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Clinical utility of pharmacogenetics-guided treatment of depression and anxiety

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ABSTRACT

Major depressive disorder (MDD) and generalized anxiety disorder (GAD) are associated with significant morbidity/mortality risk. Prolonged episodes increase impact on quality of life, risk for suicide, and harbor greater societal costs. Current management is inadequate as half of individuals do not respond to first-line therapies. Identification of an optimal treatment may hinge on exploiting interindividual genetic variability, which—in combination with other extraneous factors—is associated with heterogeneous antidepressant response. We evaluated the use of Genecept testing in an open-label trial of 468 patients, focusing on the methylenetetrahydrofolate reductase (*MTHFR*) and serotonin transporter (*SLC6A4*) genes and evaluating their plausibility as putative predictors of MDD/GAD treatment outcome. After receiving genotyping, 50.6% of clinicians made assay-congruent changes to treatment. This yielded a selective serotonin reuptake inhibitor (SSRI) discontinuation rate of 19.0% in patients with a risk *SLC6A4* genotype, and, an acute folate derivative addition rate of 41.8% in *MTHFR* risk allele carriers. After 8 weeks of treatment, patients with a risk *MTHFR* genotype that were treated with assay-guided treatment regimens—as compared to those that were not—demonstrated a greater reduction in Quick Inventory of Depressive Symptoms (QIDS-SR) and Undersøgelser (UKU) scores, and an increased quality of life score (Q-LES-Q-SF). *SLC6A4* risk patients who adhered to assay-guided treatment achieved a greater reduction in QIDS-SR and UKU scores and a statistically significant increase in Q-LES-SF scores, *versus* those that did not. Results support the utility of genotyping in the treatment of MDD/GAD and propose *SLC6A4* and *MTHFR* as biological predictors of treatment outcome.

Introduction

Varied drug response has long been recognized in the treatment of major depressive disorder (MDD) and generalized anxiety disorder (GAD). Of the approximately 3–7% of patients in the United States affected, nearly 50% fail to respond to first-line treatment regimens [1–3]. Influences such as environmental exposures, nutritional status, co-morbidities, severity of disease, and concomitant medications help to explain some unpredictable drug responses. However, genome wide association studies (GWAS) propose that genetic variation alone accounts for 42% of varied antidepressant response [4–9]. This presents an auspicious principle on which to base the delivery of personalized medicine.

Several classes of antidepressant medication have been shown to benefit individuals with MDD–selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs) are among the most widely used and well-studied [10]. SSRIs are currently the most commonly prescribed drug class for MDD treatment, though even within-class response to treatment varies considerably between patients and identification of the most appropriate medication is a continued challenge [11,12]. Antidepressant response does not show a classic Mendelian model of inheritance, but instead, a moderate number of loci—each with a small effect size—are proposed to be involved in response [13]. Pharmacogenetics research is actively attempting to link antidepressant treatment response to a portfolio of polymorphisms that correspond to brain circuitry/plasticity [14]. Theoretically, this will allow the personalization of MDD/GAD treatment by minimizing the use of 'trial-and-error' treatment. It is important to note that MDD and GAD likely have an overlapping genetic etiology [15]. This, in combination with high rates of comorbidity and ambiguity of onset, provide a strong case for treating MDD and GAD in the same manner [16–18].

Much research has focused on pharmacokinetic factors, specifically

Abbreviations: MDD, major depressive disorder; GAD, generalized anxiety disorder; QIDS-SR, Quick Inventory of Depressive Symptoms; Q-LES-Q-SF, Quality of Life Enjoyment and Satisfaction Questionnaire Short Form; UKU, Udvalg for Kliniske Undersøgelser Side Effect Rating Scale

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the liver metabolizing Cytochrome P450 (CYP450) superfamily of enzymes that is responsible for the oxidation of antidepressant medication. The *CYP450* genes mainly involved in antidepressant metabolism encode isoforms in the CYP2D6, CYP2C19, CYP2C9, and CYP3A4/5 enzymes. These genes are highly polymorphic and result in normal (EM-extensive metabolizer), abnormal (IM-intermediate, UM-ultra-rapid, and PM-poor metabolizer), or aberrant metabolizer phenotypes [19]. Metabolizer status was demonstrated to be associated with antidepressant pharmacokinetics in a number of studies and variable rates of metabolism have been shown to increase the potential for adverse drug effects and reduce rates of compliance in patients taking antidepressants [20]. Despite the fact that the Food and Drug Administration (FDA) has incorporated genetic testing information into the labeling of nineteen antidepressants, pharmacogenetics testing has not been incorporated into treatment guidelines because of a gap in consistent evidence linking testing to clinical outcomes, i.e. clinical utility [21,22].

