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PHARMACOGENETICS

Risperidone metabolic ratio as a biomarker of individual *CYP2D6* genotype in schizophrenic patients

Buster Mannheimer · Johan Holm · Larissa Koukel · Leif Bertilsson · Urban Ösby · Erik Eliasson

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Abstract
Purpose The purpose of the present study was to investigate the predictive value of the risperidone metabolic ratio for the individual *CYP2D6* genotype.
Methods The determination of risperidone, 9-hydroxyrisperidone, and *CYP2D6* genotype was performed in 89 schizophrenic patients. The receiver operator characteristic (ROC) method and the area under the ROC curve (AUC) were used to illustrate the predictive value of risperidone metabolic ratio for the individual *CYP2D6* genotype. The area under the ROC curve (AUC) was used as a global measure of this predictive value. To evaluate the proposed cutoff levels of >1 and <0.1 to identify individuals with a poor or ultrarapid *CYP2D6* genotype the sensitivity, specificity, positive predictive value and negative predictive were calculated.
Results The area under the ROC curve (AUC) for poor and ultrarapid metabolisers was 0.85 and 0.86, respectively. The sensitivity, specificity, positive predictive value and negative predictive value of a risperidone/9–OH-risperidone ratio >1 to *CYP2D6* poor metaboliser genotype were 75 %, 95 %, 60 % and 97 %, respectively. The corresponding measures for a metabolic ratio<0.1 to predict ultrarapid metabolisers were 80 %, 77 %, 18 % and 98 %.
Conclusions A metabolic ratio>1 or<0.1 may be a useful therapeutic biomarker to recommend *CYP2D6* genetic testing to guide the present or future treatment of patients in need of psychotropic drugs.

Keywords *CYP2D6* genotype · Genetic polymorphism · Therapeutic drug monitoring · Psychotropic drugs

Introduction

The frequently used antipsychotic drug risperidone undergoes cytochrome P450 2D6 (CYP2D6) -dependent hydroxylation to 9-hydroxyrisperidone. The pharmacological activity and potency of risperidone and 9-hydroxyrisperidone have been claimed to be similar [1], and the sum of the two compounds is often referred to as "active moiety". Although some studies have reported that the active moiety would not differ between subjects with different *CYP2D6* genotypes [2, 3], more recent data derived from clinical studies on the effect of CYP2D6 inhibitors instead show that risperidone accumulates in slow metabolisers without any significant decrease in the corresponding levels of 9-hydroxyrisperidone [4–9]. Polymorphic CYP2D6 metabolises a wide range of psychotropic drugs. Different genetic variants, of *CYP2D6* indeed contribute to marked inter-patient variability in the pharmacokinetics of frequently used antipsychotic drugs, with an obvious potential to impact on the clinical outcome and in particularly the risk of adverse events [10–14]. Still, *CYP2D6* genotyping has not been adopted into routine clinical practice within psychiatry but has been restricted to retrospective analyses in selected cases[15]. Knowledge of the individual CYP2D6 activity, or more specifically, the poor or ultra-rapid metaboliser genotype, is of potentially high relevance for on-going or future

B. Mannheimer (✉)
Karolinska Institutet, Department of Clinical Science and Education at Södersjukhuset, Stockholm, Sweden
e-mail: Buster.mannheimer@sodersjukhuset.se

J. Holm · L. Koukel · L. Bertilsson · E. Eliasson
Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska University Hospital Huddinge, Stockholm, Sweden

U. Ösby
Department of Molecular Medicine and Surgery, Neurogenetics Unit, and Centre for Molecular Medicine, Stockholm, Sweden

U. Ösby
Department of Psychiatry, Tiohundra AB, Norrtälje, Sweden

drug treatment in psychotic patients. Therefore, analyses of risperidone plasma levels might provide useful information in this regard. The purpose of the present study was to investigate the relationship between risperidone metabolic ratio (MR) and CYP2D6 genotype and assess the predictive value of risperidone MR to predict genotype.

