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OPTIMISATION AND INDIVIDUALISATION OF PSYCHOTROPIC THERAPIES: FOCUS ON VARENICLINE, AMISULPRIDE AND QUETIAPINE

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Prof. Bogdan Draganski

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ABSTRACT

The psychiatric disease itself is a cause of chronic metabolic complications. In addition, psychiatric patients are frequently smokers and receive psychotropic drugs worsening the risk of cardiometabolic diseases and leading to an increased mortality in comparison to the general population. The Nicotine replacement therapy (NRT) and varenicline are recommended treatments to help patients who want to quit smoking. These treatments have a modest efficacy, which may be enhanced by adapting them to the patients' individual characteristics (e.g. demographic, clinical and/or genetic). Second generation antipsychotics have a propensity for inducing cardiometabolic side effects, especially weight gain, which can be important. Optimized prescription is therefore essential in order to maximize efficacy and tolerability of treatments. This can be reached by understanding the influence of environmental factors (e.g. comedications) and patients' clinical susceptibilities such as age, gender and morbidities (e.g. cardiovascular diseases, hepatic or renal impairments) on the pharmacokinetics and pharmacodynamics of psychotropic drugs. To that purpose, this thesis aims at identifying opportunities to optimize the treatment by amisulpride and quetiapine, as well as NRT and varenicline, by the study of their pharmacokinetics, pharmacodynamics and pharmacogenetics. We identified factors influencing the effectiveness of NRT and varenicline, pharmacokinetics of varenicline, amisulpride and quetiapine as well as adverse events of amisulpride and quetiapine using standard clinical care data. In nicotine normal metabolizers determined by phenotyping or genotyping, we confirmed that varenicline have better quit rates compared to NRT. Women who smoke had higher response with varenicline over NRT. By using a population approach, varenicline pharmacokinetics was found to be influenced by body weight and a *UGT2B7* genetic polymorphism. In adult and older patients, amisulpride pharmacokinetics was influenced by age and lean body weight, and the observed hyperprolactinemia, which can contribute to metabolic complications in the long-term, was not dependent on amisulpride concentrations within the therapeutic range. Quetiapine elimination was influenced by the concomitant administration of drugs inducing cytochrome P450 3A4 (CYP3A4) and

its metabolic rate into norquetiapine was influenced by the CYP3A phenotype. Weight gain seemed to depend on quetiapine exposure. To conclude, the results of this thesis can be employed to optimize the efficacy and tolerability of NRT and varenicline in the general population and of amisulpride and quetiapine in the psychiatric population.

RÉSUMÉ

La maladie psychiatrique induit elle-même des complications métaboliques chroniques. De plus, les patients psychiatriques sont généralement fumeurs et reçoivent des médicaments psychotropes, ce qui aggrave le risque de maladies cardiométaboliques et conduit à des décès précoces par rapport à la population générale. Pour aider à arrêter de fumer, les substituts nicotiniques et la varenicline sont recommandés. Ces traitements ont une efficacité moyenne qui peut être améliorée en les adaptant aux caractéristiques individuelles des patients (ex : démographiques, cliniques et/ou génétiques). Les antipsychotiques de seconde génération induisent des effets indésirables cardiométaboliques, en particulier une prise de poids qui peut être importante. L'optimisation de la prescription est nécessaire pour maximiser l'efficacité et la tolérance des traitements. Cela peut être obtenu par la compréhension de l'influence de facteurs environnementaux (ex : comédications) et cliniques comme l'âge, le sexe et les comorbidités (ex : maladies cardiovasculaires, insuffisance rénale ou hépatique) sur la pharmacocinétique et la pharmacodynamie des médicaments psychotropes. Pour cela, le but général de cette thèse était d'identifier les possibilités d'optimisation du traitement par l'amisulpride, la quétiapine et également par les substituts nicotiniques et la varenicline par l'étude de leur pharmacocinétique, leur pharmacodynamie et leur pharmacogénétique. En utilisant des données de la pratique clinique courante, nous avons identifié des facteurs influençant l'efficacité des substituts nicotiniques et de la varenicline, la pharmacocinétique de la varenicline, de l'amisulpride et de la quétiapine et influençant également des effets indésirables de l'amisulpride et de la quétiapine. Chez les métaboliseurs normaux de la nicotine déterminés par phénotypage ou génotypage, nous avons confirmé que la varenicline permet d'obtenir de meilleurs taux d'arrêt du tabac par rapport aux substituts nicotiniques. Les fumeuses ont une meilleure réponse thérapeutique avec la varenicline qu'avec les substituts nicotiniques. En utilisant une approche de population, nous avons mis en évidence que la pharmacocinétique de la varenicline était influencée par le poids et un polymorphisme génétique de l'*UGT2B7*. Chez les adultes et les personnes âgées, la pharmacocinétique de l'amisulpride

