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Source / Izvornik: **Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2021, 104**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/10.1016/j.pnpbp.2020.110042

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:975683

1ABCB1, *ABCG2* and *CYP2D6* polymorphism effects on disposition and response to long-acting risperidone

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Key words: ABCB1, ABCG2, CYP2D6, pharmacogenetics, long-acting risperidone

Word count: 6735 (with tables and figures)

Number of figures: 1 (no colour should be used for any figures in print)

Number of tables: 6

Number of references: 68

Supplemental files: 1

Abstract

The relevance of the multidrug resistance (ABCB1) and breast cancer resistance (ABCG2) protein transporter polymorphisms for treatment with long-acting intramuscular (LAI) risperidone is largely unknown. We explored the relationship between these polymorphisms and cytochrome P450 (CYP) 2D6 genotype-predicted phenotype in their effects on drug disposition and clinical outcomes in adults with schizophrenia. In a 24-week observational study, patients initiated on LAI-risperidone (N=101) were genotyped [enzymes (*CYP2D6 dupl,*3,*4,*5,*6,*41*; *CYP3A4*22, CYP3A5*3*), transporters (*ABCG2 421C>A; ABCB1 1236C>T, 2677G>T/A, 3435C>T*)] and evaluated for steady-state (weeks 6-8) serum levels of dose-corrected risperidone, 9-OH-risperidone, risperidone+9-OH-risperidone (active moiety), and for response to treatment (PANSS, reduction vs. baseline ≥30% at week 12 and ≥45 at week 24). CYP2D6 normal/ultrarapid metabolizers (NM/UM) (vs. other) had lower risperidone (29%) and active moiety levels (24%) (9-OH-risperidone not affected). The effect on the three analytes was mild (0 to 23% reduction) in *ABCG2* wild-type homozygotes and pronounced (44-55% reduction) in *ABCG2* variant allele carriers. *ABCG2* variant had no effect on disposition in CYP2D6 "other" phenotypes, while the effect was pronounced in CYP2D6 NM/UM subjects (31-37% reduction). *ABCB1* polymorphisms had no effect on exposure to risperidone. CYP2D6 NM/UM phenotype tended to lower odds of PANSS response, *ABCG2* variant was associated with 4-fold higher odds and *ABCB1* (*1236C>T, 2677G>T/A, 3435C>T*) overall mainly wild-type genotype was associated with around 4-fold lower odds of response. In patients treated with LAI-risperidone, CYP2D6 phenotype effect on systemic exposure is conditional on the *ABCG2 421C>A* polymorphism. *ABCG2* and *ABCB1* polymorphisms affect clinical response independently of systemic risperidone disposition.

Introduction

Risperidone is a second-generation antipsychotic recommended for long-term treatment in patients with schizophrenia (Hasan et al., 2013). It is extensively metabolised to an active metabolite 9-OH-risperidone by cytochrome (CYP) 2D6 and their cumulative concentrations represent the drug's active moiety. A minor metabolic pathway is *N*-dealkylation of risperidone by CYP3A4/5 (Bork et al., 1999; Fang et al., 1999; Mannens et al., 1993). Considerable inter- and intra-individual variations in systemic exposure to the active moiety observed with immediate-release oral formulations (Aravagiri et al., 2003) are reduced with long-acting (extended-release) (LAI) injections (Eerdekens et al., 2004), which might be beneficial in respect to therapeutic response/risk of adverse effects (Mauri et al., 2018). In therapeutic drug monitoring, the reference range for the active moiety is between 50 and 150 nmol/L (20-60 ng/mL) (Hiemke et al., 2018; Schoretsanitis et al., 2017). *In vitro*, risperidone and 9-OH-risperidone are substrates (Boulton et al., 2002) and inhibitors (Wang et al. 2006) of P-glycoprotein (multidrug resistance protein 1, MDR1 or ABCB1), and inhibitors (possibly also by competition) of the human breast cancer resistance protein (BCRP or ABCG2) (Wang et al., 2008). These two efflux transporters from the adenosine triphosphate-binding cassette (ABC) superfamily are expressed in the gastrointestinal system, kidney, liver and the blood-brain barrier and affect disposition of a number of drugs (Dutheil et al., 2009; Goncalves et al., 2018; Hira and Terada, 2018; Ieiri, 2012).

Older age and reduced renal function increase exposure to the active moiety (reduced clearance), while mild hepatic dysfunction and sex have no relevant (or only mild) impact (Aichhorn et al., 2005; Feng et al., 2008; Snoeck et al., 1995; De Leon et al., 2007; Molden et al., 2016). CYP2D6 inhibitors increase exposure to risperidone (Bork et al., 1999). Drug-mediated CYP3A4 inhibition/induction have mild effects, but with oral administration, combined CYP3A4/ABCB1 inhibitors increase, while inducers reduce exposure to the active moiety (De Leon et al., 2007; Nakagami et al., 2005).

Polymorphisms in the *CYP2D6* gene can cause a complete loss of CYP2D6 activity, reduced function or hyperfunction through gene duplications (Johansson et al., 1993). The resulting phenotypes are classified as poor (PM), intermediate (IM), normal (NM) and ultrarapid metabolizers (UM) (Caudle et al., 2020) and affect exposure to risperidone: in PMs or IMs it appears higher than in NMs or UMs (Choong et al., 2013; De Leon et al., 2007; Hendset et al., 2009; Jukic et al., 2019; Kuzman et al., 2011; Locatelli et al., 2010; Vandenberghe et al., 2015). Levels of 9-OH-risperidone and of active moiety do not seem to be affected by *CYP2D6* polymorphisms (Gunes et al., 2008; Mihara et al., 2003; Roh et al., 2001; Scordo et al., 1999; Suzuki et al., 2012; Yasui-Furukori et al., 2003). The PM phenotype has been reported associated with a better clinical response (Almoguera et al., 2013), but also with more common adverse effects in patients treated with risperidone (de Leon et al., 2005), and both UM and PM phenotypes appear associated with a higher risk of treatment failure (lack of effect/adverse effects) (Jukic et al., 2019). On the other hand, in non-Asian populations CYP3A metabolizer status does not seem to have a relevant impact on exposure to or on clinical effects of risperidone (Choong et al., 2013; De Leon et al., 2007; Gunes et al., 2008; Rafaniello et al., 2018; Vandenberghe et al., 2015). The efflux transporters ABCB1 and ABCG2 might affect clinical effects of risperidone related both to systemic exposure and to central nervous system availability regulated at the blood-brain barrier. In *ABCB1* knock-out mice, brain concentrations of active moiety were markedly higher than systemic concentrations (Wang et al., 2004), while a small study in humans indicated increased D2 receptor occupancy by risperidone preferentially in the striatum vs. the pituitary upon co-administration of an ABCB1/ABCG2 inhibitor ketoconazole (Reist et al., 2012). The *ABCB1* gene is highly polymorphic and it is not completely clear which of the polymorphisms (and how) affect protein expression and function (Hodges et al., 2011; Ieiri, 2012; Wolking et al., 2015). Three loci in strong linkage disequilibrium (*1236C>T*, rs1128503; *2677G>T/A*, rs2032582, *3435C>T*, rs1045642) have been most extensively studied in respect to drug disposition (individually, or as haplotypes/genotypes). Generally, despite a lot of uncertainties (Hodges et al., 2011), T/T/T i.e., TT/TT/TT

haplotype/genotype seem to be linked to reduced transporter activity vs. C/G/C, i.e., CC/GG/CC (Wolking et al., 2015). In studies with oral risperidone, variant alleles were associated with higher exposure to risperidone/active moiety (Jovanovic et al., 2010) and reduced elimination of 9-OH-risperidone (Saiz-Rodríguez et al., 2018), particularly if combined with CYP2D6 PM phenotype (Suzuki et al., 2013; Yoo et al., 2012). However, opposing effects (Gunes et al., 2008) or no effects of these polymorphisms have also been reported (De Leon et al., 2007; Gunes et al., 2008; Vandenberghe et al., 2015; Yasui-Furukori et al., 2004). Similarly, a better clinical response (Almoguera et al., 2013; Jovanovic et al., 2010; Mi et al., 2016; Xing et al., 2006), no effect (Kastelic et al., 2010) or more common adverse events (Jovanovic et al., 2010; Kastelic et al., 2010) have been reported in patients on oral risperidone harbouring variant *ABCB1* alleles. Studies in subjects treated with LAI risperidone are rare. One study (n=42) indicated no relevant effect of these *ABCB1* loci on steady-state exposure to or safety of risperidone (Choong et al., 2013). The *ABCG2* gene is also polymorphic and the *ABCG2 421C>A* polymorphism (rs2231142), that results in reduced expression due to enhanced degradation of the variant protein (Furukawa et al., 2009; Kondo et al., 2004) and reduced transporter activity (Giacomini and Huang, 2013), is considered to be of a particular relevance in drug pharmacokinetics (Fohner et al., 2017; Giacomini and Huang, 2013; Hira and Terada, 2018). The impact of this polymorphism on exposure, efficacy and safety in adults with schizophrenia treated with oral or LAI risperidone is unknown. One study in children suggested reduced exposure to risperidone in variant allele carriers vs. wild type homozygotes (Rafaniello et al., 2018).

We aimed to explore the effects of polymorphisms in *ABCG2* (*421C>A*) and *ABCB1* (*1236C>T*, *3435C>T*, *2677G>T/A*) and their relationship to the effects of CYP2D6 phenotype on exposure to risperidone and clinical outcomes in a sample of Central-Eastern European (Croatian) adults with schizophrenia treated with LAI risperidone (microsphere) with bi-monthly intramuscular administration.

Patients and Methods

Study outline

This single-centre (Zagreb University Hospital Centre, Croatia) prospective 24-week observational study was conducted (December 2012-March 2017) in line with the Declaration of Helsinki (the 2008 version) and was approved by Institutional Ethics Committee (approval number: 23-469/2-15). Consecutive adults with schizophrenia and indication for treatment with LAI risperidone (q2w) were evaluated for (Figure 1A): a) exposure to risperidone after the 4th injection at the time of the expected peak exposure (a.m. on day 5 of week 6) and at trough (end of week 8, before 5th injection). For the specific LAI formulation used (microsphere), steady-state of active moiety has been reported to be achieved after the 4th injection (Eerdekens et al., 2004; Gefvert et al., 2005); b) symptom severity at the start and after 12 and 24 weeks of treatment; and c) severity of extrapyramidal syndrome (EPS) at week 12. Patients were genotyped for transporter polymorphisms (*ABCG2 421C>A; ABCB1 1236C>T, 2677G>T/A, 3435C>T*), and for *CYP2D6* (gene duplication (xN), **3*, **4*, **5* (whole gene deletion), **6*, **41*), *CYP3A4* (**22*) and *CYP3A5* (**3*) polymorphisms, and were classified into (predicted) CYP phenotypes: a) CYP2D6 (i) poor metabolizers (PM) carry two non-functional alleles (*CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, or *CYP2D6*6*); (ii) intermediate metabolizers (IM) carry one non-functional or two reduced function alleles (*CYP2D6*41*); (iii) normal metabolizers (NM) carry two fully functional CYP2D6 alleles or one fully functional and one reduced function allele, and (iv) ultra-rapid metabolizers (UM) with CYP2D6 gene duplications with functional alleles (Caudle et al., 2020); b) CYP3A PM, IM or NM, based on presence of loss-of-function alleles (*CYP3A4*22, CYP3A5*3*) (Elens et al., 2013). *The primary objective* was to estimate the effects of transporter polymorphisms on exposure to risperidone and to explore their potential interaction with CYP2D6. *The secondary objective* was to estimate their effects and CYP2D6 interactions on clinical outcomes (disease symptoms, EPS) (Figure 1B).