Methods

Patients and setting

The patients were recruited from psychosis-specialised psychiatric outpatient departments mainly in the Stockholm County, Sweden, as described previously [16]. Determination of risperidone, 9-hydroxyrisperidone, and *CYP2D6* genotype were performed by quality-accredited LCMS-based routine methods in the Therapeutic Drug Monitoring laboratory, Department of Clinical Pharmacology, Karolinska University Hospital Huddinge, Sweden. In six samples, the risperidone concentration was detectable but below the lowest limit of quantification and, therefore, defined as <5 nmol/L in the TDM database. In these cases, a value of 2.5 nmol/L was imputated. Routine *CYP2D6* genotyping was performed on DNA isolated from peripheral blood, and determined by PCR-based TaqMan allele discrimination methods, with commercially available assays from Applied Biosystems that discriminate between the *CYP2D6*3*, **4*, **10*, **17* and**41* allele variants and the corresponding sequence of the *CYP2D6*1* allele. Carriers of another fully functional *CYP2D6* allele (**2*) would be detected as *CYP2D6*1*, which is irrelevant with respect to predicted activity. *CYP2D6* copy number analysis (CNA) was performed in all samples, essentially according to the manufacturer's protocol (Applied Biosystems). A CNA result of 0 or 1, as compared to the normal 2, indicates *CYP2D6* gene deletion(s), and was interpreted as a homozygous (CNA=0) or heterozygous (CNA=1) carrier of the *CYP2D6*5* allele variant (see Table 1). Any extra copies of CYP2D6 (CNA>2) were interpreted as fully functional allele variants (**1 or *2*), as amplification of functional alleles is more common than that of defect alleles, such **4* or **41* [17]. Admittedly, this might carry a risk of misclassifying a few UM individuals in our study. Ultrarapid metabolisers (UM) were defined as carriers of three or more gene copies, poor metabolisers (PM) were defined as carriers of two null-alleles (**3*, **4*, and/or **5*), and intermediate metabolisers (IM) were defined as carriers of at least one partially defective allele (**10, *41, *17*) or maximally one null-allele. Individuals on concomitant treatment with drugs known to inhibit CYP2D6 were excluded. Identification of these CYP2D6 inhibitors were based on the Flockhart Cytochrome P450 Drug Interaction Table [18] with the addition of thioridazine, perphenazine and levomepromazine based on available evidence in the literature [9, 19, 20].

Table 1 CYP2D6 genotype frequencies in the study population (n=89)

CYP2D6 alleles and copy number	Number of patients	Predicted metabolic activity (% of **1/*1*)	Genotype classification	Frequency (%)
**1/*1*, n=3	5	150	UM	5.6
**1/*1*, n=2	34	100	EM	38
**1/*41*, n=2	9	75	IM	47
**1/*10*, n=2	3	60		
**1/*3*, n=2	3	50		
**1/*4*, n=2	18	50		
**1/*5*, n=1	1	50		
**41/*41*, n=2	2	50		
**4/*41*, n=2	3	25		
**5/*17*, n=1	1	25		
**5/*41*, n=1	1	25		
**5/*10*, n=1	1	10		
**3/*4*, n=2	2	0	PM	9.0
**4/*4*, n=2	5	0		
**4/*5*, n=1	1	0		

Statistical methods

All data on plasma concentrations for risperidone, 9-OH-risperidone, or the sum of them (i.e., active moiety) was related to daily dose, by forming concentration-to-dose exposure values (*C:D*). As the distribution of these concentration-to-dose values was positively skewed (skewness ranging between 1.2 and 3.3), nonparametric statistics were used. The receiver operator characteristic (ROC) method and the area under the ROC curve (AUC) were used to illustrate the predictive value of the risperidone metabolic ratio for the individual *CYP2D6* genotype. The area under the ROC curve (AUC) was used as a global measure of this predictive value [21, 22]. To evaluate the proposed cutoff levels of >1 and <0.1 for identification of individuals with a poor or ultrarapid *CYP2D6* genotype the sensitivity, specificity, positive predictive value and negative predictive were calculated. To compare the *C:D* of risperidone, 9-OH-risperidone, and active moiety, or risperidone/9-OH-risperidone-ratio among patients with different genotypes, the Kruskal Wallis test was used. P values <0.05 were considered as significant. The statistical analyses were performed using IBM SPSS 22.0.

Ethics approval

The study was approved by the Regional Ethics Committee in Stockholm, Karolinska Institutet.

