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Clinical validation of combinatorial pharmacogenomic testing and single-gene guidelines in predicting psychotropic medication blood levels and clinical outcomes in patients with depression

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ABSTRACT

We evaluated the clinical validity of a combinatorial pharmacogenomic test and single-gene Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines against patient outcomes and medication blood levels to assess their ability to inform prescribing in major depressive disorder (MDD).

This is a secondary analysis of the Genomics Used to Improve DEpression Decisions (GUIDED) randomized-controlled trial, which included patients with a diagnosis of MDD, and ≥1 prior medication failure. The ability to predict increased/decreased medication metabolism was validated against blood levels at screening (adjusted for age, sex, smoking status). The ability of predicted gene-drug interactions (pharmacogenomic test) or therapeutic recommendations (single-gene guidelines) to predict patient outcomes was validated against week 8 outcomes (17-item Hamilton Depression Rating Scale; symptom improvement, response, remission). Analyses were performed for patients taking any eligible medication (outcomes N=1,022, blood levels N=1,034) and the subset taking medications with single-gene guidelines (outcomes N=584, blood levels N=372). The combinatorial pharmacogenomic test was the only significant predictor of patient outcomes. Both the combinatorial pharmacogenomic test and single-gene guidelines were significant predictors of blood levels for all medications when evaluated separately; however, only the combinatorial pharmacogenomic test remained significant when both were included in the multivariate model. There were no substantial differences when all medications were evaluated or for the subset with single-gene guidelines. Overall, this evaluation of clinical validity demonstrates that the combinatorial pharmacogenomic test was a superior predictor of patient outcomes and medication blood levels when compared with guidelines based on individual genes.

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1. Introduction

Clinical guidelines for the treatment of major depressive disorder (MDD) suggest a selective serotonin reuptake inhibitor (SSRI) or serotonin norepinephrine reuptake inhibitor (SNRI) as a first line treatment (Gelenberg et al., 2010, Kennedy et al., 2016). However, the STAR*D trial demonstrated that only 36% of patients achieved remission after first line treatment with the SSRI citalopram, and 16% of patients experienced intolerable side-effects (Rush et al., 2006). Medication failure may be linked to a number of factors that impact efficacy or tolerability. Alterations in medication metabolism (pharmacokinetics) can cause medication blood levels to be outside the therapeutic range. Efficacy may be inhibited if medication blood levels are too low, while side-effects may be associated with elevated blood levels (Fiaturi and Greenblatt, 2019, Florio et al., 2017, Perry et al., 1994). In addition, differential pharmacodynamic effects may decrease or inhibit the biological response to a medication – impacting efficacy – or trigger a different biological response – impacting safety or tolerability (Carr and Pirmohamed, 2018).

Some factors that impact pharmacokinetics and pharmacodynamics may be considered as part of clinical decision-making, such as age, drug-drug interactions, and smoking status (Oliveira et al., 2017, Shelton, 2019, Sultana et al., 2015). Gene-drug interactions are another important consideration, as genetic variations in some pharmacokinetic or pharmacodynamic genes have been shown to impact medication efficacy or safety (Jukić et al., 2018, Mrazek et al., 2011, Phillips et al., 2018, Porcelli et al., 2012). To this end, pharmacogenomic testing has become more common in clinical practice in the U.S. (Dunnenberger et al., 2016, Cavallari et al., 2016) to support a data-driven approach to improve medication selection and, ultimately, patient outcomes for MDD.

There are many available options for pharmacogenomic testing, and it is important that tests be rigorously evaluated to ensure appropriate clinical use and patient management (Mattocks et al., 2010, Pitini et al., 2018, CDC, 2010). Studies can assess the ability of a pharmacogenomic test to improve patient outcomes when used to guide prescribing (clinical utility) and the ability to identify medications unlikely to be safe or effective (clinical validity). Importantly, clinical validation against patient outcomes enables an evaluation of all components of a pharmacogenomic test, including the genes evaluated and the derived phenotype. A more direct method to validate the pharmacokinetic component of a test is to evaluate whether changes in metabolism predicted by a test correlate with changes in medication blood levels.

Another important clinical consideration in the use of pharmacogenomics to guide treatment decisions is how different approaches compare with each other. Clinically available tests range in design (single-gene, multi-gene, weighted combinatorial approach), phenotype assignment, and gene composition (Bousman and Dunlop, 2018, Moyer and Caraballo, 2017). These differences are clinically meaningful, with a recent meta-analysis showing that the clinical utility of three individual pharmacogenomic tests ranged from no clinical benefit to a 70% improvement in outcomes among patients with MDD (Rosenblat et al., 2018). In addition, the Clinical Pharmacogenetics Implementation Consortium (CPIC) (Hicks et al., 2015, Hicks et al., 2017), Dutch Pharmacogenetics Working Group (KNMP, 2020), and other organizations (FDA, 2020, Sangkuhl et al., 2011, Huddart et al., 2020) provide pharmacogenomic guidance for many psychotropic medications commonly prescribed to treat depression. However, these guidelines are often limited to individual pharmacokinetic genes and do not account for the additive or off-setting effects of variation in multiple genes involved in medications' metabolic pathways.

Some pharmacogenomic tests consider the combined impact of variation in multiple genes on a single medication (combinatorial pharmacogenomics). The clinical utility and validity of one combinatorial pharmacogenomic test has been demonstrated in several clinical trials among patients with MDD who have at least one prior medication failure (Altar et al., 2015, Hall-Flavin et al., 2013, Hall-Flavin et al., 2012, Winner et al., 2013), including in the **G**enomics **U**sed to **I**mprove **DE**pression **D**ecisions (GUIDED) large randomized, controlled trial (Greden et al., 2019). This combinatorial pharmacogenomic test included a weighted assessment of multiple pharmacokinetic and pharmacodynamic genes to provide a single, combined phenotype for each medication. The pharmacokinetic component of this combinatorial pharmacogenomic test has also been shown to predict variation in citalopram and escitalopram blood levels for patients enrolled in the GUIDED trial (Shelton et al., 2020).