Patients

Eligible were adults (≥18 years of age) with schizophrenia (DSM-IV) in whom, at the discretion of the attending psychiatrist (investigators MŽ, MŠ), treatment with LAI risperidone was indicated, and who provided written informed consent. Patients with comorbid conditions that could hinder their understanding of the study aims and procedures, psychiatric evaluation or could impact exposure to risperidone were not included (see Supplementary Patients and Methods). Pre-study patient evaluations were carried-out within three months before inclusion.

Psychiatric and other treatments

Intramuscular LAI (microsphere formulation) risperidone (Risperdal Consta®, Janssen-Cilag) was the base antipsychotic – if there was no ongoing antipsychotic treatment at the start of the observational period, treatment started simultaneously with oral (initial 3 weeks) and LAI risperidone and continued with LAI risperidone over the remaining study period (Figure 1A). Starting doses were determined with respect to disease severity and were to remain fixed during the observed period unless intolerable adverse effects occurred. Other psychiatric treatments were prescribed at the discretion of the attending psychiatrist unaware of the patients' genotypes/phenotypes. Somatic comorbidity was treated in line with the standards of care.

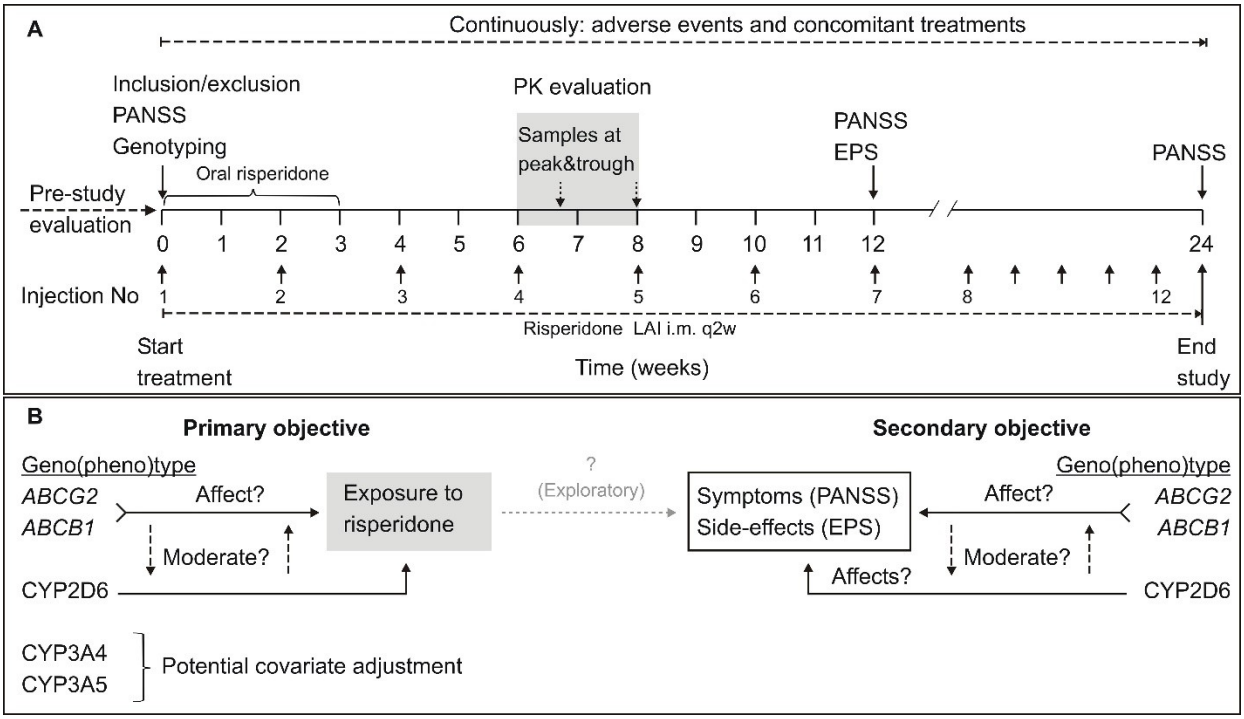

Figure 1. Outline of study procedures (A) and objectives (B). **A**. Study procedures are detailed in the text (Patients and Methods). **B**. In respect to the *primary objective*, we expected that CYP2D6 phenotype would affect systemic risperidone levels; we intended to explore potential effects of transporter polymorphisms and potential mutual moderation between transporter and CYP2D6 effects. We did not expect nor did we aim to evaluate the effect of CYP3A phenotype, but it was to be considered as a potential covariate adjustment. Regarding the *secondary objective*, we aimed to evaluate whether any of the effects of primary interest (*ABCG2* and *ABCB1* genotypes, CYP2D6 phenotype) would affect clinical outcomes, i.e., response in terms of symptoms reduction (Positive and Negative Syndrome Scale, PANSS, score) or occurrence of relevant extrapyramidal syndrome (EPS), and to explore potential mutual moderation. A dashed grey arrow indicates a naturally occurring hypothesis that the effects on exposure would reflect on the clinical outcomes. It places exposure data into a role of a mediator. However, the study was not conceived as one that would test a mediation hypothesis – there was a 4-6 week gap between pharmacokinetic blood sampling (weeks 6 and 8) and the first clinical assessment (week 12). Therefore, the potential effects of CYP2D6 phenotype and of transporter polymorphisms on clinical outcomes were evaluated as total effects (without account to exposure data at weeks 6-8). Still, an ancillary analysis was performed in which exposure data (week 6-8) and transporter genotypes were considered as independent variables and clinical response (week 12, week 24) was an outcome: it is reported only as supplementary material.

Genotyping procedures

Genomic DNA was extracted from 3 mL of whole blood using the FlexiGene DNA Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Genotyping of *ABCB1 c.1236C>T* (rs1128503), *ABCB1 c.3435C>T* (rs1045642), *ABCG2 c.421C>A* (rs2231142), *CYP2D6*3* (rs35742686), *CYP2D6*4* (rs3892097), *CYP2D6*6* (rs5030655), *CYP2D6 *41* (rs28371725), *CYP3A4*22* (rs35599367) and *CYP3A5*3* (rs776746) was performed using TaqMan® SNP Genotyping assays ID C___7586662_10, ID C___7586657_20, ID C__15854163_70, ID C__32407232_50, ID C__27102431_D0, ID C__32407243_20, ID C__34816116_20, ID C__59013445_10, and ID C__26201809_30, respectively by real-time PCR genotyping on the 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA), according to the manufacturer's instructions. Genotyping of *ABCB1 c.2677G>T/A* (rs2032582) was performed by real-time PCR genotyping on the LightCycler® instrument (Roche Diagnostics, Mannheim, Germany) (Arjomand-Nahad et al., 2004). *CYP2D6*5* whole gene deletion and *CYP2D6* gene duplications were genotyped by long-range PCR analysis on the Gene Amp PCR System 9700 (Applied Biosystems, Carlsbad, CA, USA) (Steijns and Van Der Weide, 1998; Stuven et al., 1996). Genotyping methods were implemented and validated for routine pharmacogenetics testing at the Institution.

Bioanalytical method for risperidone and 9-OH-risperidone

Serum concentrations were quantified using high-performance liquid chromatography method with a diode-array detector (Shimadzu Corporation, Kyoto, Japan) as described previously (Jovanovic et al., 2010). The analytical assay is validated for routine therapeutic drug monitoring and is included in external quality control schemes (DGKL RfB and UK NEQAS). All calibration curves were linear (R^2>0.99), with assay linearity ranges 7.6-244.0 nmol/L for risperidone and 8.0-234.0 nmol/L for 9-OH-risperidone. Lower limits of detection were 2.5 nmol/L and 3.2 nmol/L, respectively. Imprecision and inaccuracy were consistently <5.0%.

Psychiatric evaluation

Symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) by PANSS-certified raters [PANSS certification (PANSS Institute) required for participation in regulatory clinical drug trials]. Extrapyramidal syndrome was evaluated using the Simpson-Angus scale (SAS) (Simpson and Angus, 1970). All other clinical adverse events were recorded. Clinical assessors were unaware of the patients' genotype.

Outcomes

Primary outcomes were dose-corrected risperidone, 9-OH-risperidone and risperidone+9-OH-risperidone concentrations (active moiety) and the risperidone/9-OH-risperidone ratio at the expected peak and trough after the 4th injection. For data analysis, measured concentrations below the limit of quantification were set to zero. *Secondary* outcomes were clinical. PANSS reduction of 20% commonly used as a cut-off in clinical trials including patients with severe schizophrenia corresponds to clinical global impression "minimally improved", while "much improved" corresponds to reductions of 40%-50%, although this relationship somewhat changes during treatment (Leucht et al., 2005; Levine et al., 2008). Considering the characteristics of patients to be included in the study, we defined the outcome of interest as "relevant PANSS response": ≥30% PANSS reduction at 12 weeks and ≥45% at 24 weeks. Pharmacokinetic (PK, peak and trough) and PANSS response (week 12 and 24) outcomes were to be treated as paired values *per* patient. The outcome of interest regarding EPS was the proportion of patients with raw SAS score ≥3 points at week 12 (Hawley et al., 2003; Simpson and Angus, 1970).

Data analysis and sample size

Independent variables of primary interest were *ABCG2* and *ABCB1* polymorphisms, CYP2D6 phenotype, i.e., a well-known effect on exposure to risperidone (Choong et al., 2013; De Leon et al., 2007; Hendset et al., 2009; Jovanovic et al., 2010; Jukic et al., 2019; Locatelli et al., 2010; Vandenberghe et al., 2015), and their interaction terms. Regarding CYP2D6, subjects were

dichotomized as normal/ultrarapid metabolizers (NM/UM) and as "other" (IM/PM), as the two subsets differ in exposure to risperidone (Choong et al., 2013; De Leon et al., 2007; Hendset et al., 2009; Jukic et al., 2019; Kuzman et al., 2011; Locatelli et al., 2010; Vandenberghe et al., 2015). Regarding *ABCG2 421C>A*, dichotomization was to "variant allele carriers" and "wild type" (wt) homozygotes, since variant allele is associated with transporter dysfunction (Fohner et al., 2017; Giacomini and Huang, 2013). The three *ABCB1* polymorphisms were to be addressed as overall genotypes. Based on the suggested (Wolking et al., 2015) reduced transporter function associated with the overall variant-allele genotype (TT/TT/TT) vs. the overall wt genotype (CC/GG/CC), three categories were possible (wt, variant and "mixed"), unless preliminary analysis of the variant-allele effects suggested possible dichotomization (or other classification). Pharmacokinetic outcomes and proportions of PANSS responders were analysed as paired data *per* patient by fitting (hierarchical) generalized linear mixed models: the main effects model, and two models testing interactions between *ABCG2* or *ABCB1* and CYP2D6 phenotype. Supplementary Material - Supplementary Patients and Methods - elaborate fitting procedures, model building approach and considerations on type 1 error rate. Preliminary evaluation was performed to assess whether effect|time interaction could be omitted from the main models (considering a limited number of patients). The proportion of patients with raw SAS score ≥3 was analysed by logistic regression.