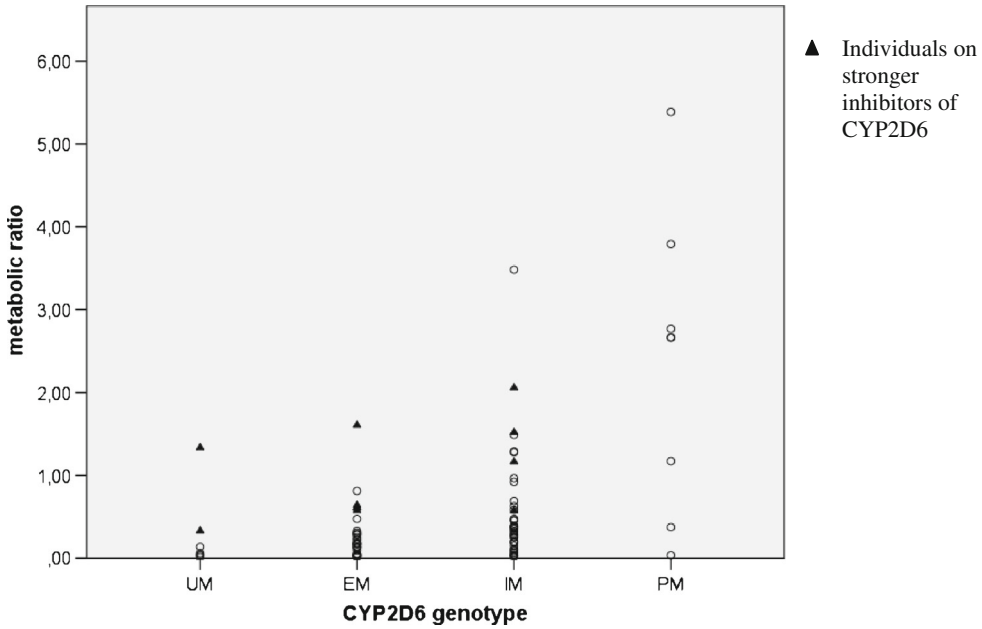

Fig. 1 Risperidone/9-OH-risperidone ratio (metabolic ratio) in patients with different CYP2D6 genotypes and in patients with stronger inhibitors of CYP2D6

Results

Initially, 775 psychotic patients were recruited. While 676 patients were not using risperidone and ten risperidone-treated patients also were prescribed a potent CYP2D6 inhibitor, 89 patients remained for further analysis in this study. The median age was 49 years (range 23–75), and 38 % were women. The median daily dose of risperidone was 3.0 mg (range 0.9–10 mg/day).

The genotypes of the *CYP2D6* variants in the 89 patients under study are given in Table 1. The most frequent genotypes were **1/*1*, **1/*4* and **1/*41* with a prevalence of 39 %, 18 % and 9 %, respectively. As shown in Table 1, the prevalence of poor metabolisers (homozygous for two null-alleles) was 9.0 % and the prevalence of ultrarapid metabolisers was 5.6 %.

The range of the risperidone/9-OH-risperidone ratio in *CYP2D6* ultrarapid metabolisers, extensive metabolisers, intermediate metabolisers and poor metabolisers (1.3, 1.6, 3.5 and 5.4 respectively) is illustrated in Fig. 1. A comparison between dose-corrected (*C:D*) concentrations of risperidone, 9-OH-risperidone, active moiety and risperidone/9-OH-risperidone ratio is shown in Table 2. Here, it is evident that the active moiety of risperidone increased significantly in a step-wise fashion when comparing the UM, EM, IM, and PM genotypes (12, 16, 17, and 25 nmol L^{-1} mg^{-1}, respectively, p=0.028). These differences were explained by an accumulation of risperidone (0.50, 1.6, 3.6 and 18 nmol L^{-1} mg^{-1}, respectively) whereas no significant genotype differences were evident in plasma concentrations of the active metabolite 9-OH-risperidone (10, 15, 13, and 8.3 nmol L^{-1} mg^{-1}, respectively).

