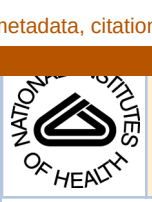

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Genetic Determinants of Variable Metabolism Have Little Impact on the Clinical Use of Leading Antipsychotics in the CATIE study

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Abstract

Purpose—To evaluate systematically in real clinical settings whether functional genetic variations in drug metabolizing enzymes influence optimized doses, efficacy, and safety of antipsychotic medications.

Methods—DNA was collected from 750 patients with chronic schizophrenia treated with five antipsychotic drugs (olanzapine, quetiapine, risperidone, ziprasidone and perphenazine) as part of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study. Doses for each of the medicines were optimized to 1, 2, 3, or 4x units in identically-appearing capsules in a double blind design. We analyzed 25 known functional genetic variants in the major and minor metabolizing enzymes for each medication. These variants were tested for association with optimized dose and other relevant clinical outcomes.

Results—None of the tested variants showed a nominally significant main effect in association with any of the tested phenotypes in European-Americans, African-Americans or all patients. Even after accounting for potential covariates no genetic variant was found to be associated with dosing, efficacy, overall tolerability, or tardive dyskinesia.

Conclusion—There are no strong associations between common functional genetic variants in drug metabolizing enzymes and dosing, safety or efficacy of leading antipsychotics, strongly

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suggesting merely modest effects on the use of these medicines in most patients in typical clinical settings.

Keywords

Pharmacogenetics; CYP 450; Drug Metabolizing Enzymes; Antipsychotics; Personalized Medicine

INTRODUCTION

Drug metabolizing enzymes (DMEs) have been a key focus of pharmacogenetics since its inception in the 1950s[1,2]. A large body of evidence collected over the proceeding decades by various independent groups shows a direct impact of functional variations in DMEs on the pharmacokinetic properties of medications metabolized by these enzymes[3,4]. This has been particularly true for psychiatric drugs[5-7]. The above observations have led many to argue that the genotypes of patients at key DME genes should be incorporated into clinical decision–making, particularly dosing[8,9]. As a result, there is an increasing number of companies and products that offer clinicians convenient ways to determine patient genotypes at key DMEs. One product in particular, the Roche AmpliChip[10], has been approved by the FDA and is being billed as of specific relevance in the use of a broad range of antipsychotics and antidepressants. Surprisingly, there are few data that clearly support the relevance of DME variation to the decisions that clinicians make in the treatment of either schizophrenia or depression.

The Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE)[11] assessed the overall effectiveness of five antipsychotics in parallel in a double blind, randomized fashion. This study design affords an important opportunity to assess how genetic variation among patients may influence clinical decisions. We therefore systematically evaluated the impact of known functional genetic variation in the major and minor DMEs for each tested drug on the doses clinicians decided to prescribe in the CATIE study. Complementary to these analyses we tested associations between these variants and drug response features related to efficacy, safety and adherence to medication. Overall we found that even validated functional variation in relevant enzymes has but marginal impact on dosing and continued use of medicines. These results suggest that DME-based genotyping tests may be of limited utility in guiding clinicians regarding the optimal use of these medicines in most patients, and that direct assessment of phenotype should be considered as an alternative strategy to optimize drug dosing in this patient population.

MATERIALS AND METHODS

Subjects

The study subjects were a subset of patients who had participated in the CATIE study, described at length elsewhere[12]. Briefly, in Phase 1, patients were randomized to treatment with one of five antipsychotic medications –perphenazine, olanzapine, quetiapine, risperidone or ziprasidone – and followed for up to 18 months or until treatment discontinuation for any reason[13]. If perphenazine as the Phase 1 treatment was discontinued, patients entered Phase 1B in which they were randomly assigned to receive olanzapine, quetiapine, or risperidone. Discontinuation of Phases 1, 1A and 1B led to Phase 2, in which randomization pathways were offered[14]: Phase 2E compared open-label clozapine treatment to double-blind treatment with olanzapine, quetiapine, or risperidone. Because of the lack of blinding, clozapine is not considered in this report; Phase 2T compared double-blind treatment with olanzapine, quetiapine, risperidone, or ziprasidone. Throughout the complex design of study phases, no subject was randomized to any medication more than once.

Patients were not allowed to take other antipsychotics while participating in the trial. However, in order to mimic real-life settings, concomitant medications for any other indications were not restricted.