The sample size was determined with respect to PK outcomes and expected prevalence of genotypes/phenotypes of primary interest. We expected: a) around 50% and 5% CYP2D6 NM and UM, respectively (i.e., around 55% NM/UM subjects) (Ganoci et al., 2017), b) around 20% *ABCG2* variant allele carriers (Klarica Domjanović et al., 2018); c) around 35-40% wt and around 20-25% variant-homozygotes at each *ABCB1* locus, i.e., around 25% overall CC/GG/CC and around 20% overall TT/TT/TT genotype (Lovrić et al., 2012). We expected 85-90% of patients to be intermediate CPY3A metabolizers (Ganoci et al., 2017). We did not expect and did not aim to evaluate CYP3A effects on exposure to risperidone, but CYP3A phenotype was to be considered as a potential adjustment if

the observed prevalence departed from the expectations, overall and across subsets of patients based on effects of primary interest. We considered that an overall mean difference (on a log-scale) of ≥±0.223 between *ABCG2* variant allele vs. wt or between *ABCB1* CC/GG/CC (or TT/TT/TT) vs. "other" could be of practical relevance (GMR ≥1.25 or ≤0.80). With two repeated measures, 4:1 ratio between patient subsets and coefficient of variation of 150%, 72 patients in the larger subset and 18 in the smaller are needed for a power of 80% to detect such a difference at two-sided alpha 0.05. Under the same assumptions, such a sample (n=90) split into 50-40 subsets (e.g., in respect to the CYP2D6 dichotomized phenotype), achieves >95% power to detect the specified difference and 82% power to detect a difference of ≥±0.182 (on a log-scale) (GMR ≥1.20 or ≤0.83). In respect to potential transporter genotypes|CYP2D6 phenotype interactions, and assuming that the ratio of CYP2D6 NM/UM and "other" phenotypes across transporter genotype subsets is 1.25:1 (e.g., 10:8 in the smaller and 40:32 in the larger subset), this sample sizes achieves 71.6% power to detect an interaction, i.e., a difference between effects of 0.470 (on a log-scale), e.g., between GMR=0.79 and a GMR=1.26 at two-sided alpha=0.1. To prevent spurious findings, observed interaction *P*-values were corrected by the false discovery rate method (see Supplementary Patients and Methods). Patient enrolment continued until the smallest (expected) ABCG2-based or ABCB1-based subset attained 18 subjects. We used SAS for Windows 9.4 software (SAS Inc., Cary, NC).

Results

Patient characteristics and raw pharmacokinetic data

A total of 101 patients were enrolled with risperidone LAI doses unchanged during the observed period (predominantly 50 mg or 37.5 mg) (Table 1). Prevalence of *ABCG2* and *ABCB1* genotypes and CYP2D6 and CYP3A phenotypes was in line with the expectations (Table 1). At the time of blood sampling, 21 patients were receiving concomitant treatments that might have inhibited CYP enzymes (primarily CYP3A4), while the use of other enzyme/transporter inhibitors/inducers was sporadic (Table 1).

Table 1. Patient demographics, genotypes, phenotypes and raw pharmacokinetic data.

Demographics & geno(pheno)types	
N	101
Age (years)	38 (20-69)
Men	57 (56.4)
ABCG2 421C>A	
CC	83 (82.1)
CA	18 (17.8)
AA	0
ABCB1 1236C>T	
CC	38 (37.6)
CT	40 (39.6)
TT	23 (22.8)
ABCB1 2677G>T/A	
GG	37 (36.6)
GT	40 (39.6)
GA	3 (3.0)
TT	21 (20.8)
AA	0
ABCB1 3435C>T	
CC	35 (34.6)
CT	33 (32.7)
TT	33 (32.7)
ABCB1 across three loci	
CC/CC/CC (Wild type homozygous)	25 (24.8)
TT/TT/TT (Variant homozygous)	20 (19.8)
CYP2D6 genotype	
*1/*1	42 (41.6)
*1/*1 xN	4 (4.0)
*1/*3	3 (3.0)
*1/*4	23 (22.8)
*1/*4 xN	4 (4.0)
*1/*41	12 (11.9)
*1/*41 xN	1 (1.0)
*3/*4	1 (1.0)
*4/*4	2 (2.0)
*4/*41	5 (4.9)
*41/*41	3 (3.0)
*5/*41	1 (1.0)
CYP2D6 overall phenotype	
Normal metabolizer (NM)	54 (53.5)
Intermediate metabolizer (IM)	35 (34.6)
Poor metabolizer (PM)	3 (3.0)
Ultrarapid metabolizer (UM)	4 (4.0)
xN	5 (4.9)
Normal + ultrarapid (NM+UM)	58 (57.5)
CYP3A4 genotype	
*1/*1	94 (93.1)
*1/*22	7 (6.9)
CYP3A5 genotype	
*1/*3	8 (7.9)
*3/*3	93 (92.1)
CYP3A overall phenotype	
Normal metabolizer (NM)	7 (6.9)
Intermediate metabolizer (IM)	88 (87.1)
Poor metabolizer (PM)	6 (5.9)
At pharmacokinetic evaluation	
Number of concomitant drugs[a]	3 (1-7)
Any CYP inhibitor[b]	21 (20.8)
Any 2D6 inhibitor[b]	4 (4.0)
Any 3A4 inhibitor[b]	18 (17.8)
Any 3A4 inductor[b]	2 (2.0)
Any ABCB1 inhibitor[b]	4 (4.0)
Any ABCB1 inductor[b]	2 (2.0)
Risperidone dose 25/37.5/50 mg	3 / 48 / 50
At expected peak (nmol/L)	
Risperidone	25.5 (0-384)
9-OH-risperidone	45.3 (15-168)
Risperidone+9-OH-risperidone	76.5 (15-442)
Risperidone dose-corrected	0.62 (0-10.2)
9-OH-risperidone dose-corrected	1.17 (0.3-4.48)
Risperidone+9-OH-risperidone dose-corrected	1.78 (0.30-11.8)
Risperidone/9-OH-risperidone ratio	0.49 (0-7.60)
At trough (nmol/L)	
Risperidone	13.7 (0-217)
9-OH-risperidone	31.8 (0-140)
Risperidone+9-OH-risperidone	46.6 (0-264)
Risperidone dose-corrected	0.32 (0-5.78)
9-OH-risperidone dose-corrected	0.75 (0-2.97)
Risperidone+9-OH-risperidone dose-corrected	1.07 (0-7.03)
Risperidone/9-OH-risperidone ratio	0.40 (0-4.63)

Data are median (range) or count (percent).

[a]Practically exclusively psychiatric drugs, since only three patients were treated with beta blockers, three with angiotensin-converting enzyme inhibitors, one with amlodipine (hypertension), and one

each with metformin (diabetes), levothyroxine (hypothyroidism), inhaled salmeterol + fluticasone propionate (asthma) and norethisterone + ethinyl estradiol (irregular vaginal bleeding)

[b]Considered were (i) for CYP2D6 inhibition: paroxetine (one patient), duloxetine (one patient), sertraline (two patients); (ii) for CYP3A4 inhibition: paroxetine (one patient), sodium valproate (18 patients); (iii) for CYP3A4 induction: carbamazepine (two patients); (iv) for ABCB1 inhibition: paroxetine (one patient), sertraline (two patients), pantoprazole (one patient); (v) for ABCB1 induction: carbamazepine (two patients). Barbiturates were not used.

Effects of CYP2D6 phenotype and transporter variants on exposure to risperidone

The preliminary analysis did not indicate any relevant effect of the *ABCB1* variants on any of the primary outcomes (individual loci or overall genotypes in different contrasts, see Supplementary Table S1-S4). Hence, it appeared justified to classify patients with respect to *ABCB1* polymorphisms as "overall wt genotype" (CC/GG/CC) and "other genotypes". Data across levels of the three independents of primary interest are summarized in Table 2. The effects of primary interest appeared consistent at peak and at trough (see Supplementary Table S5). In the main-effects models (Table 3), CYP2D6 NM/UM phenotype (vs. other) was associated with around 29% lower risperidone and around 24% lower risperidone+9-OH-risperidone (active moiety) levels, and with around 28% lower risperidone/9-OH ratio, with no apparent effect on the 9-OH-risperidone levels. Variant *ABCG2* allele (vs. wt homozygosity) tended towards mildly lower risperidone, 9-OH-risperidone and active moiety levels, but the uncertainty about these effects was high (Table 3). The overall wt *ABCB1* genotype did not differ vs. all other genotypes regarding any of the primary outcomes (Table 3). Data indicated an interaction between CYP2D6 phenotype and *ABCG2* polymorphism effects (Table 4): a) the NM/UM effect on active moiety levels were more pronounced in *ABCG2* variant allele caries than in wt homozygotes; b) in *ABCG2* variant allele carriers, NM/UM phenotype was associated with around 44% lower 9-OH levels, while no effect was apparent in wt homozygotes; c) the same trend was observed regarding risperidone levels; d) conversely, *ABCG2* variant allele was associated with around 31% to 37% lower risperidone, 9-OH-risperidone and active moiety levels in NM/UM

phenotype patients, and had no effect in patients with other CYP2D6 phenotypes (Table 4). Data did not indicate any relevant interaction between *ABCB1* overall genotype and CYP2D6 phenotype (Table 4).

Relationship between CYP2D6 phenotype, transporter variants and clinical outcomes

Total PANSS score decreased in all patients but the proportion of those with *relevant* PANSS response appeared lower with CYP2D6 NM/UM phenotype (vs. other) and in *ABCG2* wt homozygotes (vs. variant allele), both at week 12 and at week 24 (Table 5). In respect to the *ABCB1* overall genotype, patients were classified as mainly variant (4-6 variant alleles across the 3 loci), mainly wt (4-6 wt alleles) or as mixed genotypes. These three subsets were closely similar to each other and to other subsets based on overall genotype or individual loci regarding exposure to risperidone (see Supplementary Table S1-S4), but there appeared a trend of a decreasing proportion of PANSS responders with increasing presence of wt alleles (Table 5). In multivariate analysis (Table 6), CYP2D6 NM/UM phenotype tended towards lower odds of PANSS response, but the estimate was rather imprecise. Variant *ABCG2 421C>A* allele was associated with considerably higher odds of response (OR=4.03) (Table 6), while mainly wt overall *ABCB1* genotype was associated with lower odds of response (OR=0.27) (Table 6). Due to a relatively limited sample size/low number of responders, there is some uncertainty about these effects. Effects of primary interest were consistent at week 12 and week 24, and the effects of CYP2D6 phenotype were consistent across the levels of *ABCG2* and *ABCB1* genotype and *vice-versa* (see Supplementary Table S6). The proportion of patients with SAS score ≥3 appeared similar across the patient subsets based on CYP2D6 phenotype and *ABCG2* and *ABCB1* genotypes (Table 5). No independent association between the effects of primary interest and probability of SAS score ≥3 was observed (not shown).

Table 2. Key genotype, phenotype and pharmacokinetic data by CYP2D6 phenotype [normal or ultrarapid metabolizer (NM/UM) or *other*], *ABCG2 421C>A* genotype (wild type or variant allele carriage) and overall *ABCB1* genotype [wild type homozygous at all three loci (CC/GG/CC) or *other*].

