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Genetics-based population pharmacokinetics and pharmacodynamics of risperidone in a psychiatric cohort

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ABSTRACT

Background: There is a high inter-individual variability in risperidone and its active metabolite, 9-hydroxyrisperidone, plasma concentrations that might lead to suboptimal drug concentration.

Aim: Using population pharmacokinetic approach, we aimed at characterizing the genetic and non-genetic sources of variability affecting risperidone and 9-hydroxyrisperidone pharmacokinetics and relate them to common side effects.

Methods: 150 psychiatric patients (178 observations) treated with risperidone were genotyped for common polymorphisms in *NR1I2*, *POR*, *PPARα*, *ABCB1*, *CYP2D6* and *CYP3A* genes. Plasma risperidone and 9-hydroxyrisperidone were measured; clinical data and common clinical chemistry parameters were collected. Drug and metabolite concentrations were analyzed by non-linear mixed effect modelling (NONMEM®). Correlations between trough concentrations of the active moiety (risperidone plus 9-hydroxyrisperidone) and common side effects were assessed using logistic regression and linear mixed modelling.

Results: CYP2D6 phenotype explained 52% of inter-individual variability in risperidone pharmacokinetics. AUC of the active moiety was found to be 28% higher in CYP2D6 poor metabolizers compared to intermediate, extensive and ultrarapid metabolizers. No other genetic markers were found to significantly affect risperidone concentrations. 9-hydroxyrisperidone elimination was decreased by 26% by doubling of age. A correlation between trough predicted concentration of active moiety and neurologic symptoms was found ($p=0.03$), suggesting that a concentration >40 ng/ml should be targeted only in cases of insufficient or absence of response.

Conclusions: Genetic polymorphisms of *CYP2D6* play an important role in risperidone, 9-hydroxyrisperidone and active moiety plasma concentration variability, which were associated with common side effects. These results highlight the importance of a personalized dosage adjustment during risperidone treatment.

INTRODUCTION

Risperidone, an atypical antipsychotic, is widely prescribed in adults, but also in pediatric and elderly patients. Beside an overall good therapeutic efficacy, several adverse events have been described during risperidone treatment, including neurologic symptoms, hyperprolactinemia, weight gain, insulin resistance, some of which have been shown to be dose and plasma concentration dependent[1]. There is a high inter-individual variability in plasma concentration of risperidone, which can be explained by genetic (i.e. *cytochrome P450 2D6* (*CYP2D6*)) and non-genetic factors (i.e. age, sex or renal function)[2,3]. In humans, risperidone undergoes an important first pass effect and is metabolized mainly by CYP2D6[4,5] with a contribution of cytochrome 3A (CYP3A) as shown by several interaction studies with CYP3A inducers[6,7] or inhibitors[8,9]. Risperidone's main metabolite, 9-hydroxy-risperidone[4], is mainly eliminated by the kidneys [10]. Risperidone and 9-hydroxyrisperidone are generally considered to have the same pharmacodynamic properties and the sum of both substances defines the "active moiety"[11].

The *CYP2D6* genetic polymorphism has been well described with the existence of four predicted phenotypes: poor (PM), intermediate (IM), extensive (EM) or ultra rapid (UM) metabolizers[12]. A growing number of reports underline the impact of *CYP2D6* polymorphism on risperidone and 9-hydroxyrisperidone pharmacokinetics and on clinical outcomes. A significant median increase of steady-state risperidone concentration in PM patients compared to other genotypes was indeed found in a Caucasian schizophrenic cohort[13]. Moreover, discontinuation rate due to adverse reactions was found to be higher in PM carriers compared to IM, EM or UM[14]. CYP3A is the combination of 3 isoforms, CYP3A4, CYP3A5 and CYP3A7 with overlapping activities. Several genetic polymorphisms of *CYP3A* isoforms have been described, such as the *3A5*3* variant resulting in a loss of CYP3A5 protein synthesis or the *CYP 3A4*22* allele resulting in a decrease of CYP3A4 mRNA expression, polymorphisms which have been associated with differences in clinical outcome for drugs such as simvastatin and cyclosporine[15,16]. Few studies investigated the effect of *CYP3A* polymorphism on risperidone plasma concentration, with opposite results [17,18]. This

may be partly due to the fact that genotyping methods allow to capture only a small extent of the variability of CYP3A activity[19-21]. Mutations of other non-CYP genes, such as the cytochrome P450 oxydo-reductase (POR), which provides the necessary electrons to P450 activity, have also been found to influence CYP activity. *POR*28* allele was found to be associated with decreased CYP3A activities[22]. In addition, many nuclear receptors such as the pregnane X receptor (encoded by the *NR1I2* gene) or the peroxisome proliferator-activated receptor alpha (PPARα) may regulate the expression of genes involved in drug disposition, such as *CYP450* and *ABCB1* which encodes the permeability glycoprotein (Pgp), a drug transporter[23]. In-vitro experiments identified several SNPs in the promoter region of *NR1I2* which may affect *CYP3A* expression[24,25]. Recent studies also showed associations between *NR1I2* genotypes and an increased risk of delayed graft rejection[26] or side effects during treatment with long-acting risperidone[27].

Risperidone treatment can induce neurologic side effects such as akathisia, rigidity or tremor. Conflicting results were however obtained in several studies examining the relationship between risperidone or 9-hydroxyrisperidone concentrations with neurological toxicity[28-31]. Hyperprolactinemia is also a common issue for patients receiving risperidone. It may induce sexual dysfunction and, in the long term, be a risk factor for cardiovascular disorders and decreased bone mineral density[32]. A relationship between plasma concentration of risperidone or 9-hydroxyrisperidone and prolactin have been shown in several reports[33,34].

To date, several population pharmacokinetic studies on risperidone in psychiatric populations have been published indicating an influence of carbamazepine on the active moiety's plasma concentration[35], an age-related decrease of 9-hydroxyrisperidone clearance and a decrease in risperidone clearance in male patients and in patients under paroxetine or fluoxetine treatment[36]. One study found higher average active moiety plasma concentration in CYP2D6 PMs, and an association between active moiety and dystonia and parkinsonism[28].

The objectives of the present study are to characterize the population pharmacokinetics of risperidone, 9-hydroxyrisperidone and/or the active moiety in a cohort of psychiatric patients,

to study genetic and non-genetic sources of variability, and identify a threshold level associated to common side effects.