Patients were given identically appearing capsules of their assigned drug and instructed to take between 1 and 4 pills per day based on the judgment of the treating physician. Each capsule contained 8 mg perphenazine; 7.5 mg olanzapine; 1.5 mg risperidone; 200 mg quetiapine; or 40 mg ziprasidone.

The study was approved by the institutional review board at each site. Written informed consent was obtained from the patients or their legal guardians. 756 study participants consented to provide blood samples for genetic analysis (52.8% of patients who had received at least one dose of treatment in CATIE).

Candidate Gene selection and Hypotheses Tested

All five of the antipsychotics used in CATIE are extensively metabolized into less active or inactive metabolites (Table 5) and some are substrates for transporters active at the blood-brain-barrier such as PgP (*ABCB1, MDR1*)[15,16]. The relevant DMEs have been thoroughly screened for functional variation in coding and regulatory genomic regions[17] and we therefore concentrated our attention on such known polymorphisms. We have considered any known or suspected functional polymorphism with minor allele frequency greater than 2% in either Caucasians and/or African-Americans (Supplementary Materials and Methods Table S3), with the exception of *CYP2D6* gene duplications.

Genotyping

All genetic variants, with the exception of the *CYP2D6* polymorphisms, were genotyped by TaqMan fluorescence based allelic discrimination[18]. Pre-made assays offered as Assay-by-Demand were used when available, otherwise primers were designed using the Applied Biosystems (ABI) Assay-by-Design tool (Applied Biosystems, Foster City, CA). Polymerase chain reactions (PCRs) were carried out using 0.5x standard ABI protocol for a 5μl reaction volume (Supplementary Materials and Methods Table S1).

CYP2D6 genotyping was performed as described previously[19,20], and included **2, *3, *4, *5, *6, *9, *10, *17, *29* and **41*. Primer sequences (IDT, Coralville, IA) and additional details pertaining to all genotyping reactions are presented in the Supplementary Materials and Methods Table S1. *CYP2D6* allele nomenclature throughout this report uses the recently revised allele definitions as established by the *CYP2D6* nomenclature committee (http://www.cypalleles.ki.se/).

Genetic Models

Within each treatment group analyses were performed separately for each DME. The genetic models tested and the scheme by which genotypes were collapsed into phenotypes are described in the Supplementary Materials and Methods (Table S2). Generally, we coded genotype for each gene by the number of functional, active, gene copies present: 2, 1 or 0. Haplotypes were inferred with PHASE version 2.1 for genes in which 2 SNPs were genotyped (i.e. *CYP3A5, UGT1A4, FMO3, ABCB1, CYP2C8* and *CYP2C9*) to determine the number of active gene copies carried. Analyses were carried out assuming an additive model (power calculations suggest that in situations where roughly 50% of known causal variants have an additive component[21], tests assuming additivity are more powerful than unrestricted genotype tests (unpublished data))

Phenotype Definitions and Statistical Analyses

To maximize statistical power, the analyzed independent cohorts included subjects who had received one of the five medications in any treatment phase, given the fact that patients could not receive any specific drug more than once throughout the trial.

Dosing—optimized dose was defined as the average of all doses (in capsule number) prescribed from the fourth phase-visit and onward for a specific drug. A visual inspection (DBG and IG) of dose prescription data in a subset of patients indicated frequently fluctuating doses during the first three visits (i.e. a dosage 'adjustment' period) followed by relatively stable subsequent regimens. The first doses were therefore excluded from optimized dose definitions, and any patient treated for less than four visits was excluded from the dose analyses. We also considered adherence to medication based on pill-count, self-administered questionnaires and the judgment of CATIE personnel performed each visit and summarized by quartiles reflecting 0-100% adherence. Patients were included in analyses in phases for which their phase-averaged scores were at least 63%, reflecting overall "usual" (51-75% of the time) adherence to medication. Since patients may be non-compliant to medication due to either ADRs or inadequate efficacy, we tested separately the effect of DME functional variation on adherence to medication as defined above.