Also, the risperidone/9-OH-risperidone ratio increased in patients genotyped as slower in CYP2D6-dependent metabolism (UM: 0.041, EM: 0.17, IM: 0.30 and PM: 2.7 nmol L^{-1} mg^{-1}, respectively, p=0.001). To illustrate the usefulness of the risperidone/9-OH-risperidone ratio for identification of individuals being either *CYP2D6* PM or UM, ROC curves were generated (Fig. 2). The area under the

Table 2 The relationships between risperidone and 9-OH-risperidone and CYP2D6 genotype in patients on maintenance treatment. Plasma concentration-to-dose (*C/D*) ratios of risperidone, 9-OH-risperidone, risperidone/9-OH-risperidone ratio and the active moiety (sum of risperidone and 9-OH-risperidone) in 89 patients in four different *CYP2D6* genotype groups. Data are expressed as medians with 25th-75th percentiles in parenthesis and n=number of subjects

Genotype classification	Ultrarapid metabolisers (UM)	Extensive metabolisers (EM)	Intermediate metabolisers (IM)	Poor metabolisers (PM)
risperidone (nmol L^{-1} mg^{-1})[1]	0.50 (0.22-0.96) n=5	1.6 (0.63-5.1) n=32	3.6 (1.1-8.2) n=39	18 (6.4-27) n=8
9 -OH-risperidone (nmol L^{-1} mg^{-1})	10 (7.0-16) n=5	15 (8.8-22) n=31	13 (8.4-18) n=38	8.3 (5.9-13) n=8
Ratio risperidone/ 9-OH-risperidone[1]	0.04 (0.02-0.10) n=5	0.17 (0.05-0.30) n=31	0.30 (0.11-0.46) n=38	2.7 (0.57-3.5) n=8
Active moiety (nmol L^{-1} mg^{-1})[1]	12 (7.4-16) n=5	16 (11–25) n=31	17 (12–22) n=38	25 (19–37) n=8

[1] Difference in *C/D* ratios of risperidone, risperidone / 9-OH-risperidone ratio and active moiety between the different genotypes are statistically significant (p<0.001, p=0.001 and p=0.028, respectively) (Kruskal Wallis)

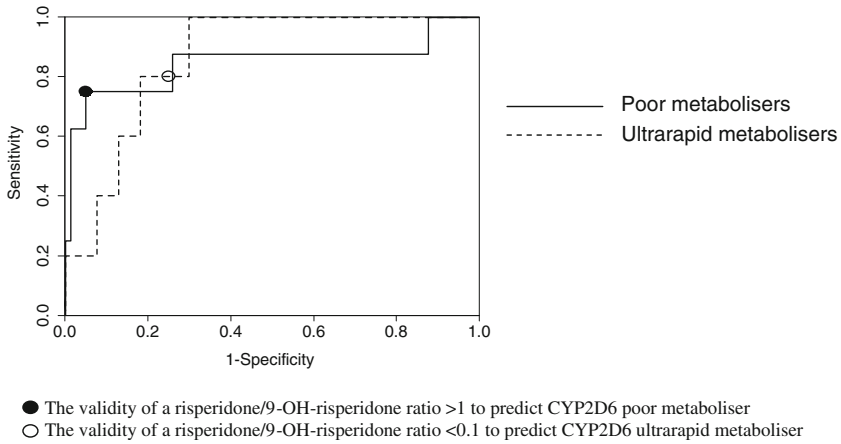

Fig. 2 Receiver operating characteristic curve used to evaluate the validity of the risperidone/9-OH-risperidone ratio to identify CYP2D6 poor and rapid metabolisers

ROC curve (AUC) for poor and ultrarapid metabolisers was 0.85 and 0.86, respectively. The sensitivity, specificity, positive predictive value and negative predictive value of a risperidone/9-OH-risperidone ratio >1 to predict *CYP2D6* poor metaboliser genotype were 75 %, 95 %, 60 % and 97 %, respectively. The corresponding measures for a metabolic ratio<0.1 to predict ultrarapid metabolisers were 80 %, 77 %, 18 % and 98 % (Fig. 3).

