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Novel drug metabolism indices for pharmacogenetic functional status based on combinatory genotyping of *CYP2C9*, *CYP2C19* and *CYP2D6* genes

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Abstract

Aims—We aim to demonstrate clinical relevance and utility of four novel drug-metabolism indices derived from a combinatory (multigene) approach to *CYP2C9*, *CYP2C19* and *CYP2D6* allele scoring. Each index considers all three genes as complementary components of a liver enzyme drug metabolism system and uniquely benchmarks innate hepatic drug metabolism reserve or alteration through CYP450 combinatory genotype scores.

Methods—A total of 1199 psychiatric referrals were genotyped for polymorphisms in the *CYP2C9*, *CYP2C19* and *CYP2D6* gene loci and were scored on each of the four indices. The data were used to create distributions and rankings of innate drug metabolism capacity to which individuals can be compared. Drug-specific indices are a combination of the drug metabolism indices with substrate-specific coefficients.

Results—The combinatory drug metabolism indices proved useful in positioning individuals relative to a population with regard to innate drug metabolism capacity prior to pharmacotherapy. Drug-specific indices generate pharmacogenetic guidance of immediate clinical relevance, and can be further modified to incorporate covariates in particular clinical cases.

Conclusions—We believe that this combinatory approach represents an improvement over the current gene-by-gene reporting by providing greater scope while still allowing for the resolution of a single-gene index when needed. This method will result in novel clinical and research applications, facilitating the translation from pharmacogenomics to personalized medicine, particularly in psychiatry where many drugs are metabolized or activated by multiple CYP450 isoenzymes.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

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Keywords

combinatory genotypes; CYP450; drug metabolism; metabolizer phenotypes

Pharmacogenetic studies investigating the human cytochrome P450 (CYP450) isoenzyme system genes have relied upon the phenotyping system of poor metabolizer, intermediate metabolizer, extensive metabolizer and ultra-rapid metabolizer to describe innate metabolizer status on a gene-by-gene basis [1–4]. However, many of the most used medications, particularly psychotropics, are metabolized by multiple CYP450 pathways, each of which, taken alone, insufficiently represents the actual metabolic pharmacokinetics. Such distributed substrate affinity suggests the need for a combinatory approach that considers *CYP2C9*, *CYP2C19* and *CYP2D6* as complementary components of a liver enzyme drug metabolism system [5–12]. Each of the three genes in the *CYP2C9*, *CYP2C19* and *CYP2D6* array contributes significantly to psychotropic metabolism and response [9,11]. *CYP2C9* is involved in the metabolism of multiple antidepressants, including fluoxetine and sertraline, for which its isoenzyme product constitutes a major metabolic pathway [10,13]. In addition, *CYP2C9* activity may be modulated by endogenous substrates such as adrenaline and serotonin, and polymorphism of *CYP2C9* has been associated with major depressive disorder [14,15]. *CYP2C19* is involved in the metabolism of citalopram, escitalopram, sertraline and several tricyclic antidepressants [13,16]. *CYP2C19* isoenzyme functionality has also recently been linked to depressive symptoms [17]. *CYP2D6* metabolizes several antipsychotic and antidepressant drugs, and is highly polymorphic with more than 60 alleles and 130 gene variations [101]. The *CYP2D6* gene product is a primary metabolizer of many neuroleptics, including haloperidol, aripiprazole, fluphenazine, risperidone and ziprasidone, as well as many common antidepressants [13,18]. Furthermore, research suggests that *CYP2D6*, like *CYP2C9*, is involved in the metabolism of endogenous substrates in the brain, and may be associated with disease vulnerability as well as certain personality and cognitive traits (e.g., increased characteristics of social cognitive anxiety in healthy, null metabolizers) [19–21].

There is substantial evidence to support the importance of *CYP2C9*, *CYP2C19* and *CYP2D6* genotypes in psychotropic management [5,8–12,16,22–33,102]. Moreover, many diagnostic CYP450 tests for drug metabolism are packaged as combinations of multiple CYP450 genes (e.g., the AmpliChip CYP450 test, which combines *CYP2D6* and *CYP2C19*) [7,16]. Leading clinical DNA-typing laboratories, such as the Mayo Clinic (Rochester, MN, USA) and our own Laboratory of Personalized Health (Hartford, CT, USA), recommend that psychiatric patients be genotyped for variants on multiple CYP450 loci, further evidence that no single gene is sufficient for thorough pharmacogenetic guidance [103,104]. Despite the widespread understanding that a multigene system is responsible for psychiatric drug metabolism, interpretation and clinical guidance based on genotype is still dominated by a gene-by-gene approach. Such single-gene analyses have yielded clinically relevant quantitative models to predict individual drug metabolism pharmacokinetics, in particular for the *CYP2D6* locus [33–36].

