomic test, due to combined effects of CYP2C19, CYP2D6, and CYP3A4. In this subset of patients, the concentration/dose ratio was 59% higher than when citalopram had no gene-drug interaction ($p = 0.02$).

Multivariate analysis adjusted for age and smoking status was performed to evaluate the linear relationship between the pharmacogenomic test and individual genes with citalopram metabolism. This analysis showed that CYP2C19 alone was a significant predictor of

### Table 1

Baseline Patient Demographics. Clinical and demographic characteristics for patients taking citalopram or escitalopram at screening in the GUIDED trial.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Citalopram (N=96)</th>
<th>Escitalopram (N=95)</th>
<th>Total (N=191)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>51 (13.4)</td>
<td>48.3 (14.4)</td>
<td>49.7 (14.0)</td>
</tr>
<tr>
<td>Min, Max</td>
<td>19, 73</td>
<td>18, 90</td>
<td>18, 90</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>69 (72%)</td>
<td>68 (72%)</td>
<td>137 (72%)</td>
</tr>
<tr>
<td>Male</td>
<td>27 (28%)</td>
<td>27 (28%)</td>
<td>54 (28%)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>6 (6%)</td>
<td>3 (3%)</td>
<td>9 (5%)</td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
<td>90 (94%)</td>
<td>92 (97%)</td>
<td>182 (95%)</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 (13%)</td>
<td>13 (14%)</td>
<td>25 (13%)</td>
</tr>
<tr>
<td>No</td>
<td>84 (88%)</td>
<td>82 (86%)</td>
<td>166 (87%)</td>
</tr>
</tbody>
</table>
citalopram blood levels (Table 3). This was true when CYP2C19 phenotypes were made using CPIC guidelines (p=0.006) or the pharmacogenomic test phenotype assignments (p=0.01). An evaluation of CYP2D6 alone showed that the gene was not significant when using CPIC phenotyping (p=0.84); however, CYP2D6 was significant when the pharmacogenomic test phenotype assignments were used (p=0.03; Table 3). Analysis of the combinatorial pharmacogenomic test alone showed that the test was a significant predictor of citalopram blood levels (p=0.0003, Table 3). The F statistic for combinatorial pharmacogenomic testing was 1.7 to 2.9-times higher than for the individual genes, showing that the combinatorial pharmacogenomic test explained more variance in citalopram blood levels than the individual genes. The relative improvement of combinatorial pharmacogenomic testing could not be compared to CYP2D6 (CPIC-defined phenotypes) because the F statistic for CYP2D6 was not significantly different from zero.

Multivariate analyses that incorporated the individual genes and the combinatorial pharmacogenomic test were also performed. For both CYP2C19 and CYP2D6, the individual genes were not significant in multivariate analysis that included the pharmacogenomic test (Table 4). This was true both when CPIC phenotypes were used and when the pharmacogenomic test phenotype assignments were used. Conversely, the combinatorial pharmacogenomic test remained highly significant in multivariate analyses that included CYP2C19 and CYP2D6 (Table 4). This demonstrates that, even in the presence of individual gene phenotypes, the combinatorial pharmacogenomic test explained an additional, significant portion of the variance in concentration/dose ratios relative to CYP2C19 or CYP2D6 alone, with substantially higher predictive power than the individual genes.

3.4. Extrapolation to Clinical Testing Population

The distribution of phenotype combinations for CYP2C19, CYP2D6, and CYP3A4 in this cohort was similar to the distribution observed in the clinical testing population for the combinatorial pharmacogenomic

Fig. 1. Citalopram and Escitalopram Blood Levels According to Individual Gene Phenotypes from CPIC and the Combinatorial Pharmacogenomic Test. Boxplots of the log-transformed concentration/dose ratios according to phenotype for CYP2C19, CYP2D6, and CYP3A4. The median (thick horizontal line), interquartile range (box), plus/minus 1.5×interquartile range (vertical lines) are shown, with outliers shown as individual dots. Phenotypes were evaluated based on the assignments from the combinatorial pharmacogenomic test and using CPIC guidelines for SSRIs (only available for CYP2C19 and CYP2D6).
test (Table 2). This indicates that the trends observed in the sub-set of patients from the GUIDED trial are applicable to a broader clinical population. When phenotypes that were observed here and in the clinical population were considered, 70,486 patients in the clinical testing population (6.9%) received clinically actionable pharmacogenomic information related to reduced metabolism for citalopram. Among these patients, 19,345 patients (1.9%) were CYP2C19 poor metabolizers and would have received similar guidance based on CPIC guidelines for citalopram (Hicks et al., 2015). However, the remaining 51,141 (5.0%) patients had a combined phenotype that resulted in significant gene-drug interactions with reduced metabolism and would not have received clinical guidance based on current CPIC guidelines because they were not CYP2C19 poor metabolizers.