Univariate analysis was used to test main effects of each candidate gene as the independent variable versus optimized dose by way of least-squares regression. A multivariate model was constructed using mixed stepwise regression, incorporating the following covariates: age, gender, race (White, Black or other), weight at baseline or phase initiation, maximum weight change throughout the relevant phase, trail phase, phase duration (measured by number of visits), PANSS at baseline or phase initiation, PANSS change throughout the relevant phase, phase-averaged CGJMA score, concomitant medications categorized by their inhibitory or inductive effects on the study drugs, summarized in the Supplementary Materials and Methods Table S4), smoking status, occurrence of severe ADRs, TD status during the phase, antipsychotic treatment prior to study initiation, years since first treated with antipsychotics, alcohol and drug abuse. When more than one enzyme was known to metabolize a study drug the genotype status of the relevant DMEs was incorporated into the model as a potential covariate to account for gene-gene correlations. Criteria for model construction required each covariate to satisfy entry and exit p-values of 0.1 or less. Once the covariate model was developed we compared it to the same model incorporating the relevant genetic effect using the least squares test, as implemented in JMP IN[22]. The final model was then tested for gene-by-race interaction and if a significant effect was detected analyses were performed in each ethnic group independently.

Safety and efficacy assessment—We tested associations between relevant DME gene variants and treatment discontinuation due to safety or inefficacy reasons. These analyses included all patients treated in phases 1, 1A, 1B, 2E or 2T, with the exception of patients discontinuing treatment due to "patient decision" or "subject advocate discontinuation". Additionally, patients discontinuing treatment due to lack of efficacy before the 4th phase-visit were excluded from the efficacy tests. Analysis was performed in a similar manner to that described for the dosing end-point.

Because of the expectation that poor metabolizers or individuals with reduced DME activity may be at a higher risk to experience dose dependent ADRs and that extensive metabolizers are more likely to show lower efficacy, we constituted two different tests:

a) discontinuation due to ADRs versus full phase completion

b) discontinuation due to inadequate efficacy versus full phase completion

Tardive Dyskinesia—TD has been suggested to be a dose dependent ADR associated with antipsychotic treatment, particularly "conventional", typical drugs such as perphenazine. TD status was determined based on the Schooler-Kane criteria for probable TD[23], for which patients were tested at baseline, every 3 months thereafter, and at phase end[24]. Analyses were performed with the case group comprised of patients who were positive for the probable TD criteria at least twice throughout their participation in the trial, and the control group including all individuals who never met these criteria. Patients who met the TD criteria only once during the trial were excluded from analyses (N=74). It should be mentioned that while drug treatment immediately prior to CATIE participation was included in the multivariate analysis (as described in the dosing section above), we had no access to complete records of medical history.

Analyses were performed by R version 2.3.0[25] and by JMP IN version 5.1[22] separately and independently within each drug cohort. Hidden population stratification within each ethnicity was not explored in detail. Simple Genomic Control methods were not pursued due to the lack of any significant results. More sophisticated correction methods (e.g. EIGENSTRAT[26]) which could correct for false negatives, require an extremely large number of additional genotypes (>5,000) to be effective.

RESULTS

Genotyping results

All genotype calls were determined independently by at least two researchers and ambiguous calls were re-genotyped or discarded. Genotyping failure rates were below 3%. *CYP2C19*3* showed a very low minor allele frequency (as expected) and was excluded from all analyses. Upon testing for Hardy-Weinberg Equilibrium (HWE), one variant out of a total of 16 TaqMan based assays had a p-value <0.05 (*CYP2C19*2*, p=0.035), due to a deficiency of heterozygotes. It was re-genotyped, and the same genotypes for all samples were obtained. Allele frequencies were compared to published data and confirmed to match expected values (Supplementary Materials and Methods Table S1). Among the 10 genotyped *CYP2D6* variants, HWE analysis was significant for the *5 deletion allele (Fisher's exact test p=0.0016) in Caucasians (out of 20 tests in total), the deviation again being due to a deficiency of heterozygotes. The observed rate of significant p-values was consistent with that expected under the null hypothesis. These samples, along with 24 randomly picked samples, were re-genotyped independently in a separate blinded laboratory (AG) and were identically called.

Association Results

Optimized dose—We tested association between optimized dose (Figure 1) and genotype within each relevant treatment group (Table 1).

None of the tested variants showed a significant main effect (p<0.05) in association with optimized dose in European-American, African-Americans or all patients (Table 2). Even after accounting for potential covariates no genetic factors were found to be associated with dosing (Table 2).