variait avec l'âge et la masse maigre, et l'hyperprolactinémie observée, qui peut contribuer aux complications métaboliques à long terme, n'était pas dépendante des concentrations thérapeutiques d'amisulpride. L'élimination de la quétiapine était influencée par l'administration concomitante de médicaments induisant le cytochrome P450 3A4 (CYP3A4) et le taux de métabolisation en norquétiapine était influencé par le phénotype du CYP3A. La prise de poids semblait dépendre de l'exposition à la quétiapine. Pour conclure, les résultats de cette thèse peuvent être utilisés pour optimiser l'efficacité et la tolérance des substituts nicotiniques et de la varenicline dans la population générale et de l'amisulpride et de la quétiapine dans la population psychiatrique.

RÉSUMÉ LARGE PUBLIC

Optimisation et individualisation des traitements psychotropes : études sur les substituts nicotiniques, la varenicline, l'amisulpride et la quétiapine

La maladie psychiatrique induit des complications métaboliques chroniques. Ces maladies métaboliques peuvent être aggravées lorsque les patients fument et/ou reçoivent des médicaments dits psychotropes agissant sur le cerveau (par ex : amisulpride et quétiapine) et peuvent conduire à des décès plus précoces que dans la population générale. Les substituts nicotiniques et la varenicline sont recommandés pour aider à arrêter de fumer. Ces traitements ont une efficacité moyenne qui peut être améliorée en choisissant le bon traitement et la bonne dose pour chaque patient en fonction de ses caractéristiques individuelles (ex : âge, poids, génétique). Certains psychotropes induisent une prise de poids qui peut être importante. L'efficacité et la tolérance des traitements peuvent être améliorées par la compréhension de l'influence de certains facteurs (ex : autres médicaments administrés, âge, sexe et maladies cardiovasculaires ou rénales) sur l'exposition aux psychotropes et donc leurs effets. Cette thèse avait pour but d'identifier les facteurs influant l'exposition et donc l'effet de l'amisulpride, de la quétiapine, des substituts nicotiniques et de la varenicline. Nous avons confirmé que l'effet de la varenicline sur l'arrêt du tabac est meilleur que celui des substituts nicotiniques chez les personnes éliminant normalement la nicotine et chez les fumeuses. Nous avons mis en évidence que l'exposition à la varenicline dépendait du poids et de la génétique d'une enzyme, l'UGT2B7. Chez les adultes et les personnes âgées, l'exposition à l'amisulpride variait avec l'âge et le poids, et l'augmentation observée du taux de prolactine n'était pas dépendante de la quantité d'amisulpride dans le sang. L'élimination de la quétiapine dépendait de l'activité des enzymes de la famille du cytochrome P450 3A. La prise de poids observée sous quétiapine semblait dépendre de la quantité de quétiapine dans le sang. Pour conclure, les résultats de cette thèse peuvent être utilisés pour optimiser l'efficacité et la tolérance des substituts nicotiniques et de la varenicline dans la population générale, de l'amisulpride et de la quétiapine dans la population psychiatrique.

SCIENTIFIC COMMUNICATIONS

PUBLICATIONS IN PEER-REVIEWED JOURNALS

- **Glatard A**, Guidi M, Dobrinas M, Cornuz J, Csajka C, Eap CB. Influence of body weight and UGT2B7 polymorphism on varenicline exposure in a cohort of smokers from the general population. Eur J Clin Pharmacol. 2019;75(7):939-949

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ORAL PRESENTATION

- Glatard A, Guidi M, Delacrétaz A, Dubath C, Levier A, Conus P, von Gunten A, Csajka C and Eap C.B. Amisulpride dose adaptation with age and lean body weight in adult and elderly psychiatric patients. Swiss Association of Public Health Administration and Hospital Pharmacists – GSASA congress. Fribourg, Switzerland. 15 November, 2018

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- Glatard A, Guidi M, Dobrinas M, Cornuz J, Csajka C and Eap CB. Varenicline exposure: influence of *UGT2B7* polymorphism and relationship with abstinence in a cohort of smokers from the general population. Symposium Clinical Pharmacy Switzerland. Basel, Switzerland. 14 September, 2016.