To more broadly evaluate the clinical validity of this combinatorial pharmacogenomic test, we assessed its ability to predict patient outcomes and medication blood levels for patients enrolled in the GUIDED trial. This included a comparison to CPIC single-gene recommendations based on CYP2C19 or CYP2D6.

2. Methods

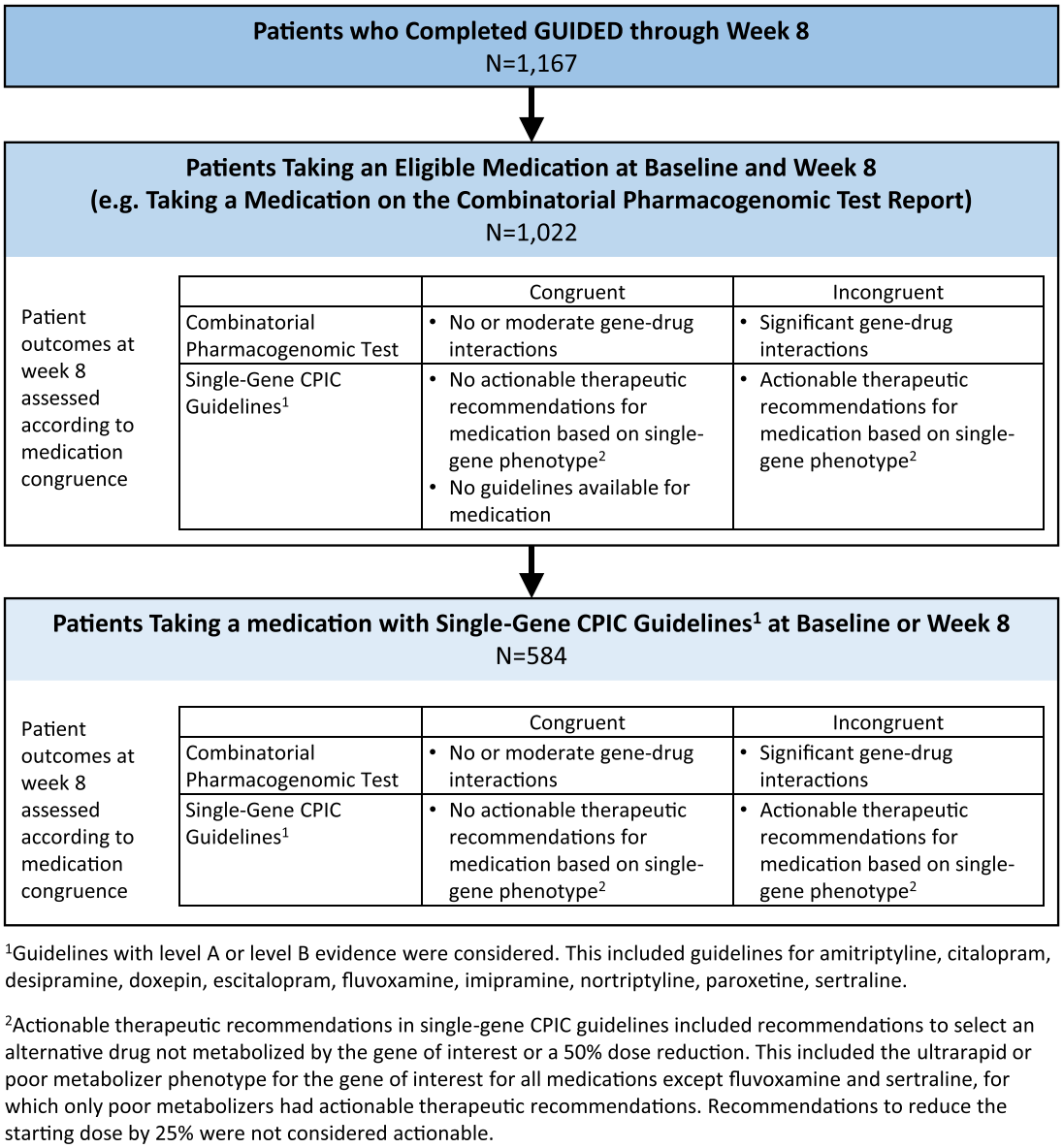
2.1. Cohort

All patients were enrolled in the GUIDED trial and provided written informed consent for participation (NCT02109939). GUIDED was a patient- and rater-blinded, randomized, controlled trial (approved by Copernicus Group independent review board INC1-14-012). The GUIDED trial has been previously described in detail (Greden et al., 2019). GUIDED was conducted across 60 psychiatry and primary care centers in the U.S. between 2014 and 2017. Patients were eligible if they had a diagnosis of MDD, a score of $\geq$11 on the self-rated and clinician-rated 16-item Quick Inventory of Depressive Symptomatology Scale at screening and baseline. An MDD medication history log was completed at the screening visit. To be eligible for the GUIDED study, patients were also required to have had at least one failed psychotropic medication trial (inadequate response after 6 weeks of treatment or intolerable side-effects) within the current depressive episode, though this did not have to be the current medication trial. Patients were randomized between screening and baseline to receive combinatorial pharmacogenomic-guided care or treatment as usual (TAU). Patients and raters were blinded to arm until after week 8. Providers were blinded to pharmacogenomic test reports for patients in TAU until after week 8.

All patients were taking at least one psychotropic medication at screening and patients in both arms were prescribed medication during according to standard care (TAU) or with access to the combinatorial pharmacogenomic test report (intervention arm). Patients were not eligible for inclusion in the GUIDED trial if medication changes were made between screening and baseline; however, medication changes could be made at any point at or after the baseline visit. Because GUIDED was a clinical utility study designed to represent clinical practice, medication dose, medication duration, and concomitant medications were uncontrolled and medication changes were not mandated.