Differences in metabolic ratios of psychotropics have been characterized by a *CYP2D6* semi-quantitative gene dose model and compared with conventional predicted phenotypes (poor metabolizer, intermediate metabolizer, extensive metabolizer and ultra-rapid metabolizer) [37]. Semi-quantitative gene dose model performed slightly better than the conventional model when predicting the individual's metabolic ratios of venlafaxine, risperidone and fluoxetine. The boost in performance of the was attributed, in part, to the quantitative model's ability to score alleles separately and distinguish *CYP2D6*-deficient alleles from functional or null alleles. However, it was applied only to *CYP2D6*, with the

exclusion of other CYP450 genes relevant to psychotropic drug metabolism. Other similar semi-quantitative gene score models are accurate when applied to drugs that depend on *CYP2D6* as the primary metabolic pathway (e.g., tamoxifen) [33], but many psychiatric drugs depend on multiple CYP450 substrates for metabolism or activation [11]. In a pilot study of psychiatric inpatients, we found very high rates of gene alterations compared with a cardiovascular population using a combinatory approach [38]. Our clinical applications study demonstrates that combinatory and drug-specific analyses yielded more significant correlations with clinical measures of metabolic syndrome than did any single gene index [39].

This article describes a scoring system based on a novel method of analysis of *CYP2C9*, *CYP2C19* and *CYP2D6* using combinatory genotypes. The four indices were created as quantitative representations matching various empirical genotype–phenotype observations made over the course of over 5 years of clinical CYP450 genotyping. We have found scientific and clinical relevance supporting each index. We demonstrate an immediate utility of the indices by presenting the distributions and rankings of index scores in a psychiatric population of 1199 patients. Charting the index rankings enables the visualization of an individual in the context of a wider population, which, in itself, is a clinically informative benchmark. Furthermore, we use the indices for subpopulation comparisons. We demonstrate that the combinatory indices provide informative and actionable quantitative (index values) and qualitative (ranking charts) representations of CYP450 drug metabolism capacity as measured by gene polymorphisms and their effect on liver enzyme functionality. Finally, we describe drug-specific indices, which merge an individual's combinatory index scores with a drug's pharmacokinetic properties to provide clinicians an additional predictive tool for drug selection and management. Together, these novel drug metabolism indices provide a quantitative foundation upon which researchers and clinicians may build precise research analytics, enhance drug response through more informed selection, diminish side effects, increase adherence and reduce inpatient length of stay [39]. Our novel combinatory and drug-specific metabolism benchmarking models will facilitate the translation of genetic biomarker discovery and research to clinical practice by both improving and simplifying the research, analytical and translational processes [40,41].

Materials & methods

Sample collection

Blood samples were collected from 1199 psychiatric referrals over a period of over 5 years (July 2005–September 2010). The patients were referred from the community for CYP450 diagnostic genotyping as part of their clinical care because of efficacy or safety problems related to their medications. This population was used to create rankings and distributions of the combinatory CYP450 indices. Within this group 62% were female and 37% were male, and 1% of samples reported no gender. The average patient age was 37 years at the time of clinical referral with ages ranging from 5 to 90 years. A total of 173 patients (14%) did not report ethnicities. Of reported ethnicities, 92% were Caucasian, 5% were Hispanic, 2% were African–American and less than 1% were Asian or ‘other’. Ethnicities were reported by the clinician requesting the DNA typing.

Single nucleotide polymorphism assays

CYP450 DNA typing data were obtained on *CYP2C9*, *CYP2C19* and *CYP2D6*. Blood samples were collected into tubes containing either EDTA or citrate, and were extracted from lymphocytes using the Qiagen EZ-1 robotic DNA isolation procedure (Qiagen, Germantown, MD, USA). DNA typing was performed at the Genomas Laboratory of Personalized Health (LPH) at Hartford Hospital (CT, USA). LPH is a high-complexity

clinical DNA testing center licensed by the Connecticut Department of Public Health (CL-0644) and certified by the Centers for Medicare and Medicaid Services (ID #07D1036625) under Clinical Laboratory Improvement Amendments.