Safety and efficacy—Treatment response phenotypes reflecting safety and efficacy were tested by contrasting discontinuation counts due to unacceptable side effects (ADRs) and inadequate efficacy versus successful phase completion (Table 3), respectively. No nominally significant associations (p<0.05) were detected between the tested variants and occurrence of ADRs in univariate analysis. When exploring potential confounding effects via logistic regression analysis, few nominally significant associations were detected. The

only association recorded (p=0.013) in the safety test suggested a correlation between the lower activity variants in the *FMO3* gene and higher incidence of ADRs in African-Americans treated with olanzapine (N=59). No association was detected with this variant in either the whole cohort or European-Americans alone. In the efficacy test we recorded two marginally significant associations between the severely decreased activity genetic variants in *CYP3A5* and higher efficacy of quetiapine (p=0.02) and risperidone (p=0.04). None of these results survives correction for multiple testing.

Tardive dyskinesia—Neither univariate nor regression analyses (Table 4) found the exhibition of TD to be significantly associated ($p<0.05$) with the lower activity genetic variants of the *CYP2D6* and/or *CYP1A2* genes.

Medication Adherence Assessment

We considered the effect of 23 different drug-DME combinations (Table 5) on two summary indices of medication adherence (the mean of the treating clinician's global judgment scores; and the median proportion of pills taken) using non-parametric and parametric analyses. These two indices showed an inverse correlation (Spearman $\rho=-0.55$). None of the tests performed remained significant after adjustment for multiple testing (92 non-independent tests). The most significant finding observed was for *ABCB1* in subjects who had received risperidone: non-parametric p=0.004 for clinician judgment based adherence (False Discovery Rate[27] (FDR)-adjusted p=0.19) and non-parametric p=0.04 for pill count (FDR-adjusted p=0.36). Even if significance had been achieved, the magnitude of the effect was very small: a median of 96% of pills taken for the *ABCB1* haplotype "0" (coding scheme available in the Supplementary Materials and Methods Table S2) versus 91% for all other *ABCB1* haplotype variants.

DISCUSSION

Despite the growing enthusiasm for using genetic diagnostics to guide clinical decision making there remains scant evidence supporting the clinical utility of currently known genetic differences among patients. For DME variants in particular there has been a strong push toward their incorporation into the clinical use of antipsychotics, antidepressants and other medicines. Here we have evaluated how known functional variation in all the major DMEs for five antipsychotic drugs influences their clinical use in the context of a randomized clinical trial. Despite the relative homogeneity of clinical decision making in this setting (in comparison, for example, to care outside of a trial setting) and a comprehensive set of clinical and genetic covariates tested we find no strong associations between the relevant genetic variants and the safety or efficacy of the medicines or even with their optimized doses. These results do not, of course, rule out modest effects or potential impact of gene-gene and gene-environment synergies not well understood yet, that may overall be clinically meaningful. Power analyses[28] suggest, however, that our study was well-powered to detect minimum genetic effects of between 6-20% of variance in the dosing phenotype, as well as minimum genotype relative risks of between 1.6-2.7 for the discontinuation tests. This suggests that in normal clinical settings DMEs variation has, at best, modest effects on the use of these medicines.

A few inherent limitations in the current study should be noted. First, pharmacogenetic variability in drug biotransformation is most likely to have clinical consequence when the clearance of the drug is primarily or solely dependent on a single pathway. While CYP2D6 is the lead determinant of perphenazine clearance, alternative pathways may play important roles (Table 5). As patients differ in their individual complement of hepatic and intestinal CYPs, the clinical impact of *CYP2D6* genotype and phenotype will vary depending on the

relative importance of CYP2D6- *vs* non-CYP2D6-mediated clearance pathways. For example, when expressed relative to the administered dose, median steady state concentrations of perphenazine were almost double in CYP2D6 PMs compared to EMs, but overlapped in range[29]. Secondly, risperidone and its CYP2D6-dependent metabolite, 9-hydroxyrisperidone, are considered to have similar pharmacologic activity[30] and are jointly referred to as the active moiety. While a relationship between *CYP2D6* genotype and phenotypes, such as steady state serum risperidone concentration/dose ratios and risperidone/9-hydroxyrisperidone ratios, has been established by several groups and has potentially been associated with ADRs[31], there appears to be no such relationship between genotype and total active moiety[32]. On the other hand, any pathway affecting the latter (*e.g., N*-dealkylation by CYP3A[33], Table 5) potentially confers prognostic value. Thirdly, the use of genotype data to predict drug clearance and dose requirements or toxicity is particularly difficult when enzyme activity demonstrates considerable variability in a particular population and is inducible. For example, phenotypic differences in CYP1A2 activity are more apparent in smokers[34] (compelling us to perform analyses conditioned on smoking status and consider smoking as a covariate in multiple regression models). However, it is unclear what is the quantitative importance of the polymorphic pathway in the overall disposition of a drug of interest[35]. Fourthly, interpretation and application of genotype information is also compounded by knowledge of the likely functional consequences of the genotyped SNP (or variants in linkage equilibrium) on the specific drugs of interest in specific populations. One example is the *CYP2D6*17* allele, which demonstrates "reduced" activity towards dextromethorphan, bufuralol and debrisoquine, relative to the reference *CYP2D6*1* alleles, but has been reported to convey increased activity towards other medications, such as haloperidol[36] and risperidone[37] in specific populations. Lastly, recently published data indicates the potential of variants not explored in the current report to affect drug response phenotypes, such as the recently reported *CYP2C19*17*[38]. The above phenomena, if extends to other CYPs and substrates, may further obfuscate genotype-phenotype relationships.