Here we present a secondary analysis of the GUIDED trial to evaluate the clinical validation of the combinatorial pharmacogenomic test and single-gene CPIC guidelines. The ability to predict variation in patient outcomes for both the combinatorial pharmacogenomic test and single-gene CPIC guidelines was validated against patient outcomes at the blinded week 8 endpoint. As an analysis of clinical validity to evaluate whether taking medications with predicted gene-drug interactions were correlated with worse outcomes, study arm was not pertinent. As such, patients were pooled from both arms to evaluate clinical validity based on actual medication changes. Patients were included in the outcomes analysis if they completed the study through week 8 and were taking at least one eligible medication at baseline and week 8 (Fig. 1). Eligible medications were those included on the combinatorial pharmacogenomic test report at the time of analysis (**Supplemental Table 1**).

The ability of the combinatorial pharmacogenomic test and single-gene CPIC guidelines to predict changes in medication metabolism



[1]Guidelines with level A or level B evidence were considered. This included guidelines for amitriptyline, citalopram, desipramine, doxepin, escitalopram, fluvoxamine, imipramine, nortriptyline, paroxetine, sertraline.

[2]Actionable therapeutic recommendations in single-gene CPIC guidelines included recommendations to select an alternative drug not metabolized by the gene of interest or a 50% dose reduction. This included the ultrarapid or poor metabolizer phenotype for the gene of interest for all medications except fluvoxamine and sertraline, for which only poor metabolizers had actionable therapeutic recommendations. Recommendations to reduce the starting dose by 25% were not considered actionable.

Fig. 1. Patient flow chart for outcomes analysis. Definitions for medication congruence according to the combinatorial pharmacogenomic test or single-gene CPIC guidelines are also included.

was validated against medication blood levels from patients enrolled in GUIDED. Patients were included in the blood levels analysis if they met study eligibility criteria at the screening visit and had blood level and dose information for an eligible medication (Supplemental Figure 1). Blood samples were collected at the screening visit. Medication blood levels were not returned to patients or clinicians as part of the GUIDED study.

The subset of patients enrolled in GUIDED who were taking at least one medication in CPIC guidelines with level A (evidence is high or moderate in favor of changing prescribing) or level B (evidence is weak with little conflicting data) evidence at baseline and/or week 8 was also evaluated. Level A or B evidence was required for inclusion in this analysis based on the CPIC definition that this level of evidence supports "prescribing action recommended; alternative therapies or dosing are highly likely to be effective and safe" (CPIC, 2019). A total of 10 eligible medications met this criterion (amitriptyline, citalopram, desipramine, doxepin, escitalopram, fluvoxamine, imipramine, nortriptyline, paroxetine, sertraline) (CPIC, 2020). Details of which medications were included in each analysis are in **Supplemental Table 1**.

2.2. Pharmacogenomic testing

All patients were tested with the GeneSight Psychotropic test (Assurex Health Inc., Mason, OH), which included an evaluation of the genotypes of 59 alleles and variants across 6 pharmacokinetic genes (*CYP1A2*: -3860G>A, -2467T>delT, -739T>G, -729C>T, -163C>A, 125C>G, 558C>A, 2116G>A, 2473G>A, 2499A>T, 3497G>A, 3533G>A, 5090C>T, 5166G>A, 5347C>T; *CYP2C9*: *1, *2, *3, *4, *5, *6; *CYP2C19*: *1, *2, *3, *4, *5, *6, *7, *8, *17; *CYP3A4*: *1, *13, *15A, *22; *CYP2B6*: *1, *4, *6, *9; *CYP2D6*: *1, *2, *2A, *3, *4, *5 (gene deletion), *6, *7, *8, *9, *10, *11, *12, *14, *15, *17, *41, gene duplication) and 2 pharmacodynamic genes (*HTR2A*: -1438 G >A; *SLC6A4*: L, S) (Jablonski et al., 2018).

A combined phenotype was assigned based on a previously validated weighted assessment of individual gene phenotypes (Altar et al., 2015, Assurex Health, 2020). The pharmacokinetic genes were weighted based on the relative contribution of each enzyme to medication metabolism to account for any additive or off-setting effects. This was combined with the pharmacodynamic weights to provide a combined phenotype for

each individual medication. The combined phenotype was used to categorize medications according to the level of gene-drug interactions (no, moderate, or significant gene-drug interactions).

For analyses of single-gene CPIC guidelines, the individual gene phenotypes for CYP2C19 and CYP2D6 (ultra-rapid, rapid, extensive (normal), intermediate, or poor metabolizers) were assigned as described in CPIC guidelines (Caudle et al., 2019, Hicks et al., 2015, Hicks et al., 2017) using genotype data obtained in the course of combinatorial pharmacogenomic testing (**Supplemental Table 2**). No separate single-gene testing was performed, and no single-gene reports were provided.

2.3. Patient outcomes

The primary assessment in the GUIDED trial was the 17-item Hamilton depression scale (HAM-D17) at the blinded week 8 visit. Symptom improvement (percent decrease in HAM-D17 from baseline to week 8), response (≥50% decrease in HAM-D17 from baseline to week 8), and remission (HAM-D17 ≤7 at week 8) were evaluated. The ability of the pharmacogenomic test and single-gene CPIC guidelines to predict variability in patient outcomes at week 8 according to medication congruence was assessed here.

A medication was considered congruent if prescribing that medication aligned with an individual's pharmacogenomic information. The specific definition of medication congruence for the combinatorial pharmacogenomic test and single-gene CPIC guidelines is provided in Fig. 1.For the combinatorial pharmacogenomic test, medications with no or moderate gene-drug interactions were considered congruent and medications with significant gene-drug interactions were considered incongruent. For single-gene CPIC guidelines, medications with no actionable therapeutic recommendations (typically normal and intermediate metabolizers for the gene of interest) were considered congruent and medications with actionable therapeutic recommendations (typically ultrarapid and poor metabolizers for the gene of interest) were considered incongruent. For the analysis of all eligible medications, those that did not have CPIC guidelines with Level A or Level B evidence at the time of this analysis were considered congruent with CPIC guidelines based on the absence of actionable guidance.

Medication congruence was assessed at baseline and week 8. Patients were categorized for analysis according to medication congruence as: 1. change from incongruent at baseline to congruent at week 8, 2. change from congruent at baseline to incongruent at week 8, or 3. no change in medication congruence from baseline to week 8. This analysis was performed for all eligible medications as well as for the subset of medications with CPIC guidelines.

