Phenotypic Assessment of Drug Metabolic Pathways and P-Glycoprotein in Patients Treated With Antidepressants in an Ambulatory Setting

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ABSTRACT

Objective: Drug-metabolizing enzymes (DMEs), such as cytochrome P450 (CYP) enzymes, and transporters have emerged as major determinants of variability in drug metabolism and response. This study investigated the association between CYP and P-glycoprotein activities and plasma antidepressant concentration in an outpatient clinical setting. Secondary outcomes were antidepressant efficacy and tolerance. We also describe phenotypes in patients treated with antidepressants and evaluate the tolerance of a minimally invasive phenotyping approach.

Methods: From January 2015 to August 2015, 64 patients on a stable antidepressant regimen underwent a simultaneous assessment of steady-state antidepressant concentration and DME (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A) and P-glycoprotein transporter activity using a cocktail phenotyping approach. Psychiatric diagnoses were in accordance with *DSM-5*.

Results: We observed a high proportion of subjects (> 20%) with reduced activity of CYP2C19, CYP2D6, CYP3A4, and P-glycoprotein. As expected, higher CYP activity for major metabolic pathways was associated with lower concentration of the parent compound (CYP2C19 and escitalopram, $P = .025$; CYP2D6 and fluoxetine, $P < .001$; CYP2C19 and sertraline, $P = .001$), higher concentration of the metabolite (CYP2D6 and *O*-desmethylvenlafaxine, $P = .007$), and higher metabolite-to–parent drug ratio (CYP2C19 and escitalopram, $P = .03$; CYP2D6 and fluoxetine, $P < .001$; CYP2C19 and sertraline, $P = .048$; CYP2B6 and sertraline, $P = .006$). Phenotyping also highlighted the relevance of a minor metabolic pathway for venlafaxine (CYP3A4). Insufficient response and adverse reactions to antidepressants were not significantly associated with plasma antidepressant concentration, DME, or P-glycoprotein activity. Tolerance of the phenotypic test in ambulatory settings was found to be excellent.

Conclusions: The phenotypic assessment of DMEs and a transporter is a valuable, well-tolerated method to explore the interindividual variability in drug disposition in clinical settings. The method is able to account for the inhibitory activity of antidepressants themselves and for polymedication, which is frequent in this population of refractory depressed patients.

Trial Registration: ClinicalTrials.gov identifier: NCT02438072

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The identification of reliable markers of interindividual variability in the response to specific antidepressants is an important issue for patients diagnosed with major depressive disorder, as 20% to 45% of patients do not achieve the goal of antidepressant treatment.[1,2]

In the absence of clearly identified drug action mechanism markers, the drug-metabolizing enzymes (DMEs), such as cytochrome P450 (CYP) enzymes, and transporters have emerged as major explorable determinants of variability in drug disposition and response.[3] Recent studies have highlighted an increasing interest in developing a personalized psychopharmacotherapy based on metabolic and transport activity data; this could reduce treatment failure, provide cost savings, and improve treatment adherence in psychiatric patient populations.[3,4]

Genotyping has been largely used for the prediction of CYP enzyme family and P-glycoprotein (P-gp) transporter activity.[5,6] Undoubtedly, genetic variations of CYP and P-gp activity contribute to the variability of antidepressant pharmacokinetics (PK), efficacy, and adverse drug reactions (ADRs). However, variability, for the majority of DMEs, cannot be accounted for solely by genotype.[7] For some enzymes and transporters, the relationship between genotype and phenotype has not been established, and genotyping does not allow measuring the influence of environmental factors such as drug-drug interactions, which are frequent in the psychiatric population.[8] Phenotyping provides an in vivo measure of drug metabolism and transport at a given time. In addition, phenotyping presents a straightforward means to explore the impact of medication itself on current DME activity, information that would help in the choice of additional treatments and dose individualization.

Phenotyping methods have recently been developed.[9,10] Following the administration of a low-dose probe-drug cocktail, assessment of the ratio of metabolite-to-probe concentrations in 1- and 3-point dried blood spot samples allows direct and simultaneous measures of the activity of several

Clinical Points

- The development of personalized medicine in psychiatry is challenging. Drug-metabolizing enzymes have emerged as major explorable determinants of variability in drug disposition and response.
- Phenotypic assessment of drug-metabolizing enzymes is a valuable, well-tolerated method by which to explore interindividual variability in drug disposition in clinical settings.
- Integrated into a multimodal approach, phenotyping undoubtedly has a place in achieving personalized care of patients being treated for refractory depression.