	CYP2D6 phenotype		*ABCG2 421C>A* genotype		*ABCB1* overall genotype	
	NM or UM	Other	Wild type	Variant allele	CC/GG/CC	Other
N	58	43	83	18	25	76
Age	38 (20-69)	40 (20-67)	38 (20-69)	41 (21-69)	37 (20-56)	39.5 (20-69)
Men	31 (53.5)	26 (60.5)	48 (57.8)	9 (50.0)	14 (56.0)	43 (56.6)
CYP2D6 NM or UM phenotype	---	---	46 (55.4)	12 (66.7)	16 (64.0)	42 (55.3)
ABCG2 421C>A variant allele	12 (20.7)	6 (13.9)	---	---	6 (24.0)	12 (15.8)
ABCB1 wild type (CC/GG/CC)	16 (27.6)	9 (20.9)	19 (22.9)	6 (33.3)	---	---
CYP3A NM/IM/PM phenotype	4/ 51 (87.9) /3	3/ 37 (86.1) /3	6/ 73 (88.0) /4	1/ 15 (83.3) /2	2/ 19 (76.0) /4	5/ 69 (90.8) /2
Using any CYP inhibitor	13 (22.4)	8 (18.6)	16 (19.3)	5 (27.8)	3 (12.0)	18 (23.7)
At peak (nmol/L/mg dose)						
Risperidone	0.47 (0.25-0.73)	0.77 (0.50-1.44)	0.65 (0.34-0.96)	0.38 (0.27-1.04)	0.59 (0.24-1.39)	0.63 (0.34-0.95)
Values BLQ	5 (8.62)	3 (6.99)	6 (7.23)	2 (11.11)	2 (8.00)	6 (7.89)
9-OH-risperidone	1.06 (0.75-1.48)	1.32 (0.84-1.66)	1.20 (0.80-1.63)	0.82 (0.70-1.13)	1.09 (0.79-1.38)	1.19 (0.76-1.66)
Values BLQ	0	0	0	0	0	0
Risperidone+9-OH-risperidone	1.56 (1.12-2.18)	2.36 (1.59-3.32)	1.87 (1.36-2.80)	1.24 (1.09-2.41)	1.78 (1.14-2.52)	1.82 (1.22-2.52)
Risperidone/9-OH-risperidone ratio	0.44 (0.25-0.58)	0.75 (0.30-1.40)	0.49 (0.27-0.78)	0.50 (0.28-1.09)	0.49 (0.24-1.56)	0.49 (0.29-0.78)
At trough (nmol/L/mg dose)						
Risperidone	0.28 (0.00-0.41)	0.35 (0.25-0.69)	0.35 (0.21-0.55)	0.25 (0.08-0.37)	0.39 (0.22-0.72)	0.30 (0.11-0.46)
Values BLQ	15 (25.9)	6 (13.98)	17 (20.5)	4 (22.2)	4 (16.00)	17 (22.4)
9-OH-risperidone	0.75 (0.52-1.09)	0.75 (0.52-1.15)	0.75 (0.53-1.19)	0.76 (0.44-0.99)	0.77 (0.59-1.17)	0.75 (0.51-1.13)
Values BLQ	1 (1.72)	0	1 (1.20)	0	0	1 (1.32)
Risperidone+9-OH-risperidone	1.07 (0.71-1.40)	1.06 (0.82-1.84)	1.06 (0.79-1.76)	1.07 (0.66-1.31)	1.19 (1.01-1.61)	1.03 (0.74-1.70)
Risperidone/9-OH-risperidone ratio	0.29 (0.00-0.51)	0.54 (0.30-1.03)	0.42 (0.22-0.59)	0.28 (0.13-0.55)	0.42 (0.28-0.60)	0.40 (0.18-0.59)

Data are median (range for age, lower-upper quartile for pharmacokinetic data) or count (percent).
BLQ – below the limit of quantification; CYP3A phenotypes – NM, normal metabolizer; IM, intermediate metabolizer; PM, poor metabolizer

Table 3. Effects of CYP2D6 phenotype [normal or ultrarapid metabolizer (NM/UM) vs. *other*)], *ABCG2 421C>A* genotype [variant allele carriage (var) vs. wild type (wt)] and overall *ABCB1* genotype [wild type homozygous (CC/GG/CC) vs. *other*] on dose-corrected risperidone, 9-OH-risperidone and risperidone+9-OH-risperidone concentrations, and the risperidone/9-OH-risperidone ratio: summary of the main-effects models.[a]

	Risperidone		9-OH-risperidone		Risperidone +9-OH-risperidone		Risperidone/9-OH ratio	
	GMR (95%CI)	*t(df);P*	GMR (95%CI)	*t(df);P*	GMR (95%CI)	*t(df);P*	GMR (95%CI)	*t(df);P*
Effects of primary interest								
CYP2D6 NM/UM	0.71 (0.56-0.89)	$-2.98_{(95)}$; 0.004	0.92 (0.76-1.13)	$-0.78_{(95)}$;0.438	0.76 (0.62-0.93)	$-2.62_{(95)}$;0.010	0.72 (0.56-0.92)	$-2.68_{(95)}$;0.009
ABCG2 variant	0.84 (0.62-1.13)	$-1.17_{(95)}$; 0.244	0.81 (0.62-1.05)	$-1.62_{(95)}$;0.108	0.77 (0.59-1.01)	$-1.91_{(95)}$;0.059	0.93 (0.68-1.29)	$-0.40_{(95)}$;0.692
ABCB1 CC/GG/CC	1.09 (0.83-1.42)	$0.63_{(95)}$; 0.528	0.97 (0.77-1.22)	$-0.30_{(95)}$;0.767	1.06 (0.83-1.34)	$0.47_{(95)}$;0.638	1.14 (0.85-1.52)	$0.89_{(95)}$;0.377
Adjustments								
Sample time: peak	1.37 (1.17-1.61)	$3.93_{(100)}$;<0.001	1.52 (1.37-1.68)	$8.04_{(100)}$;<0.001	1.71 (1.55-1.89)	$10.9_{(100)}$;<0.001	1.03 (0.88-1.21)	$0.36_{(100)}$;0.723
Age	1.00 (0.99-1.01)	$0.09_{(95)}$; 0.928	1.00 (0.99-1.01)	$0.01_{(95)}$;0.955	1.00 (0.99-1.01)	$0.14_{(95)}$;0.888	1.00 (0.99-1.01)	$0.43_{(95)}$;0.670
CYP inhibitor	1.06 (0.80-1.39)	$0.39_{(95)}$; 0.695	0.86 (0.67-1.10)	$-1.24_{(95)}$;0.217	0.85 (0.66-1.09)	$-1.28_{(95)}$;0.202	1.19 (0.88-1.60)	$1.17_{(95)}$;0.246
Effects of primary interest adjusted for multiplicity[b]								
CYP2D6 NM/UM	0.71 (0.53-0.94)	0.013	0.92 (0.72-1.18)	0.819	0.76 (0.59-0.98)	0.031	0.72 (0.53-0.97)	0.027
ABCG2 variant	0.84 (0.58-1.21)	0.566	0.81 (0.59-1.11)	0.292	0.77 (0.55-1.07)	0.170	0.93 (0.63-1.37)	0.953
ABCB1 CC/GG/CC	1.09 (0.79-1.51)	0.894	0.97 (0.73-1,28)	0.986	1.06 (0.79-1.42)	0.949	1.14 (0.81-1.61)	0.728

[a] Model for each outcome included effects of primary interest (CYP2D6 phenotype, *ABCG2* and *ABCB1* genotypes) and adjustments (time, age and use of any CYP inhibitor). Effects of primary interest were consistent at both peak and trough (see Supplementary Table S5), hence interaction terms with time (peak, trough) were not included in the final models.

[b] Estimates ($\widehat{\beta}$), estimated covariance matrix [$Cov(\widehat{\beta})$] and degrees of freedom from each model were retained and used to adjust the observed confidence intervals and *P*-values (adjustment for the number of tests about the 3 effects of primary interest) by the stepdown logical simulation method (*post-hoc* multiplicity adjustment to additionally control type I error).

Table 4. Summary of multivariate models testing interactions between the effects of CYP2D6 phenotype [normal or ultrarapid metabolizer (NM/UM) vs. *other*)] and *ABCG2 421C>A* genotype [variant allele carriage vs. wild type (wt)] or overall *ABCB1* genotype [wild type homozygous (CC/GG/CC) vs. *other*] on dose-corrected risperidone, 9-OH-risperidone and risperidone+9-OH-risperidone concentrations, and the risperidone/9-OH-risperidone ratio. *P*-values for interaction terms were adjusted by false discovery rate method (FDR=5%), and adjusted *P*-values ≤0.1 were considered indicative of a true interaction. All models included adjustments for age, time (peak or trough) and use of any CYP inhibitor (not shown).

	Risperidone	9-OH-risperidone	Risperidone +9-OH-risperidone	Risperidone/9-OH ratio
	GMR (97.5%CI)	GMR (97.5%CI)	GMR (97.5%CI)	GMR (97.5%CI)
CYP2D6|*ABCG2*	*$F_{(1,94)}$=3.07, P=0.083, Adj. P=0.110*	*$F_{(1,94)}$=4.86, P=0.029, Adj. P=0.078*	*$F_{(1,94)}$=4.38, P=0.039, Adj. P=0.078*	*$F_{(1,94)}$=0.24, P=0.629, Adj. P=0.629*
CYP2D6 NM/UM at *ABCG2* variant	0.45 (0.24-0.86)	0.56 (0.32-0.98)	0.47 (0.26-0.83)	0.63 (0.31-1.26)
CYP2D6 NM/UM at *ABCG2* wt	0.77 (0.58-1.02)	1.02 (0.80-1.30)	0.83 (0.65-1.07)	0.74 (0.54-1.00)
ABCG2 variant at CYP2D6 NM/UM	0.69 (0.45-1.05)	0.65 (0.46-0.94)	0.63 (0.43-0.92)	0.88 (0.56-1.38)
ABCG2 variant at CYP2D6 *other*	1.18 (0.68-2.06)	1.17 (0.72-1.91)	1.12 (0.68-1.84)	1.03 (0.56-1.88)
CYP2D6|*ABCB1*	*$F_{(1,94)}$=3.85, P=0.053, Adj. P=0.212*	*$F_{(1,94)}$=0.14, P=0.709, Adj. P=0.709*	*$F_{(1,94)}$=0.36, P=0.551, Adj. P=0.709*	*$F_{(1,94)}$=1.37, P=0.245, Adj. P=0.490*
CYP2D6 NM/UM at *ABCB1* wt	0.48 (0.28-0.81)	0.86 (0.54-1.39)	0.68 (0.42-1.11)	0.55 (0.31-0.99)
CYP2D6 NM/UM at *ABCB1 other*	0.80 (0.60-1.07)	0.94 (0.73-1.22)	0.79 (0.60-1.03)	0.78 (0.57-1.06)
ABCB1 wt at CYP2D6 NM/UM	0.89 (0.61-1.30)	0.93 (0.66-1.31)	1.00 (0.71-1.42)	1.00 (0.66-1.51)
ABC1 wt at CYP2D6 *other*	1.49 (0.93-2.40)	1.02 (0.67-1.55)	1.16 (0.75-1.79)	1.40 (0.84-2.33)

Table 5. Patient characteristics and clinical outcomes by CYP2D6 phenotype [normal/ultrarapid metabolizer (NM/UM) or *other*)], *ABCG2 421C>A* genotype [variant allele or wild type (wt)] and *ABCB1* overall genotype (based on predominance of variant/wt alleles) [a].