More recently, candidate gene studies aimed to detect an association between antidepressant response and catecholaminergic genes. The serotonin transporter gene (*SLC6A4*) is an obvious candidate as the serotonin transporter is the primary site of SSRI action [20]. A common 44-bp insertion/deletion polymorphism—referred to as the long (L_A) or short (S) forms of *SLC6A4*, respectively—was shown to impact transcription and ultimately levels of the serotonin transporter [23]. In addition to the S allele, a variant of the L allele in which the adenine (L_A) has been replaced with guanine (L_G), is also associated with reduced serotonin transporter levels and is functionally comparable to the S allele [24,25]. Patients with variant transporter translation exhibit lower remission rates, increased side effects, and intolerance to SSRIs [26]. Further, the S and L_G alleles of *SLC6A4* have been correlated with depression and anxiety-related symptomology and antidepressant response in numerous studies [23,27–31]. For example, a study of 36 patients suggested an association between fluoxetine response and *SLC6A4* genotype and identified S allele carriers as being at risk for developing insomnia and agitation with treatment [32]. Poor response to citalopram was associated with S/S genotypes [33]. Finally, a recent meta-analysis reported an associative model between SSRI response (OR: 1.58; 95% CI: 1.16–2.16, p = .004) and remission (OR: 1.53; 95% CI: 1.14–2.04, p = .004) in Caucasian *SLC6A4* L_A allele carriers [34].

Methylenetetrahydrofolate reductase (MTHFR) is a rate limiting enzyme in the production of L-methylfolate; L-methylfolate is a critical regulatory molecule in the synthesis of monoamine neurotransmitters associated with mood regulation (i.e. dopamine, norepinephrine, and serotonin) [35]. Although the *MTHFR* gene has not been directly linked to antidepressant response, numerous studies have identified a modest association with depression symptomology and disease [36–39]. Two *MTHFR* polymorphisms, C677T and A1298C, result in diminished enzyme activity, and moreover the T allele of C677T has been associated with decreased L-methylfolate levels [40]. As MDD has an established association with low serum folate levels [38,40], folate augmentation in patients unresponsive to SSRI/SNRI treatment improved patient adherence [41,42]. Further, a meta-analysis of 15,315 participants reported a significant relationship between folate status and depression (OR: 1.55; 95% CI: 1.26–1.91; p < .05) [38]. L-Methylfolate has been efficaciously used as an adjunctive therapy for patients with inadequate or poor SSRI response and was shown to improve adherence and decrease cost of care [41,42]. Therefore, an indirect link to *MTHFR* polymorphisms and antidepressant treatment outcome is likely.

Pharmacogenetic testing has the potential to reduce antidepressant discontinuation due to adverse events and increase overall efficacy. Ideally, pharmacogenetics would inform individualized decisions by identifying DNA variants that predict outcomes. Promising evidence, including increased quality of life and reduced depression/anxiety scores, were reported with assay-guided treatment of MDD patients [43]. To date, only two randomized controlled trials (RCTs) have been conducted to investigate the impact of pharmacogenetics testing on antidepressant outcome [44,45]; one reported a two-fold increase in depression symptom relief while the other reported a greater chance of disease remission with pharmacogenetics testing usage (2.52-fold; 95% CI: 1.71–3.73; Z: 4.66, p < .0001). Despite these promising results, a systematic review of guided-treatment *versus* usual care deemed current evidence inconclusive and condemns the widespread use of pharmacogenetics testing at the onset of MDD treatment [46].

The utility of pharmacogenetics testing remains unclear though—in part because of a relative lack of RCTs and an abundance of small cohort, statistically under powered studies—because the method by which pharmacogenetic testing influences clinical treatment is not well-established [47,48]. We therefore examined data from a naturalistic study of a commercial pharmacogenetic test to characterize how likely clinicians were to make test-concordant medication changes, and whether outcomes improved when assay-congruent medication regimens were implemented. As a means to thoroughly address this gap in the literature and realistically assess the utility of pharmacogenetics testing in the treatment of MDD/GAD, we aimed to (i) determine if pharmacogenetics testing influenced clinician decision-making and prescribing patterns, and, (ii) identify putative genetic predictors of treatment outcome.