The Tag-It™ Mutation Detection assays (Luminex Corporation, Austin TX) were utilized for DNA typing of 5, 7 and 18 alleles in genes *CYP2C9*, *CYP2C19* and *CYP2D6*, respectively, as previously described [38,42]. These assays employed PCR to amplify selectively the desired gene without co-amplifying pseudogenes or other closely related sequences. In addition, the assay employs a PCR strategy to amplify fragments characteristic of unique genomic rearrangements in order to detect the presence of the deletion and duplication alleles in these genes. The assays use multiplexed Allele Specific Primer Extension to identify small nucleotide variations including single base changes and deletions of one or three bases on the Luminex xMAP™ system [42].

Allele categorization

We classified the alleles into clinically distinct categories for each of the three genes examined as null, deficient, functional or ultra-rapid based on well-defined molecular properties of the altered genes [18,43]. Null alleles lack any enzymatic activity because the altered gene does not produce a functional protein. Such null alleles include gene deletions, frame-shift mutations, stop codons and splicing defects. Deficient alleles have sub-functional enzymatic activity due to nucleotide substitutions that result in amino acid changes in the protein, and these variants may manifest subnormal enzymatic activity for some drug substrates. The functional allele refers to the non-variant allele (*1) and enzymatically functional (*CYP2D6**2) with enzymatic activity is considered normal. For the purposes of this paper, we will refer to all functional alleles as ‘reference’ (REF) alleles. A REF allele denotes a lack of variation (i.e., *1) or a functional variant (i.e., *CYP2D6**2). A REF genotype is a combination of two REF alleles, resulting in a ‘REF’ gene locus. In this manuscript we will refer to such individuals as having ‘noncarrier’ genotypes. Ultra-rapid alleles exhibit increased enzymatic activity as a consequence of either gene duplication or a promoter polymorphism. The *CYP2D6**2a allele was assigned a value of 1.5 given that it is a promoter polymorphism that has been associated with increased isoenzyme synthesis [9,11,44]. *CYP2D6**2a homozygotes have been classified as ultra-rapid metabolizers. In our system, a *CYP2D6**2a*2a individual would receive the same ultra-rapid score of 3.0 as a *1Dup*2 individual. We consider a *CYP2D6* score of 3.0 or greater to be associated with *CYP2D6* ultra-rapid metabolism. Table 1 lists alleles detected in this patient cohort and their enzyme activity classification as determined by the Human Cytochrome P450 Allele Nomenclature Committee. To determine the combinatory non-REF frequency across all three genes examined, we used the genotype data for each patient in each population and tallied the number of subjects who had non-REF alleles in 3, 2, 1 or 0 genes.

Conventions for naming the alleles according to the Human Cytochrome P450 (CYP) Allele Nomenclature Committee have been followed in this research [101]. Accordingly, all variant alleles contain nucleotide changes that have been shown to affect transcription, splicing, translation, post-transcriptional or posttranslational modifications, or result in at least one amino acid change.

CYP450 combinatory indices

In order to correlate clinically relevant data with a quantitative representation of an individual's CYP450 metabolic phenotype, a series of indices were constructed. The following are the four indices:

- Drug metabolism reserve index

- Drug metabolism alteration index
- Allele alteration index (number of non-REF alleles)
- Gene alteration index (number of noncarrier gene loci)

All four indices assign a numeric value to CYP450 alleles depending on their mutant phenotype. Table 2 shows how all alleles and genotypes are scored in the four different indices. Allele scoring is based on literature review and does not always correspond exactly with the enzyme activity as shown in Table 1 (e.g., *CYP2C9**3, *4, *5 are scored as zero metabolism reserve owing to the high degree of enzyme deficiency relative to the *CYP2C9**2 and the reference *CYP2C9**1 alleles). The first three listed indices result from the summation of the two allele scores in the *CYP2C9*, *CYP2C19* and *CYP2D6* genes and are shown at the top of Table 2. The final index considers both alleles per gene when scoring the genotype with all REF combinations receiving a score of zero and every other combination being scored as one (Table 2, bottom). A gene-specific index can be calculated for any of the three genes by adding the allele scores for only that gene. Two gene combinatory indices can also be calculated by omitting the undesired gene from the summation of the allele scores.

The drug metabolism reserve index (metabolic reserve)

The drug metabolism reserve index is designed to represent a series of discrete CYP450 metabolic phenotypes from null (index = 0) to ultra-rapid ($6.5 < \text{index} \leq 7.5$). Since deficient and null alleles are scored lower than REF alleles, the lower the index the greater the innate metabolic deficiency. Similarly, as ultra-rapid alleles are scored higher than REF, the presence of these alleles contributes a greater value to the total metabolic index.