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- Glatard A, Guidi M, Dubath C, Grosu C, Laaboub N, Delacrétaz A, von Gunten A, Conus P, Csajka C and Eap C.B. Amisulpride: Real-World Evidence of Dose Adaptation and Effect on Prolactin levels using PK/PD modeling. 28th Annual Meeting of the Population Approach Group in Europe. 11 - 14 June, 2019

- Glatard A, Guidi M, Delacrétaz A, Dubath C, Conus P, von Gunten A, Eap C.B and Csajka C. Population pharmacokinetic model of amisulpride for dosage individualization in psychiatric patients. 27th Annual Meeting of the Population Approach Group in Europe. 29 May - 1 June, 2018

- Glatard A, Guidi M, Dobrinas M, Cornuz J, Csajka C and Eap C.B. Varenicline exposure is associated with abstinence from smoking in a cohort of smokers from the general population. 26th Annual Meeting of the Population Approach Group in Europe. Budapest, Hungary. 6-9 June, 2017

- Glatard A, Guidi M, Dobrinas M, Cornuz J, Csajka C, Eap CB. Influence of UGT2B7 polymorphism on varenicline clearance in a cohort of smokers from the general population. 25th Annual Meeting of the Population Approach Group in Europe. Lisbon, Portugal. 7-10 June, 2016.

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ABBREVIATIONS

5-HT	Serotoninergic receptors
95%CI	95% of Confidence Interval
ACC	American College of Cardiology
ADME	Absorption, Distribution, Metabolism, Excretion
AGNP	Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie
AIC	Akaike's Information Criterion
AUC	Area Under the Curve
BMI	Body Mass Index
b.i.d.	bis in die
BW	Body Weight
CAR	Continuous Abstinence Rate
C_{av}	Average concentrations over 24h
CER-VD	Ethics Committee of Vaud
CL	Drug clearance
CL/F	Apparent drug clearance
CLCRCG	Creatinine Clearance estimated by the Cockcroft-Gault formula
Cmax	Maximum plasma concentration
$Cmin_{SS}$	Trough concentrations at steady-state
CO	Carbon monoxide
CPD	Cigarettes Per Day
Cpred	Model-based predicted plasma concentration
CrI	Credible Interval
CV	Coefficient of Variation
CYP	Cytochrome P450
$D_{2/3}$	Dopamine receptor 2 or 3
DNA	DeoxyriboNucleic Acid
EBE	Empirical Bayesian Estimate
EDTA	EthyleneDiamineTetraAcetate
E_{max}	Maximal achievable effect value
EPS	Extra-Pyramidal Symptoms
F	Absolute oral bioavailability
FDA	Food and Drug Administration
FOCE	First-Order Conditional Estimation
FOCEI	First-Order Conditional Estimation with Interaction
FTND	Fagerström Test for Nicotine Dependence
GFR	Glomerular Filtration Rate
H1	Histaminergic receptor 1
HDL	High-density lipoprotein
Het-Alt	Heterozygous for the reference/alternative allele
Hom-Alt	Homozygous for the alternative allele
HR	Hazard Ratio
ICD-10	International Classification of Diseases – 10^{th} edition
IIV	InterIndividual Variability
iPGP	Permeability-glycoprotein inhibitors
IQR	InterQuartile Range
IR	Immediate Release
k_a	First-order absorption rate
K_i	Inhibitor constant
Kin	Zero order input rate
Kout	First-order degradation rate
LBW	Lean Body Weight
$M_{1/3}$	Muscarinic receptor 1 or 3
MAF	Minor Allele Frequency
MDRD	Modification of Diet in Renal Disease formula
MPE	Mean Prediction Error
mRNA	messenger RiboNucleic Acid
nAchR	Nicotinic Acetylcholine Receptor
NMR	Nicotine-Metabolite Ratio
NM	Nicotine Normal Metabolizers