METHODS

Study design and participants:

Participants were selected from three observational pharmacogenetic studies whose main inclusion criteria were the prescription of second generation antipsychotic drugs. 35 patients were included in a first cross-sectional study as detailed previously[37]. 78 patients were included during a longitudinal study conducted at the psychiatric department of the Lausanne University Hospital, with data collection during the first year of treatment. 37 patients came from a retrospective study conducted from 2010 to 2011 in two out-patients centers of the psychiatric department of Lausanne University Hospital. Blood samples for pharmacokinetic measurements were collected after a median (interquartile range, IQR) time of 13h(4h), in steady-state conditions (at least one week under constant dose), with variable duration of treatment (median duration of treatment: 7.3 months (IQR: 33.5 months)). A median of one sample (range 1-3) of risperidone and 9-hydroxyrisperidone was collected per patient.

In addition to the exact time of last drug intake and blood sampling, the following informations were recorded at the same time than the blood samples drawn for pharmacokinetic measurements : gender, age, race, grapefruit consumption, smoking status, renal and liver function tests (creatinine, ASAT, ALAT), creatinine clearance (CL_{creat}, estimated by the Modification of Diet in Renal Disease (MDRD)[38] and Salazar-Corcoran[39] formulas), co-medications, including possible co-prescription of a second generation antipsychotic (classified as strong or moderate CYP2D6 inhibitors, CYP3A4 inducers and Pgp inhibitors or inducers) and several genetic polymorphisms[40-45]. Side effects for the 78 subjects from the longitudinal study were classified according to the Udvalg for Kliniske Undersøgelser (UKU) side effect rating scale and categorized as absent, light, medium or severe[46]. All investigated side effects were reported on the same day that the blood samples drawn for pharmacokinetic measurements. They were classified into 5 groups: neurologic (akathisia, rigidity and tremor), gastro-intestinal (constipation, increased/reduced salivation and nausea), cardiovascular (hypertension, hypotension and lower-extremity edema), central nervous system (asthenia, sleepiness) and sexual side effects (diminished sexual desire,

ejaculatory dysfunction, erectile dysfunction and orgasmic dysfunction). All three studies were approved by local ethics committees (Geneva and Lausanne), and written informed consents (also for genetic analysis) were obtained from all patients or from their legal representatives.

Plasma concentration determinations:

All blood samples were collected in EDTA containing tubes. After centrifugation, plasma samples were stored at -20°C until routine analysis. Risperidone concentrations were determined by a HPLC-MS method as described previously[27]. Prolactin, creatinine, aspartate aminotransferase (ASAT) and alaninetransaminase (ALAT) concentrations were determined by immunoassay on an Abbott Axsym system (Abbott, Wiesbaden, Germany). Concerning prolactin, only blood samples drawn between 8h00 and 10h30 AM were analysed.

Genotyping:

Genomic DNA was extracted from EDTA blood samples with the FlexiGene DNA extraction kit (QIAGEN, Hombrechtikon, Switzerland) according to the manufacturer's protocol. The following SNPs were detected by real-time polymerase chain reaction according to manufacturer's instructions (ABI PRISM 7000; Applied Biosystems, Rotkreuz, Switzerland) : *CYP2D6*3* (*rs35742686*), *CYP2D6*4* (*rs3892097*), *CYP2D6*6* (*rs5030655*), *CYP3A4*1B* (*rs2740574*), *CYP3A4 intron 7* (*rs4646437*)C>T, *CYP3A4* (*rs35599367*)G>A, *CYP3A5*3* (*rs776746*), *CYP3A7*1C* (*2262T>A and 2270T>G*), *POR*28* (*rs1057868*), *NR1I2* (*rs1523130*), *NR1I2* (*rs2472677*), *NR1I2* (*rs7643645*), *NR1I2* (*rs2276707*), *PPARA (rs4253728), ABCB1* 2677G>T (*rs2032582*), *ABCB1* 61A>G (*rs9282564*), *ABCB1* 1199G>A (*rs2229109*), *ABCB1* 3435C>T (*rs1045642*), *ABCB1* 1236C>T (*rs1128503*). Detection of duplication/multiplication of *CYP2D6*xN* were performed by long PCR, as previously described[47] and gene deletion *CYP2D6*5 (Hs0001001_cn)* was analyzed by TaqMan copy number assay. In each set of analyses, controls DNAs with known genotypes were included.

Model-based pharmacokinetic analysis

Structural and error model:

Since metabolite formation occurs rapidly *via* first pass metabolism and systemic biotransformation of risperidone[4], a simultaneous parent drug/metabolite pharmacokinetic analysis was conducted. A one-compartment model with first-order absorption and elimination was used for both risperidone and 9-hydroxyrisperidone, using a linear conversion from risperidone to 9-hydroxyrisperidone (Figure 1). In order to account for the pre-systemic metabolism, the fraction (FR) of risperidone dose converted during the first pass effect was estimated. The absorption rate constants k_{12} and k_{13} were defined respectively as (1-FR)*k_a and FR*k_a, with k_a representing the total absorption rate constant. Because of the limited amount of measurements in the absorption phase, k_a was fixed to 3.1 h^{-1} to achieve risperidone peak plasma concentration as reported in the literature [48,35,36]. Since risperidone was administered orally, risperidone and 9-hydroxyrisperidone pharmacokinetic parameters represent apparent values. Owing to identifiability problems, both compounds were assumed to have the same apparent volume of distribution (V). Inter-patient variability of all the pharmacokinetic parameters but FR was described by exponential errors following a log-normal distribution. The logit of FR was used to constrain individual FR to vary between 0 and 1 and its inter-individual variability calculated as previously reported[49]. Correlations between pharmacokinetic parameters were investigated. Finally, several error models were compared to describe the intra-patient (residual) variability for both drug and metabolite. The correlation between risperidone and 9-hydroxyrisperidone concentration measurements was tested using the L2 function in NONMEM®.

Covariate model:

The covariate analysis was performed using a stepwise insertion/deletion approach. Visual inspection of the correlation between post hoc individual estimates of the pharmacokinetic parameters and the available factors was first conducted. Potentially influential covariates were then incorporated sequentially in the model using linear or non-linear functions as appropriate (categorical variables coded as 0 and 1, a fixed effect was associated to each of

the ethnic group within the population, continuous covariates were centered on their median value or to 80 ml/min for CL_{creat}). Sequential analysis of the impact of genetic polymorphisms on pharmacokinetic parameters was conducted categorizing patients into genotypic groups, or in the case of CYP2D6, into predicted phenotypic groups. Parameters values were then estimated for each genotypic/phenotypic group (rich model) or for further regrouped (reduced model) sub-populations.