Drug metabolism alteration index (metabolic alteration)

As shown in Table 1, the *1 and the *CYP2D6**2 alleles represent the reference alleles and are associated with a normal metabolic capacity. In this index, we capture any departure from REF alleles on any of the *CYP2C9*, *CYP2C19* and *CYP2D6* genes, and score it according to the method in Table 2. Given that the REF allele is assigned a score of 0, any alteration, whether deficient or ultra-rapid is scored higher than zero as the index measures the absolute difference. More severe mutations are assigned a greater difference from the REF allele.

Allele alteration index (number of non-REF alleles)

This index depends on a binary scoring of each individual allele. In this case, the index represents the sum of each of the six alleles, designated as either REF (*1, *CYP2D6**2) or non-REF (all other alleles). Thus, a score of zero denotes a patient who has a noncarrier genotype across all three genes, while a score of six indicates a patient with two non-REF alleles on all three genes.

Gene alteration index (number of noncarrier gene loci)

The gene alteration index was created to represent a broad overview of gene alterations, as opposed to allelic mutations. Like the allele alteration index, this scoring method utilizes binary scoring by dichotomizing the presence of any non-REF allele on a gene and two copies of a REF allele. Therefore, an individual with a score of three has at least one non-REF allele on all three genes, while an individual with a score of zero carries only REF alleles.

Metabolic ranking curves

The metabolic ranking curves were derived using the same ‘metabolic ranking’ formula for all indices.

$$\frac{\sum_{\{i|x_i < x\}} N_i + \frac{1}{2} \sum_{\{i|x_i = x\}} N_i}{\sum_{i=1}^j N_i}$$

In this formula, j represents the number of levels in the index (length of N). N is the array of counts per index level, and x is the index value for the individual.

Drug-specific indices

The drug-specific indices utilize coefficients to weigh relative metabolic contributions of *CYP2C9*, *CYP2C19* and *CYP2D6* depending on a drug’s pharmacokinetic properties. These coefficients (α , β , and γ for *CYP2C9*, *CYP2C19* and *CYP2D6*, respectively) are assigned a value of 1.0 if the isoenzyme is considered a major metabolic pathway, a lesser value if it is a minor pathway, or a value of 0.0 if the isoenzyme is not involved in the metabolism of the particular drug. The index value (V) for a given drug (d) and index (i) are calculated according to the formula below:

$$v = \alpha_d(CYP2C9_i) + \beta_d(CYP2C19_i) + \gamma_d(CYP2D6_i)$$

Results

The results demonstrate that there is wide variation and high prevalence of combinatory alterations in the *CYP2C9*, *CYP2C19* and *CYP2D6* genes. Figure 1 shows the frequencies of polymorphisms across multiple genes. Of the 1199 patients genotyped, 89 (7.4%) were noncarriers for *CYP2C9*, *CYP2C19* and *CYP2D6*. 492 (41%) were polymorphic on only one of the three genes. Of those 492, individuals with mutations on the *CYP2D6* gene accounted for 395 (80%). Patients with polymorphisms across two of the three genes made up 45% of the population (539 individuals); 6.6% (79) of the samples had at least one polymorphic allele in all three genes tested. Greater than 50% of the population carried polymorphisms in either two or all three of the *CYP2C9*, *CYP2C19* and *CYP2D6* genes. These results correlate directly with the gene alteration index shown in Figure 2D.

Figure 2 displays histograms that depict the distributions and counts for each combinatory index. Figure 2a shows the histogram for the drug metabolism reserve index. The median index value is 5.0 and the upper-boundaries for the first and third quartiles are 4.5 and 5.5, respectively. Figure 2b shows the drug metabolism alteration index histogram. In this data set, the median value is 1.5 and the first and third quartile boundaries are 1.0 and 2.0, respectively. The median values in Figure 2C and Figure 2D are 2.0 and 1.0, respectively. The mean values for the metabolic reserve, metabolic alteration, allele alteration and gene alteration indices are 5.05, 1.44, 1.88 and 1.48, respectively. Examples of the distributions for all CYP450 combinatory indices are shown in Figure 2. Metabolic ranking curves, as calculated from the distributions by the metabolic ranking formula, are shown in Figure 3 for all four indices. In each index distribution, the median value correlates to the 50% value of the metabolic ranking curve. The same ranking curves are recreated in Figure 4 and

indicate, as examples, the placements of four individuals with distinct combinatory genotypes on each of the four indices.