DMEs and P-gp.[9] Such a combined method has never been used routinely in clinical settings.[11] The first aim of this study was therefore to systematically and prospectively explore the association between the activities of several DMEs and P-gp, assessed by a phenotypic method, and plasma antidepressant concentrations in a naturalistic clinical setting in a population of depressed patients. Antidepressant efficacy and tolerance were also recorded at the time of phenotyping. The second aim was to describe the distribution of DME phenotypes in patients currently being treated with antidepressants. The third aim was to evaluate the tolerance of a minimally invasive phenotypic determination method in ambulatory settings.

METHODS

Subjects

This cross-sectional naturalistic study was conducted in the Division of Psychiatric Specialties, Department of Mental Health and Psychiatry, Geneva University Hospitals (Geneva, Switzerland), a center specialized in the management of refractory mood disorders.

Adult outpatients aged from 18 to 70 years and currently treated with antidepressants were invited to participate. Patients were required to be on a stable psychotropic medication regimen for at least 6 weeks. Patients were not included if any of the following conditions was present: (1) renal impairment (creatinine clearance below 60 mL/min) or hepatic impairment (aspartate amino transferase or alanine amino transferase above 3-fold the upper limit of the reference range), (2) documented sensitivity to any of the phenotypic cocktail substrate probes, (3) electrocardiogram showing long QT interval (> 0.46 seconds), and (4) current pregnancy or intent to get pregnant.

Patients were included after giving their written informed consent and were assessed from January 2015 to August 2015. This study was approved by the local ethics committee of the Canton of Geneva (ID: 14–051) and the Swiss Agency for Therapeutic Products (Swissmedic) and registered at ClinicalTrials.gov (NCT02438072).

Study Design

The definitions of major and minor metabolic pathways of antidepressants were based on Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry (Arbeitsgemeinschaft Neuropsychopharmakologie und Pharmakopsychiatrie [AGNP]).[12]

Assessment of the efficacy and ADRs of antidepressants. The referring psychiatrist was asked to record diagnoses in accordance with the *DSM-5*.[13] The Montgomery-Asberg Depression Rating Scale (MADRS), a 10-item questionnaire, was used to measure the severity of depression.[14] Remission was defined by a score lower than or equal to 10, and insufficient response, by a score greater than or equal to 20, that is, persistent moderate to severe symptoms usually leading to a change of medication after an adequate trial.[15] ADRs were systematically elicited from the patients and recorded.

Phenotypic assessment. The visit took place on an outpatient basis after an overnight fast. Subjects were required to abstain from caffeine-containing products (coffee, tea, chocolate, energy drinks) for at least 24 hours before the study session. Upon the patient's arrival, a pill count was performed to assess compliance by comparing the number of doses observed in the drug boxes with the number of doses that were expected to remain if full compliance had been maintained since inclusion. The consumption of St John's wort products and/or grapefruit within the 2 weeks preceding the study was recorded. Comedications, including somatic, were classified as inhibitors or inducers based on a tool developed by the Division of Clinical Pharmacology and Toxicology, Geneva University Hospitals.[8,16,17] At arrival, 20–30 hours after the last intake of antidepressant, venous blood samples were collected for the measurement of plasma antidepressant concentration. Subjects then received oral capsules containing low-dose cocktails of the probes. The first contained a combination of substances (caffeine 50 mg, flurbiprofen 10 mg, dextromethorphan 10 mg, midazolam 1 mg), and the second and third capsules contained either fexofenadine 25 mg or bupropion 20 mg. An additional capsule of omeprazole 10 mg was given. Capillary blood samples were obtained by pricking the fingertip using contact-activated lancets (BD Microtainer; BD, Franklin Lakes, New Jersey) at 2, 3, and 6 hours following cocktail administration. At each time point, 3 blood drops were dropped onto a blotting paper (903 S&S, Whatman; Sigma-Aldrich, St Louis, Missouri).

Analytic Method

The quantification of the administered drugs and their metabolites was performed using a previously validated HPLC-MS/MS method.[9] The DME activities were assessed by specific metabolite/probe concentration ratios (metabolic ratios [MRs]) determined in the sample taken 2 hours after cocktail administration.