Parameter estimation and model selection:

Risperidone and 9-hydroxyrisperidone data were fitted by use of the first-order conditional estimation (FOCE) method with interaction using NONMEM® version 7.2[50] with the PsN-Toolkit version 3.5.3[51]. Concentrations below the quantification limit of the assay were replaced by LOQ/2 (M6 method[52]). The log-likelihood ratio test, based on changes in the OFV value (ΔOFV), was employed to discriminate between hierarchical models. Since a ΔOFV between any two models approximates a χ^2 distribution, a decrease of the objective function was considered statistically significant if it exceeded 3.84 ($p<0.05$) or 6.63 ($p<0.01$) for 1 additional parameter in model-building and backward-deletion steps respectively. Diagnostic goodness-of-fit plots and precision of the pharmacokinetic parameters were also used to assess the reliability of the results.

Model evaluation and assessment:

Final model stability was assessed by means of the bootstrap method implemented in PsN[51], generating two-thousand datasets by re-sampling from the original dataset. Mean parameters values with their 95% confidential interval ($CI_{95\%}$) were compared with the original model estimates. The predictive performance of the final pharmacokinetic model was evaluated by calculation of the normalized prediction distribution errors (NPDEs)[53]. In addition, visual predictive checks stratified on CYP2D6 phenotype were performed for model validation. Figures were generated with GraphPad Prism® (Version 6.00 for Windows, GraphPad Software, USA).

Simulations:

Simulations of 1000 individuals for each CYP2D6 phenotype based on the final model with variability were conducted to derive average $AUC_{0\text{-}24}$ with 95% prediction intervals ($PI_{95\%}$) for risperidone, 9-hydroxyrisperidone and the active moiety (AUC_{RISP}, AUC_{9OHR}, $AUC_{active\ moiety}$= AUC_{RISP}+ AUC_{9OHR}). Differences in average $AUC_{0\text{-}24}$ between metabolic groups were analyzed by one-way ANOVA with the Bonferroni correction (STATA Version 12, StataCorp, College Station, USA).

Concentration-adverse events relationship:

Relation between UKU reported side effects and final model-predicted trough plasma concentration of risperidone, 9-hydroxyrisperidone and active moiety ($C_{min\text{-}RISP}$, $C_{min\text{-}9OHR}$, $C_{min\text{-}active\ moiety}$) were investigated by logistic regression analyses. Side effect ratings were dichotomized in absent versus presence while including side-effect reported at the last visit. Relation between prolactin and $C_{min\text{-}RISP}$, $C_{min\text{-}9OHR}$ and $C_{min\text{-}active\ moiety}$ were investigated by linear mixed effect model using the “nlme” package of R[54]. Statistically significant results were considered as $p \leq 0.05$.

RESULTS

Demographic:

A total of 178 sample concentrations were available from 150 included patients, receiving risperidone at a total dose ranging from 0.5 mg to 8 mg daily given either once (n=21 in the morning, n=106 in the evening) or twice (n=51) daily. All concentrations were measured in steady-state conditions and 47 concentrations were on trough. The measured concentrations for drug and metabolite ranged respectively between 0-62 ng/ml and 0.5-69 ng/ml. Median prolactin concentration of 31 µg/l and 85 µg/l were observed in male and females, respectively. Three patients treated with haloperidol, levomepromazine and pipamperone and two patients with a prolactin level of 345 µg/l (previous co-administration of pipamperone) and 238 µg/l (breast-cancer) were discarded from the prolactin analysis. Other characteristics of the study population are presented in table 1. In our population with completed UKU rating scale, 43%, 40%, 7%, 45% and 30% of the patients reported neurologic, autonomic, cardiovascular, psychic and sexual dysfunction side effects, respectively. 9 patients were discarded from the side effect analysis (biperiden=11, haloperidol=5, levomepromazine=1, pipamperone=1, with some patients receiving more than one of these 4 drugs at the same time). Numbers and frequencies of all side effects are described in eTable 1. For all Caucasian patients, genotype frequencies are in Hardy Weinberg equilibrium. Genetic characteristics of patients are presented in eTable 2.

Structural model:

The model illustrated in figure 1 adequately described risperidone and 9-hydroxyrisperidone data. However, because of the important pre-systemic metabolism, the metabolic rate constant (k_{23}) could not be properly estimated and, therefore, fixed to 0. Association of inter-individual variability on FR ($\Delta OFV=-22.2$; $p=2.4\cdot10^{-6}$) in addition to risperidone clearance (CL_{RISP}) markedly improved the model fit, which was further improved by inclusion of an inter-patient variability on 9-hydroxyrisperidone clearance (CL_{9OHR}) ($\Delta OFV=-38.3$; $p=6.1\cdot10^{-10}$) but not on V ($\Delta OFV=-3.6$; $p=0.06$). Allowing for a correlation between FR and CL_{9OHR} ($\Delta OFV=$-

54.9; $p=1.3\cdot10^{-13}$) and, successively, between FR and CL_{RISP} ($\Delta OFV=-7.5$; $p=6.3\cdot10^{-3}$) resulted in a noticeable improvement of the model fit. Residual intra-patient variability on both risperidone and 9-hydroxyrisperidone was satisfactorily described using a proportional error model. Drug and metabolite concentration measurements were found to be significantly correlated ($\Delta OFV=-6.3$; $p=0.01$). The parameter estimates with inter-patient variability (CV%) of the basic pharmacokinetic model were a CL_{RISP} of 3.8 L/h (42%), a V of 290 L, a CL_{9OHR} of 5.8 L/h (44%), a FR of 89% (19%) and a k_a fixed to 3.1 h^{-1}.

Covariate analysis:

Univariate analyses testing the influence of the non-genetic covariates on risperidone pharmacokinetics revealed a significant impact of age and sex on both FR ($\Delta OFV\leq-6.5$; $p\leq0.01$) and CL_{9OHR} ($\Delta OFV\leq-8.2$; $p\leq4.0\bullet10^{-3}$). All the remaining demographic, environmental and physiologic characteristics were not associated with the drug pharmacokinetics ($\Delta OFV>-2.7$; $p>0.10$). Because of the high correlation between CL_{9OHR} and FR, only the effects of sex and age on CL_{9OHR} were retained for further investigations ($\Delta OFV>-2.2$; $p>0.14$). Multivariate analyses showed that solely age influenced CL_{9OHR} ($\Delta OFV=-2.9$; $p=0.08$ with respect to the model with age and sex on CL_{9OHR}). A decrease of 27% in CL_{9OHR} with doubling age as compared to the median population age (39 years) was observed.