To assess the linear relationship between congruency category and outcomes, congruency category was coded numerically (details in **Supplemental Methods**). The association of the independent variable (the numerically coded congruency status) with each outcome was determined using ANCOVA for both the combinatorial pharmacogenomic test and the single-gene CPIC guidelines individually. Superiority analyses involved including the numerically coded congruency status of both the combinatorial pharmacogenomic test and that of the CPIC guidelines as independent variables in the same model and determining their associations with each outcome using ANCOVA. Baseline HAM-D17 score was included as a covariate in all patient outcome analyses. All p-values were two sided and findings were considered statistically significant if $p<0.05$. All analyses were performed using R.3.6.3.

2.4. Medication blood levels

Medication blood concentrations were quantified as previously described for citalopram and escitalopram (Shelton et al., 2020). In brief, drug concentrations from 10μL of lysed whole blood were determined by Precera Bioscience, Inc. (Franklin, TN, USA) using liquid chromatography with tandem mass spectrometry and medication specific m/z transitions. The time since last dose and concomitant medications were uncontrolled. Blood levels were log base 10 transformed and mean centered at zero to control for medication-specific differences.

The ability of the pharmacogenomic test and CPIC guidelines to predict variation in medication blood levels was assessed. Medications were categorized according to whether the combinatorial pharmacogenomic test or individual gene phenotypes based on CPIC guidelines predicted a substantial change in medication metabolism (**Supplemental Figure 1**). For the combinatorial pharmacogenomic test, this included medications with significant gene-drug interactions predicted to increase or decrease medication metabolism. For single-gene CPIC guidelines, this included medications with actionable therapeutic recommendations based on an ultrarapid or poor metabolizer phenotype in the gene of interest. These changes were categorized as: 1. significant increase in medication metabolism (decrease in medication blood levels expected), 2. significant decrease in medication metabolism (increase in medication blood levels expected), or 3. no or moderate change in medication metabolism (no or minimal change in medication blood levels expected).

To test the statistical differences between test categories and the log-transformed mean-centered concentration/dose ratios, ANCOVA tests were run using categorical variables that represent the three changes in metabolism described above. To test the linear relationship between the three test categories generated by the combinatorial pharmacogenomic test or by CPIC guidelines, ANCOVA tests were run with a numeric variable representing the directional predicted change in metabolism (details in **Supplemental Methods**). The ANCOVAs with numerically-transformed genetic variables were run for both tests individually to find the variance described by each test and another, single ANCOVA was run including both tests to discover the unique variance explained by each test. Age, smoking status, and sex were included as covariates in all analyses. All p-values were two sided and findings were considered statistically significant if $p<0.05$. All analyses were performed using R.3.6.3.

3. Results

3.1. Patient outcomes

A total of 1,022 patients were taking at least one eligible medication at baseline and week 8 (40 total medications). This included a subset of 584 patients who were taking at least one medication with single-gene guidelines at baseline or week 8 (Fig. 1). Baseline demographics and disease characteristics did not vary between the groups (Table 1). Overall patient outcomes at week 8 are summarized in **Supplemental Table 3**. We evaluated whether any clinical or demographic variables were associated with overall outcomes (**Supplemental Table 4**). Only smoking status (response and remission) and age (response; **Supplemental Figure 2**) were significant.

There was a significant correlation between patient outcomes at week 8 and medication congruence with the combinatorial pharmacogenomic test, where congruent medications had no or moderate predicted gene-drug interactions. Patients who changed from incongruent medications at baseline to congruent medications at week 8 experienced the largest improvement in HAM-D17 scores when all medications were considered (35.2%) and within the subset of medications with single-gene CPIC guidelines (36.4%; Fig. 2**A**). Conversely, patients who changed from congruent medications at baseline to incongruent medications at week 8 experienced the smallest improvement in HAM-D17 scores both for all medications (21.0%) and for the subset of medications with single-gene guidelines (17.3%; Fig. 2**A**).

The same correlation trends were observed for response and remission: response and remission rates correlated with changes in medication congruence from baseline to week 8 in the analysis of all medications and the subset of medications with single-gene guidelines

Table 1
Baseline characteristics.

		Patients Included in Outcomes Analysis		Patients Included in Blood Levels Analysis	
		All Medications*	Subset with Single-Gene Guidelines	All Medications*	Subset with Single-Gene Guidelines
Total		1022	584	746	311
Age (years)	Mean (SD)	48.6 (14.4)	48.3 (14.6)	49.5 (14.2)	49.1 (14.5)
Sex, n (%)	Female	720 (70.5%)	407 (69.7%)	549 (73.6%)	232 (74.6%)
	Male	302 (29.5%)	177 (30.3%)	197 (26.4%)	79 (25.4%)
Ethnicity, n (%)	Hispanic or Latino	72 (7.0%)	46 (7.9%)	41 (5.5%)	18 (5.8%)
	Not Hispanic or Latino	950 (93.0%)	538 (92.1%)	705 (94.5%)	293 (94.2%)
Ancestry, n (%)	White	839 (82.1%)	467 (80.0%)	629 (84.3%)	249 (80.1%)
	Black	140 (13.7%)	92 (15.8%)	85 (11.4%)	44 (14.1%)
	Asian	21 (2.1%)	14 (2.4%)	15 (2.0%)	10 (3.2%)
	American Indian or Alaskan Native	6 (0.6%)	2 (0.3%)	4 (0.5%)	0
	Other or Multiple	16 (1.6%)	9 (1.5%)	13 (1.7%)	8 (2.6%)
Smoker, n (%)	Yes	163 (15.9%)	95 (16.3%)	113 (15.1%)	48 (15.4%)
	No	859 (84.1%)	489 (83.7%)	633 (84.9%)	263 (84.6%)
HAM-D17 Scores	Mean (SD)	21.2 (4.2)	21.2 (4.2)	21.0 (4.2)	20.9 (4.3)
Psych Co-morbidities, n (%)	General anxiety Disorder	168 (16.4%)	81 (13.9%)	136 (18.2%)	52 (16.7%)
	Panic disorders/ social phobia	168 (16.4%)	81 (13.9%)	131 (17.6%)	58 (18.6%)
	Post-traumatic stress disorder	53 (5.2%)	26 (4.5%)	44 (5.9%)	19 (6.1%)
Failed Medication Trials	Mean (SD)	3.6 (3.1)	3.4 (2.8)	3.8 (3.2)	3.3 (2.6)
PGx Report Category, n (%)**	No GDI	245 (24.0%)	85 (14.6%)	352 (47.2%)	199 (54.7%)
	Moderate GDI	504 (49.3%)	364 (62.3%)	248 (33.2%)	113 (31.0%)
	Significant GDI	273 (26.7%)	135 (23.1%)	146 (19.6%)	52 (14.3%)