This increase in the proportion of patients identified as having clinically actionable gene-drug interactions is largely due to the inclusion of CYP2D6 and CYP3A4 into citalopram guidance as part of this combinatorial pharmacogenomic test. For example, the most frequent phenotype combination that resulted in a report category of “significant gene-drug interaction with reduced metabolism” for citalopram was CYP2C19 intermediate metabolizer, CYP2D6 poor metabolizer, and CYP3A4 normal metabolizer. In the clinical testing population, 38.2% (26,919/70,486) of patients for whom citalopram was in the “significant gene-drug interaction with reduced metabolism” report category had this phenotype combination (Table 2). For these patients, this report classification is a direct reflection of the inclusion of CYP2D6 into citalopram guidance in the combinatorial pharmacogenomic test.

4. Discussion

The use of pharmacogenomics to personalize treatment may improve treatment selection, and ultimately outcomes, for patients with MDD. With the growing number and types of available pharmacogenomic tests, it is important that tests be subjected to robust validation to ensure appropriate clinical use. This is especially important in MDD, where previous studies have shown that pharmacogenomic tests are different from each other and must be evaluated separately (Bousman and Dunlop, 2018). The utility of the combinatorial pharmacogenomic test used here has been previously evaluated (Hall-Flavin et al., 2013; Hall-Flavin et al., 2012; Winner et al., 2013), most recently in the GUIDED trial (Greden et al., 2019). The test has also been previously validated (Altar et al., 2015); however, a direct evaluation of the ability of the test to predict medication blood levels has not been possible due to limited available data. Using data from the GUIDED trial, we were able to validate the combinatorial pharmacogenomic test against citalopram and escitalopram blood levels.

An important consideration in pharmacogenomic testing is genotype and phenotype assignments, as this informs clinical recommendations for dose adjustments or whether a medication with a different mechanism of action should be considered. In this study, the concentration/dose ratios for citalopram and escitalopram were correlated with the CYP2C19 phenotypes recommended by CPIC guidelines and assigned by the combinatorial pharmacogenomic test. However, there was no difference between CYP rapid and normal metabolizers. The genotypes that are classified differently between CPIC and the combinatorial pharmacogenomic test (CYP2C19*2/*17 and CYP2C19*1/*17) were not found to be a significant predictor of concentration/dose ratios. These data demonstrate that the CYP2C19 phenotype calls made by the combinatorial pharmacogenomic test are better predictors of citalopram and escitalopram blood levels than phenotype assignments from CPIC.

Additional considerations in pharmacogenomic testing include which genes to test and the combined effect of relevant phenotypes for each medication. The metabolic pathway for citalopram is largely regulated by CYP2C19 and CPIC guidelines provide clinical recommendations for citalopram prescribing based on CYP2C19 phenotypes alone (Hicks et al., 2015). However, CYP2D6 and CYP3A4 are also involved in citalopram metabolism. Citalopram and escitalopram blood levels were significantly different between the gene-drug interaction categories from the combinatorial pharmacogenomic test based on the combined effect of CYP2C19, CYP2D6, and CYP3A4. Consideration of CYP2C19 alone, as done by CPIC, would miss any relevant contribution from these genes.

Although there are limited data available describing the relationship between es/citalopram blood concentrations and medication efficacy and tolerability, it is commonly implied by CPIC and others that a 50% change in expected blood concentration is clinically actionable. For CYP2C19 poor metabolizers, there is an implied risk of adverse events based on the significant increase in concentration relative to normal metabolizers (Hicks et al., 2015). In addition, there is evidence of es/citalopram dose-related QT prolongation and risk of arrhythmia with a 50% increase in blood levels (Funk and Bostwick, 2013). Based on this direct or implied evidence, CPIC and FDA offer clinical guidance for CYP2C19 poor metabolizers to reduce es/citalopram dose by 50% (Hicks et al., 2015; U.S. Food & Drug Administration, 2019; U.S. Food and Drug Administration, 2017). Furthermore, the significant decrease in concentration for CYP2C19 ultrarapid metabolizers relative | R.C. Shelton, et al. Psychiatry Research 290 (2020) 113017