Co-administration of strong and moderate Pgp and CYP2D6 inhibitors affected both FR ($\Delta OFV\leq-6.1$; $p\leq0.01$) and CL_{RISP} ($\Delta OFV\leq-4.0$; $p \leq 0.05$), while CYP3A4 did not impact risperidone pharmacokinetics ($\Delta OFV\geq-1.0$; $p\geq0.32$). Multivariate analyses allowed discarding the effect of inhibitors on CL_{RISP} while keeping it on FR ($\Delta OFV\geq-0.8$; $p\geq0.37$). Only CYP2D6 inhibitors on FR were finally retained ($\Delta OFV=-1.8$; $p=0.19$ with respect to the model with both CYP2D6 and Pgp inhibitors on FR) and were found to decrease FR by 5% and 9% if categorized as moderate or strong inhibitors respectively.

The effect of common polymorphisms in *CYP2D6, CYP3A4/5/7, POR, NR1I2* and *ABCB1* on CL_{RISP}, CL_{9OHR} and FR showed that the predicted CYP2D6 phenotypes had by far the most important influence on FR ($\Delta OFV=-82.6$; $p=8.5\bullet10^{-18}$), CL_{RISP} ($\Delta OFV=-31.8$; $p=5.8\bullet10^{-7}$) and CL_{9OHR} ($\Delta OFV=-30.3$; $p=1.2\bullet10^{-6}$). Carriers of the *CYP3A4 rs35599367* GA allele were found

to have a CL_{9OHR} 30% lower than the GG individuals (ΔOFV=-7.0; p=8.0•10^{-3}). *NR1I2 rs1523130* and *NR1I2 rs2276707* polymorphisms, respectively, affected CL_{RISP} and FR (ΔOFV=-8.7; p=0.01 and ΔOFV=-7.5; p=0.02). A reduced model in which the *NR1I2 rs1523130* CT and TT or *NR1I2 rs2276707* CT and TT individuals were grouped characterized as well the influence of *PXR rs1523130* on CL_{RISP} or *NR1I2 rs2276707* on FR (ΔOFV≥0.01; p=1.0), suggesting a recessive effect of the gene. CL_{RISP} was found to be 33% higher in *NR1I2 rs1523130* CT/TT than in CC individuals, while FR was 6% higher in *NR1I2 rs2276707* CT/TT with respect to CC individuals. No other genetic polymorphisms were significant (ΔOFV≥-5.7; p≥0.06).

Multivariate combination of the influential genetic polymorphisms showed that only the CYP2D6 phenotype remained significant. Moreover, the influence of this phenotype on FR captured its previously observed effect on both CL_{RISP} and CL_{9OHR} (ΔOFV≥-3.6; p≥0.31) and markedly stabilized the model fit, allowing for the estimation of k_{23}. No statistical significant difference was observed between CYP2D6 UM and EM compared to the rich model in which a FR was assigned to each CYP2D6 phenotypic group (ΔOFV=0.10; p=0.76). Estimated average FRs were 92% for EM/UM (n=99), 86% for IM (n=41) and 19% (n=10) for PM individuals. CYP2D6 polymorphisms and inhibitors on FR and age on CL_{9OHR} remained statistically significant in the multivariate analysis and backward deletion step. Estimated average fraction of risperidone dose converted into 9-hydroxyrisperidone were 93%, 85% and 8.8% for EM/UM, IM and PM patients, suggesting that IMs and PMs have, respectively, a decrease of 8% and of 91% in FR compared to EM/UM individuals. Co-administration of moderate and strong CYP2D6 inhibitors decreased FR by 4% and 19%. Predicted CYP2D6 phenotypes and CYP2D6 inhibitors intake explained altogether 52% of initial FR inter-patient variability. Moreover, our results show that age doubling with respect to the median age of the population reduced CL_{9OHR} by 26%, explaining 26% of its inter-patient variability. The final model parameters' estimates and bootstrap estimations are given in table 2. The model was considered reliable since the obtained parameter estimates laid within the bootstrap $CI_{95\%}$. NPDE analysis confirmed that the model adequately described the observed data. Dose-

normalized concentration-time plots of risperidone and 9-hydroxyrisperidone with $PI_{95\%}$ for EM/UM and PM patients aged of population median age is shown in figure 2.

Simulations

Model-based simulations were performed to estimate and compare AUC_{RISP}, AUC_{9OHR} and $AUC_{active\ moiety}$ for individuals characterized by different CYP2D6 metabolic strengths (figure 3). Simulated average AUC_{RISP} indicates a risperidone exposure 8 and 1.8 times higher in PMs and IMs compared to EM patients (eTable3). Both AUC_{RISP} and AUC_{9OHR} were found to vary significantly within individuals carrying different CYP2D6 phenotype ($p<0.0001$). EM/UMs and IMs $AUC_{active\ moiety}$ are not significantly different ($p=0.99$), while $AUC_{active\ moiety}$ was 28% ($CI_{95\%}$:24-32%) higher in PMs compared to CYP2D6 EM/UM or IM individuals ($p<0.0001$).

Concentration-adverse event relationship:

No associations were observed between CYP2D6 predicted phenotype and/or inhibitor with reported side effects. Autonomic, cardiovascular, psychic and sexual sides effects were not associated with $C_{min\text{-}active\ moiety}$ (eFigure 1). However, the presence of neurologic symptoms were associated with $C_{min\text{-}active\ moiety}$ ($p=0.03$, figure 4). In particular, only the severity of tremor was associated with $C_{min\text{-}active\ moiety}$ ($p=0.01$, eFigure 2). By correcting for age and gender, an increase of $C_{min\text{-}active\ moiety}$ was associated with developing neurologic symptoms ($p=0.03$). In the neurologic group, $C_{min\text{-}active\ moiety}$ increase was associated with tremor and akathisia ($p=0.01$, $p=0.02$, respectively, table3) but not with rigidity. Logistic regression plot indicates a 70% and more probability to develop neurologic symptoms at $C_{min\text{-}active\ moiety}$ 40 ng/ml and higher (figure 4).

Prolactin was found to be more elevated in PM, IM or in presence of CYP2D6 inhibitor but only in women ($p=0.04$) (eFigure 3). Mixed linear model corrected by age revealed a correlation between prolactin concentration and $C_{min\text{-}9OHR}$ ($\beta=2.36$ ug/l, $P_{adjusted}=0.04$) and $C_{min\text{-}active\ moiety}$ ($\beta=2.03$ ug/l, $P_{adjusted}=0.03$) in women (table4). No significant associations were observed between prolactin concentrations and sexual dysfunction side effects in both genders (data not shown).