Testing for discrepancies in the distributions and rankings between subgroups of our population yielded no significant results. The four combinatory index distributions did not differ significantly by age group. However, there was a trend for younger (<20 years) and older (>60 years) patients to have a greater average gene alteration index (mean = 1.54, combined) compared with other patients (mean = 1.45; $p = 0.08$). No significant difference in any of the indices was found between the male and female subgroups.

Discussion

Presented here are four indices of drug metabolism based on combinatory CYP450 polymorphisms: the drug metabolism reserve index, drug metabolism alteration index, allele alteration index and gene alteration index, as well as drug-specific indices derived from a combination of the drug metabolism indices and pharmacokinetic properties of particular drugs. This approach to *CYP2C9*, *CYP2C19* and *CYP2D6* analyses provides clinically relevant overarching and drug-specific measures of liver enzyme metabolism capacity. Currently, we have chosen *CYP2C9*, *CYP2C19* and *CYP2D6* because they each play a critical role in psychotropic metabolism, they have been linked with endogenous metabolism of neuro-active substrates, they have each been associated with depressive symptoms or personality traits, and their allelic variations, interindividual differences and resulting enzyme functions are well-documented [17,19,45,46]. By contrast, other isoenzymes known to play a role in the metabolism of many psychotropics, for instance those coded by *CYP1A2* and *CYP3A4* have been excluded due to the lack of sufficient evidence at present to characterize interindividual variations and relevance to drug metabolism [47]. Moreover, the susceptibility to, and interactions with various endogenous and exogenous cofactors, inhibitors and inducers are not adequately understood for these two genes, complicating the translation of the metabolic reserve or alteration score to clinical guidance. Nonetheless, the combinatory indices are designed such that addition or subtraction of any gene (e.g., *CYP1A2*, *CYP3A4*) or allele (e.g., *CYP2C19*17*) may be straightforward and simple, following an allele scoring method analogous to the one described in this study.

There may be several clinically relevant applications of the CYP450 combinatory drug metabolism indices [39,41]. Index scores can be easily calculated by following the allele specifications in Table 2. A large dataset of CYP450 genotypes can be quantified producing distributions for each index, which can then be used to compare combinatory polymorphism frequencies between populations. In this study, we found no significant difference within sub-populations, though this is not surprising given that the study population was made up of primarily Caucasian psychiatric referrals from local hospitals and, therefore, may be relatively homogenous. Significant differences may most likely emerge when comparing our population to nonpsychiatric patients, as seen when comparing gene alteration in a psychiatric versus a cardiology cohort [38]. Breaking down the gene polymorphism combinations provides further insight into the nature of the alterations, as in Figure 1. Given the varying impact of polymorphism across the three genes studied, different gene alteration combinations have distinct clinical consequences. For example, 41% of the study sample had a *CYP2C9* deficiency as well as a *CYP2C19* and/or *CYP2D6* non-REF allele, resulting in increased disease risk as well as compromised metabolic capacity. A total of 51.6% of the sample have multiple gene alterations, yielding either significantly altered drug metabolism, increased risk of disease due to endogenous drug metabolism, or both [48].

The metabolic ranking curves can be used to determine where an individual fits in the index distribution, such as the four patients in Figure 4. Such placement provides the clinician with

a clearer understanding of the patient's metabolic status in relation to the median (50%) individual and provides a level of clinical guidance that significantly augments single gene scores [39]. The placement of four distinct individuals on the ranking curves as seen in Figure 4 highlights the inherent value in benchmarking metabolic capacity prior to pharmacotherapy. For instance, individual W demonstrates that an entirely noncarrier combinatory genotype corresponds to relatively rapid metabolic capacity, as seen in the top 20th percentile ranking on the drug metabolism reserve index in Figure 4a. Intuitively, a clinician may expect a noncarrier individual to metabolize CYP450-dependent psychotropics with 'average' pharmacokinetics. However, given that this genotype is not common (as seen by its ranking in the bottom 5% of the alteration indices in Figure 4b–D), we do not predict this outcome. Individual X carries two deficient *CYP2D6* alleles (*41 double carrier), an illustration of approximately 'average' status for all four indices. For patient Y, the drug metabolism reserve index is average, but the alteration is high. In this example, the *CYP2D6* double-carrier status of the promoter polymorphism allele *2a results in ultra-rapid *CYP2D6* metabolism. However, the null metabolizer status for the *CYP2C19* gene offsets the combinatory score, giving the appearance of an 'average' metabolizer (Figure 4a). The alteration scores for patient Y provide a warning that CYP450-dependent drugs should be administered with caution, as seen by their ranking in the upper 10% of the alteration indices (Figure 4b & C). Finally, individual Z represents a case of low reserve and high alteration, spread across all three genes. Individuals with a gene alteration index score of three may need particularly careful monitoring for side effects because of what could be described as a 'Drug Sensitivity Syndrome'. The rankings in Figure 4 predict that individual Z would be such a case because he is on the extremes of all of the indices, in particular the drug metabolism reserve and the gene alteration scores. A published case report described a patient with a drug metabolism reserve ranking in the 0–1 percentile, with a metabolic reserve index of two [48]. Benchmarking this individual, as seen in Figure 4, would have immediately red-flagged that patient as being potentially difficult to manage. A clinician could then proceed using a drug-specific index to determine which drugs would be most dangerous or ineffective, or, conversely, well-suited for that individual [39].