Table 2
Phenotype Combinations in GUIDED and the Clinical Testing Population. Comparison of phenotypes from the combinatorial pharmacogenomic test in the subset of patients from the GUIDED trial with information on citalopram or escitalopram blood levels and in the clinical testing population.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>GUIDED (N=191)</th>
<th>Clinical Testing Population (N=1,014,267a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19</td>
<td>CYP2D6</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>normal</td>
<td>intermediate</td>
<td>normal</td>
</tr>
<tr>
<td>intermediate</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>normal</td>
<td>poor</td>
<td>normal</td>
</tr>
<tr>
<td>intermediate</td>
<td>poor</td>
<td>normal</td>
</tr>
<tr>
<td>normal</td>
<td>normal</td>
<td>intermediate</td>
</tr>
<tr>
<td>intermediate</td>
<td>intermediate</td>
<td>normal</td>
</tr>
<tr>
<td>ultrarapid</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>intermediate</td>
<td>ultrarapid</td>
<td>normal</td>
</tr>
<tr>
<td>normal</td>
<td>intermediate</td>
<td>intermediate</td>
</tr>
<tr>
<td>normal</td>
<td>poor</td>
<td>intermediate</td>
</tr>
<tr>
<td>poor</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>intermediate</td>
<td>normal</td>
<td>intermediate</td>
</tr>
<tr>
<td>intermediate</td>
<td>intermediate</td>
<td>normal</td>
</tr>
<tr>
<td>poor</td>
<td>intermediate</td>
<td>normal</td>
</tr>
<tr>
<td>ultrarapid</td>
<td>poor</td>
<td>normal</td>
</tr>
</tbody>
</table>

a Only includes patients with phenotype combinations that were observed in this sub-set of patients from the GUIDED trial.
to normal metabolizers is implied to increase the risk of therapy failure (Hicks et al., 2015; Rudberg et al., 2008). As such, CPIC guidelines recommend consideration of an alternative medication that is not primarily metabolized by CYP2C19 for CYP2C19 ultrarapid metabolizers (Hicks et al., 2015).

Although CPIC and FDA guidance for citalopram is based on CYP2C19, consideration of the weighted, combined effect of all relevant phenotypes is critical to making appropriate clinical decisions. For example, the majority of patients for whom citalopram and escitalopram was subject to significant gene-drug interactions with decreased metabolism according to the combinatorial pharmacogenomic test were not CYP2C19 poor metabolizers. Still, there was a >50% increase in citalopram concentration/dose ratios for these patients relative to patients with no gene-drug interactions. Similarly, nearly a third of all CYP2C19 intermediate metabolizers had significant gene-drug interactions for citalopram and escitalopram according to the combinatorial pharmacogenomic test due to the combined effects of CYP2C19, CYP2D6, and CYP3A4, with a 59% increase in concentration/dose ratios. This shows that not only did the combinatorial pharmacogenomic test accurately identify patients appropriate for a dose reduction by existing CPIC and FDA standards, it also identified patients who would not have been identified by CYP2C19 testing alone.

There is very little data directly equating medication blood levels with safety or efficacy. However, Greden et al. demonstrated that patients in the GUIDED trial who remained on medications with significant gene-drug interactions from baseline to week 8 had higher rates of adverse events and poorer outcomes relative to those who changed to medications with no/moderate gene-drug interactions (Greden et al., 2006).

Fig. 2. Examining the Link Between Combined Phenotype and Citalopram and Escitalopram Blood Level Prediction. (A) The combinations of CYP2C19, CYP2D6, and CYP3A4 phenotypes are shown according to the combinatorial pharmacogenomic report category, which was based on the combined phenotype. (B) Boxplot of the log-transformed concentration/dose ratios according to combinatorial pharmacogenomic report category. The median (thick horizontal line), interquartile range (box), plus/minus 1.5xinterquartile range are shown, with outliers shown as individual dots.
Table 3
Evaluation of Individual Genes and the Combinatorial Pharmacogenomic Test to Predict Variance in Citalopram and Escitalopram Blood Levels. Multivariate analyses were adjusted for clinical factors and separately evaluated the ability of each gene and the combinatorial pharmacogenomic test to predict variance in citalopram and escitalopram concentration/dose ratios.