Materials and methods

Patient cohort

A post-hoc analysis was performed on genotyping and outcomes data from a previously conducted clinical trial (ClinicalTrails.gov: NCT01507155) [43]. Original study design stipulated that adult patients must be diagnosed with a psychiatric disorder by a mental health care specialist who, for the purpose of this trial, ordered Genecept pharmacogenetic testing (n = 1024). Study participants were required to have the ability to complete electronic informed consents and be able to comprehend/complete online questionnaires. For the present study, primary diagnoses other than MDD (n = 297) or GAD (n = 171) were excluded. There were 468 patients in total evaluated in this analysis. Each of the 468 patients were evaluated by the clinician-reported outcome scales, but just 86 (18.4%) patients completed all of the patient-reported outcome questionnaires at each time point. Only patients that had full and complete data sets were included in this study (observed cases analysis). Clinicians were defined as mental health care professionals with the ability to prescribe medication and order a pharmacogenetics test, i.e. possession of a valid national provider identifier (NPI) number and prescribing privileges.

Genecept reporting

All clinicians were given information about trial design and goals and were willing clinicial participants. Clinicians were provided with a Genecept Report (Genomind King of Prussia, PA, USA) for each study participant at a one-month follow-up visit (to baseline visit). The report included genotyping results for ten genes (*SLC6A4*, *MTHFR*, 5HT2C, *COMT*, *CACNA1C*, *DRD2*, *ANK3*, *CYP2D6*, *CYP2C19*, *CYP3A4*) and details the implications of each genetic result on the use of a variety of FDA approved medications in the following classes: antidepressants, mood stabilizers/anticonvulsants, typical antipsychotics, atypical antipsychotics, anxiolytics, stimulants, nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics.

Genotypic processing

Of the ten genes that the Genecept Assay addressed, *SLC6A4* and *MTHFR* were the genes directly associated with antidepressant treatment. As the assay was designed to assess how one would respond to a variety of drug classes, limiting the evaluation to just genes that effect response to antidepressants was adequate for the purposes of this

Table 1
Participant demographics and allele frequencies.

	SLC6A4 WT L_A/L_A (n = 125)	*SLC6A4* Risk L_A/L_G, L_A/S, L_G/L_G, L_G/S, S/S (n = 334)	p-value	*MTHFR* WT CC (n = 195)	*MTHFR* Risk C/T, T/T (n = 272)	p-value
Mean Age (years)	43.94	41.38	.094	43.43	41.05	.055
Gender (Male: Female)[A]	31:90	105:203	.090	53:130	89:170	.232
No. Previous Trials	3.49	3.22	.423	3.27	3.36	.975
No. Medication Changes	165	396	.289	180	392	< **.0001***
No. Congruent Treatment Regimens[B]	74	157	**.016***	121	115	< **.0001***
CGI-S (Baseline)	2.50	2.35	.640	3.62	3.67	.968
CGI-I (Follow Up)	3.62	3.65	.188	2.46	2.35	.207

[A]Study participants that listed gender as 'unknown' are as follows: *SLC6A4* WT = 4; *SLC6A4* Risk = 21; *MTHFR* WT = 12; *MTHFR* Risk = 13. [B]Number of participants who were prescribed treatment regimens congruent with Genecept Pharmacogenetics Testing. Mann-Whitney U Test used to compare age, previous trials, medication changes, CGI-S, and CGI-I; two-sample Z-Test used to compare gender and congruency proportions; *p-value < .05. All numbers are mean values unless otherwise noted.

analysis. Patients were binned into groups based on their *SLC6A4* genotypes and then again separately by their *MTHFR* genotypes. For both genes, bins were dependent on phenotypic association with SSRI response. For *SLC6A4*, bins consisted of either 'wild-type (WT)' (n = 125) or 'at risk for poor SSRI response' (n = 334) genotypes (*SLC6A4* WT: L_A/L_A; *SLC6A4* risk: L_G/L_G, L_G/S, L_G/L_A, L_A/S, S/S). Similarly, for *MTHFR,* bins consisted of either 'WT' (n = 195) or 'at risk for poor treatment outcome' (n = 272) genotypes (*MTHFR* WT: C/C; *MTHFR* risk: C/T, T/T). See Table 1.

Outcome measures

Outcomes data were collected at three separate time points: *baseline* (standard MDD/GAD treatment), *1-month* from assay results received, and *3-month* follow-up. Subjects were stratified into groups based on whether their clinician's treatment choice (between receiving assay results and 3-month follow-up) was congruent with Genecept assay results. Three patient-reported scales were utilized to measure outcomes: The Quick Inventory of Depressive Symptoms (QIDS-SR) [49], the Quality of Life Enjoyment and Satisfaction Questionnaire Short Form (Q-LES-Q-SF) [50], and the Udvalg for Kliniske Undersøgelser Side Effect Rating Scale (UKU) [51]. Each of the questionnaires was made available and administered through an online portal. The QIDS-SR scale ranges from 0 to 27, with 0 indicating no depression. The Q-LES-Q-SF scale ranges from 0 to 100, with higher scores indicating a greater satisfaction with life. The UKU scale measures the degree of side effects and ranges from 0 to 100, with 0 indicating low side effects. Clinicians simultaneously reported a Clinical Global Impression (CGI) score for severity at baseline (CGI-S) and for improvement at 3-months (CGI-I).