Quantitative measures explaining CYP450 liver enzyme capacity simplify statistical procedures without compromising the accuracy of the results. In fact, using CYP450 combinatory indices, and especially the drug-specific index, may be superior in accuracy and utility to phenotype descriptors of poor metabolizer through ultra-rapid metabolizer owing to the resolution and scope of this approach [39]. A similar quantitative scoring system, recently developed for the *CYP2D6* gene, was found to be useful in guiding tamoxifen pharmacotherapy [33]. The specificity of this system in conjunction with the ability to consider concomitant inhibitory medications leads to greater utility in predicting an individual's tamoxifen response. Similarly, our proposed quantitative model facilitates consideration of drug interactions by allowing for appropriate, evidence-based modifications to index values in the presence of strong CYP450 inhibitors and/or inducers. The gene coefficients of the drug-specific index yield an analogous scoring system for single or primary metabolic pathways such as tamoxifen; however, our combinatory approach is better suited for psychotropic medications that depend on multiple CYP450 pathways.

Emerging research has highlighted the complexity of epistatic interactions as well as drug-gene induction, inhibition and regulation in the CYP450 gene family [47,49]. It may be that single-gene systems represent an oversimplification of CYP450 genetic and metabolic mechanisms. This conclusion is corroborated by our clinical observations that patients with a greater gene alteration index are often the most complex clinical cases, marked by increased morbidity, multiple drug trials, adverse drug effects and increased lengths of stay. Moreover, our clinical applications study demonstrates that the combinatory and drug-

specific indices consistently improve clinical correlations when compared with single-gene indices [39].

Our model, in its unmodified form, has numerous limitations and should be integrated with the comprehensive clinical scenario of each patient. For instance, the model does not account for demographic, pharmacologic, epigenetic or disease co-factors that are well known to be relevant to drug selection and dosing. Furthermore, the indices, including the drug-specific indices, do not consider various individual drug types such as prodrugs or those with active metabolites. In the case of a prodrug, such as codeine, the relationship between dose adjustment and drug-specific index value must be inverted relative to an active compound. Similarly, if evidence exists of a decrease in side effects in the presence of a gene variant, the researcher or clinician must consider this relationship when interpreting the clinical meaning of the index value. Finally, our single-nucleotide polymorphism array is not comprehensive, most notably lacking the *CYP2C19*17* allele associated with increased isoenzyme activity.

Owing to the quantitative nature of the indices, additional single nucleotide polymorphisms (or new evidence for genotype–phenotype relationships) and various cofactors can easily be modeled into predictive dosing formulas or clinical pharmacogenetic software tools. For example, strong CYP inhibitors or inducers can be incorporated into the drug-specific indices by respectively subtracting or adding an appropriate, evidence-based value from the relevant gene components of the drug-specific index formula. Even epigenetic factors could be modeled into the indices by modifying gene coefficients to reflect epigenetic influences. These limitations underscore the importance of using the indices as either high-level illustrative benchmarks of overall metabolic capacity, or modifying the indices to consider the cofactors present in a particular clinical case. For instance, algorithms and clinical software may be developed to integrate individual metabolism function, demographic covariates, and drug interactions with the four metabolism indices to generate an individualized drug selection and dosing guidance utility.