Reviewing the above articulated considerations, evidence for clinical utility of routine genotyping of genes involved in drug biotransformation and transport likely will first be limited to situations where a single, polymorphically expressed pathway is the primary route of elimination in most treated patients, the drug is metabolized to inactive metabolites and has a narrow range. More direct measurement of drug biotransformation phenotype, as supplements to our estimations from genotype data, is currently being evaluated in a subset of the CATIE subjects.

In summary, our results suggest that genotype information alone is a poor predictor of antipsychotic drug disposition and response in a clinical situation. Caution is warranted when using diagnostic products such as the Roche AmpliChip in absence of a clear association between the tested variants and relevant clinical responses. More generally, taken together with the report of no significant association between functional DME variants and citalopram response in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial[39], these results may suggest that the importance of genetically controlled variability in drug pharmacokinetics may often be modest. While improvements in prediction of phenotypes by usage of genotype information are constantly being refined[40,41], prospective data demonstrating the utility of *a priori* DME genotype assessment for optimizing treatment response are extremely limited. In this context, it should be mentioned that variation in genes related to a medication's mode of action may often have larger effects than variants in genes influencing metabolism[42,43], though it is too early to be certain how general this will prove. What does appear likely is that if variation in genes governing the pharmacodynamic properties of drugs does not prove to be more important than those

governing pharmacokinetics, germline pharmacogenetics will have at best a very modest clinical utility in many therapeutic areas, and specifically in psychiatry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Dr. Goldstein had full access to all of the data in the study and takes full responsibility for the integrity of the data and the accuracy of the data analysis.

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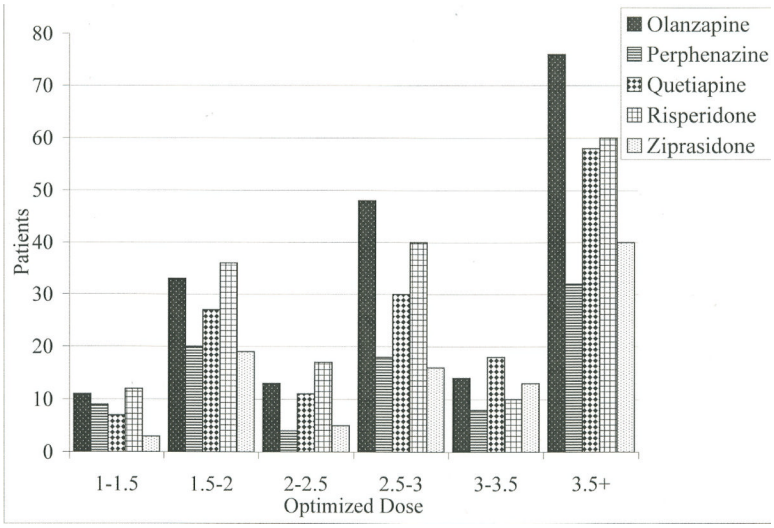

Figure 1.
Histogram displaying the distribution of optimized doses (presented in number of pills taken) in each of the five tested treatment groups