Tracking clinical treatment regimens

Clinicians reported primary diagnoses for each patient at the baseline time point. Clinicians simultaneously logged each patient's treatment regimen. Changes to treatment regimens were reported in electronic surveys administered at each subsequent time point. Time point data was compared and treatment choices were considered congruent if they (i) discontinued an assay-indicated risk medication, or, (ii) initiated a medication indicated as a therapeutic option. Incongruent decisions included continuing or initiating treatment using an assay-indicated risk medication or failing to initiate an indicated therapeutic option. In many cases, the clinicians made more than a single change to their patient's treatment. Collectively, these changes were considered congruent only if each of the changes met the criteria listed above for congruency. If at least one of the changes included administering or continuing use of a treatment deemed as a risk by the assay, then the treatment regimen was considered incongruent with the report.

Statistical analysis

Two-way repeated-measures analysis of variance (ANOVA) models, with Tukey post-hoc tests, were used to analyze the relationship between risk genotypes, assay congruency, and treatment outcome. This analysis evaluated whether changes to outcome measures—QIDS-SR, Q-LES-Q-SF, or UKU—were the result of independent variables of treatment action (relative to genotype) and of time, or to their interaction. Analyses were performed using R version 3.3.2 (http://cran.r-project.org). A two-sample Z-test was used to detect a difference between congruency proportion measures. Additionally, two major comparisons were investigated. Of the subjects carrying the *MTHFR* risk allele, a comparison between folate-supplemented SSRI/SNRI treatment and SSRI/SNRI treatment without folate supplementation was made. For participants with a risk genotype for *SLC6A4*, those taking an SSRI were compared to those alternatively prescribed a miscellaneous antidepressant/SNRI. Miscellaneous antidepressants included bupropion, mirtazapine, and nefazodone.

Results

Data from 468 participants was binned based on *SLC6A4* and *MTHFR* genotypes. Participant demographics are summarized in Table 1. The mean participant age is 42-years. There is no significant difference in mean age, gender ratio, or number of previous medications/changes between genotype groups. Similarly, CGI-S and CGI-I scores were comparable across all genotypes. Clinicians made assay-congruent changes in drug regimens significantly more in the treatment of *SLC6A4* risk patients than in *SLC6A4* WT patients ($p < .016$). Similarly, clinicians made assay-congruent changes in drug regimens significantly more in the treatment of *MTHFR* risk patients than in *MTHFR* WT patients ($p < .0001$).

Pharmacogenetics testing warranted assay-congruent changes to MDD/GAD treatment regimens

After receiving assay results, 83.6% (391) of clinicians made a change to their patient's treatment regimen while 16.5% (77) did not. Throughout the duration of the 3-month trial, 50.6% of patients were maintained on treatment regimens congruent with assay results (Fig. 1A). Omitting drug additions and discontinuation, the distribution of prescribed medications over the 3-month trial was examined.

At baseline, SSRIs were the most prescribed treatment regimen (43.8%) as compared to SNRIs (22.0%), miscellaneous antidepressants (19.4%), and folate derivatives (8.97%). The rate of SSRI prescription decreased by 17.6% over the 3-month trial period, while the rate of folate derivative supplementation increased 431% (Fig. 1B).

Drug additions and discontinuations were considered a drastic clinician-initiated changes in treatment and were separately evaluated