Activities of P-gp and DMEs, as measured by AUC_{0-6h} and MRs, respectively, were classified as induced/ultrarapid metabolism/activity, normal/extensive metabolism, intermediate metabolism (for CYP2C19 and CYP2D6 only), or inhibited/poor metabolism, based on ranges previously determined in a healthy population.[9] Steady-state

Table 1. Sociodemographic and Clinical Characteristics (N = 64)

Characteristic	Values[a]
Age, median (range), y	49 (22–70)
Female	40 (62.5)
Smoker	35 (54.7)
Psychiatric diagnosis	
Recurrent depressive disorder (F33)	27 (42.2)
Depressive episode (F32)	20 (31.3)
Bipolar affective disorder (F31)	17 (26.6)
Comorbid diagnoses	
Attention-deficit/hyperactivity disorder	9 (14.1)
Borderline personality disorder	7 (10.9)
Anxiety disorder	3 (4.7)
Current depressive episode started more than 12 months ago	48 (75.0)
Currently in remission (MADRS ≤ 10)	24 (37.5)
Adverse events documented	17 (26.6)
No comedication (psychotropic or other)	9 (14.1)
Psychotropic comedication	
Tranquilizers and hypnotics	36 (56.3)
Antipsychotics	25 (39.1)
Other antidepressants (trazodone)	13 (20.3)
Lithium	5 (7.8)

[a]Expressed as n (%) unless otherwise noted.
Abbreviation: MADRS = Montgomery-Asberg Depression Rating Scale.

plasma concentrations of antidepressants and their active metabolites were quantified using liquid chromatography coupled to tandem mass spectrometry in the routine settings.

Data Analysis

Categorical and continuous variables were described using frequency tables (n, %) and median (range), respectively. Spearman rank correlation coefficients were used to test the associations between DME and P-gp phenotyping indices, antidepressant MRs (metabolite/parent concentration ratio, corrected for molar concentrations), and antidepressant concentrations [normalized for daily dose, assuming linear PK, ie, normalized concentration = measured concentration × (median dose/actual dose)]. Because we tested a limited number of a priori hypotheses about major and minor metabolic pathways, no adjustment for multiple testing was performed. Correlations were not tested if n < 5 (mirtazapine, paroxetine). Fisher exact tests were used to compare proportions of patients with insufficient response and ADRs in independent groups. Factors associated with insufficient response and ADRs were further investigated using multivariate binary logistic regression models and tested using Wald tests. Statistics were computed using SPSS version 22 (IBM Corporation; Armonk, New York). All tests were 2-tailed, with significance level at .05.

RESULTS

Patient Characteristics

A total of 67 patients were enrolled in the study. Three dropped out: 1 decided to stop the medication, 1 experienced a manic episode, and 1 was unable to continue due to an anxiety disorder.

Patient description is presented in Table 1. Median age was 49 years (range, 22–70 years). Most patients presented with recurrent depressive disorders (n = 27, 42.2%). Nineteen patients (29.7%) had comorbid diagnoses, among which attention-deficit/hyperactivity disorder was the most frequent (n = 9, 14.1%). For a majority of patients, the current depressive episode started more than 12 months prior to their participation (n = 48, 75.0%). More than half (n = 34, 53.1%) had been prescribed at least 3 antidepressants successively during the current depressive episode.

Only 37.5% of patients (n = 24) were considered in remission after at least 6 weeks of stable treatment with psychotropic medication. Response was insufficient in 35.9% (n = 23; median MADRS score = 27; range, 20–41). Twenty-seven percent of patients (n = 17) reported ADRs related to their antidepressant; 4 described gastrointestinal side effects (diarrhea, constipation, or epigastralgia), 4 neurologic side effects (somnolence, pronounced asthenia, and/or cognitive impairment), 3 irritability and/or sleep disorders, 2 dry mouth, and 4 anorexia, bulimia, or weight gain.

Drug Treatment

Antidepressant treatments are reported in Table 2. All patients but the 3 on mirtazapine received an antidepressant with CYP-inhibitory properties. Pill count was checked in all patients except for 2 who forgot to bring their medications; both declared full compliance.

Comedication was frequent (85.9%). The most frequent psychotropic comedications were tranquilizers and hypnotics (56.3%) and antipsychotics (39.1%) (Table 1). One patient used St John's wort until 2 weeks before the phenotypic assessment. Several patients were receiving drugs mainly for cardiovascular and metabolism disorders (antihypertensive medication: n = 7, antidiabetic drug: n = 3, lipid-lowering agents: n = 4, analgesics: n = 5). One of them was treated with irbesartan, a P-gp and CYP2C9 inhibitor.