Abbreviations: GDI, Gene-drug interactions; HAM-D17, 17-item Hamilton depression rating scale; PGx, pharmacogenomic
*The list of all eligible medications is provided in Supplemental Table 1
**For patients taking more than one medication, this represents the most severe test report category

(Fig. 2**B** and **C**). The highest response and remission rates were observed for those patients who changed from medications that were incongruent with the combinatorial pharmacogenomic test to congruent medications.

In multivariate analysis adjusted for baseline HAM-D17 score, the combinatorial pharmacogenomic test was a significant predictor of symptom improvement when patients taking any medication included on the test report were evaluated (F=9.3, p=0.002; Table 2). Similarly, the combinatorial pharmacogenomic test was a significant predictor of response (χ^2=4.4, p=0.036) and remission (χ^2=5.0, p=0.025) for patients taking any medication included on the test report (Table 2). When only the subset of medications with single-gene guidelines were evaluated, the combinatorial pharmacogenomic test remained a significant predictor of symptom improvement (F=7.7, p=0.006), response (χ^2=4.0, p=0.046), and remission (χ^2=4.1, p=0.043) at week 8 (Table 2).

In contrast, there was no apparent correlation between patient outcomes at week 8 and congruence with the single-gene guidelines. For the analysis of all medications and the subset of medications with single-gene guidelines, symptom improvement at week 8 ranged from 25% to 35% regardless of the change in congruence with single-gene guidelines (Fig. 2**A**). Similar findings were observed for response and remission, with no correlation between response and remission rates and congruence with single-gene guidelines (Fig. 2**B** and **C**).

Multivariant analysis showed that congruence with single-gene guidelines was not a significant predictor of symptom improvement (F=0.02, p=0.883), response (χ^2=0.02, p=0.892), or remission (χ^2=0.06, p=0.802) at week 8 when patients taking any eligible medication were included (Table 2). Similarly, when only the subset of medications with single-gene guidelines was evaluated, the single-gene guidelines were not a significant predictor of symptom improvement (F=0.1, p=0.754), response (χ^2=0.13, p=0.718), or remission (χ^2=0.03, p=0.873) at week 8 (Table 2).

Multivariate analysis that included both the combinatorial pharmacogenomic test and single-gene guidelines showed that only the combinatorial pharmacogenomic test was significant for the evaluated patient outcomes. This was observed both for the analysis of all medications and the subset of medications with single-gene guidelines (Table 3).

3.2. *Blood levels*

A total of 746 patients taking at least one medication on the combinatorial pharmacogenomic test report at screening had blood level and dose information available. Some patients were taking more than one medication, resulting in a total of 1,034 blood levels assessments for 32 medications (**Supplemental Figure 1**). A subset of 311 patients were taking at least one medication with single-gene guidelines from CPIC (372 blood levels assessments for 10 medications). The baseline demographics and disease characteristics did not vary between the full cohort and the subset of patients taking medications with single-gene guidelines (Table 1). Age, sex, and smoking status were significantly associated with the log10 concentration/dose ratios (p=6.33×10^{-5}, p=2.72×10^{-4}, p=0.04, respectively; **Supplemental Figure 3**).

The log10 concentration/dose ratios were associated with the change in medication metabolism predicted by the combinatorial pharmacogenomic test and by the single-gene guidelines for all medications (Fig. 3**A**). Relative to patients with no/moderate predicted change in metabolism, there was a significant decrease in medication blood levels when the pharmacogenomic test predicted a significant increase in metabolism (B=-0.11, p=0.029). The same trend was observed with the single-gene guidelines, though it did not reach statistical significance (B=-0.09, p=0.068). A significant increase in medication blood levels was observed when a significant decrease in metabolism was predicted by either the combinatorial pharmacogenomic test (B=0.18, p=1.12×10^{-6}) or the single-gene guidelines (B=0.20, p=0.042; Fig. 3**A**). In this case, the relative increase in blood levels was similar for both; however, the combinatorial pharmacogenomic test predicted a significant decrease in metabolism for 98 patients compared to only 13 patients for the single-gene guidelines. This reflects the larger number of medications for which the test provides guidance as well as differences in the single-gene and combinatorial approach.

When the subset of medications with single-gene guidelines was assessed, both the combinatorial pharmacogenomic test and the single-