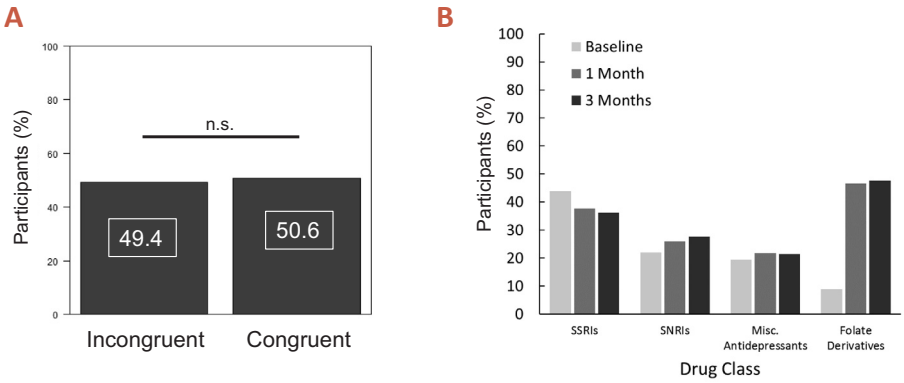

Fig. 1. Genecept pharmacogenetics testing guides changes in prescribed treatment regimens of MDD/GAD patients. (A) Percentage of prescribed treatment regimens that are congruent/not congruent with Genecept pharmacogenetics testing recommendations. Incongruent prescription regimens were defined as the addition/discontinuation of at least one treatment that countered assay results. The two proportions are not significantly different based on results of a Z-test (p = .984). (B) Percentage of participants prescribed SSRIs, SNRIs, misc. antidepressants, or folate derivatives at baseline (light gray), 1-month- assay results received (gray), or 3-month follow-up (dark gray).

Table 2
Proportion of discontinued and/or added SSRI and folate derivative treatment regimens. All numbers are percentages (%). Bolded percentages represent the largest proportion in each action group.

Action	Baseline > 1-Month	1-Month > 3-Months
SSRI		
Discontinue	**19.0**	10.8
Add	3.80	4.11
Folate Derivative		
Discontinue	4.76	2.29
Add	**41.8**	4.00

(Table 2). The most frequently discontinued class of medication was SSRIs, with a 19.0% discontinuation rate from baseline to assay-results received (1-month) and an additional 10.8% discontinuation rate from 1-month to 3-month follow-ups. Of these, 79.5% of patients had an *SLC6A4* risk genotype and were at greater risk for SSRI intolerance/poor response. Overwhelmingly, folate derivatives were the most frequently added drug class with a 41.8% addition rate from baseline to assay-results received (1-month) and an additional 4.00% addition rate from 1-month to 3-month follow-ups. Of these, 95.5% of patients were *MTHFR* T allele carriers (i.e. *MTHFR* risk genotype).

SLC6A4 genotype and MTHFR genotype serve as putative biological predictors of antidepressant outcome

As a means to further assess the influence of individual genotypes on clinician action, treatment regimen comparisons amongst groups were conducted at the 1-month time point. Clinician actions were quantified in reference to SSRIs and stratified into *start* (begin SSRI when previously not taking), *stop* (remove SSRI from the treatment regimen), and *continue* (treatment with SSRI as before). Of the 125 *SLC6A4* WT patients, clinicians stopped prescribing SSRIs to patients 6.40% of the time, though, of the 334 *SLC6A4* risk patients, clinicians stopped prescribing 9.28% of the time (Fig. 2). Similarly, a lower percentage (1.50%) of *SLC6A4* risk patients began an SSRI treatment regimen than *SLC6A4* WT patients (3.20%). The possession of a T allele distinguishes *MTHFR* genotypes into a *MTHFR* WT—normal—or *MTHFR* risk genotype (i.e. patients that demonstrate an improved SSRI/SNRI outcome with folate derivative supplementation). Therefore, focus on clinician action pertaining to folate derivatives identified a greater proportion of *MTHFR* risk patients (62.5%; n = 272) who began folate derivative supplementation than *MTHFR* WT patients (4.10%; n = 195). Clinicians stopped folate derivative supplementation for 0% of *MTHFR* risk patients, but continued supplementation for 11.4%; this is in contrast to the 4.62% of *MTHFR* WT patients. In sum, 95.5% of patients that began a folate derivative supplement had a *MTHFR* risk genotype.

To gauge the clinical utility of Genecept pharmacogenetics testing, *SLC6A4* and *MTHFR* genotypes were correlated to patient-reported treatment outcomes over the three aforementioned time points (Fig. 3). Outcomes between carriers of the *MTHFR* risk genotypes that initiated/maintained folate derivative supplementation were compared to those that did not receive supplementation. The percent reduction of both the QIDS-SR (Fig. 3A) and UKU (Fig. 3C) scores was modestly higher in the group that received folate derivatives (QIDS-SR: 34.0% *versus* 20.1%; UKU: 37.3% *versus* 21.2%, respectively). These associations, however, failed to reach statistical significance (QIDS-SR: $F(1,90) = 0.88$, $p = .35$; UKU: $F(1,90) = 1.34$, $p = .25$, respectively). Patients in the *MTHFR* risk group that did not receive a folate derivative showed a slight improvement in Q-LES-Q-SF scores (15.9% *versus* 10.6%), though this also failed to reach statistical significance ($F(1,90) = 0.78$, $p = .38$) (Fig. 3B).