	CYP2D6 phenotype		*ABCG2 421C>A* genotype		*ABCB1* genotype (predominant allele)] [a]		
	NM/UM	Other	Wild type	Variant allele	Mainly variant	Mixed	Mainly wild type
N	58	43	83	18	28	35	38
Age (years)	38 (20-69)	40 (20-67)	38 (20-69)	41 (21-69)	39.5 (21-69)	40 (27-60)	36 (20-69)
Men	31 (53.5)	26 (60.5)	48 (57.8)	9 (50.0)	15 (53.6)	21 (60.0)	21 (55.3)
CYP2D6 NM or UM	---	---	46 (55.4)	12 (66.7)	15 (53.6)	17 (48.6)	26 (68.4)
ABCG2 421C>A variant allele	12 (20.7)	6 (13.9)	---	---	4 (14.3)	5 (14.3)	9 (23.7)
ABCB1 variant/mixed/wt	15/17/26 (44.8)	13/18/12 (27.9)	24/30/29 (34.9)	4/5/9 (50.0)	---	---	---
Additional antipsychotic	33 (56.9)	28 (65.1)	50 (60.2)	11 (61.1)	16 (57.1)	24 (68.6)	21 (55.3)
Additional benzodiazepine	37 (63.8)	30 (69.8)	55 (66.3)	12 (66.7)	17 (60.7)	27 (77.1)	23 (60.5)
Additional mood stabilizer	14 (24.1)	9 (20.9)	17 (20.5)	6 (33.3)	8 (28.6)	8 (22.9)	7 (18.4)
Additional antidepressant	5 (8.6)	1 (2.3)	4 (4.8)	2 (11.1)	3 (10.7)	2 (5.7)	1 (2.6)
Dose (mg) (25/37.5/50)	2 / 24 / 32	1 / 24 / 18	3 /41 / 39	0 / 7 / 11	0 / 12 / 16	1 / 20 / 14	2 / 16 / 20
Peak dose-corrected AM	1.56 (1.12-2.18)	2.36 (1.59-3.32)	1.87 (1.36-2.80)	1.24 (1.09-2.41)	1.75 (1.22-2.47)	1.85 (1.43-2.52)	1.78 (1.14-2.72)
Trough dose-corrected AM	1.07 (0.71-1.40)	1.06 (0.82-1.84)	1.06 (0.79-1.76)	1.07 (0.66-1.31)	1.04 (0.75-1.81)	1.05 (0.77-1.86)	1.12 (0.80-1.56)
Baseline total PANSS score	129 (91-177)	128 (106-157)	129 (108-177)	126 (91-150)	125 (106-153)	127 (112-177)	131 (91-157)
PANSS response at week 12							
Percent change vs. baseline	-24.5 (-12, -46)	-25 (-18.5, -47)	-24.8 (-17, -42)	-25.3 (-12, -47)	-25.2 (-19, -46)	-25.0 (-15, -47)	-24.3 (-12, -38)
Reduction ≥30%	10 (17.2)	10 (23.3)	12 (14.5)	8 (44.4)	7 (25.0)	7 (20.0)	6 (15.8)
PANSS response at week 24							
Percent change vs. baseline	-42 (-21, -61)	-41 (-28, -62)	-41 (-27, -60)	-42.6 (-21, -62)	-43.5 (-27, -61)	-41.0 (-22, -62)	-40.0 (-21, -57)
Reduction ≥45%	16 (27.6)	13 (30.2)	22 (26.5)	7 (38.9)	13 (46.4)	9 (25.7)	7 (18.4)
Antimuscarinics introduced	23 (39.7)	15 (34.9)	33 (39.8)	5 (27.8)	11 (39.3)	10 (28.6)	17 (44.7)
SAS score at 3 months	0 (0-15)	0 (0-11)	0 (0-12)	0 (0-15)	0 (0-13)	0 (0-10)	0 (0-15)
SAS score ≥3	17 (29.3)	12 (27.9)	24 (28.9)	5 (27.8)	9 (32.1)	9 (25.7)	11 (29.0)

PANSS – positive & negative syndrome scale; SAS – Simpson Angus scale. Data: median [range; Q1-Q3 for active moiety (AM)], count (%).

[a]Overall genotype classified as: "(mainly) variant" - all three loci variant homozygous (n=20) or 1-2 wt alleles (n=8); "(mainly) wild type" - all wt homozygous (n=25) or 1-2 variant alleles (n=13); "mixed" - all other overall genotypes. Exposure to risperidone across these subsets was closely similar, and it was similar to other subsets based on overall genotype or individual *ABCB1* loci (Supplementary Table S1-S4).

Table 6. Effects of CYP2D6 phenotype [normal/ultrarapid metabolizer (NM/UM) vs. *other*], *ABCG2 421C>A* genotype [variant allele carriage vs. wild type (wt)] and *ABCB1* overall genotype (based on predominance of variant/wt alleles)[a] on the probability of relevant PANSS reduction (≥30% at 12 weeks, ≥45% at 24 weeks).

	OR (95%CI)	*t(df); P*	Adjusted CI, *P*
Effects of primary interest			
CYP2D6 NM/UM vs. other phenotypes	0.54 (0.20-1.50)	$-1.19_{(93)}$; 0.236	0.16-1.88; 0.236
ABCG2 variant vs. wild type	4.03 (1.15-14.1)	$2.21_{(93)}$; 0.029	0.87-18.6; 0.083
ABCB1 linear trend (mainly wt – mainly variant)	0.27 (0.08-0.91)	$-2.15_{(93)}$; 0.034	0.06-1.19; 0.083
Adjustments			
Dose 50 mg (vs. lower)	2.07 (0.78-5.49)	$1.48_{(93)}$; 0.143	--
Age (years)	0.96 (0.91-1.01)	$-1.55_{(93)}$; 0.123	--
Men	0.19 (0.06-0.59)	$2.90_{(93)}$; 0.005	--
Time: week 24 vs. week 12	1.95 (0.90-4.23)	$1.71_{(93)}$; 0.090	--

[a]see footnote to Table 5

Estimated effects of primary interest (CYP2D6 phenotype, *ABCG2* genotype and overall *ABCB1* genotype) are given as obtained in the fitted model and also with *post-hoc* adjustment for multiplicity (the same method as explained in a footnote to Table 3; see also Supplementary Patients and Methods).

The initial model included also an adjustment for baseline PANSS score, however, estimated effect was closely around 0 (OR=1.0), with t-value <0.4 and P >0.700, and was hence removed. Models testing interactions between the effects of primary interest and time (week 12, week 24), and between each of the transporter genotypes and CYP2D6 phenotype indicated consistent effects of primary interest at week 12 and at week 24, and of CYP2D6 phenotype at different levels of *ABCG2* and *ABCB1* genotypes and *vice-versa* (see Supplementary Table S6).

PANSS – Positive and Negative Syndrome Scale

Discussion

The main present pharmacokinetic finding is the interaction between the *ABCG2 421C>A* variant allele and CYP2D6 NM/UM phenotype in their effects on exposure to risperidone, 9-OH-risperidone and active moiety at presumed steady-state (Eerdekens et al., 2004; Gefvert et al., 2005) in patients with schizophrenia treated with parenteral risperidone. The overall effect of the *ABCG2* variant is mild, but in patients with CYP2D6 NM/UM phenotype, it amounts to 31%-37% lower levels of all analytes vs. the wild type (wt) (with other CYP2D6 phenotypes, effect ~0). Another facet of this interaction is that in *ABCG2* wt homozygotes, the effect of CYP2D6 NM/UM phenotype is mild (~20% lower risperidone and active moiety levels vs. other phenotypes), but the combination with *ABCG2* variant results in 55% lower risperidone, 44% lower 9-OH-risperidone and 53% lower active moiety levels, with maintained relationship considering the metabolic ratio. This additive effect of the *ABCG2* variant appears practically relevant: a mild effect is turned into 50% lower bioavailability, suggesting two subsets among CYP2D6 NM/UM patients in whom proposed dose-adjustments [26] would result in different exposures. The site of the *ABCG2* variant allele effect remains unknown (gastrointestinal system is by-passed). Closely similar risperidone/9-OH-risperidone ratio in variant allele carriers and wt homozygotes (GMR around 1.0) at both levels of the CYP2D6 phenotype (NM/UM and *other*) suggests that both active components are comparably affected. The observation is limited by a rather small CYP2D6 NM/UM+*ABCG2* variant allele patient subset, but it provided 12 samples with consistent effects at peak and at trough, controlled for *ABCB1* genotype, CYP inhibitor use, age (statistical adjustment), renal and hepatic function (exclusion criteria), CYP3A phenotype (85-88% IM across the four patient subsets), and ABCG2, ABCB1 and CYP inducer or transporter inhibitor use (a few patients overall).

Regarding the complexity of *ABCB1* polymorphisms, we did not intend to explore their potential effects in detail, but the used approach (each considered individually or in different combinations)

supports a view that *ABCB1* genotype has no robust effect on systemic exposure to risperidone in patients treated with parenteral formulations.

The observations regarding clinical outcomes share common limitations and strengths with the pharmacokinetic analysis. CYP2D6 NM/UM phenotype tended towards lower odds of a relevant PANSS reduction, but estimates were imprecise (leaving a rather high level of uncertainty). They were based on 116 observations in NM/UM subjects and 86 in subjects with other phenotypes suggesting that imprecision was (at least in part) due to variability of the NM/UM effect, not only to a limited sample. Effects of transporter polymorphisms, on the other hand, can be claimed with reasonable certainty. While odds ratios might not be too intuitive for interpretation, adjusted estimated probabilities of a clinically relevant response (model in Table 6) suggest a probability of 47.0% for *ABCG2* variant allele carriers vs. 18.0% for wt homozygotes (difference 29.0%, 95%CI 2.4 to 55.9), and a probability of 18.8% in subjects with mainly wt overall *ABCB1* genotype (and presumed normal transporter activity) vs. 45.8% in patients with mainly variant overall genotype (and presumed reduced activity) (difference -27.0%, 95%CI -52.2 to 0.00). These seem to be practically relevant effects. Their mechanisms remain unclear, and the observations are in a way counterintuitive: CYP2D6 NM/UM phenotype was associated with lower exposure and tended to less response, but *ABCG2* variant allele was associated with lower exposure and better response, while overall mainly wt *ABCB1* genotype was not associated with exposure to risperidone, and was associated with poorer clinical response. This indicates a possibility that the clinical effects of studied transporter polymorphisms were due to their effects on CNS availability of risperidone active moiety. The theoretical background is supportive in this sense: *ABCB1* limits CNS availability of the risperidone active moiety (Wang et al., 2004), hence overall wt genotype (presumably associated with normal function vs. reduced with other genotypes (Wolking et al., 2015) might have resulted in lower CNS availability and less response to treatment. A similar function has been suggested for *ABCG2* (Reist et al., 2012), hence variant allele, associated with reduced transporter function (Fohner et al., 2017;

Giacomini and Huang, 2013), could have resulted in higher CNS availability and better clinical response. As a further support of such a possibility: in an ancillary analysis (Supplementary Table S7), patients were classified based on (presumed) overall (ABCG2 and ABCB1) transporter activity, as low transporter activity [ABCG2 low activity (variant allele) + ABCB1 low activity (overall genotype other than mainly wt) (n=9)], high transporter activity [ABCG2 high activity (wt) + ABCB1 high activity (overall mainly wt genotype) (n=29)] and intermediate overall transporter activity (high-low ABCG2-ABCB1 combinations) (n=63). With adjustment for age, sex, dose (50 mg or lower), time (week 12 or 24) and dose-corrected concentration of active moiety at weeks 6-8 (mean of the peak and trough values), estimated probability of relevant PANSS response was 63.1% in patients with low overall transporter activity, 21.3% in patients with intermediate and 11.7% in patients with high activity (OR=12.9, 95% CI 2.10-69.5 for low vs. high, and OR=6.02, 95%CI 1.25-28.9 for low vs. intermediate) (see Supplementary Table S7).

In conclusion, present results suggest a seemingly relevant (by extent) pharmacokinetic interaction between CYP2D6 phenotype and *ABCG2 421C>A* variant allele, but indicate that the relationship between systemic exposure to risperidone and factors affecting it (like, e.g., CYP2D6 phenotype), and clinical effects is not straightforward and might depend on a number of factors, including the activity of ABCB1 and ABCG2 transporters not related to systemic bioavailability. The observed effects of *ABCG2 421C>A* polymorphism and of overall *ABCB1* genotype (*1236C>T*, *3435C>T*, *2677G>T/A*) on clinical response to parenteral risperidone seem practically relevant. Present data suggest the impact of these polymorphisms on CNS availability of risperidone, but this is just a hypothesis. Also, at this point, it is unclear how would awareness about the *ABCG2*/*ABCB1* genotype be relevant for the optimization of treatment with parenteral risperidone.