Discussion

In our study cohort, risperidone / 9-OH-risperidone ratio was useful as a biomarker to predict individuals with poor or ultrarapid *CYP2D6* genotype. Using the previously proposed cutoff value of 1[8] it was evident that 75 % (6/8) of patients above this value were lacking functional CYP2D6 on a genetic basis. On the other hand, 97 % (70/72) of non-poor metabolisers were correctly classified as such having a risperidone metabolic ratio≤1. This information may be clinically useful, in particular considering on-going or future treatment with CYP2D6 drug substrates. In these cases, a risperidone result indicative of a *CYP2D6* PM genotype might facilitate in dose selection and potentially reduce the risk of adverse drug reactions related to high plasma concentrations [23]. Also, confirmatory genotyping may be considered. The results from the present investigation also suggest that low metabolic ratios (<0.1) might be a potential biomarker for the UM genotype, even though this needs to be validated in a larger number of patients.

Our results are well in line with the findings presented by De Leon and co-workers, showing that 95 % (19 / 20) of poor metabolisers had a metabolic ratio >1 as compared to 13 % (34 / 257) for the rest of the patients [8]. In our material, patients on concomitant treatment with established CYP2D6 inhibitors (n=10) were excluded from the final analysis of the relationship between risperidone metabolic ratio and *CYP2D6* genotype. However, in a retrospective analysis, we found that stronger inhibitors of CYP2D6 convert converted five out of nine patients, irrespective of genotype, to a poor metaboliser phenotype; i.e. ,as here defined by a risperidone metabolic ratio>1. Among the patients on established CYP2D6 inhibitors, concomitant paroxetine (n=2) or levomepromazine (n=5) were associated with the

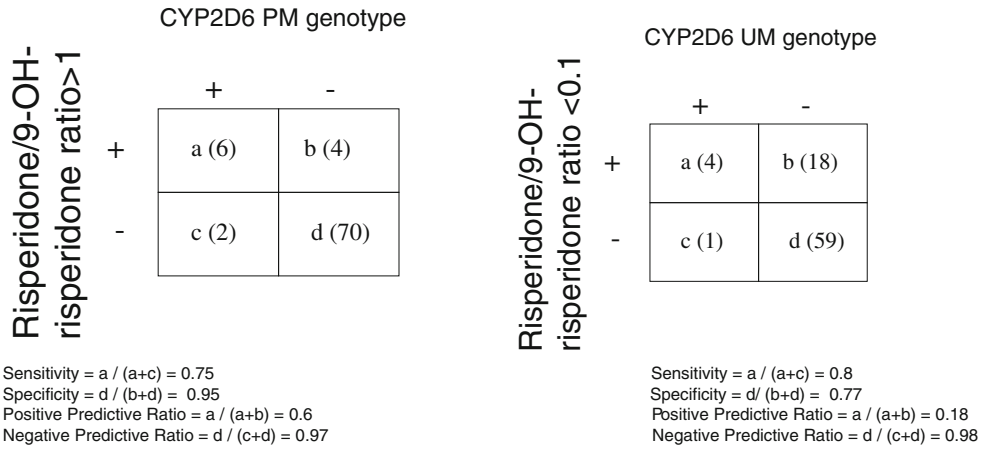

Fig. 3 Validity of the risperidone/9-OH-risperidone ratio to predict CYP2D6 genotype in 82 patients

highest risperidone MR (>1), despite the fact that one of these subjects were genotyped as *1/*1, n=3 and another one as extensive metaboliser *1/*1, n=2 (data not shown). This agrees well with previous results on the impact of CYP2D6 inhibitors and also the validity of 1 as the cut-off for PM definition in risperidone metabolic ratio [9].

Limitations

In the current study, information on time between dosing and the risperidone sampling was lacking. This may be important as the half life of risperidone and 9-OH-risperidone differ leading to a variable ratio depending on sampling time in relation to last dose intake. This may have contributed to increased inter-individual variability in our material and a risk of underestimating the sensitivity and specificity of the metabolic ratio as a biomarker as well as the importance of genotype for the level of active moiety.

In summary, we confirm that the *CYP2D6* genotype is important for differences in the active moiety of risperidone and may, therefore, impact on the clinical outcome during risperidone treatment. This also strengthens the indication to perform TDM in patients that are co-dispensed drugs known to interact with CYP2D6-dependent drug metabolism, or in patients that develop pronounced adverse drug reactions at standard doses. Furthermore, a metabolic ratio>1 or<0.1 may be a useful therapeutic biomarker to recommend *CYP2D6* genetic testing to guide the present or future treatment of patients in need of psychotropic drugs.