Similarly, when assessing *SLC6A4* risk patients treated with SSRIs *versus* those that received SNRIs/miscellaneous antidepressants, no significant difference between groups was found when comparing QIDS-SR or UKU scores (QIDS-SR: 21.5% *versus* 33.6%; UKU: 27.0% *versus* 32.04%)) QIDS-SR: $F(1,90) = 2.27$, $p = 0.14$; UKU: $F(1,90) = 0.47$, $p = 0.49$) (Fig. 3D,F). Interestingly, a significant increase in quality of life, as per the Q-LES-Q-SF score, was detected in *SLC6A4* risk patients treated with SNRI/miscellaneous antidepressants *versus* SSRIs (17.5% *versus* 3.97%; $F(1,90) = 12.2$, $p = .00076$, Odds Ratio = 3.55, Cohen's d = 0.6989) (Fig. 3E).

Discussion

Study results suggest that receiving Genecept pharmacogenetic testing improves MDD/GAD patient outcomes as measured through patient-reported scales for depression, side effect severity, and quality of life. Intriguingly, improvements in quality of life scores were significantly higher in *SLC6A4* risk patients receiving SNRI/miscellaneous antidepressants *versus SCL6A4* risk patients receiving SSRIs (Fig. 3). Amongst the 468 patients tested, 50.6% of their clinicians prescribed assay-congruent treatment regimens, decreased SSRI prescription rate by 17.6%, and, increased SNRI prescription rate by 25.2%, miscellaneous antidepressant rate by 9.89%, and folate derivative prescription rate by 431% (Figs. 1 and 2). At baseline, we did not find a significant difference between participant demographics or clinical global impression scores for *SLC6A4* WT *versus* risk patients, nor *MTHFR* WT *versus* risk patients (Table 1). Over the course of the 3-month clinical trial, SSRIs represented the most discontinued drug class though discontinuation was higher in patients with *SLC6A4* risk genotypes. Similarly, folate derivatives represented the most added drug class throughout the trial though addition was higher in patients with risk *MTHFR* risk genotypes (Table 2).

The question of whether genetic markers can help predict response to medication has major implications for personalizing treatment for depression and anxiety. A genome-wide complex trait analysis estimated the contribution of common polymorphisms to antidepressant response to be 42% (SE = 0.18; p = .009) in patients with MDD [4].

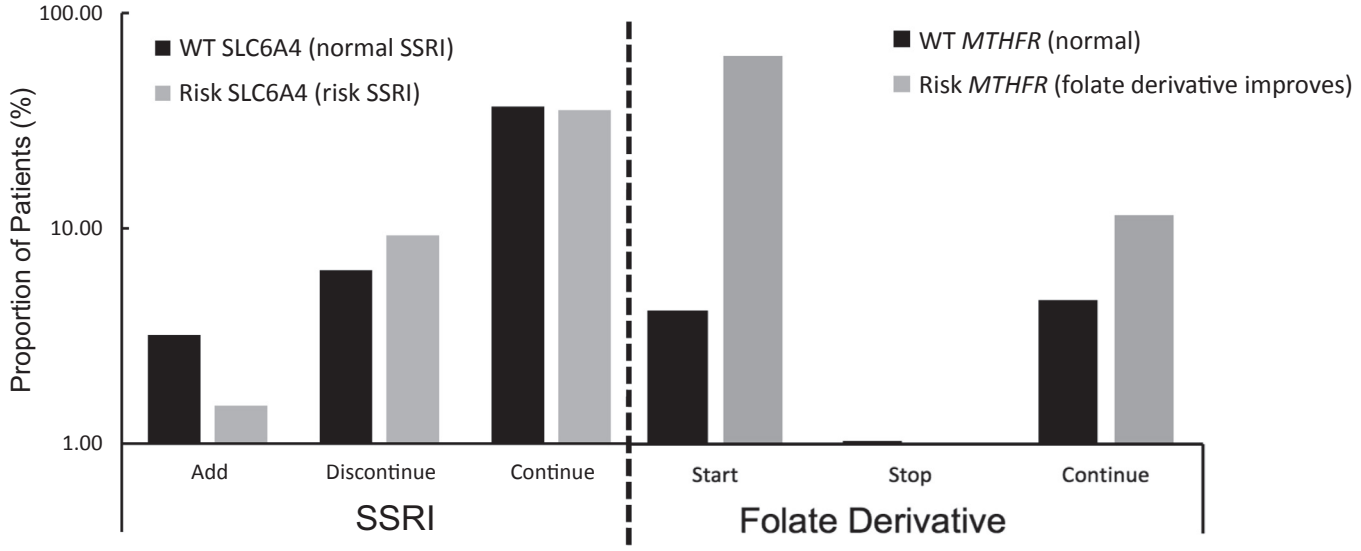

Fig. 2. *SLC6A4* and *MTHFR* genotyping predict prescription regimens in MDD/GAD patients. Percentage of prescription treatment regimens categorized by clinician action of either *start* (first prescribed), *stop* (ceased prescribing), or *continue* (treatment as before). Results are from 1-month-assay results received. Light blue bars represent WT *SLC6A4* normal response SSRI genotypes. Blue bars represent *SLC6A4* genotypes attributed to risk of poor response with SSRIs. Light green bars represent WT *MTHFR* normal response genotypes. Green bars represent *MTHFR* improved response to folate supplementation genotypes.