<table>
<thead>
<tr>
<th>Variables included in Model</th>
<th>F Statistic</th>
<th>p-value</th>
<th>Relative Increase in F statistic for the Combinatorial PGx Test&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combinatorial PGx Test</td>
<td>13.3</td>
<td>0.0003</td>
<td>n/a</td>
</tr>
<tr>
<td>CYP2C19 Alone&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.8</td>
<td>0.006</td>
<td>1.7</td>
</tr>
<tr>
<td>CYP2C19 Alone&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.8</td>
<td>0.01</td>
<td>2.0</td>
</tr>
<tr>
<td>CYP2D6 Alone&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.04</td>
<td>0.84</td>
<td>n/a</td>
</tr>
<tr>
<td>CYP2D6 Alone&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.6</td>
<td>0.03</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Abbreviations: PGx, Pharmacogenomic; n/a, not applicable
<sup>a</sup> All models included patient age and smoking status
<sup>b</sup> Relative increase was calculated as (F statistic for Combinatorial PGx Test)/ (F statistic for Individual Gene)
<sup>c</sup> Phenotypes assigned using CPIC guidelines were used
<sup>d</sup> Phenotypes assigned as part of combinatorial pharmacogenomic testing were used
<sup>e</sup> Relative increase could not be evaluated because the F statistic for CYP2D6 was not significantly different from zero

In summary, the ability to accurately predict medication blood levels is an important measure of validity for pharmacogenomics testing. Combinatorial pharmacogenomic testing that incorporates CYP2C19, CYP2D6, and CYP3A4 was a superior predictor of citalopram and escitalopram blood levels compared to individual genes. The ability to accurately predict clinically significant changes (≥ 50%) in medication blood levels is critical to minimizing the risk that a medication will not be safe or effective. With this combinatorial pharmacogenomic test, more patients were identified as appropriate candidates for clinically actionable dosing changes for citalopram and escitalopram from comprehensive and predictive information compared to single-gene testing and CPIC classifications.

Role of the Funding Source

Individuals from Myriad Neuroscience are included as authors and were involved in the study design, data collection and analysis, writing of the report, and decision to submit the article for publication.

CRediT authorship contribution statement

Richard C. Shelton: Conceptualization, Investigation, Writing - review & editing, Visualization, Supervision. Sagar V. Parikh: Conceptualization, Investigation, Writing - review & editing, Visualization. Rebecca A. Law: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. Anthony J. Rothschild: Investigation, Writing - review & editing. Michael E. Thase: Investigation, Writing - review & editing.

Table 4
Evaluation of the Additional Variance in Citalopram and Escitalopram Blood Levels Using the Combinatorial Pharmacogenomic Test to Predict Variance in Citalopram Blood Levels. Multivariate analyses were adjusted for clinical factors and included individual genes and the combinatorial pharmacogenomic test.

<table>
<thead>
<tr>
<th>Variables included in Model&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Individual Gene F Statistic</th>
<th>p-value</th>
<th>Combinatorial PGx Test F Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19&lt;sup&gt;a&lt;/sup&gt; and Combinatorial PGx Test</td>
<td>2.5</td>
<td>0.12</td>
<td>7.7</td>
<td>0.006</td>
</tr>
<tr>
<td>CYP2C19&lt;sup&gt;a&lt;/sup&gt; and Combinatorial PGx Test</td>
<td>0.21</td>
<td>0.65</td>
<td>6.4</td>
<td>0.01</td>
</tr>
<tr>
<td>CYP2D6&lt;sup&gt;a&lt;/sup&gt; and Combinatorial PGx Test</td>
<td>0.97</td>
<td>0.33</td>
<td>14.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>CYP2D6&lt;sup&gt;a&lt;/sup&gt; and Combinatorial PGx Test</td>
<td>0.87</td>
<td>0.35</td>
<td>9.3</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Abbreviations: PGx, Pharmacogenomic
<sup>a</sup> All models included patient age and smoking status
<sup>b</sup> Phenotypes assigned using CPIC guidelines were used
<sup>c</sup> Phenotypes assigned as part of combinatorial pharmacogenomic testing were used
Declaration of Competing Interest