Steady-State Plasma Antidepressant Concentration and Metabolic Ratio

As shown in Table 2, the concentrations of antidepressants and their metabolites were highly variable between patients. Based on Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry (Arbeitsgemeinschaft Neuropsychopharmakologie und Pharmakopsychiatrie, AGNP) 17.2%, 71.9%, and 10.9% of concentrations were below, within and above the therapeutic range, respectively.[12] For antidepressant MRs, 11.8%, 62.7%, and 25.5% of ratios were below, within, and above the usual range, respectively, with the usual range being defined as the range expected to include 68% of values.[12]

Tolerance of the Phenotyping Cocktail and Phenotype Distribution

No subject reported any severe ADR after cocktail administration. Eight of them reported a transient and discrete dizziness; 6 had headache once during the day, and, among those, 1 suffered from headache before the cocktail administration; none had nausea or vomiting.

Table 2. Antidepressant Dose, Plasma Concentration, and Metabolic Ratio

Antidepressant Drug	n	Dose (mg/d) Median	Dose (mg/d) Range	Concentration (ng/mL) Median	Concentration (ng/mL) Range	Concentration Below/Within/Above Therapeutic Reference Range, n[a]	Ratio Metabolite/Parent[b] Median	Ratio Metabolite/Parent[b] Range	Ratio Low/Normal/High Compared With Usual Range, n[c]
Bupropion	12	300	150–300	23.4	12.2–53.5				
Hydroxybupropion[d]	12			634.1	373.3–1,414.0		25.8	12.5–60.6	0/11/1
Total[e]	12			650.1	385.5–1,461.0	0/12/0			
Duloxetine	7	60	30–120	34.2	5.0–185.3	3/2/2			
Escitalopram	10	20	5–30	22.0	7.4–86.0	3/6/1			
N-desmethylescitalopram	9[f]			12.7	3.7–18.6		0.60	0.07–1.00	3/6/0
Fluoxetine	11	20	20–60	91.8	8.9–298.8				
Norfluoxetine[d]	11			145.3	68.2–363.2		1.48	0.49–7.97	1/5/5
Total[e]	11			209.4	77.1–620.2	1/8/2			
Mirtazapine	3	45	30–60	34.2	32.1–40.9	0/3/0			
N-desmethylmirtazapine	3			40.0	16.8–40.9		1.26	0.44–1.31	0/1/2
Paroxetine	4	25	20–40	26.8	15.5–38.2	2/2/0			
Sertraline	10	150	25–200	44.1	3.4–398.0	1/8/1			
Norsertraline	10			86.4	9.6–336.0		1.84	0.89–3.00	2/8/0
Venlafaxine	7	150	150–300	20.2	0–112.6				
O-desmethylvenlafaxine[d]	7			192.3	23.7–553.1		8.32[g]	5.17–16.39	0/1/5[g]
Total[e]	7			204.8	23.7–665.7	1/5/1			

[a]According to the Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry (AGNP).[12]
[b]Metabolic ratios were corrected for molar concentrations.
[c]According to the range that contains 68% of values determined under normal conditions.[12]
[d]Active metabolite.
[e]Total refers to the sum of parent compound and active metabolite.
[f]One missing value (concentration of metabolite not determined).
[g]One missing value (concentration of parent compound undetectable).

The phenotype distributions are reported in Table 3. In the whole sample, which included patients with and without comedication, the normal/extensive metabolizer phenotype was the most frequently observed (from 56.3% to 68.8%), except for CYP2D6 (only 35.9%). Among the patients with a normal/extensive phenotype for CYP2C9, CYP2D6, CYP3A, and P-gp activities, 40.9%, 95.7%, 36.4%, and 77.8%, respectively, received 1 or several comedications with inhibitory effects. Among patients with intermediate or inhibited/poor phenotype for CYP2C9, CYP2C19, and P-gp, more than half received 1 or several inhibitors. Of patients with intermediate and poor CYP2D6 metabolism, 92% and 100%, respectively, were currently treated with CYP2D6 inhibitors.