Declarations of interest: none

Author contributions

Lana Ganoci: Conceptualization, Methodology, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing. **Vladimir Trkulja:** Conceptualization, Software, Validation, Formal analysis, Writing - Original Draft, Visualization, Writing - Review & Editing. **Tamara Božina:** Investigation, Data Curation, Writing - Review & Editing. **Maja Živković:** Methodology, Investigation, Writing - Review & Editing. **Marina Šagud:** Investigation, Writing - Review & Editing. **Mila Lovrić:** Methodology, Resources, Writing - Review & Editing. **Nada Božina:** Conceptualization, Methodology, Resources, Writing - Original Draft, Supervision, Writing - Review & Editing.

Role of the funding source

This publication was co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).

Supplementary material

Supplementary Patients and Methods. Patient exclusion criteria, additional information on data analysis and considerations on type 1 error rate

Supplementary Table S1-S4. Pharmacokinetic data across different subsets of patients based on genotypes at *ABCB1* loci.

Supplementary Table S5. Tests of interactions with time – pharmacokinetic outcomes.

Supplementary Table S6. Tests of interactions with time and between CYP2D6 phenotype and transporter genotypes – proportion of PANSS responders.

Supplementary Table 7. Ancillary analysis of PANSS response

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ABCB1, *ABCG2* and CYP2D6 *polymorphism* effects on disposition and response to long-acting risperidone

Supplementary material

Supplementary Patients and Methods contains supplementary information on methods employed in the study: (i) list of patient exclusion criteria (ii) additional information on data analysis and considerations on the type I error rate.

Supplementary Table S1 to S4. Tables summarize *primary outcomes* (dose-corrected risperidone, 9-OH-risperidone, risperidone+9-OH risperidone [active moiety] levels and risperidone/9-OH-risperidone ratio) across different patient subsets defined in respect to genotypes at the three investigated *ABCB1* loci. **Table S1** – by individual locus, all genotypes. **Table S2** - by individual locus categorized as "wild type" (wt) or "variant allele carriage". **Table S3** - by overall genotype (all three loci) categorized as "overall wt" (CC/GG/CC) vs. "all other", or as "overall variant" (TT/TT/TT) vs. "all other" genotypes, and by subsets based on the number of wt alleles across all three loci (0, 1-3, 4-5 or 6). **Table S4** - by overall genotype categorized into three levels: "overall wt", "overall variant" and "other" (mixed) genotypes; or as "overall mainly wt" (all six wt alleles or up to 1-2 variant alleles), "overall mainly variant" (all six variant alleles or up to 1-2 wt alleles) and "other" (mixed) genotypes.

Supplementary Table S5. Summarizes models testing interactions between effects of primary interest (CYP2D6 phenotype, *ABCG2/ABCB*1 genotypes) and time (peak or trough).

Supplementary Table S6. Summarizes models fitted to probability of relevant PANSS response, testing interactions between the effects of primary interest (CYP2D6 phenotype, *ABCG2* and *ABCB1* overall genotype) and time (week 12, week 24), and interactions between each of the transporter genotypes with CYP2D6 phenotype.

Supplementary Table S7. Ancillary analysis of PANSS response.

Supplementary Patients and Methods

List of exclusion criteria

Exclusion criteria were defined as comorbid conditions that could hinder patient's understanding of the study aims and procedures, psychiatric evaluation or could impact exposure to risperidone:

- Any form of intellectual disability, catatonia or aggressive behavior
- Substance dependence, or substance abuse (except for nicotine of caffeine) within the past three months
- Uncontrolled hypertension, history of symptomatic coronary/peripheral artery disease, stroke or transitory ischemic attack
- Any neurodegenerative disease
- Chronic heart failure, estimated glomerular filtration rate <60 mL/min/1.73 m^2
- Liver cirrhosis / symptomatic liver failure (Child-Pugh class B or C), active hepatitis
- Malignant disease or HIV infection

Data analysis and considerations on the type I error rate

Primary outcomes (ln-transformed dose-corrected risperidone, 9-OH-risperidone, risperidone+9-OH [active moiety] and risperidone/9-OH ratio, paired data *per* patient) were each (separately) analyzed by fitting (hierarchical) generalized linear mixed models (normal distribution, identity link, maximum likelihood with Gauss-Hermit quadrature, unstructured covariance, between-within degrees of freedom). All three effects of primary interest (CYP2D6, *ABCG2*, *ABCB1*) were included in each model, with further adjustment for age (known to affect exposure to risperidone [6-8]), time of blood sampling (peak or trough) and, as suggested by the data – for the use of any CYP inhibitor at the time of blood sampling. These effects were kept in all models regardless of their (lack of) association with the outcomes. Potential interference of renal or liver dysfunction was controlled by exclusion criteria. Sex was not considered since know not to relevantly affect pharmacokinetics of risperidone [7, 8]. Potential interaction between time and effects of primary interest was also evaluated and since the effects appeared consistent at both peak and at trough, the interaction term was removed from the final models (due to a limited number of subjects). The fact that the four pharmacokinetic outcomes are closely related (metabolism of risperidone gives rise to 9-OH-risperidone; the two represent active moiety; effects on one analyte will reflect also on their ratio) and each is analyzed separately, might raise the question of multiplicity, i.e., a possibility that, for example, one detects a spurious independent (adjusted) effect of CYP2D6 phenotype (the only one of

the independent variables of primary interest for which we *a priori* expected to see an effect) on one (or more) of the four outcomes. In this respect, we adopted reasoning based on biological plausibility and the concept of serial gatekeeping by testing ordered hypotheses as depicted in Figure A. The reasoning was as follows: a) CYP2D6 NM/UM phenotype has been repeatedly shown to affect exposure to risperidone (lower levels vs. IM/PM) [4-9], hence this was the only effect that we *a priori* expected to observe in the current study (considering the sample size and the expected prevalence of phenotypes): (i) primarily in respect to risperidone/9-OH ratio; (ii) and also in respect to risperidone; (iii) we were uncertain about the effect on risperidone+9-OH-risperidone levels (active moiety), but the planned sample size would still provide around 80% power to detect a smaller effect (e.g., around 20%) and (iv) we expected no effect on the levels of 9-OH risperidone; b) consequently, we considered the effect of CYP2D6 phenotype as a "lead" in a set of 4 ordered hypotheses: (i) if the 1st null hypothesis (H1) about the independent effect on the risperidone/9-OH ratio was not rejected – this would signal the need to reconsider study validity (genotyping, analyte measurements, need to increase the sample). However, if rejected, α could be transferred to the next hypothesis (H2) about the effect on risperidone exposure; (ii) a failure to reject it would have the same meaning as in the case of H1, but if H2 is rejected, α could be transferred to the next hypothesis (H3) about the effect on exposure to active moiety; (iii) if H3 was not rejected, only data on risperidone and risperidone/9-OH ratio would be used for inference, and data regarding active moiety and 9-OH would be provided as descriptive only. However, if H3 is rejected, α could be transferred to H4 (about the effect on 9-OH) (Figure A). Hence, we linked the "destiny" of the study results to the effects of CYP2D6: it could be reconsidered and maybe completely disregarded (if H1 and H2 not rejected) or, if H1-H3 rejected, each of the four multivariate models evaluating main effects (on the four PK outcomes) of the 3 effects of primary interest would be evaluated at the overall alpha=0.05.

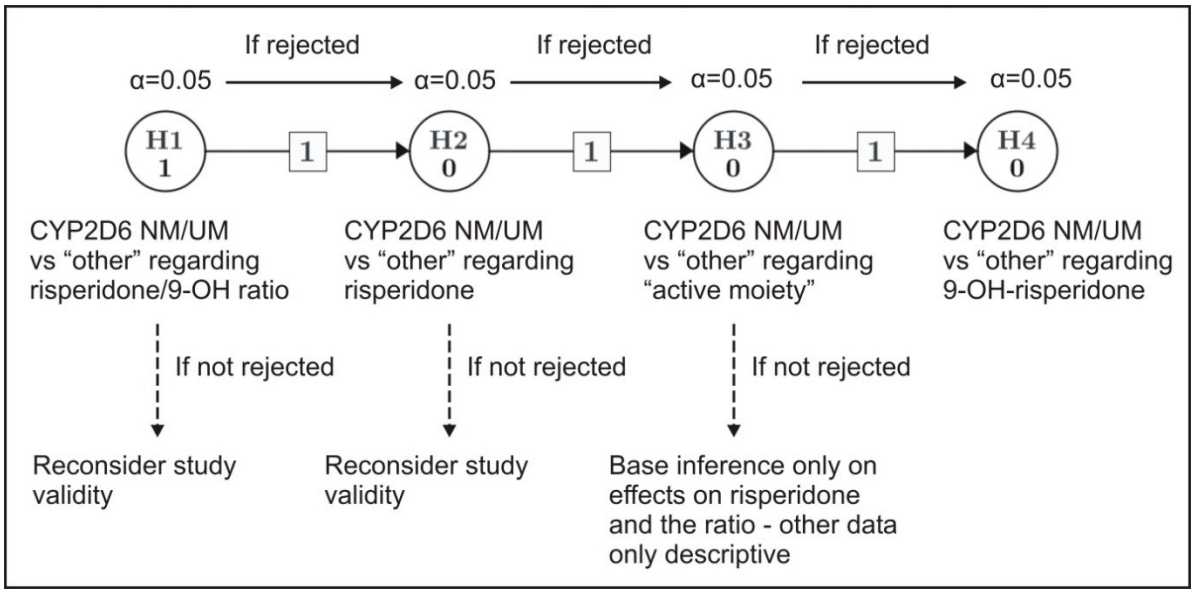

Figure A. Outline of the serial gatekeeping approach based on the independent effects of CYP2D6 phenotype on the four outcomes illustrating exposure to risperidone. 1, 0 = *a priori* assigned weights to individual hypotheses; 1 – transferred weight. Hypothesis testing/α-propagation scheme was generated using package gMCP in R software. In this order, multivariate models were fitted to primary (pharmacokinetic) outcomes.

However, in multivariate models (e.g., generalized linear mixed models used to analyze exposure data), the problem of multiplicity can also occur and result in spurious detection of "effects" of some of the several included independent variables [10]. All four of the main-effects testing models fitted to the present data included the same independents, with a focus on 3 of the primary interest (CYP2D6 phenotype, *ABCG2* and *ABCB1* genotype). To prevent spurious findings of their effects, after fitting each of the main-effects models, estimates ($\widehat{\beta}$), estimated covariance matrix [$Cov(\widehat{\beta})$] and degrees of freedom were retained and used to adjust the observed confidence intervals and *P*-values (adjustment for the number of tests about the 3 effects of primary interest) by the stepdown logical simulation method [10]. After the main-effects model, two additional (same independents) models were fitted to each PK outcome: one additionally testing the interaction between CYP2D6 and *ABCG2* genotype, and one testing the interaction between CYP2D6 and *ABCB1* genotype. To avoid spurious interaction findings, the 4 *P*-values obtained for the 2D6|*ABCG2* interactions (one for each PK outcome) and those for the 2D6|*ABCB1* interactions were adjusted by the false discovery rate method (FDR at 5%) – adjusted $P \leq 0.1$ was considered to signal an interaction that might be considered real.

Contrast estimates arising from the interaction terms are reported with 97.5% CIs which equals Bonferroni adjustment.