Dr. Shelton has received research funding from Acadia Pharmaceuticals, Alkermes, Inc., Allergan, Assurex Health, Avanir Pharmaceuticals, Cerecor, Inc., Genomind, Intracellular Therapies, Janssen Pharmaceutica, Otsuka Pharmaceuticals, and Takeda Pharmaceuticals. Dr. Shelton has served as a consultant for Acadia Pharmaceuticals, Allergan Inc., Cerecor, Inc., Janssen Pharmaceutica, Lundbeck A/S, Takeda Pharmaceuticals. Dr. Parikh has received research funding from the Ontario Brain Institute, the Canadian Institutes of Health Research, the James and ETHEL Flinn Foundation. Dr. Parikh has served as a consultant for Assurex Health. Dr. Parikh has received honoraria from Mensante Corporation, Takeda, and the Canadian Network for Mood and Anxiety Treatments (CANNAT). Dr. Parikh has equity in Mensante. Ms. Law is employed by Myriad Neuroscience (formerly Assurex Health). Dr. Rothschild has received research support from Allergan, AssureRx, Janssen, the National Institute of Mental Health, Takeda, Eli-Lilly, and Pfizer; Consultant: Alkermes, Eli Lilly and Company, GlaxoSmithKline, Myriad Genetics, Pfizer, SageTherapeutics, and Sanofi-Aventis. Dr. Rothschild receives royalties for the Rothschild Scale for Antidepressant Tachyphylaxis (RSAT)*; Clinical Manual for the Diagnosis and Treatment of Psychotic Depression, American Psychiatric Press, 2009; The Evidence-Based Guide to Antipsychotic Medications, American Psychiatric Press, 2010; The Evidence-Based Guide to Antidepressant Medications, American Psychiatric Press, 2012, and UpToDate®. Dr. Thase has received research support from Assurex Health, Acadia, Agency for Healthcare Research and Quality, Alkermes, Avanir, Forest, Intracellular, Janssen, National Institute of Mental Health, Otsuka, Patient-Centered Outcomes Research Institute, Takeda. Dr. Thase has served as a consultant for Acadia, Akili, Alkermes, Allergan (Forest, Naurex), AstraZeneca, Cerecor, Eli Lilly, Fabre-Kramer, Gerson Lehrman Group, Guidepoint Global, Johnson & Johnson (Janssen, Ortho-McNeil), Lundbeck, MedAvante, Merck, Muksha®, Nestlé (PamLab), Novartis, Otsuka, Pfizer, Shire, Sunovion, Takeda. Dr. Thase receives royalties from American Psychiatric Press, Guilford Publications, Herald House, W.W. Norton & Company, Inc. Dr. Dunlop has received research support from Acadia, NIMH, Sage, Assurex Health, Axsome, Janssen, and Takeda. Dr. Dunlop has served as a consultant for Assurex Health and Aptinyx. Dr. DeBattista has received research support from Acclaim Psychopharmacology, Stanley Medical Research Institute, the National Institute of Mental Health, NeoSync Inc., The Taylor Family Institute for Innovative Psychiatric Research, The August Busch IV Foundation, and the Barnes-Jewish Hospital Foundation. Dr. Conway has received speaking fees from Bristol-Myers Squibb and Otsuka Pharmaceuticals. Dr. Conway has served as a research design consultant for LivaNova. Dr. Conway is a part-time employee of the John Cochran Veterans Administration Hospital in St. Louis. Dr. Forestor has received research funding from the National Institutes of Health, Rogers Family Foundation, Spier Family Foundation, Assurex Health, Eli Lilly, and Biogen. Dr. Forestor has served as a consultant for Biogen. Dr. Macaluso has conducted clinical trials research as principal investigator for Acadia, Alkermes, Allergan, Assurex Health, Eisai, Lundbeck, Janssen, Naurex/Aptinyx, and Neurim; all study contracts and payments were made to Kansas University Medical Cancer Research Institute. Mr. Hain is employed by Myriad Neuroscience (formerly Assurex Health). Dr. Aguilar is employed by Myriad Neuroscience (formerly Assurex Health). Dr. Brown is employed by Myriad Genetics. Dr. Lewis is employed by Myriad Neuroscience (formerly Assurex Health). Dr. Jablonski is employed by Myriad Neuroscience (formerly Assurex Health). Dr. Gredeen has served as a scientific advisor for Janssen Pharmaceutical, Naurex (Allergan) Pharmaceutical, Cerecor Pharmaceutical, NeuralStatm, Sage Therapeutics and Genomi. Dr. Gredeen has received reimbursement as a speaker for Assurex Health in 2014. All work done as an unpaid consultant to Assurex and Myriad.

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Supplementary materials


References

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