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Declaration of potential conflicts of interest The authors declare no conflicts of interest.

References

1. van Beijsterveldt LE et al (1994) Regional brain distribution of risperidone and its active metabolite 9-hydroxy-risperidone in the rat. Psychopharmacology (Berlin) 114(1):53–62
2. Olesen OV et al (1998) Serum concentrations and side effects in psychiatric patients during risperidone therapy. Ther Drug Monit 20(4):380–384
3. Scordo MG et al (1999) Cytochrome P450 2D6 genotype and steady state plasma levels of risperidone and 9-hydroxyrisperidone. Psychopharmacology (Berlin) 147(3):300–305
4. Alfaro CL et al (2000) CYP2D6 inhibition by fluoxetine, paroxetine, sertraline, and venlafaxine in a crossover study: intraindividual variability and plasma concentration correlations. J Clin Pharmacol 40(1):58–66
5. Spina E et al (2001) Plasma concentrations of risperidone and 9-hydroxyrisperidone during combined treatment with paroxetine. Ther Drug Monit 23(3):223–227
6. Spina E et al (2002) Inhibition of risperidone metabolism by fluoxetine in patients with schizophrenia: a clinically relevant pharmacokinetic drug interaction. J Clin Psychopharmacol 22(4):419–423
7. Bondolfi G et al (2002) The effect of fluoxetine on the pharmacokinetics and safety of risperidone in psychotic patients. Pharmacopsychiatry 35(2):50–56
8. Leon J et al (2007) A study of genetic (CYP2D6 and ABCB1) and environmental (drug inhibitors and inducers) variables that may influence plasma risperidone levels. Pharmacopsychiatry 40(3):93–102
9. Mannheimer B et al (2008) Impact of multiple inhibitors or substrates of cytochrome P450 2D6 on plasma risperidone levels in patients on polypharmacy. Ther Drug Monit 30(5):565–569
10. Dahl ML (2002) Cytochrome p450 phenotyping/genotyping in patients receiving antipsychotics: useful aid to prescribing? Clin Pharmacokinet 41(7):453–470
11. Cartwright AL et al (2013) Pharmacogenetics of risperidone: a systematic review of the clinical effects of CYP2D6 polymorphisms. Ann Pharmacother 47(3):350–360
12. Llerena A et al (2013) Pharmacogenetics of clinical response to risperidone. Pharmacogenomics 14(2):177–194
13. Fleeman N et al (2011) Cytochrome P450 testing for prescribing antipsychotics in adults with schizophrenia: systematic review and meta-analyses. Pharmacogenomics J 11(1):1–14
14. Ingelman-Sundberg M et al (2007) Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther 116(3): 496–526
15. Sjoqvist F, Eliasson E (2007) The convergence of conventional therapeutic drug monitoring and pharmacogenetic testing in personalized medicine: focus on antidepressants. Clin Pharmacol Ther 81(6):899–902
16. Ghotbi R et al (2010) Carriers of the UGT1A4 142 T>G gene variant are predisposed to reduced olanzapine exposure–an impact similar to male gender or smoking in schizophrenic patients. Eur J Clin Pharmacol 66(5):465–474
17. Sachse C et al (1997) Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 60(2):284–295
18. Flockhart, D., Drug interactions: Cytochrome P450 Drug Interaction Table. Indiana School of Medicine 2007. Available at: http://medicine.iupui.edu/flockhart/table.htm Accessed August 28, 2013.
19. Jerling M, Bertilsson L, Sjoqvist F (1994) The use of therapeutic drug monitoring data to document kinetic drug interactions: an example with amitriptyline and nortriptyline. Ther Drug Monit 16(1):1–12
20. Yasui N et al (1995) Inhibition of trazodone metabolism by thioridazine in humans. Ther Drug Monit 17(4):333–335
21. Grzybowski M, Younger JG (1997) Statistical methodology: III. Receiver operating characteristic (ROC) curves. Acad Emerg Med 4(8):818–826
22. Faraggi D, Reiser B (2002) Estimation of the area under the ROC curve. Stat Med 21(20):3093–3106
23. Wilkinson GR (2005) Drug metabolism and variability among patients in drug response. N Engl J Med 352(21):2211–2221