Secondary outcome – proportion of patients with relevant PANSS reduction (week 12, week 24) – was analyzed by fitting (hierarchical) generalized linear mixed models with binary distribution, logit link, maximum pseudo-likelihood estimation with subject-specific expansions, unstructured covariance and between-within degrees of freedom. The main-effects model included the 3 effects of primary interest and further adjustments for age, sex, risperidone dose and time of assessment. Potential interactions between the 3 effects of primary interest and time were also evaluated, and since the effects were consistent at week 12 and at week 24, the interaction term was removed from the final models. Also, baseline PANSS score, included in initial models, was subsequently removed (all *t*-values <0.4, effects closely around 0 [OR=1.0]). The estimated effects and associated *P*-values for CYP2D6 phenotype and *ABCG2* and *ABCB1* phenotypes were *post-hoc* adjusted in the same way as for the primary outcomes. Two additional models were fitted to test the 2D6|*ABCG2* and 2D6|*ABCB1* interaction, respectively. Since there were no indications of relevant interactions, the interaction *P*-values were not further adjusted. Generalized linear models were fitted by PROC GLIMMIX and *post-hoc* adjustments were performed by PROC PLM in SAS for Windows 9.4 (SAS Inc., Cary, NC).

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Table S1-S4 – data on dose-corrected risperidone, 9-OH-risperidone, risperidone+9-OH risperidone (active moiety) levels (nmol/L) and risperidone/9-OH-risperidone ratio summarized (median, quartiles) across patient subsets based on different considerations of genotypes across the three analyzed *ABCB1* loci.

Table S1. By individual loci, all genotypes. Trends across the genotypes were evaluated by the Jonckheere-Terpstra test

	1236 C>T			Trend	*2677 G>T/A*			Trend	*3435 C>T*			Trend
	CC	CT	TT	z; *P*	GG	GT/A	TT	z; *P*	CC	CT	TT	z; *P*
N	38	40	23		37	43	21		35	33	33	
Day 5 (peak)												
Risperidone	0.59	0.62	0.66	0.375;	0.65	0.62	0.65	-0.727;	0.55	0.76	0.50	-0.365;
	(0.30-1.09)	(0.34-0.96)	(0.25-0.94)	0.708	(0.28-1.14)	(0.35-0.87)	(0.25-0.97)	0.467	(0.27-0.80)	(0.5-1.14)	(0.25-0.77)	0.715
9-OH	1.09	1.14	1.26	1.09;	1.13	1.08	1.35	1.122;	1.06	1.38	1.10	0.964;
	(0.77-1.52)	(0.72-1.60)	(0.88-2.20)	0.275	(0.79-1.54)	(0.68-1.54)	(0.98-2.22)	0.262	(0.76-1.33)	(1.03-2.06)	(0.66-1.63)	0.335
Risp.+9-OH	1.78	1.77	1.91	0.378;	1.87	1.70	1.94	0.307;	1.61	2.33	1.46	0.262;
	(1.15-2.72)	(1.18-2.52)	(1.25-2.49)	0.706	(1.23-3.02)	(1.16-2.39)	(1.46-2.99)	0.759	(1.11-2.34)	(1.67-3.05)	(1.162-36)	0.793
Risp./9-OH	0.49	0.54	0.33	-0.900;	0.49	0.57	0.31	-1.230;	0.49	0.53	0.44	-0.917;
	(0.27-0.97)	(0.30-0.80)	(0.21-0.75)	0.368	(0.28-1.08)	(0.31-0.85)	(0.17-0.64)	0.219	(0.29-0.85)	(0.32-1.02)	(0.25-0.76)	0.359
Day 14 (trough)												
Risperidone	0.34	0.31	0.32	-0.550;	0.35	0.31	0.29	-0.966;	0.32	0.35	0.29	-0.764;
	(0.22-0.6)	(0.14-0.46)	(0-0.51)	0.582	(0.21-0.69)	(0.22-0.46)	(0.0-0.53)	0.334	(0.21-0.57)	(0.22-0.54)	(0-0.46)	0.445
9-OH	0.75	0.77	0.74	0.060;	0.77	0.74	0.78	-0.101;	0.73	0.91	0.69	0.200;
	(0.51-1.16)	(0.52-1.08)	(0.54-1.22)	0.952	(0.53-1.19)	(0.50-1.05)	(0.55-1.32)	0.919	(0.50-1.04)	(0.55-1.27)	(0.44-1.02)	0.842
Risp.+9-OH	1.12	1.03	1.06	-0.620;	1.18	1.02	1.06	-0.774;	1.07	1.19	0.97	-0.430;
	(0.83-1.58)	(0.76-1.62)	(0.69-1.84)	0.535	(0.83-1.71)	(0.74-1.41)	(0.72-1.84)	0.439	(0.75-1.45)	(0.89-1.92)	(0.69-1.78)	0.667
Risp./9-OH	0.41	0.42	0.30	-0.737;	0.41	0.48	0.23	-0.947;	0.43	0.40	0.26	-1.335;
	(0.26-0.57)	(0.21-0.69)	(0-0.58)	0.461	(0.27-0.57)	(0.23-0.70)	(0-0.52)	0.344	(0.27-0.58)	(0.23-0.62)	(0.0-0.60)	0.182

Data did not indicate any univariate association between the genotypes at individual loci and primary outcomes.

Table S2. By individual loci, categorized as "wild-type" (wt) or variant allele carriage. Potential differences are evaluated by the Mann-Whitney test.

	1236 C>T		U-test	*2677 G>T/A*		U-test	*3435 C>T*		U-test
	CC (wt)	T-carrier	z; *P*	GG (wt)	T/A-carrier	z; *P*	CC (wt)	T-carrier	z; *P*
N	38	63		37	64		35	66	
Day 5 (peak)									
Risperidone	0.59 (0.30-1.09)	0.65 (0.31-0.96)	0.256; 0.798	0.65 (0.28-1.13)	0.62 (0.33-0.87)	0.564; 0.573	0.55 (0.27-0.80)	0.67 (0.33-1.01)	-0.860; 0.390
9-OH	1.09 (0.77-1.52)	1.20 (0.77-1.65)	-0.715; 0.474	1.13 (0.79-1.54)	1.18 (0.76-1.63)	-0.247; 0.805	1.06 (0.76-1.33)	1.23 (0.80-1.76)	-2.023; 0.043
Risp.+9-OH	1.78 (1.15-2.72)	1.85 (1.21-2.50)	-0.147; 0.883	1.87 (1.22-3.02)	1.78 (1.20-2.47)	0.268; 0.789	1.61 (1.11-2.34)	1.89 (1.40-2.95)	-1.734; 0.083
Risp./9-OH	0.49 (0.27-0.97)	0.48 (0.28-0.78)	0.347; 0.728	0.49 (0.28-1.08)	0.48 (0.26-0.78)	0.314; 0.754	0.49 (0.29-0.85)	0.46 (0.25-0.78)	0.357; 0.721
Day 14 (trough)									
Risperidone	0.34 (0.22-0.60)	0.31 (0-0.46)	0.581; 0.561	0.35 (0.21-0.69)	0.31 (0.22-0.46)	0.871; 0.384	0.32 (0.21-0.57)	0.31 (0.09-0.47)	0.344; 0.731
9-OH	0.75 (0.51-1.16)	0.75 (0.52-1.14)	0.162; 0.899	0.77 (0.53-1.19)	0.74 (0.52-1.08)	0.606; 0.544	0.73 (0.50-1.04)	0.81 (0.53-1.23)	-0.949; 0.342
Risp.+9-OH	1.13 (0.83-1.58)	1.03 (0.74-1.71)	0.719; 0.472	1.18 (0.82-1.71)	1.03 (0.74-1.63)	1.069; 0.285	1.07 (0.75-1.45)	1.06 (0.78-1.84)	-0.343; 0.732
Risp./9-OH	0.41 (0.26-0.57)	0.40 (0.0-0.62)	0.415; 0.678	0.41 (0.27-0.57)	0.40 (0.10-0.62)	0.244; 0.807	0.43 (0.27-0.58)	0.36 (0.05-0.60)	0.911; 0.363

Table S3. By overall (all three loci) *ABCB1* genotypes categorized as "wild type homozygous" (wt) vs. all other, as "variant homozygous" (var) vs. all other, and across subsets based on the number of wt alleles across the three loci. Potential differences are evaluated by the Mann-Whitney test and trends by the Jonckheere-Terpstra test.

	Overall wild type vs. all other			Overall variant homozygous vs. all other			Number of wt alleles across the three loci				
	CC/GG/CC (wt)	All other	U-test z; *P*	TT/TT/TT (var)	All other	U-test z; *P*	0	1-3	4-5	6	Trend z; *P*
N	25	76		20	81		20	32	24	25	
Day 5 (peak)											
Risperidone	0.59 (0.24-1.40)	0.64 (0.34-0.95)	-0.208; 0.978	0.65 (0.25-0.99)	0.62 (0.33-0.96)	0.388; 0.688	0.65	0.67	0.54	0.59	-0.179; 0.858
9-OH	1.09 (0.79-1.37)	1.20 (0.76-1.66)	-0.952; 0.341	1.31 (0.98-2.22)	1.09 (0.76-1.56)	1.820; 0.069	1.31	1.24	1.03	1.09	-1.895; 0.058
Risp.+9-OH	1.78 (1.14-2.52)	1.81 (1.22-2.52)	-0.311; 0.756	1.92 (1.46-3.25)	1.78 (0.16-2.51)	0.895; 0.371	1.92	1.92	1.62	1.78	-1.243; 0.214
Risp./9-OH	0.49 (0.24-1.56)	0.49 (0.29-0.78)	0.110; 0.912	0.32 (0.21-0.65)	0.49 (0.30-0.85)	-1.782; 0.075	0.32	0.60	0.50	0.49	0.857; 0.391
Day 14 (trough)											
Risperidone	0.39 (0.22-0.72)	0.30 (0.11-0.46)	1.328; 0.184	0.31 (0.0-0.54)	0.32 (0.21-0.47)	-0.651; 0.515	0.31	0.36	0.29	0.39	0.791; 0.429
9-OH	0.77 (0.59-1.17)	0.75 (0.50-1.13)	0.515; 0.606	0.74 (0.55-1.35)	0.75 (0.51-1.12)	-0.469; 0.639	0.74	0.89	0.53	0.77	-0.631; 0.528
Risp.+9-OH	1.19 (1.01-1.61)	1.03 (0.74-1.70)	1.326; 0.185	1.01 (0.70-1.84)	1.07 (0.80-1.56)	-0.106; 0.915	1.01	1.19	0.80	1.19	0.188; 0.851
Risp./9-OH	0.42 (0.28-0.60)	0.40 (0.18-0.59)	0.664; 0.507	0.26 (0.0-0.53)	0.42 (0.23-0.62	-1.490; 0.136	0.26	0.44	0.43	0.42	1.141; 0.254

Data did not indicate any univariate association between individual loci or overall genotypes and the primary outcomes.

Table S4. By overall *ABCB1* genotypes categorized as "wild type homozygous" (wt) (CC/GG/CC), "variant homozygous" (var) (TT/TT/TT) and all other, i.e., "mixed" genotypes; or as "overall predominantly wild type" with all six wt alleles or up to 1-2 variant alleles, "overall predominantly variant" with all six variant alleles or up to 1-2 wt alleles, or all other, i.e., "mixed" genotypes. Potential trends (any trend) were evaluated using the Jonckheere-Terpstra test.