DISCUSSION

To our knowledge, this is the first population pharmacokinetic study which included a broad range of patients of different ages, with an extensive analysis of genetic markers, and with a concentration-effect analysis. CL_{RISP} and CL_{9OHR} were estimated to be of 4.6 l/h and 6 l/h, in the same range of previously published studies[35,28]. A first pass effect of 92% for EM/UM and of 19% for PM patients was calculated in the present study, highlighting the strong influence of *CYP2D6* genetic polymorphism on first pass effect. Other non-CYP2D6 genetic determinants were found to significantly influence risperidone and/or 9-hydroxyrisperidone disposition in univariate analysis and, although discarded in the multivariate model, these results must be further investigated in larger population samples. A decrease of 4% and 19% in the first pass effect upon co-administration of weak (methadone, citalopram, duloxetine, venlafaxine and sertraline[40]) and strong (levomepromazine, haloperidol, paroxetine or fluoxetine[40]) CYP2D6 inhibitors was observed. These results are in agreement with studies showing that paroxetine increases the plasma concentration of risperidone[55], that patients with paroxetine or levomepromazine as co-medications have a significant higher risperidone concentration-dose ratio than patients without co-medication[56], and that the prescription of fluoxetine as co-medication increases the active moiety by 50% to 75%[57,58]. One can mention that there are discrepant data on the inhibition potential of haloperidol, some defining it as a strong inhibitor[40] and other as a weak inhibitor[41]. However, no differences were observed when comparing the pharmacokinetic model with haloperidol classified as a strong versus weak CYP2D6 inhibitor (data not shown).

As demonstrated by several other studies[28,59-61], CYP2D6 activity plays the most important role in risperidone disposition resulting in low concentrations of risperidone and high concentrations of 9-hydroxyrisperidone in patients with a high CYP2D6 activity. Simulations showed that *CYP2D6* genotype affects risperidone and 9-hydroxyrisperidone exposures with a direct influence on the active moiety. Thus, although no statistical differences were found in $AUC_{active\ moiety}$ between EM/UMs and IMs, a significant difference was found between PMs and EM/UM/IM. Non-significant increase of $AUC_{active\ moiety}$ was observed in a single dose

study with healthy volunteers[62]. Although some studies found no differences in steady state active moiety plasma concentration between *CYP2D6* genotypes[60,30,63,13], an increase of 27% in the active moiety plasma concentration was observed in another study[59], which is in agreement with the present finding. Negative results could be due to small patient samples and insufficient numbers of PM patients. In addition, an influence of PM status on the active moiety concentrations is in agreement with the results of a study including 325 stabilized outpatients receiving risperidone, with PM having 3.1 fold higher risk of moderate to severe adverse drug reaction[14].

Age, by reflecting renal function, is a well-known factor affecting CL_{9OHR}[2,4,36]. Nevertheless, no significant associations were found between CL_{9OHR} and renal function. This may be due to the age heterogeneity (median(IQR):39(24) years old) of our cohort. Furthermore, 25% of our population is obese and thus these renal indicators might be not appropriate. A non-significant correlation between CL_{9OHR} and the renal function, estimated by the Salazar-Corcoran[39] formula suitable for obese subjects, was however found.

Neurologic symptoms such as akathisia, rigidity or tremor, mainly caused by an antagonism of striatal dopamine D_2 receptors, are frequent side effects in patients under risperidone treatment. Several studies investigated the relationship between neurologic symptoms and plasma concentration, with positive[64,1,28,27] or negative results[30,29,31]. In the present study, $C_{min\text{-}active\ moiety}$ was significantly associated with akathisia, tremor and combined with rigidity. Interestingly, akathisia was not associated with average plasma concentration in a previous published population pharmacokinetic study[28]. C_{min} of 9-hydroxyrisperidone was not found to be associated with any neurologic side effects, which could be tentatively explained by a lower affinity to D_2 receptor and a higher affinity to 5-HT2A receptor compared to risperidone[65]. This is also in agreement with prospective studies reporting a decrease of neurologic symptoms after switching from risperidone to 9-hydroxyrisperidone (paliperidone)[66,67], although more studies are needed to validate the strategy of switching from risperidone to paliperidone in case of poor tolerability. Logistic regression analysis indicated that a $C_{min\text{-}active\ moiety}$ over 40 ng/ml is associated with more than 70% risk to develop

neurologic symptoms (figure 3). Because 40 ng/ml is the median value of the proposed therapeutic range (20-60 ng/ml)[68], this suggests that the upper range of the therapeutic window should only be targeted in cases of insufficient or absence of response.

Prolactin concentration was associated with 9-hydroxyrisperidone C_{min} in women. 9-hydroxyrisperidone is characterized by a longer half-life and a higher hydrophilicity than risperidone, which is important considering that the pituitary lies outside the blood-brain barrier [69-71,34]. Estrogens lead to an increase of lactotrophic cells in the pituitary and a decrease of D_2 receptor synthesis[72], which may confer to women a higher sensibility to prolactin release induced by anti D_2 drugs[73].

Several limitations of the present study have to be mentioned. No effect of CYP3A inducers and of Pgp inhibitors as co-medication could be observed, which may be explained by an insufficient number of patients under such treatments. Secondly, side effect scales were only recorded for the longitudinal study during which scales were missing for several patients, which could therefore lead to a reporting bias.

Strengths of the present study include a naturalistic design, multiple observations over time and a broad age and BMI range which may therefore increase the clinical validity of the pharmacokinetics results. In addition, patients with co-medications known to induce neurologic symptoms or to increase prolactin concentration were excluded from the pharmacodynamic analysis.

CONCLUSION:

This analysis underlines the importance of CYP2D6 activity status on first pass effect and of age on metabolite clearance. Due to this important variability, therapeutic drug monitoring should be used to adjust drug dosage with the aim, to target in a first step, and after a careful evaluation of the clinical situation, the lower therapeutic window range (20-40 ng/ml) which is less associated with neurologic side effects. These results highlight the importance of a personalized approach, including both genetic (i.e. before introduction of treatment) and therapeutic drug monitoring (during treatment) data, when treating patients with risperidone.

Key Points:

- There is an important influence of CYP2D6 phenotype on risperidone disposition and an influence of age on 9-hydroxyrisperidone elimination.
- Increase in prolactin concentration and neurologic side effects were correlated to the active moiety plasma concentration.
- In order to reduce neurologic side effects, concentration of the active moiety higher than 40 ng/ml should be targeted only in cases of insufficient or absence of therapeutic response.