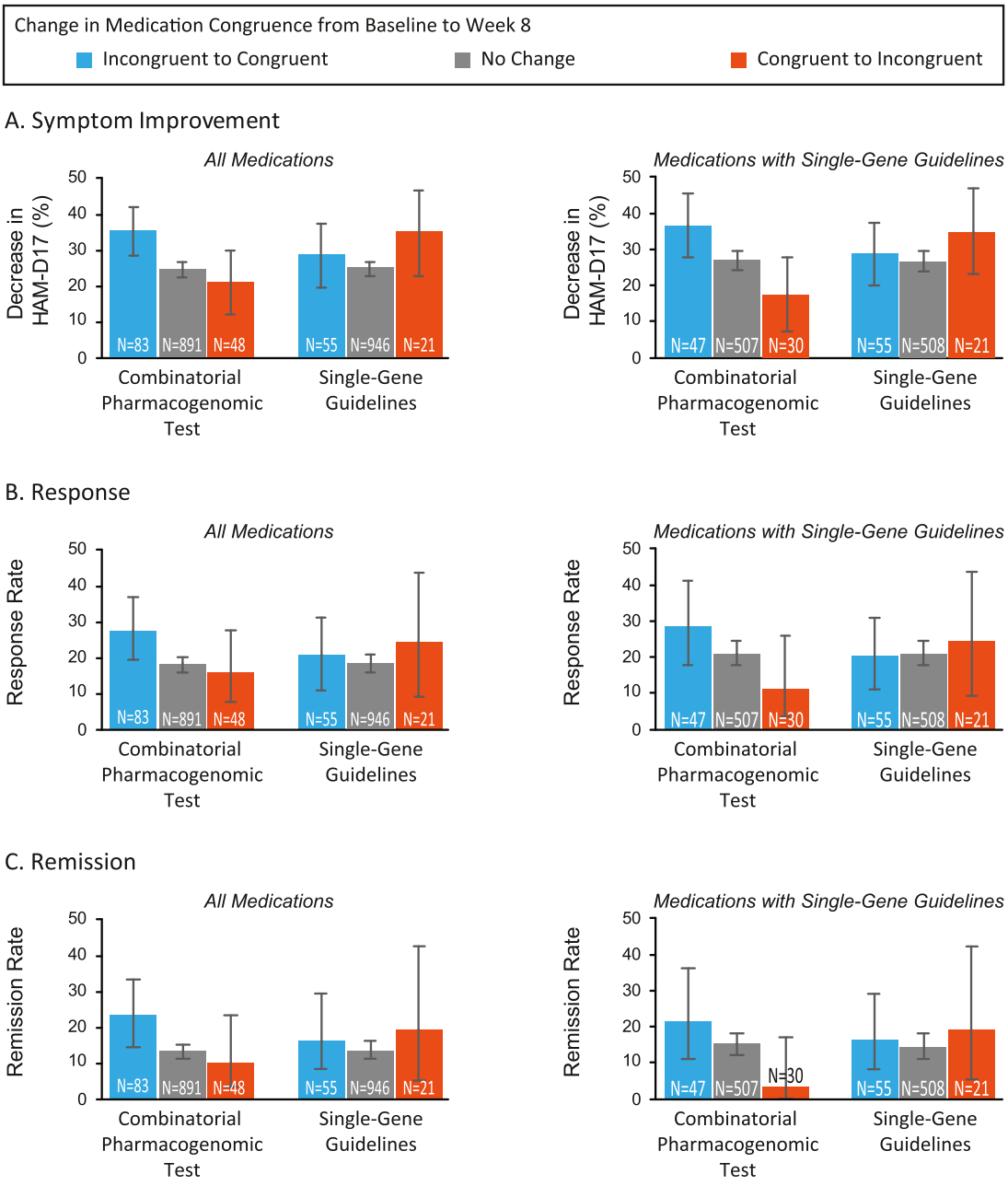


Fig. 2. Patient Outcomes According to Medication Congruence. Symptom improvement, response rate, and remission rate are shown according to medication congruence with the combinatorial pharmacogenomic test or single-gene CPIC guidelines at baseline and week 8. Symptom improvement plots include mean 95% confidence intervals. Response and remission rate plots show exact 95% confidence intervals.

gene guidelines were associated with significant changes in the log10 concentration/dose ratios corresponding with the predicted change in metabolism (Fig. 3**B**). In this subset, the combinatorial pharmacogenomic test predicted that fewer patients would have a significant increase in metabolism than the single-gene guidelines (16 patients versus 56 patients); however, the decrease in blood levels was approximately twice as large for the combinatorial pharmacogenomic test compared to single-gene guidelines. Conversely, the combinatorial pharmacogenomic test predicted that more patients would have a significant decrease in metabolism compared to the single-gene test (36 patients versus 13 patients) with a similar increase in medication blood levels for both (Fig. 3**B**). In this subset analysis, both the combinatorial pharmacogenomic test and single-gene guidelines provide guidance for all included medications and differences reflect the combinatorial versus single-gene approach.

In multivariate analysis adjusted for age, sex and smoking status, both the combinatorial pharmacogenomic test ($F=29.3$, $p=7.55\times10^{-8}$) and single-gene guidelines ($F=6.7$, $p=0.010$) individually were significant predictors of variance in medication blood levels when all medications were considered (Table 4). The F-statistic for the combinatorial pharmacogenomic test was 4-times larger than for single-gene guidance, showing that the pharmacogenomic test predicted more variance in medication blood levels than the single-gene guidelines. Similar trends were observed when the subset of medications with single-gene CPIC guidelines were assessed. Both the combinatorial pharmacogenomic test ($F=31.4$, $p=4.06\times10^{-8}$) and single-gene guidelines ($F=9.9$, $p=0.002$) were significant predictors, with the combinatorial pharmacogenomic test explaining a larger amount of variance in medication blood levels (Table 4).

Multivariate analysis that included both the combinatorial

Table 2
Separate Evaluation of the Combinatorial Pharmacogenomic Test and Single-Gene Guidelines to Predict Patient Outcomes. The individual models shown here included either the combinatorial pharmacogenomic test *or* single-gene CPIC guidelines. All models were adjusted for baseline HAM-D17 score.

Outcome	Combinatorial Pharmacogenomic Test F-Statistic or χ^2	P-Value	Single-Gene Guidelines F-Statistic or χ^2	P-Value
Patients Taking Any Medication on the Combinatorial Pharmacogenomic Test Report (N=1,022)				
Symptom Improvement	9.3	0.002	0.02	0.883
Response	4.4	0.036	0.02	0.892
Remission	5.0	0.025	0.06	0.802
Patients Taking Medications with Single-Gene Guidelines (N=584)				
Symptom Improvement	7.7	0.006	0.1	0.754
Response	4.0	0.046	0.13	0.718
Remission	4.1	0.043	0.03	0.873

Table 3
Combined Evaluation of the Combinatorial Pharmacogenomic Test and Single-Gene Guidelines to Predict Patient Outcomes. The combined models shown here included both the combinatorial pharmacogenomic test and single-gene CPIC guidelines. All models were adjusted for baseline HAM-D17 score.