The purpose of the drug metabolism reserve and alteration indices is to create a DNA-based clinically relevant and physiologically accurate representation of liver enzyme metabolic function through a combinatory analysis of *CYP2C9*, *CYP2C19* and *CYP2D6* single nucleotide polymorphisms and haplotypes. The four combinatory indices serve to benchmark an individual's metabolic capacity, providing a clinician with a broad picture of their patient's inherent pharmacokinetic status relative to a larger population prior to choosing a medication. The indices differ from liver function or plasma concentration tests in that they are independent from temporary environmental conditions and covariates, and they provide innately predictive, as opposed to reactive, utility. The drug-specific indices match single-drug pharmacokinetic properties with individual genotypes to provide clinically relevant drug selection and dosing support. Together, these innovations provide a platform for adoption and application of personalized medicine by simplifying and improving analytical processes. Furthermore, they may support clinically meaningful decision support tools for personalized drug selection. Ultimately, the CYP450 combinatory drug metabolism indices should assist in both the research, driving novel genotype–phenotype correlations, and the translation of those findings to individualized clinical treatment decisions.

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Executive summary

- Many of the most utilized medications, particularly psychotropics, are metabolized by multiple CYP450 pathways, each of which, in isolation may inadequately account for a drug's pharmacokinetic properties. Distributed substrate affinity would benefit from a combinatory approach that considers *CYP2C9*, *CYP2C19* and *CYP2D6* as integrative components of a hepatic enzyme system for drug metabolism.
- We genotyped 1199 psychiatric patients referred to the Genomas Laboratory of Personalized Health at Hartford Hospital (Hartford, Connecticut USA) for a total of 30 polymorphisms in the *CYP2C9*, *CYP2C19* and *CYP2D6* genes (5, 7 and 18 allelic loci, respectively).
- There was wide variation and high prevalence of combinatory alterations in the *CYP2C9*, *CYP2C19* and *CYP2D6* genes. Of the 1199 patients genotyped, 7.4% were not polymorphic in any of the three genes, 41% were polymorphic in one gene, 45% were polymorphic in two genes and 6.6% were polymorphic in all three genes. Greater than 50% of the population carried polymorphisms in either two or all of the *CYP2C9*, *CYP2C19* and *CYP2D6* genes.
- We developed four novel drug metabolism indices from the combinatory genotyping data and scored them for each patient, creating distributions and rankings of innate drug metabolism capacity to which individuals can be compared.

Drug metabolism reserve index (metabolic reserve)

- This index is designed to represent a series of discrete CYP450 metabolic phenotypes from null (index = 0) to ultra-rapid ($6.5 < \text{index} \leq 7.5$). The higher the index, the greater the innate drug metabolism capacity of the individual is. The median value for the drug metabolism reserve index value is 5.0 (mean of 5.05) and the upper-boundaries for the first and third quartiles are 4.5 and 5.5, respectively.

Drug metabolism alteration index (metabolic alteration)

- This index quantifies any departure from functional reference (REF) alleles on any of the three genes and measures the absolute difference. More severe mutations are assigned a greater difference from the REF allele. The median value for the drug metabolism alteration index was 1.5 (mean of 1.44) and the first and third quartile boundaries are 1.0 and 2.0, respectively.

Allele alteration index (number of non-REF alleles)

- This index represents the sum of each of the six alleles, designated as either REF or non-REF (all other alleles). A score of 0 denotes a patient who has a non-carrier genotype across all three genes; a score of 6, a patient with two non-REF alleles on all three genes. The median value for the allele alteration index was 2.0 (mean of 1.88).

Gene alteration index (number of non-carrier gene loci)

- This index utilizes binary scoring by dichotomizing the presence of any non-REF allele on a gene and two copies of a REF allele. An individual with a score of three has at least one non-REF allele on all three genes. An individual with a score of 0 carries only REF alleles. The median values for the gene alteration index was 1.0 (Mean of 1.48).

- Quantitative measures explaining CYP450 liver enzyme capacity simplify statistical procedures without compromising the accuracy of the results. CYP450 combinatory indices for innate drug metabolism function may be superior in accuracy and utility to phenotype descriptors of metabolizer status (e.g., poor, intermediate, extensive and ultra-rapid) due to the resolution and scope of this approach. In the companion paper, the combinatory drug metabolism indices are applied to clinical correlations in the assessment of dyslipidemia in hospitalized psychiatric patients treated with various psychotropics. Patients with the least metabolic reserve index and greatest alteration indices were found to have the most marked dyslipidemia.

Figure 1. Combinatory polymorphisms in *CYP2C9*, *CYP2C19* and *CYP2D6* genes from a population of 1199 psychiatric referrals.

Figure 2. Distributions of the four CYP450 combinatory drug metabolism indices in a population of 1199 psychiatric referrals
(A) Drug metabolism reserve index, **(B)** drug metabolism alteration index, **(C)** allele alteration index and **(D)** gene alteration index.