Association Between Phenotype, Plasma Antidepressant Concentration, and Antidepressant MR

Table 4 reports correlations between CYP/P-gp activities determined by the probe drug cocktail assay and plasma antidepressant concentrations and MRs. As expected, higher CYP activity for major metabolic pathways was associated with lower concentration of the parent compound for escitalopram (CYP2C19, $r_s = -0.70$, $P = .025$), fluoxetine (CYP2D6, $r_s = -0.94$, $P < .001$), and sertraline (CYP2C19, $r_s = -0.89$, $P = .001$). CYP2D6, involved in the *O*-desmethylation of venlafaxine, and CYP3A, involved in its *N*-desmethylation, had opposite effects on the concentration of the active metabolite *O*-desmethylvenlafaxine (CYP2D6, $r_s = 0.89$, $P = .007$; CYP3A, $r_s = -0.82$, $P = .023$). The following CYP metabolite/probe ratios correlated positively with the antidepressant MRs: CYP2C19 for escitalopram, CYP2D6 for fluoxetine, and both CYP2B6 and CYP2C19 for sertraline. In addition, a higher *N*-desmethylcitalopram–to-citalopram ratio was associated with higher P-gp activity (ie, lower fexofenadine AUC_{0-6h}; $r_s = -0.78$, $P = .013$).

Association Between Phenotype, Insufficient Response, and ADRs

The proportion of patients with insufficient response (MADRS ≥ 20) did not differ according to whether concentration was below, within, or above the therapeutic range (Table 5). It also did not differ according to P-gp phenotype, postulated to influence blood-brain barrier penetration of antidepressants. When plasma concentration and P-gp phenotype were considered together as potential predictors of insufficient response, no significant association was observed (logistic regression, Wald tests $P > .05$). Insufficient response was slightly more frequent in patients treated with more than 3 antidepressants for the current depressive episode compared to those who had received 1 or 2 drugs (44.1% vs 26.7%, $P = .19$).

The proportion of patients with ADRs related to antidepressants did not differ according to concentration being below, within or above the therapeutic range or to P-gp phenotype (Table 5). No significant association was observed when the 2 potential predictors were considered together (logistic regression, Wald tests $P > .05$). Small sample size did not allow investigation of the influence of specific CYP activities on response and adverse reactions to each antidepressant drug.

Table 3. Cytochrome P450 (CYP) and P-Glycoprotein (P-gp) Phenotype

	Phenotype Distribution		Inhibitor Present		Inducer Present	
	n	%	n	%	n	%
CYP1A2						
Induced	23	35.9	11	47.8	15	65.2[a]
Normal	36	56.3	22	61.1	18	50.0[a]
Inhibited	5	7.8	2	40.0	2	40.0[a]
CYP2B6						
Induced	26	40.6	0	0.0		
Normal	37	57.8	3	8.1		
Inhibited	1	1.6	0	0.0		
CYP2C9						
Induced	16	25.0	9	56.3		
Normal	44	68.8	18	40.9		
Inhibited	4	6.3	2	50.0		
CYP2C19						
UM	1	1.6	1	100.0		
EM	36	56.3	7	19.4		
IM	10	15.6	5	50.0		
PM	17	26.6	12	70.6		
CYP2D6						
UM	0	0.0				
EM	23	35.9	22	95.7		
IM	25	39.1	23	92.0		
PM	16	25.0	16	100.0		
CYP3A4						
Induced	7	10.9	5	71.4	1 patient with topiramate	
Normal	44	68.8	16	36.4		
Inhibited	13	20.3	4	30.8		
P-gp						
Induced	5	7.9	3	60.0	1 patient with topiramate	
Normal	36	57.1	28	77.8		
Inhibited	22	34.9	12	54.5		

[a]Refers to the percentage of smokers.
Abbreviations: EM = extensive metabolizer, IM = intermediate metabolizer, PM = poor metabolizer, UM = ultrarapid metabolizer.

DISCUSSION

This study primarily describes the correlations between antidepressant concentration, antidepressant efficacy, and ADRs and the activity of 6 DMEs and 1 transporter, simultaneously assessed by a phenotypic method, in a naturalistic psychiatric outpatient setting.