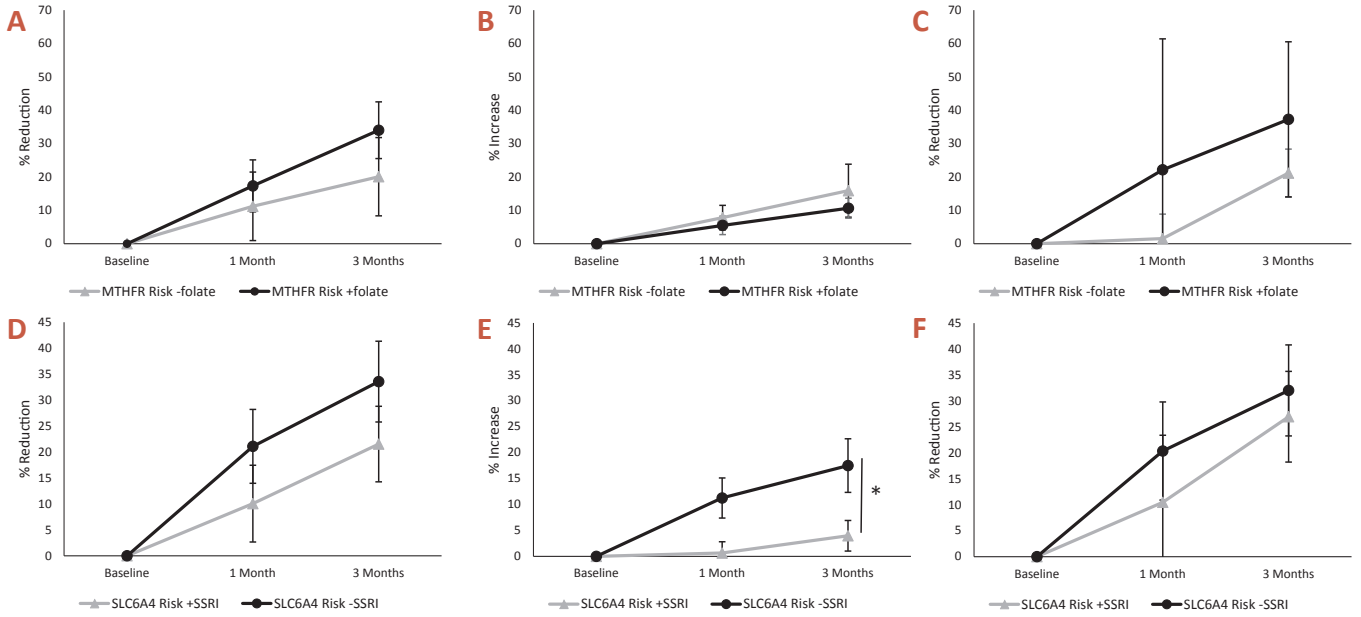

Fig. 3. Prescription regimens congruent with Genecept pharmacogenetics testing resulted in improved patient outcomes in patients with risk genotypes. Reduction/increase in (A) quick inventory of depression symptoms (QIDS-SR) (B) quality of life (Q-LES-Q-SF) and (C) Undersøgelser side effect (UKU) scores from baseline to 1-month (Genecept results received), and from baseline to 3-month follow-up. Black line represents *MTHFR* T allele carriers that are expected to have greater symptom reduction with folate supplementation of SSRI or SNRI treatment and are supplementing with a folate derivative (N = 9). Gray line represents *MTHFR* T allele carriers that are expected to have greater symptom reduction with folate supplementation of SSRI or SNRI treatment and are not supplementing with a folate derivative (N = 25). Reduction/increase in (D) quick inventory of depression symptoms (QIDS-SR) (E) quality of life (Q-LES-Q-SF) and (F) Undersøgelser side effect (UKU) scores from baseline to 1-month (Genecept results received), and from baseline to 3-month follow-ups. Black line represents *SLC6A4* risk allele carriers that are at risk for poor SSRI response and are not being treated with a SSRI (N = 19). Gray line represents *SLC6A4* risk allele carriers that are at risk for poor SSRI response and are being treated with a SSRI (N = 28). Statistical significance determined using repeated-measures analysis of variance (ANOVA) models and subsequent Tukey post hoc tests. Black vertical line represents *p-value $< .05$. Comparisons not shown are not significant (n.s.). Error bars represent standard error.