Table 1

Description and Outcome Measures of Patients in the Optimized Dose Cohorts

Characteristic	Olanzapine	Quetiapine	Risperidone	Ziprasidone	Perphenazine
Patients – OD cohort	195	151	175	96	91
Phase 1/1A	139	104	118	57	91
Phase 1B	17	22	12	0	0
Phase 2	39	25	45	39	0
Age – Mean yr ±SD	40.67±11.28	41.41±11.45	40.69±11.27	40.9±10.07	40.5±10.61
Sex – no. (%)					
Male	141 (72.3)	117 (77.5)	131 (74.9)	74 (77.1)	64 (70.3)
Female	54 (27.7)	34 (22.5)	44 (25.1)	22 (22.9)	27 (29.7)
Race – no. (%)					
Caucasian	124 (63.6)	106 (70.2)	117 (66.8)	61 (63.5)	58 (63.7)
African-American	63 (32.3)	39 (25.8)	53 (30.3)	29 (30.2)	27 (29.7)
Other	8 (4.1)	6 (4)	5 (2.9)	6 (6.3)	6 (6.6)
BL antipsychotic medications [$] – no. (%)					
Olanzapine	71 (36.4)	43 (28.5)	50 (28.6)	23 (23.9)	28 (30.8)
Quetiapine	19 (9.7)	11 (7.3)	14 (8)	10 (10.4)	3 (3.3)
Risperidone	38 (19.5)	39 (25.8)	42 (24)	26 (27.1)	26 (28.6)
Perphenazine	0	7 (4.6)	5 (2.9)	1 (1)	1 (1.1)
Ziprasidone	11 (5.6)	8 (5.3)	9 (5.1)	3 (3.1)	2 (2.2)
None	47 (24.1)	35 (23.2)	38 (21.8)	28 (29.2)	25 (27.5)
Treatment disc. - no. (%)					
Ineffectiveness	25 (12.8)	49 (32.5)	45 (25.7)	23 (24)	19 (20.9)
ADRs	28 (14.4)	17 (11.3)	15 (8.6)	9 (9.4)	12 (14.3)
Administrative decision	8 (4.1)	5 (3.3)	4 (2.3)	4 (4.2)	1 (1.1)
Patient decision	10 (5.1)	19[#] (12.5)	15 (8.6)	15 (15.6)	7 (7.7)
Treatment completion	124 (63.6)	61 (40.4)	96 (54.9)	45 (46.9)	51 (56)
TD status – no. (%)					
Probable	42 (21.5)	34 (22.5)	30 (17.1)	21 (21.9)	20 (22)
Definite	8 (4.1)	10 (6.6)	6 (3.4)	3 (3.1)	6 (6.6)
Weight (Lb)					
Mean BL	197.7±48.33	200.29±45.54	199.65±52.67	201±45.93	191.57±47.73
Mean change/month	1.05±1.95	0.28±3.39	0.26±2.4	−0.53±3.13	−0.05±2.05
Max phase change	16.3±13.79	15.95±11.88	16.6±14.2	16.1±13.04	17.12±12.76
Concomitant medications – no. (%)					
Inhibitors**	34 (17.4)	31 (20.5)	28 (16)	21 (21.9)	14 (15.4)
Inducers**	12 (6.2)	5 (3.3)	5 (2.9)	3 (3.1)	–
PANSS score					
BL	75.10±19.1	74.1±16.7	75.7±16.8	71.1±14.8	71±17.6
Change	−10.6±17.04	−3.9±18.6	−9.4±18.8	−2.7±18.9	−8.7±19.3

Characteristic	Olanzapine	Quetiapine	Risperidone	Ziprasidone	Perphenazine
Smoking – no. (%)	131 (67.2)	105 (69.5)	124 (70.9)	74 (77.1)	66 (72.5)
Cigarettes/day – Mean ± SD	12.11±12.6	10.54±11.24	12.5±12	11.92±11.09	12.9±12.9
Alcohol abuse – no.** **(%)**	16 (8.2)	19 (12.6)	19 (10.9)	6 (6.3)	6 (6.6)
Drug abuse – no. (%)	20 (10.3)	20 (13.2)	22 (12.6)	13 (13.5)	12 (13.2)
No. visits/phase – Mean ± SD					
Phase I/IA	14.48±5.15	11.8±5.44	13.68±5.25	12.21±5.52	13.1±5.6
Phase IB	9.88±3.95	10.77±3.9	10±3.54	–	–
Phase II	13.35±5.17	7.12±3.89	12.27±5.25	8.23±3.62	–
Optimized dose - Mean ± SD	3.04±0.88	3.06±0.88	2.91±0.9	3.14±0.86	2.88±0.96
Phase-averaged adherence to medication score – Mean ± SD	84.83±5.43	84.05±6.12	84.7±5.37	83.83±6.15	84.9±5.55
Years treated with antipsychotics – Mean ± SD	14.69±11.76	13.23±10.28	13.18±11.47	13.75±10.67	13.37±10.57
Anticholinergic treatment – no. (%)					
BL	42 (21.5)	31 (20.5)	36 (20.6)	19 (19.8)	17 (18.7)
During phase	52 (26.7)	29 (19.2)	38 (21.7)	21 (21.9)	25 (27.5)

Abbreviations: OD, optimized dose; SD, standard deviation; BL, baseline; disc, discontinuation; ADRs, adverse drug reactions; TD, tardive dyskinesia.