Outcome	Combinatorial Pharmacogenomic Test F-Statistic or χ^2	P-Value	Single-Gene Guidelines F-Statistic or χ^2	P-Value
Patients Taking Any Medication on the Combinatorial Pharmacogenomic Test Report (N=1022)				
Symptom Improvement	9.4	0.002	0.15	0.695
Response	4.5	0.034	0.099	0.754
Remission	5.0	0.026	0.004	0.947
Patients Taking Medications with Single-Gene CPIC Guidelines (N=584)				
Symptom Improvement	7.9	0.005	0.38	0.539
Response	4.2	0.041	0.35	0.556
Remission	4.1	0.044	0.004	0.947

pharmacogenomic test and single-gene guidelines showed that only the combinatorial pharmacogenomic test remained a significant predictor of blood levels when all medications on the test report were included ($F=25.0$, $p=6.71x10^{-7}$; **Table 4**). This was also observed when the subset of medications with single-gene guidelines was evaluated, with only the combinatorial pharmacogenomic test remaining significant ($F=22.8$, $p=2.64x10^{-6}$; **Table 4**).

4. Discussion

As the field of pharmacogenomics has evolved, multiple methods have been developed to identify gene-drug interactions and then translate that information into clinical practice. Many professional societies and other agencies include guidance for a limited number of medications (FDA, 2020, CPIC, 2019, KNMP, 2020). Although this guidance largely focuses on individual genes, the FDA cites the need to evaluate "the combined effects of different genotypes" in their guidance on clinical drug interaction studies (U.S. Department of Health and Human Services Food and Drug Administration, 2020). In addition, some testing laboratories employ a combinatorial approach with a weighted assessment of multiple genes. In order to ensure appropriate clinical care, pharmacogenomic tests must have a robust body of evidence demonstrating their clinical validity and clinical utility (Mattocks et al., 2010, Pitini et al., 2018, CDC, 2010). This is especially important with the growing number of available tests and the lack of consensus about what genes to include and what approach to employ (single-gene versus combinatorial).

We evaluated patient outcomes at week 8 according to whether medications at baseline and week 8 aligned with guidance from the combinatorial pharmacogenomic test or with single-gene guidelines. This provides a direct assessment of the clinical validity for improving patient outcomes by following the provided guidance. In this analysis, only the combinatorial pharmacogenomic test was significantly correlated with all evaluated patient outcomes (symptom improvement, response, and remission). These findings support that improved patient outcomes were achieved when prescribing was consistent with the gene-drug interactions predicted by the combinatorial pharmacogenomic test. The similar performance of the combinatorial pharmacogenomic test for all medications and the subset of medications with single-gene guidelines indicates that the pharmacogenomic test provides more meaningful guidance for a larger group of medications than is addressed in current guidelines. In contrast, therapeutic recommendations in single-gene guidelines had no association with patient outcomes. This highlights a clinically relevant benefit of combinatorial pharmacogenomic testing to improve outcomes among this difficult to treat population of patients with depression who have prior medication failures.

Variation in medication blood levels has had implied impacts on medication efficacy and, ultimately, patient outcomes. Because many professional societies focus on the impact of pharmacokinetic genes, there has been a significant focus on the ability to predict gene-drug interactions that have a clinically meaningful impact on medication blood levels. Combinatorial pharmacogenomic testing integrates an assessment of multiple pharmacokinetic genes weighted based on the metabolic pathway for an individual medication. As outlined by the PharmGKB metabolic pathways for individual medications (Huddart et al., 2020, Sangkuhl et al., 2011), the impact of multiple enzymes in a medication's metabolic pathway provides a more complete picture of overall metabolism. The combinatorial pharmacogenomic test evaluated here was a significant predictor of variation in medication blood levels for the 32 eligible medications with blood level information from the GUIDED study. These findings support that gene-drug interactions predicted by the combined, weighted assessment of multiple genes accurately predicted decreased blood levels due to increased metabolism, and vice versa. This included the prediction of a 50% variation in medication blood levels when significant gene-drug interactions were predicted. This magnitude of variation in medication blood levels is largely considered clinically actionable based on implications of medication efficacy or safety.

We also evaluated the ability of single-gene guidelines from CPIC to predict variation in medication blood levels. CPIC provides actionable guidelines for 10 antidepressants based on alterations in CYP2C19 or CYP2D6. For some medications, such as amitriptyline, CPIC guidelines do include a joint consideration of both CYP2C19 and CYP2D6 (Hicks et al., 2017). However, this guidance does not account for differences in the efficacy of secondary pathways or the relative importance of CYP2C19 and CYP2D6 in amitriptyline metabolism as identified by PharmGKB and others (PharmGKB, 2019, Breyer-Pfaff, 2004).

Although, single-gene guidelines were a significant predictor of variation in medication blood levels when assessed alone, multivariate analysis that included both methods (single-gene guidelines and combinatorial pharmacogenomic testing) showed that only the combinatorial pharmacogenomic test remained a significant predictor of medication blood levels. This indicates that the combinatorial pharmacogenomic test accounts for all medication blood level variance addressed by single-gene guidelines and adds significant, independent information. This finding was true when all eligible medications were considered as well as when the subset of medications with single-gene guidelines were considered. In addition, the similar performance in the analysis of all medications and the subset with guidelines highlights the fact that the addition of medications without single-gene guidelines did not dilute the performance of the combinatorial pharmacogenomic test. Collectively, these findings support that although existing CPIC