Further supporting the principle that antidepressant response is a complex trait with substantial genetic influence is the low clinical efficacy of antidepressant medication– one in three patients does not fully recover from depression even after several treatment trials [52,53]. However, two separate genome-wide analyses (GWA) focusing on pharmacodynamic genes did not detect a single SNP significantly associated with SSRI response [5,6]. This suggests that the effects of genetic variation on antidepressant response are multifaceted and warrant the use of observational trials to simultaneously evaluate genetic components that, in combination, wield an implication about the larger whole.

Genecept pharmacogenetic testing evaluates multiple pharmacodynamic and pharmacokinetic genes relevant to psychiatric treatment outcome and is therefore a plausible avenue to personalize MDD drug treatment for an individual. In a retrospective study of health claims data, authors report enhanced medication adherence and outpatient cost savings with Genecept assay-guided treatment [54]. A subsequent naturalistic study reported improved patient outcomes with Genecept

assay-guided treatment for a mixed-diagnosis patient cohort [43]. However, these previous studies failed to isolate collective biomarkers specific to the clinical utility of MDD/GAD treatment.

Emphasis on patients with genetic predispositions to treatment resistant depression (TRD) is of grave importance and the most profound indications for the use of pharmacogenetic testing are in TRD cases [14,46]. For this group of patients it is particularly important to determine the supposed adequacy and outcome of treatment; 10–30% of the depressive population receiving treatment do not respond adequately to antidepressants [55,56]. This is especially troublesome as the burden of TRD is substantial: chronic unremitting depressive disease confers personal suffering and disability and carries an associated employee healthcare cost double that of nonresistant employees [57]. Patients with *MTHFR* risk genotypes represent a population of patients at risk for poor SSRI response. This is supported by the concept that deficient folate levels impact catecholaminergic pathways [35] and can correspondingly be seen in our reported evidence: *MTHFR* risk patients demonstrated a significantly increased number of medication changes (i.e. failed medication trials) (Table 1). Fundamentally, *SLC6A4* risk patients also represent a patient population at risk for poor SSRI response due to the mode of action of SSRIs and their reliance on adequate serotonin transporter levels [33].

As in most studies including patients with MDD, heterogeneity between individual patients limits the power of genetic analyses. Discordances in cohort samples, including differences in diagnosis criteria and treatment regimens, further increase this phenotypic heterogeneity. Thus, the overall cohort is likely to include subgroups with unique susceptibility factors for clinical depression contingent on unidentified biological factors and environmental aspects not controlled for (*e.g.* chaotic home-life or dietary choices). Additional study limitations include a lack of ancestral data and limited statistical power due to a relatively undersized patient cohort. As the power to detect associations was low, corrections of multiple testing were not performed, as these would increase the probability of producing false negative results. Lastly, hard endpoints on which to base clinical success, such as suicide or hospitalization, are infrequently encountered in psychiatric prospective studies of short follow-up duration [58]. In an attempt to rectify this constraint, data were collected at three separate time points over a 3-month time period, each taking into account surrogate hard-endpoints more appropriate for a short-term trial. Future studies should aim to perform longer-term evaluations, incorporate hard-endpoints, and report data on 'time to effectiveness' to gauge the duration of time to treatment success/failure for antidepressant medications.

An important issue often unaddressed by studies evaluating pharmacogenetic testing is the issue of whether reported genetic information leads to better clinical outcomes (i.e. having clinical utility). The present study sought to address this by determining whether testing warranted not only changes in clinician prescribing behavior, but specifically assay-congruent changes. Moreover, this study identified distinct genetic components relevant to MDD/GAD treatment outcome—as opposed to a grouping of known pharmaco-psychiatric genes—and reports *SLC6A4* and *MTHFR* as putative biological predictors of quality of life.

Conclusions

This study reports *SLC6A4* and *MTHFR* gene-outcome associations and correlates the use of Genecept pharmacogenetics testing to improved clinical outcomes in the treatment of MDD/GAD. This information is especially pertinent to the treatment of patients with *SLC6A4* and *MTHFR* risk genotypes, as these patients are prone to treatment-resistance and are at a greater risk for of experiencing primary medication failure. When treating chronic depression patients, clinicians strive to manage symptomology and increase overall quality of life as full disease remission is rare [56]. Results demonstrate significantly improved quality of life scores in risk patients treated with an assay-congruent regimen.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pmip.2017.11.001.

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