Table 2

Association Analysis of all Relevant DME Genetic Effects on Optimized Dose in Univariate and Covariance Analyses

Drug group	Gene & variant	Main effect		Analysis of covariance
		Effect size †	p-value	p-value
Perphenazine	CYP2D6_combined	β = +0.33	0.30	0.22
	CYP1A2*1F	β= +0.17	0.30	*
	CYP2C8_combined	β= −0.33	0.13	*
	CYP2C9_combined	β= −0.08	0.71	*
	CYP2C19*2	β= −0.001	0.99	*
Risperidone	CYP2D6_combined	β = −0.07	0.64	0.17
	CYP3A5_combined	β= −0.12	0.24	0.49
	CYP3A4*1B	β= −0.06	0.53	*
	ABCB1_combined	β= −0.12	0.20	0.81
Olanzapine	CYP1A2*1F	β= −0.15	0.11	0.08
	FMO3_combined	β= −0.05	0.56	0.54
	UGT1A4_combined	β= +0.07	0.58	*
	CYP3A5_combined	β= −0.002	0.98	*
	CYP1A4*1B	β= +0.08	0.35	*
	CYP2D6_combined	β = −0.06	0.64	*
Quetiapine	CYP3A5_combined	β= +0.03	0.79	0.46
	CYP3A4*1B	β= +0.05	0.60	*
	ABCB1_combined	β= −0.001	0.99	*
	CYP2D6_combined	β = −0.04	0.78	*
	CYP2C9_combined	β= −0.01	0.94	*
Ziprasidone	CYP3A5_combined	β= −0.15	0.21	0.34‡
	CYP3A4*1B	β= −0.1	0.40	*
	ABCB1_combined	β= +0.02	0.88	*

β is the slope of the linear regression model defined by $Y = \alpha + \beta \times X$, where Y is optimized dose and X is the genetic factor tested. Annotation of "gene_combined" relates to the sum functionality of the gene as reflected by the two haplotypes individuals carry (defined in Supplementary Materials and Methods, Table 3).

*Asterisks denote variants which were considered as potential covariates in the step-wise regression model.

Nominal p-values are reported.

†Main effects are registered in reference to the lower/absent enzymatic activity genotypes, expecting the effect to be positive (i.e. lower activity genotypes are expected to settle on lower optimized doses).

‡Race-by-gene interaction term was statistically significant (p=0.04) in the ziprasidone cohort, but a multivariate analysis in each race group separately yielded p= 0.36 and 0.12 for African-Americans and Caucasians, respectively.

Table 3

Association Analysis of DME Genetic Effects with Safety and Efficacy Phenotypes in Each of the Tested Drug Cohorts: (a) Olanzapine; (b) Perphenazine; (c) Quetiapine; (d) Risperidone; (e) Ziprasidone

(a) Olanzapine

		CYP1A2 *1F			p-value (Pearson/ Fisher's exact)	p-value (L-R test)	FMO3			p-value (Pearson/ Fisher's exact)	p-value (L-R test)
Genotype		LL	HL	HH			MM	MN	NN		
Safety test	Disc. Due to ADRs	5	25	28	0.92	0.42	16	22	21	0.24	0.73*
	Treatment completion	13	52	58			21	58	45		
Efficacy test	Disc. Due to inadequate efficacy	4	6	15	0.22	0.51	4	13	8	0.89	0.78
	Treatment completion	13	52	58			21	58	45		

(b) Perphenazine

		CYP2D6			p-value (Pearson/ Fisher's exact)	p-value (L-R test)
Genotype		**PM**	**IM**	**EM**		
Safety test	Disc. Due to ADRs	1	5	18	0.13	0.93
	Treatment completion	0	5	46		
Efficacy test	Disc. Due to inadequate efficacy	0	0	20	0.31	0.09
	Treatment completion	0	5	46		