Correlations between plasma concentration and DME activity, determined by the probe drug cocktail assay in this study (Table 4), were significant for the main metabolic pathways of the individual antidepressants as previously described in the literature.[18–22]

In addition, the cocktail revealed metabolic or transport pathways that were only suggested by in vitro studies, highlighting their role in determining plasma drug concentrations of individual antidepressants. Escitalopram MR but not escitalopram concentration was correlated with P-gp activity, suggesting that *N*-desmethylescitalopram may reduce P-gp transport. Besides the well-described role of CYP2D6 in fluoxetine metabolism,[19,20] we observed that CYP2C9 activity might also be involved in determining the concentrations of fluoxetine and its active moiety (fluoxetine + norfluoxetine), although correlations were at the limit of significance. The relationship between CYP2C9 and CYP2C19 activity based on genotype and fluoxetine concentration has remained controversial until now, probably due to large interindividual variation within genotype.[19,20,23,24] Recent data suggest that CYP2C9 would be more involved in fluoxetine metabolism than CYP2C19.[19] The phenotypic determination was in keeping with both CYP2B6 and CYP2C19 contributing to catalyze sertraline *N*-demethylation.[22,25–27] Venlafaxine is mainly metabolized by CYP2D6 to *O*-desmethylvenlafaxine (active metabolite) and by CYP2C19 and CYP3A to *N*-desmethylvenlafaxine (inactive metabolite). Consequently, in our study, the concentrations of *O*-desmethylvenlafaxine and the level of the active moiety (venlafaxine + *O*-desmethylvenlafaxine) were positively correlated with CYP2D6 activity and negatively correlated with the activity of CYP3A; thus, CYP2D6 and CYP3A activities may be involved in drug response.[28–30] Due to the limitations of a naturalistic study and a small number of patients on individual antidepressants, we failed to show an effect of CYP1A2 and CYP2D6 on duloxetine concentration. With the exception of escitalopram, we did not observe a role of P-gp activity on plasma antidepressant concentrations. P-gp has mostly been described in the literature as a good candidate in determining brain concentrations,[31–34] and phenotyping assesses its peripheral activity.

In this study, associations between antidepressant concentrations and effects were not significant in psychiatric settings in which doses were adjusted according to clinical response.[35] The study was conducted in depressed patients with significant illness chronicity, and 30% had psychiatric comorbid conditions. Therefore, in this population, nonresponse to antidepressant treatment was unlikely to be determined by plasma concentration alone. The prevalence of ADR was low, as might be expected with the newer antidepressants prescribed here.[36]

This study demonstrated that more than 30% of patients referred to a center for refractory mood disorders showed phenotypic variations of the CYP 1A2, 2C9, 2C19, and 3A4 metabolic activity. The proportion went up to 60% for CYP2D6 activity. Forty percent displayed variation in the transporter P-gp activity. These findings are in accordance with a high prevalence of patients expressing multiple allelic variations potentially yielding diversity in drug metabolism phenotype when explored with a genetic approach.[37]

We observed a high proportion of subjects (> 20%) with reduced activity of CYP2D6 but also CYP2C19, CYP3A, and P-gp. In view of the allelic frequencies of known polymorphisms conferring low activity, we suggest a major impact of psychotropic agents themselves on CYP metabolic or P-gp transport capacity.[38] Obviously, without genetic data on enzyme activity, this interpretation should be taken with caution. This consideration mainly concerns CYP2D6 (64% of patients were intermediate or poor metabolizers); in fact, CYP2D6 phenoconversion is documented in the literature as common in patients being treated for depression and depends on the antidepressant used.[39–42] However, it appears from our

Table 4. Correlations Between Antidepressant Concentration, Antidepressant Metabolic Ratio, Cytochrome P450 (CYP) and P-Glycoprotein (P-gp) Phenotyping Indices (Major and Minor Metabolic Pathways in Dark and Light Gray, Respectively [a])

		CYP1A2		CYP2B6		CYP2C9		CYP2C19		CYP2D6		CYP3A4		P-gp	
Concentration[b]	n	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
Duloxetine	7	0.21	.65							−0.61	.15				
Escitalopram	10							−0.70	.025	0.41	.24	−0.04	.91	0.54	.11
N-desmethylescitalopram/escitalopram	9[c]							0.72	.030	−0.27	.49	0.29	.44	−0.78	.013
Fluoxetine	11			−0.13	.71	−0.60	.053	0.59	.056	−0.94	<.001				
Norfluoxetine	11			0.26	.43	−0.02	.95	0.04	.92	0.23	.50				
Fluoxetine + norfluoxetine	11			−0.12	.73	−0.53	.095	0.60	.053	−0.51	.11				
Norfluoxetine/fluoxetine	11			0.03	.94	0.51	.11	−0.59	.058	0.95	<.001				
Sertraline	10			−0.48	.16	0.12	.75	−0.89	.001	−0.44	.20				
Norsertraline/sertraline	10			0.79	.006	−0.42	.23	0.64	.048	0.56	.09				
Venlafaxine	7							0.00	1.00	0.54	.22	−0.75	.052	0.04	.94
O-desmethylvenlafaxine	7							−0.57	.18	0.89	.007	−0.82	.023	0.46	.29
Venlafaxine + *O*-desmethylvenlafaxine	7							−0.57	.18	0.89	.007	−0.82	.023	0.46	.29
O-desmethylvenlafaxine/venlafaxine	6[d]							−0.03	.96	0.31	.54	0.49	.33	0.43	.40