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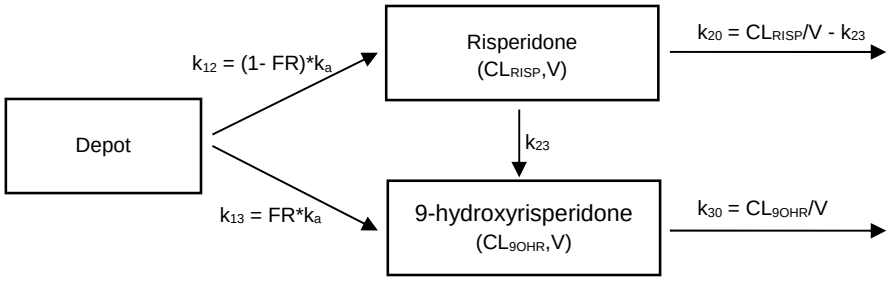

Figure 1: Compartmental model used to describe risperidone and 9-hydroxyrisperidone plasma concentration-time profiles. FR, fraction of the dose converted into metabolite; CL_{RISP} and CL_{9OHR}, mean apparent risperidone and 9-hydroxyrisperidone clearances; V, mean apparent volume of distribution; k_a, total absorption rate constant; k_{12} and k_{13} absorption rate constants from depot to, respectively, risperidone and 9-hydroxyrisperidone compartments; k_{23}, metabolic rate constant; k_{20} and k_{30}, risperidone and 9-hydroxyrisperidone elimination rate constants.

Table 1: Summary of the demographic parameters.

Variable	Last observation
Sex, male; n / total n (%)	82 /150 (55%)
Age, years; Median (IQR)	39 (24)
BMI, kg/m^2; Median (IQR)	25.9 (8.5)
Prevalence of overweight[a]; n / total n (%)	32 /144 (22%)
Prevalence of obesity[a]; n / total n (%)	39 /144 (27%)
Smoker	74 /143 (52%)
Ethnic group; n / total n (%)	
Caucasian	122 /150 (81%)
Asian	4 /150 (3%)
Arab	3 /150 (2%)
African	6 /150 (4%)
Other	15 /150 (10%)
Psychiatric diagnosis[b]; n / total n (%)	
Bipolar disorders	17 /150 (11%)
Depression	21 /150 (14%)
Drug addiction	3 /150 (2%)
Organic	7 /150 (5%)
Other	10 /150 (7%)
Psychotic disorders	79 /150 (53%)
Schizoaffective disorders	11 /150 (7%)
Unknown	2 /150 (1%)
Risperidone dose, mg/day; n / total n (%)	
< 2	68 /150 (45%)
2.1 - 4	65 /150 (43%)
4.1 - 6	13 /150 (9%)
> 6	4 /150 (3%)
Δ Time between last dose and blood sampling, h; Median (IQR)	13 (4)
CYP2D6 weak inhibitors[c]; n / total n (%)	48 /150 (32%)
CYP2D6 strong inhibitors[d]; n / total n (%)	7 /150 (5%)
CYP3A strong inducers[e]; n / total n (%)	2 /150 (1%)
Pgp inhibitors[f]; n / total n (%)	21/150 (14%)
Grapefruit	4 /150 (3%)
Prolactin	
Male, ug/L	31 (25)
Female, ug/L	85 (85)
High prolactin levels[g]; n / total n (%)	54 /93 (58%)
Aspartate aminotransferase, U.l; Median (IQR)	24 (11)
Alanine aminotransferase, U.l; Median (IQR)	19 (17)
Estimated creatinine clearance, mL/min; Median (IQR)	115 (48)

[a] Patient is considered as overweight when BMI is equal or higher than 25 kg/m2 and less than 30 kg/m2. Patient is considered as obese when BMI is equal or higher than 30 kg/m2.

[b] Diagnoses were establish following the International Classification of Disease, 10th Revision (ICD-10).

[c] duloxetine=2 / citalopram=21 / methadone=6 / sertraline=12 / venlafaxine=7.

[d] haloperidol=3 / levomepromazine=2 / paroxetine=2.

[e] oxcarbazepine=1 / topiramate=1.

[f] lansoprazole=1 / methadone=6 / paroxetine=2 / sertraline=12.

[g] Prolactin plasma levels higher than 50 µg/l for women and higher than 40 µg/l for men are considered as high prolactin levels.

Table 2: Final population pharmacokinetic parameter estimates and their bootstrap evaluations.

Parameter	Population mean		Bootstrap evaluation	
	Estimate	SE[a] (%)	Estimate	$CI_{95\%}$
$logitFR_{EM/UM}$	2.6	9	2.6	(2.1;3.2)
$\theta_{CYP2D6\ PM}$	-5.0	45	-6.3	(-21.1;-3.9)
$\theta_{CYP2D6\ IM}$	-0.85	23	-0.87	(-1.21;-0.51)
$\theta_{INHCYP2D6\ M}$	-0.51	31	-0.54	(-1.02;-0.15)
$\theta_{INHCYP2D6\ S}$	-1.5	20	-1.5	(-2.99;0.10)
CL_{RISP} (l/h)	4.6	17	4.8	(3.4;6.2)
V (l)	250	21	270	(178;414)
k_a (h^{-1})	3.1	--	3.11	--
CL_{9OHR} (l/h)	6.1	6	6.2	(5.4;7.1)
θ_{AGE} (%)	-26	20	-26	(-34;-16)
k_{23} (h^{-1})	$4.9 \cdot 10^{-3}$	64	$5.0 \cdot 10^{-3}$	$(1.8;8.1) \cdot 10^{-3}$
$IIV^b_{logitFR}$ (CV%)	132	11	131	(99;157)
IIV^b_{CLRISP} (CV%)	41	37	38	(13;55)
IIV^b_{CL9OHR} (CV%)	32	14	33	(19;46)
$\omega^c_{logitFR,\ CLRISP}$ (CV%)	-42	50	-34	(-281;11)
$\omega^c_{logitFR,\ CL9OHR}$(CV%)	85	11	84	(77;88)
σ^d_{RISP} (CV%)	41	11	46	(32;52)
σ^d_{9OHR} (CV%)	37	10	44	(31;44)
Correlation risperidone/9-hydroxyrisperidone (%)	9	156	26	(-14;44)

logitFR, logit transformation of fraction of the dose converted into metabolite defined in Eq 1; CL_{RISP}, mean apparent risperidone clearance; V, mean apparent volume of distribution; k_a, total absorption rate constant; CL_{9OHR}, mean apparent 9OHR clearance; k_{23}, metabolic rate constant; UM, EM, IM and PM ultra-, extensive, intermediate, poor CYP2D6 metabolizers; INHCYP2D6 M and INHCYP2D6 S, moderate and strong CYP2D6 inhibitors.