(c) Quetiapine

		CYP3A5			p-value (Pearson/ Fisher's exact)	p-value (L-R test)
Genotype		**MM**	**MN**	**NN**		
Safety test	Disc. Due to ADRs	26	11	9	0.54	0.08
	Treatment completion	41	13	8		
Efficacy test	Disc. Due to inadequate efficacy	33	11	5	0.9	0.02

(c) Quetiapine

		CYP3A5			p-value (Pearson/ Fisher's exact)	p-value (L-R test)
	Treatment completion	41	13	8		

(d) Risperidone

		CYP2D6			p-value (Pearson/ Fisher's exact)	p-value (L-R test)	CYP3A5			p-value (Pearson/ Fisher's exact)	p-value (L-R test)
Genotype		**PM**	**IM**	**EM**			**MM**	**MN**	**NN**		
Safety test	Disc. Due to ADRs	2	7	27	0.08	0.44	24	6	7	0.11	0.65
	Treatment completion	5	7	87			63	28	8		
Efficacy test	Disc. Due to inadequate efficacy	1	3	42	0.91	0.64	24	17	5	0.42	0.04
	Treatment completion	5	7	87			63	28	8		

(e) Ziprasidone

		CYP3A5			p-value (Pearson/ Fisher's exact)	p-value (L-R test)
Genotype		**MM**	**MN**	**NN**		
Safety test	Disc. Due to ADRs	19	8	5	0.6	0.77
	Treatment completion	33	9	5		
Efficacy test	Disc. Due to inadequate efficacy	13	7	3	0.5	0.08
	Treatment completion	33	9	5		

Abbreviations: Disc, Discontinuation; M, Mutant/lower activity allele; N, normal/higher activity allele; L, lower inducibility variant; H, higher inducibility allele; L-R, logistic regression.

* Race-by-gene interaction term for FMO3 in the Olanzapine cohort was statistically significant in the safety test (p=0.05). Each race group was therefore analyzed separately, with the African-American group (N=59) reaching p=0.013.

Table 4

Association Analysis of *CYP2D6* Phenotype and *CYP1A2* Genotype with Tardive Dyskinesia (TD) in Univariate Analysis and Logistic Regression (L-R)

Cohort	Test	CYP2D6			Univariate p-value	L-R test p-value (*racexgene p-value*)	CYP1A2*1F			Univariate p-value (*smokers only*)
		EM	**IM**	**PM**			**HH**	**LH**	**LL**	
All	TD positive	113	24	5	0.15	0.66 *(0.1)*	65	60	16	0.55 *(0.45)*
	TD absent	441	59	27			259	228	45	
Caucasian	TD positive	71	14	5	0.05	0.33	44	39	6	0.77 *(0.41)*
	TD absent	307	26	22			187	153	18	
African American	TD positive	39	7	0	0.62	0.16	19	17	10	0.57 *(0.74)*
	TD absent	115	28	4			59	66	24	

Pearson's p-values are reported for the univariate analysis, accept for *CYP2D6* association analysis in African-Americans, where Fisher's exact test p-value is listed.

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; H, higher inducibility allele; L, lower inducibility allele.

Table 5

Antipsychotic Drugs Analyzed and the DMEs that Metabolize Them

	Drug	Major DME	Secondary DME	Minor DME1	Minor DME2	Minor DME3	Transporter	Glucuronidase
1	**Olanzapine**	CYP1A2	FMO3	CYP2D6	CYP3A4 & CYP3A5			UGT1A4
2	**Quetiapine**	CYP3A4 & CYP3A5		CYP2D6	CYP2C9		ABCB1	
3	**Risperidone**	CYP2D6	CYP3A4 & CYP3A5				ABCB1	
4	**Ziprasidone**	AOX1*	CYP3A4 & CYP3A5				ABCB1	
5	**Perphenazine**	CYP2D6	CYP1A2	CYP2C9	CYP2C19	CYP2C8		

Abbreviations: DME, drug metabolizing enzyme; CYP, cytochrome P450; AOX1, aldehyde oxidase 1; FMO3, flavin-containing monoxigenase 3; ABCB1, p-glycoprotein (MDR1); UGT1A4, uridine 5′-diphosphate glucuronosyl transferase 1A4.

*There are no common functional variants in AOX1 and inhibitors and inducers of the enzyme do not affect ziprasidone pharmacokinetics[44,4536, 37]. AOX1 was thus not analyzed in this report.