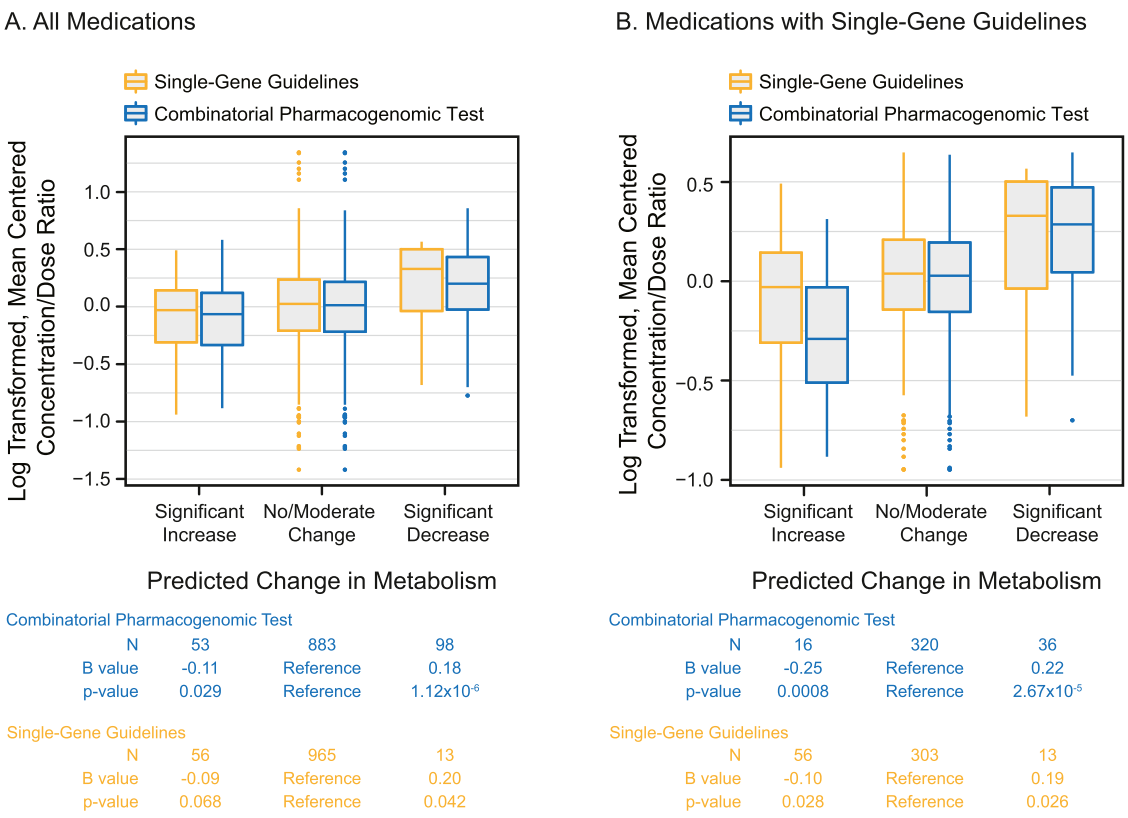


Fig. 3. Medication Blood Levels According to Predicted Changes in Metabolism. Boxplots of the log10-transformed, mean-centered concentration/dose ratios according predicted changes in metabolism based on single-gene CPIC guidelines or the combinatorial pharmacogenomic test. The median (thick horizontal line), interquartile range (box), plus/minus 1.5xinterquartile range (vertical lines) are shown, with outliers shown as individual dots. B values correspond to the effect size relative to the reference, representing the effect of the predicted change in metabolism on the concentration/dose ratio produced by a linear regression model that corrects for age, sex, and smoking status.

Table 4
Evaluation of the Combinatorial Pharmacogenomic Test and Single-Gene Guidelines to Predict Variance in Medication Blood Levels. Analyses were performed for the combinatorial pharmacogenomic test alone, single-gene CPIC guidelines alone, and for the combinatorial pharmacogenomic test and single-gene CPIC guidelines together. All models were adjusted for age, sex and smoking status. Individual models included either the combinatorial pharmacogenomic test *or* single-gene guidelines. Combined models included both the combinatorial pharmacogenomic test and single-gene guidelines.

	Combinatorial Pharmacogenomic Test		Single-Gene Guidelines	
Model	F-Statistic	P-Value	F-Statistic	P-Value
Blood Levels for Patients Taking Any Medication on the Combinatorial Pharmacogenomic Test Report (N=1,034)				
Individual Models	29.3	$7.55x10^{-8}$	6.7	0.010
Combined Model	25.0	$6.71x10^{-7}$	2.5	0.116
Blood Levels for Patients Taking Medications with Single-Gene Guidelines (N=372)				
Individual Models	31.4	$4.06x10^{-8}$	9.9	0.002
Combined Model	22.8	$2.64x10^{-6}$	1.7	0.190

guidelines related to CYP2C19 and CYP2D6 provide some information about changes in metabolism, combinatorial pharmacogenomic testing (e.g. a weighted assessment of multiple genes associated with the pharmacokinetics for a specific medication) was a superior assessment.

There were some limitations of this analysis. GUIDED was not a controlled pharmacokinetics study. As such, blood levels were not controlled for time of intake or medication adherence. Further, patient inclusion was not limited by concomitant medications (e.g., medications known to impact drug metabolism). In addition, blood levels were only collected at screening and could not be directly correlated with outcomes or adverse events.

In summary, this evaluation of clinical validity shows that only the combinatorial pharmacogenomic test was significantly associated with improved patient outcomes. In addition, the combinatorial pharmacogenomic test was a superior predictor of medication blood levels across a larger group of medications relative to guidelines focused on only CYP2C19 and CYP2D6. These data suggest that the superior ability of combinatorial pharmacogenetic testing to predict variation in medication blood levels may result in improved patient outcomes. Given the variety of approaches employed in pharmacogenomic testing between professional societies, government associations, and testing laboratories, it is critical that tests have robust evidence of validity and utility. Collectively, the data presented here provide compelling evidence of the clinical validity of the combinatorial pharmacogenomic test in a large cohort of patients with MDD who have at least one prior medication failure.

Declaration of Competing Interest

Dr. Rothschild has received research support from Allergan, Assurex Health, Janssen, the National Institute of Mental Health, Otsuka, Eli-Lilly, Pfizer, and the Irving S. and Betty Brudnick Endowed Chair in Psychiatry. Dr. Rothschild is a consultant for GlaxoSmithKline, Janssen, Myriad Genetics, and SageTherapeutics. 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. Parikh** has

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Individuals from Myriad Neuroscience and Myriad Genetics, Inc. 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.

Supplementary materials

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