	Overall wild type or overall variant or mixed				Overall predominantly wild type or predominantly variant or mixed			
	CC/GG/CC (wt)	Mixed	TT/TT/TT (var)	Trend z; *P*	Predominant var	Mixed	Predominant wt	Trend z; *P*
N	25	56	20		28	35	38	
Day 5 (peak)								
Risperidone	0.59 (0.24-1.40)	0.62 (0.35-0.94)	0.65 (0.25-0.99)	0.199; 0.842	0.64 (0.25-0.89)	0.65 (0.350.96)	0.59 (0.29-1.09)	-0.375; 0.707
9-OH	1.09 (0.79-1.37)	1.10 (1.72-1.65)	1.31 (0.98-2.22)	1.653; 0.098	1.21 (0.83-2.03)	1.19 (0.71-1.63)	1.09 (0.76-1.44)	-1.038; 0.299
Risp.+9-OH	1.78 (1.14-2.52)	1.76 (1.17-2.52)	1.92 (1.46-3.25)	0.727; 0.467	1.75 (1.22-2.47)	1.85 (1.43-2.52)	1.78 (1.14-2.72)	-0.353; 0.724
Risp./9-OH	0.49 (0.24-1.56)	0.52 (0.32-0.80)	0.32 (0.21-0.65)	-1.149; 0.250	0.39 (0.21-0.95)	0.53 (0.31-0.78)	0.49 (0.29-0.97)	0.763; 0.445
Day 14 (trough)								
Risperidone	0.39 (0.22-0.72)	0.30 (0.21-0.44)	0.31 (0.0-0.54)	-1.279; 0.201	0.31 (0.0-0.50)	0.32 (0.22-0.46)	0.31 (0.21-0.60)	0.270; 0.787
9-OH	0.77 (0.59-1.17)	0.74 (0.49-1.08)	0.74 (0.55-1.35)	-0.056; 0.956	0.76 (0.55-1.14)	0.79 (0.52-1.25)	0.75 (0.50-1.07)	-0.478; 0.632
Risp.+9-OH	1.19 (1.01-1.61)	1.03 (0.74-1.49)	1.01 (0.70-1.84)	-1.005; 0.315	1.04 (0.75-1.81)	1.05 (0.77-1.86)	1.12 (0.80-1.56)	0.103; 0.918
Risp./9-OH	0.42 (0.28-0.60)	0.42 (0.23-0.62)	0.26 (0.0-0.53)	-1.325; 0.185	0.35 (0.0-0.61)	0.40 (0.23-0.65)	0.42 (0.26-0.56)	0.622; 0.534

Data did not indicate any univariate association between overall genotypes and the primary outcomes.

Table S5. Testing "effect|time"[a] interactions regarding exposure (dose-corrected) to risperidone, 9-OH-risperidone, risperidone+9-OH-risperidone (active moiety), and regarding risperidone/9-OH-risperidone ratio.

	Risperidone		9-OH-risperidone		Risperidone + 9-OH		Risperidone/9-OH ratio	
Model for	GMR (95%CI)	*P*	GMR (95%CI)	*P*	GMR (95%CI)	*P*	GMR (95%CI)	*P*
CYP2D6 interaction with time *adjustment: age, use of CYP inhibitors, ABCG2 & ABCB1 genotype*								
CYP2D6|time	---	0.247	---	0.424	---	0.362	---	0.340
NM/UM effect at peak	0.59 (0.45-0.78)	---	0.89 (0.71-1.11)	---	0.73 (0.58-0.91)	---	0.66 (0.50-0.89)	---
NM/UM effect at trough	0.74 (0.54-1.02)	---	0.96 (0.77-1.21)	---	0.79 (0.63-0.98)	---	0.78 (0.58-1.04)	---
ABCG2 interaction with time *adjustment: age, use of CYP inhibitors, CYP2D6 phenotype and ABCB1 genotype*								
ABCG2|time	---	0.610	---	0.407	---	0.858	---	0.162
Variant allele effect at peak	0.90 (0.63-1.29)	---	0.75 (0.56-1.01)	---	0.76 (0.56-1.02)	---	1.11 (0.76-1.62)	---
Variant allele effect at trough	0.81 (0.56-1.16)	---	0.84 (0.63-1.13)	---	0.78 (0.58-1.04)	---	0.82 (0.56-1.21)	---
ABCB1 interaction with time *adjustment: age, use of CYP inhibitors, CYP2D6 phenotype and ABCG2 genotype*								
ABCB1|time	---	0.560	---	0.149	---	0.265	---	0.618
CC/GG/CC effect at peak	1.01 (0.73-1.39)	---	0.89 (0.68-1.15)	---	0.99 (0.76-1.29)	---	1.16 (0.83-1.63)	---
CC/GG/CC effect at trough	1.12 (0.81-1.55)	---	1.05 (0.81-1.37)	---	1.12 (0.86-1.47)	---	1.06 (0.75-1.48)	---
CYP inhibitor use interaction with time *adjustment: age, CYP2D6 phenotype, ABCG2 and ABCB1 genotype*								
CYP inhibitor|time	---	0.522	---	0.934	---	0.317	---	0.791
CYP inhibitor effect at peak	0.97 (0.69-1.36)	---	0.85 (0.65-1.12)	---	0.88 (0.66-1.16)	---	1.19 (0.84-1.71)	---
CYP inhibitor effect at trough	1.10 (0.78-1.54)	---	0.84 (0.64-1.11)	---	0.78 (0.59-1.03)	---	1.13 (0.79-1.62)	---

[a]"Effect" pertains to genotypes and phenotypes of primary interest - CYP2D6 phenotype [classified as "normal or ultrarapid metabolizer" (NM+UM), n=58 or "other", n=43], *ABCG2 421C>A* genotype [wild type (wt) homozygous, n=83 or variant allele carriage, n=18], overall *ABCB1* genotype [wild type homozygous (CC/GG/CC), n=25 or "other", n=76] - and to use of any CYP inhibitor (yes, n=21 or no, n=80). "Time" pertains to timing of blood sampling – peak or trough.

Data indicated consistent effects of primary interests (CYP2D6 phenotype, *ABCG2/ABCB1* genotype) on the primary outcomes at both peak and at trough.

Table S6. Summary of multivariate models fitted to probability of relevant PANSS response testing interactions between each of the effects of primary interest - CYP2D6 phenotype [normal/ultrarapid metabolizer (NM/UM) or *other*)], *ABCG2 421C>A* genotype [variant allele or wild type (wt)] and *ABCB1* overall genotype (based on predominance of variant/wt alleles) [a] – and time (week 12 or week 24); and also interactions between CPY2D6 phenotype and *ABCG2* genotype or overall *ABCB1* genotype. Each of the five fitted models included adjustments for age, sex, dose 50 mg (vs. lower) and effects of primary interest not included in the interaction term. For brevity, shown are only interaction term *P*-values and contrasts derived from the interaction terms.

Model for	OR (97.5%CI)	*P*
CYP2D6 phenotype interaction with time		
CYP2D6|time	---	0.695
CYP2D6 NM/UM vs. other phenotypes at 12 weeks	0.45 (0.12-1.75)	---
CYP2D6 NM/UM vs. other phenotypes at 24 weeks	0.62 (0.18-2.10)	---
***ABCG2 421C>A* interaction with time**		
ABCG2|time	---	0.180
ABCG2 variant allele vs. wild type at 12 weeks	8.09 (1.60-40.8)	---
ABCG2 variant allele vs. wild type at 24 weeks	2.42 (0.45-13.1)	---
***ABCB1* overall genotype interaction with time**		
ABCB1|time	---	0.502
ABCB1 linear trend (mainly wild type – mainly variant) at 12 weeks	0.49 (0.10-2.47)	---
ABCB1 linear trend (mainly wild type – mainly variant) at 24 weeks	0.17 (0.04-0.75)	---
CYP2D6 – *ABCG2* interaction		
CYP2D6|*ABCG2*	---	0.751
CYP2D6 NM/UM vs. other phenotypes at *ABCG2* wild type	0.58 (0.19-1.79)	---
CYP2D6 NM/UM vs. other phenotypes at *ABCG2* variant	0.38 (0.03-4.34)	---
ABCG2 variant vs. wild type at CYP2D6 NM/UM	3.42 (0.68-17.2)	---
ABCG2 variant vs. wild type at CYP2D6 other phenotypes	5.24 (0.65-41.7)	---
CYP2D6 – *ABCB1* interaction		
CYP2D6|*ABCB1*	---	0.961
CYP2D6 NM/UM vs. other phenotypes at *ABCB1* mainly wild type	0.48 (0.08-2.93)	---
CYP2D6 NM/UM vs. other phenotypes at *ABCB1* mainly variant	0.59 (0.18-1.94)	---
ABCB1 linear trend (mainly wild type - mainly variant) at CYP2D6 NM/UM	0.35 (0.09-1.37)	---
ABCB1 linear trend (mainly wild type - mainly variant) at CYP2D6 *other*	0.43 (0.09-2.21)	---

[a]Overall genotype classified as: "(mainly) variant" - all three loci variant homozygous (n=20) or 1-2 wt alleles (n=8); "(mainly) wild type" - all three loci wt homozygous (n=25) or 1-2 variant alleles (n=13); "mixed" - all other overall genotypes. Exposure to risperidone across these three subsets was closely similar, and it was similar to other subsets based on overall genotype or individual *ABCB1* loci (see Supplementary Table S1-S4).

Data indicated no relevant interaciton between the effects of primary interest and time, and between transporter polymorphisms and CYP2D6 phenotype.

Table S7. Ancillary analysis of PANSS repsonse. Patients were classified in respect to the presumed overall (combination of ABCG2 and ABCB1) transporter activity, based on genotypes as: (i) low transporter activity (*ABCG2* variant allele + *ABCB1* overall genotype other than mainly wild-type). Response was seen in 6/9 and in 5/9 patients at weeks 12 and 24, respectively; (ii) high transporter activity (*ABCG2* wt + *ABCB1* overall mainly wt genotype). Response was seen in 4/29 and 5/29 patients at weeks 12 and 24, respectively; (iii) Intermediate transporter activity (wild type – non-wild type *ABCG2-ABCB1* combinations). Response was seen in 10/63 and 19/63 of patients at weeks 12 and 24, respectively. A generalized linear mixed model (as in the main data analysis) was fitted to probability of relevant response with adjustment for age, sex, dose 50 mg (vs. lower), time and dose-corrected active moiety measured at weeks 6-8 (mean of the peak and trough values). In the subsequent models, interactions were tested between overall transporter activity and time, and between overall transporter activity and active moiety levels – transporter activity effects were consistent at week 12 and 24, and there was no indication of the overall transporter activity|active moiety concentration interaction. Adjusted estimated probabilities of response were obtained by the inverse link function.

	OR (95.0% CI)	*t(df); P*
Low overall transporter activity (ABCG2 low + ABCB1 low) vs. high	12.9 (2.15-77.3)	$t_{(94)}$=2.84; 0.006
Low overall transporter activity vs. intermediate	6.31 (1.26-31.5)	$t_{(94)}$=2.28; 0.025
Active moiety concentration	0.99 (0.67-1.48)	$t_{(94)}$=-0.03; 0.977
Age	0.97 (0.92-1.02)	$t_{(94)}$=-1.32; 0.191
Men	0.21 (0.07-0.64)	$t_{(94)}$=-2.78; 0.007
Dose 50 mg (vs. lower)	1.93 (0.71-5.22)	$t_{(94)}$=1.31; 0.192
Week 24 vs. week 12	1.93 (0.90-4.18)	$t_{(94)}$=1.70; 0.092

Estimated adjusted probabilities: a) Low overall transporter activity 63.1% (95%CI 27.7 – 88.4); b) Intermediate overall transporter activity 21.3% (95%CI 13.0 – 32.8); c) High overall transporter activity 11.7% (95%CI 4.8 – 25.8).