FINAL MODEL:

$TVlogitFR = logitFR_{EM/UM} + \theta_{CYP2D6\ PM} * I_{CYP2D6\ PM} + \theta_{CYP2D6\ IM} * I_{CYP2D6\ IM} + \theta_{INHCYP2D6\ M} * I_{INHCYP2D6\ M} + \theta_{INHCYP2D6\ S} * I_{INHCYP2D6\ S}$

$TVCL_M = CL_{9OHR} * (1 + \theta_{AGE} * (AGE - MAGE)/MAGE)$

where $I_{CYP2D6\ PM}$=1 for PMs 0 otherwise, $I_{CYP2D6\ IM}$=1 for IMs 0 otherwise, $I_{INHCYP2D6\ M}$=1 and $I_{INHCYP2D6\ S}$=1 respectively for moderate and strong CYP2D6 inhibitors intake 0 otherwise, MAGE= 37.3 years.

[a] Standard errors of the estimates (SE) defined as SE/estimate and expressed as percentages.

[b] Interpatient variability defined as CVs (%).

[c] Correlation between pharmacokinetic parameters expressed as a CVs (%)

[d] Residual intrapatient variability expressed as a CVs (%)

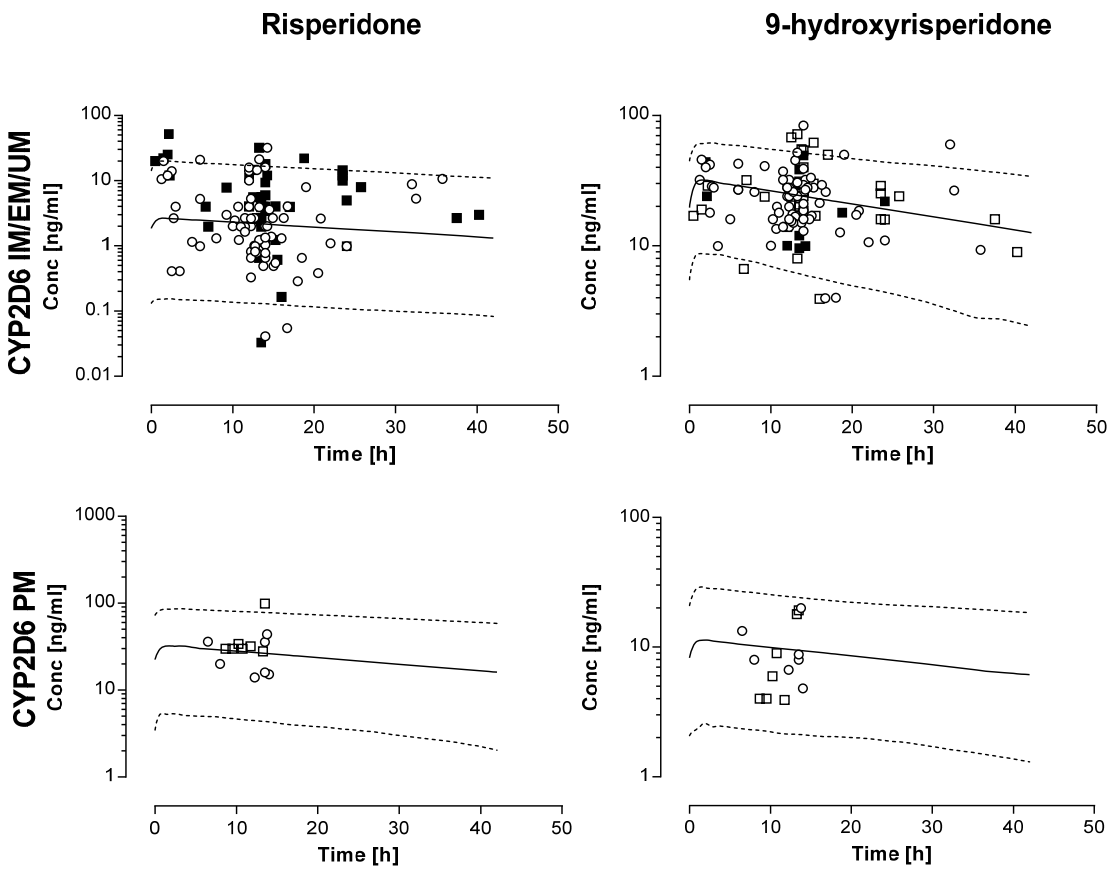

Figure 2: Concentration-time plots of risperidone (upper graphs) and 9-hydroxyrisperidone (lower graphs) with $PI_{95\%}$ for intermediate, extensive, ultra rapid (CYP2D6 IM/EM/UM) and poor (CYP2D6 PM) CYP2D6 metabolizers (empty and filled squares represent patient receiving moderate and strong CYP2D6 inhibitors; circles all the remaining individuals).

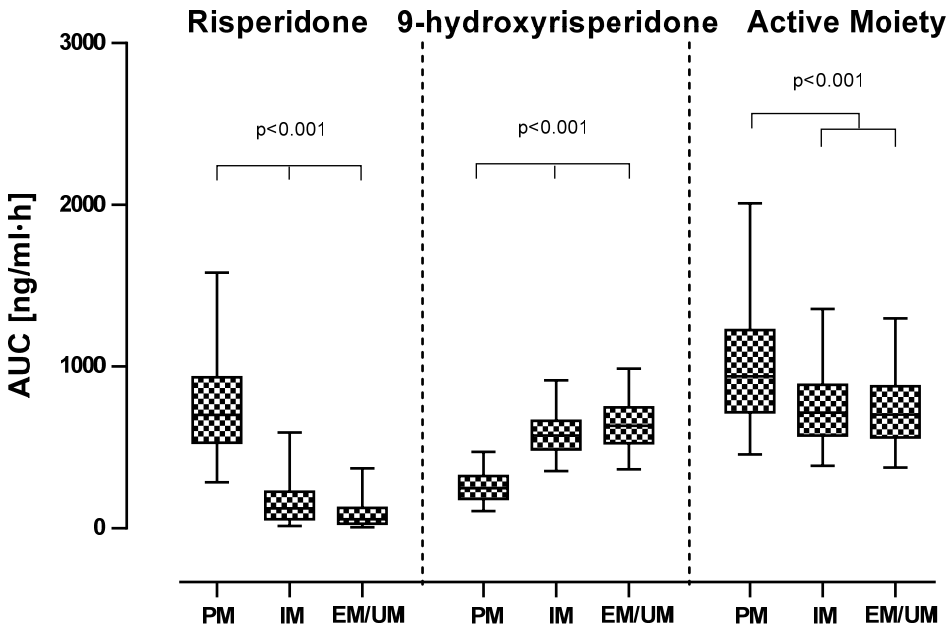

Figure 3: Simulated average estimates of AUC_{0-24} for risperidone, 9-hydroxyrisperidone and the active moiety with their $PI_{95\%}$ for PM (poor metabolizers), IM (intermediate metabolizers) and EM/UM (extensive metabolizers/ultrarapid metabolizers) individuals.