[a]Major and minor metabolic pathways are in accordance with Hiemke et al.[12]
[b]Concentration was dose-normalized ; metabolic ratios were corrected for molar concentrations.
[c]One missing value (concentration of metabolite not determined).
[d]One missing value (concentration of parent compound undetectable).

results that CYP3A, CYP2C19, and P-gp inhibition should also be considered in this population. Although DeVane et al[43] showed that venlafaxine, sertraline, and fluoxetine do not affect CYP3A function per se, the function of this enzyme in the given population may be significantly altered by the frequent comedication with more than 1 inhibitor, including quetiapine (n = 18). In several cases, patients had normal or induced CYP or P-gp activity despite receiving drugs with inhibitory effect, probably due to environmental factors.[44,45] This observation illustrates the variability in the inhibitory effect of drugs among individuals and the difficulty of predicting whether a theoretical drug interaction would be seen in a given patient.

Differences in CYP drug metabolic capacity, whether genetically determined or due to phenoconversion, can affect clinical outcome in patients treated with drugs substantially metabolized by CYPs, which is a common situation. This could be particularly relevant in the cases of prodrugs that must be bioactivated, such as tramadol, codeine, tamoxifen, clopidogrel, or drugs metabolized by CYP and/or transported by P-gp, such as rivaroxaban, metoprolol, warfarin, and valproic acid. Phenotypic methods provide a clinically relevant opportunity to take into account the frequent use of polymedication occurring in real-life settings due to the presence of multiple physical and mental health comorbidities, as well as lifestyle factors such as diet, smoking, alcohol use, and recreational use of psychoactive substances.

This study does however have several limitations, partly due to its naturalistic setting. The compliance control by pill count is a limit. The small number of patients on each antidepressant was associated with limited statistical power. Small sample size also precluded exploring the relationships between CYP activity and treatment effects or ADRs of specific antidepressants. The cross-sectional study design did not allow any possible causal relationship to be assessed, in particular with respect to the influence of antidepressant medication on phenotyping indices and the role of clinical response and ADRs on antidepressant dose adjustments. Generalizability of results might be limited, because the study focused on a sample of chronic, polymedicated patients referred to a center specialized in the management of treatment-resistant depression. Moreover, the variability in the inhibitory effect of antidepressants was probably influenced by genetic factors that should be further evaluated.

Table 5. Associations Between Antidepressant Concentration, P-Glycoprotein (P-gp) Phenotype, Insufficient Response, and Adverse Events

	Total	Insufficient Response (MADRS ≥ 20)			Adverse Events		
	n	n	%	*P*[a]	n	%	*P*[a]
Concentration (N = 64)							
Below therapeutic range	11	3	27.3	0.50	1	9.1	0.26
Within therapeutic range	46	16	34.8		15	32.6	
Above therapeutic range	7	4	57.1		1	14.3	
P-gp phenotype (N = 63)							
Induced	5	2	40.0	0.92	2	40.0	0.68
Normal	36	14	38.9		10	27.8	
Inhibited	22	7	31.8		5	22.7	

[a]Fisher exact test.
Abbreviation: MADRS = Montgomery-Asberg Depression Rating Scale.

In conclusion, this descriptive, naturalistic study provides important insights into the relationship between CYP activity assessed by a phenotypic method and antidepressant concentrations. Phenotyping potentially represents a highly valuable tool in this outpatient population given that it is simple, well tolerated, fast, and inexpensive (US $200); it could potentially account for the inhibitory effect of antidepressants themselves and for the effects of polymedication. Repeating testing might be considered whenever comedications change.

Integrated into a multimodal approach, which combines genotyping of metabolic enzymes and drug targets and therapeutic drug monitoring, phenotyping of metabolic activity undoubtedly has a place in achieving personalized care of patients being treated for refractory depression.

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