Figure 3. Ranking curves of the four CYP450 combinatory drug metabolism indices in a population of 1199 psychiatric referrals

The rankings are calculated using the 'ranking formula' from the distributions seen in Figure 2. Individuals can visualize their metabolic ranking by locating the point associated with their index value (x-axis) for a particular index and determining the relative percentile ranking (y-axis) corresponding to that point. **(A)** Drug metabolism reserve, **(B)** drug metabolism alteration ranking, **(C)** allele alteration ranking and **(D)** gene alteration ranking.

Figure 4. Placements of four individuals (W,X,Y,Z) with distinct genotypes on the ranking curves of the four CYP450 combinatory drug metabolism indices in a population of 1199 psychiatric referrals shown in Figure 3

The genotypes of individuals W, X, Y and Z are as follows; W: *CYP2C9* *1*1, *CYP2C19* *1*1, *CYP2D6* *1*1; X: *CYP2C9* *1*1, *CYP2C19* *1*1, *CYP2D6* *41*41; Y: *CYP2C9* *1*1, *CYP2C19* *2*2, *CYP2D6* *2a*2a; Z: *CYP2C9* *1*3, *CYP2C19* *1*2, *CYP2D6* *1*4.

(A) Drug metabolism reserve ranking, (B) drug metabolism alteration ranking, (C) allele alteration ranking and (D) gene alteration ranking.

Table 1

CYP2C9, *CYP2C19* and *CYP2D6* gene variants tested in this study.

Gene	Allele	Gene nucleotide change	Effect	Enzyme activity
<i>CYP2C9</i>	*1	None	None	Normal
	*2	3608 C>T	R144C	Decreased
	*3	42614 A>C	I359L	Decreased
	*4	42615 T>C	I359T	Decreased
	*5	42619 C>G	D360E	Decreased
	*6	10601 delA	Frameshift	None
<i>CYP2C19</i>	*1	None	None	Normal
	*2	19154 G>A	Splicing defect	None
	*3	17948 G>A	W212X	None
	*4	1 A>G	GTG initiation codon	None
	*5	90033 C>T	R433W	None
	*6	12748 G>A	R132Q	None
	*7	19294 T>A	Splicing defect	None
	*8	12711 T>C	W120R	None
<i>CYP2D6</i>	*1	None	None	Normal
	*1XN	Gene copy number (N)	N gene copies	Increased
	*2	1661 G>C	None	Normal
	*2a	-1584 C>G	Promoter	Increased
	*2XN	Gene copy number (N)	N gene copies	Increased
	*3	2549 delA	Frameshift	None
	*4	1846 G>A	Splicing defect	None
	*4XN	Gene copy number (N)	N gene copies	None
	*5	Gene deletion	CYP2D6 del	None
	*6	1707 delT	Frameshift	None
	*7	2935 A>C	H324P	None
	*8	1758 G>T	G169X	None
	*9	2615 2617 delAAG	K281del	Decreased
	*10	100 C>T	P34S	Decreased
	*11	883 G>C	Splicing defect	None
	*12	124 G>A	G42R	None
	*14	1758 G>A	G169R	None
	*15	137_138 insT	Frameshift	None
	*17	1023 C>T	T107I	Decreased
	*41	2988 G>A	Splicing defect	Decreased

The 'Gene' column denotes the gene carrying the variants. The 'Allele' column shows the specific allele, named according to the Human Cytochrome P450 Allele Nomenclature Committee [101]. The 'Nucleotide Change' column details the change in nucleotide associated with the particular allele. The 'Effect' explains the resultant effect on the polypeptide and the 'enzyme activity' shows the enzymatic activity as a result of the variant allele.

del: Deleted; ins: Inserted; N: A gene copy number exceeding 1.

Table 2

Allele scoring for four novel drug metabolism indices.

CYP2C9	CYP2C19	CYP2D6	Metabolic reserve	Metabolic alteration	Allele alteration
*1	*1	*1,*2	1	0	0
*2		*9,*10,*17,*41	0.5	0.5	1
		*2a	1.5	0.5	1
		*1XN,*2XN	2	1	1
*3,*4,*5,*6	*2,*3,*4,*5,*6,*7,*8	*3,*4,*6,*7,*8,*11,*12,*14,*15,*5,*4XN	0	1	1
CYP2C9	CYP2C19	CYP2D6	Gene alteration		
*1*1	*1*1	*1*1,*1*2,*2*2	0		
All other allele combinations	All other allele combinations	All other allele combinations	1		