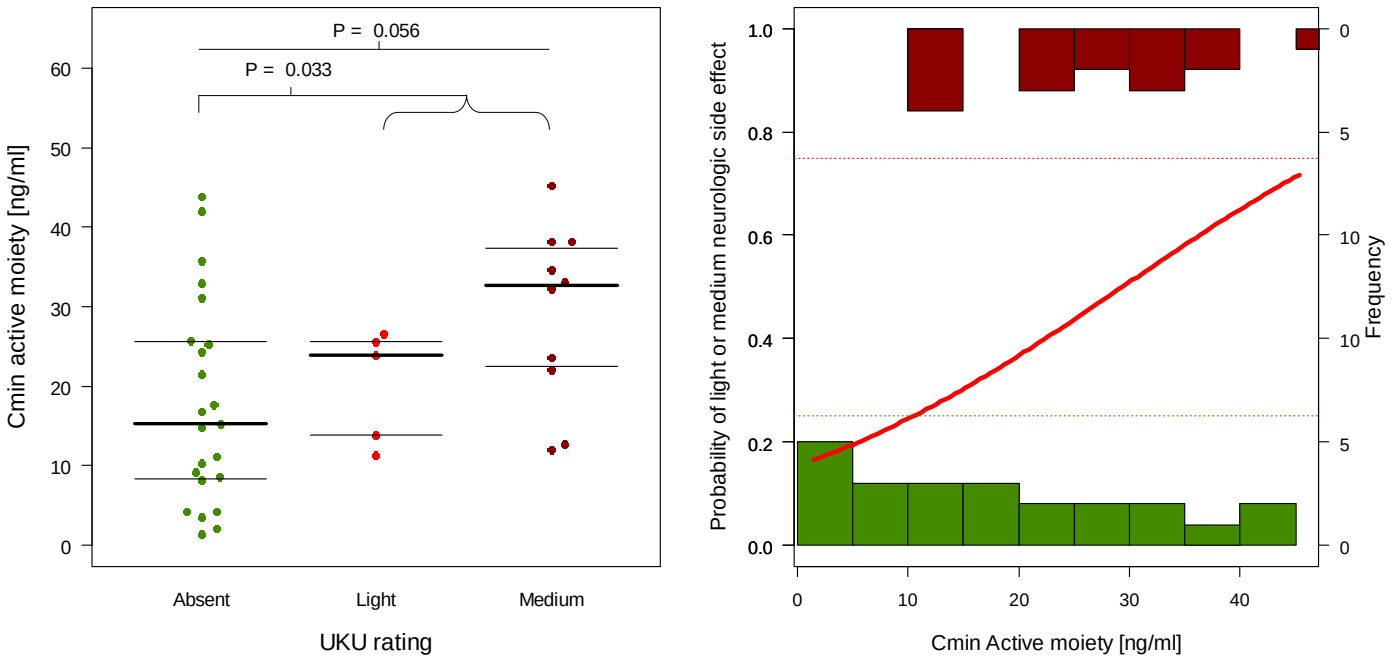

Figure 4: The left figure represents minimal concentration of active moiety in relation to the severity of neurologic side effects group. The bold horizontal line shows the median value and the two other lines represent the upper and lower quartile. Patients having co-medication possibly inducing neurologic symptoms (haloperidol, levomepromazine, pipamperone and biperiden) were excluded from analysis. On the right panel, the lower and upper histogram represents the frequency of patients without (green histogram) and with (red histogram) sides effect, respectively, over Cmin. Probability of developing light or medium extrapyramidal symptoms (left y axis) related to minimal active moiety concentration is represented by the red regression curve. Green and red dotted lines represent a 25% and 75% probability to develop extrapyramidal symptoms.

Table 3: Logistic regression fitted for neurologic and their related side effect over Cmin.

Side effect	C_{min} [ng/ml]	Estimate (se)	P-Value
Neurologic			
	Risperidone	0.021 (0.01)	**0.05**
	9-hydroxyrisperidone	0.008 (0.008)	0.3
	Active moiety	0.015 (0.006)	**0.03**
Akathisia			
	Risperidone	0.014 (0.01)	0.2
	9-hydroxyrisperidone	0.013 (0.008)	0.1
	Active moiety	0.015 (0.006)	**0.02**
Rigidity			
	Risperidone	0.018 (0.009)	0.06
	9-hydroxyrisperidone	-0.007 (0.007)	0.3
	Active moiety	0.002 (0.006)	0.7
Tremor			
	Risperidone	0.023 (0.01)	**0.03**
	9-hydroxyrisperidone	0.012 (0.008)	0.1
	Active moiety	0.018 (0.006)	**0.01**

Logistic regression adjusted by age and gender made on last observation. Patients having co-medication possibly inducing neurologic symptoms (haloperidol, levomepromazine, pipamperone) and biperiden were excluded from analysis.

Table 4: Linear mixed effect model fitted on prolactin concentration over Cmin.

Population[a]	n. obs (n subjects)		Increase of prolactin concentration for 1 ng/ml of Cmin.	P-Value
All sample[b]	87 (76)			
		Risperidone	1.69	**0.04**
		9-hydroxyrisperidone	1.39	**0.004**
		Active moiety	1.28	**0.002**
Gender stratification[c]:				
Men	48 (42)			
		Risperidone	0.98	0.3
		9-hydroxyrisperidone	0.53	0.2
		Active moiety	0.55	0.2
Women	39 (34)			
		Risperidone	2.56	0.1
		9-hydroxyrisperidone	2.36	**0.04**
		Active moiety	2.03	**0.03**

[a]3 patients were deleted from analysis because of presence of co-medication possibly inducing hyperprolactinemia (haloperidol, levomepromazine, pipamperone). All blood samples were drawn before 10:30AM.

[b]Results were obtained by fitting a linear mixed model controlling for age and sex.

[c]Results were obtained by fitting a linear mixed model controlling for age.