NONMEM®	Non Linear Mixed Effect Modelling software
NPDE	Normalized Prediction Distribution Errors
NRT	Nicotine Replacement Therapy
OCT	Organic Cation Transporter
OFV	Objective Function Value
OR	Odds-Ratio
q.d.	quaque die
PD	Pharmacodynamics
P-gp	Permeability-glycoprotein
ppm	parts per million
PK	Pharmacokinetics
PsN	Perl-speaks-NONMEM
pvcVPC	prediction- and variability-corrected Visual Predictive Check
PXR	Pregnane X Receptor
Ref	Reference allele group
RCT	Randomized Clinical Trials
RMSE	Root Mean Square prediction Error
RSE	Residual Standard Error
SGA	Second Generation Antipsychotics
SM	Nicotine Slow Metabolizers
SMD	Standardized Mean Difference
SNP	Single Nucleotide Polymorphism
TDM	Therapeutic Drug Monitoring
T_{max}	Time when maximum plasma concentration is reached
UGT	UDP glucuronosyltransferase
V	Volume of distribution
V/F	Apparent volume of distribution
VPC	Visual Predictive Check
wGRS	weighted Genetic Risk Score
XR	Extended release

CHAPTER I. GENERAL INTRODUCTION

I.1 The psychiatric population: a high-risk population for metabolic co-morbidities and death

The psychiatric population is vulnerable. Compared to the general population, psychiatric patients are recognized to have a life expectancy reduced by 10-15 years[1, 2] and more importantly in men than in women (reduction by 15.9 and 13.6 years, respectively).[2] This higher mortality risk is due to suicides (41%) but mostly to natural causes (59%).[3] Higher mortality risk due to natural causes could be related to poor self-care and diet, sedentary lifestyle, substance abuse including excessive smoking and the simple inability to get good medical care.[2] In a retrospective analysis in Western Australia, cardiovascular diseases were the main cause of excess deaths (35%).[4] In an American cohort of more than 1'000'000 patients with schizophrenia, cardiovascular diseases had the highest mortality rate (403.2 per 100'000 person-years).[5, 6] This is due to the high prevalence of risk factors in schizophrenic patients: obesity, hypertension, dyslipidemia, glucose intolerance and more cigarette smoking than the general population.[7] In a meta-analysis involving 25'690 patients with schizophrenia, the overall rate of metabolic syndrome was 32.5% (95%CI = 30.1%-35.0%). The metabolic syndrome is defined by disturbances of waist adiposity, insulin resistance, increased blood pressure, high levels of triglyceride and low levels of high-density lipoprotein (HDL) cholesterol.[8] In the population included in the meta-analysis, the prevalence of each contributor to the metabolic syndrome was high: 47% of patients were overweight, 20% had hyperglycemia, 39% had hypertriglyceridemia, 43% had low HDL cholesterol levels and 39% had high blood pressure. Additionally, 54% were smokers.

Therefore, the increased frequencies of metabolic disturbances and smoking behavior highlight the need for an integrated somatic and psychiatric care for patients with psychotic disorders. In particular, adequate interventions for restraining metabolic disturbances and for smoking cessation are important in this population.

I.2 Second-generation antipsychotics: psychiatric care inducing adverse events and notably cardiometabolic complications

Second-generation antipsychotics have become the cornerstone of pharmacological treatment of psychoses and schizophrenia because of their superiority on first-generation antipsychotics with regard to safety profile, notably a reduced risk of extrapyramidal adverse effects.[9] Second-generation antipsychotics like risperidone, clozapine, olanzapine, quetiapine are characterized by a dual pharmacological action: dopaminergic D_2 receptor antagonism and serotoninergic (5-HT) antagonism among other interactions with muscarinic, adrenergic and histaminergic receptors as well. The antagonism of the 5-HT_{2A} receptor give the property of most atypical antipsychotic and is responsible for reducing extrapyramidal symptoms.

Atypical antipsychotics propensity for metabolic disturbances is well recognized, and many can induce significant weight gain, diabetes and dyslipidemia.[10] In a meta-analysis including 212 clinical trials and 43'000 participants, clozapine and olanzapine have the highest potency to induce body weight gain followed by quetiapine, risperidone and paliperidone while amisulpride, aripiprazole and lurasidone are associated with the lowest risk as illustrated in **Figure 1.1.**[10, 11]

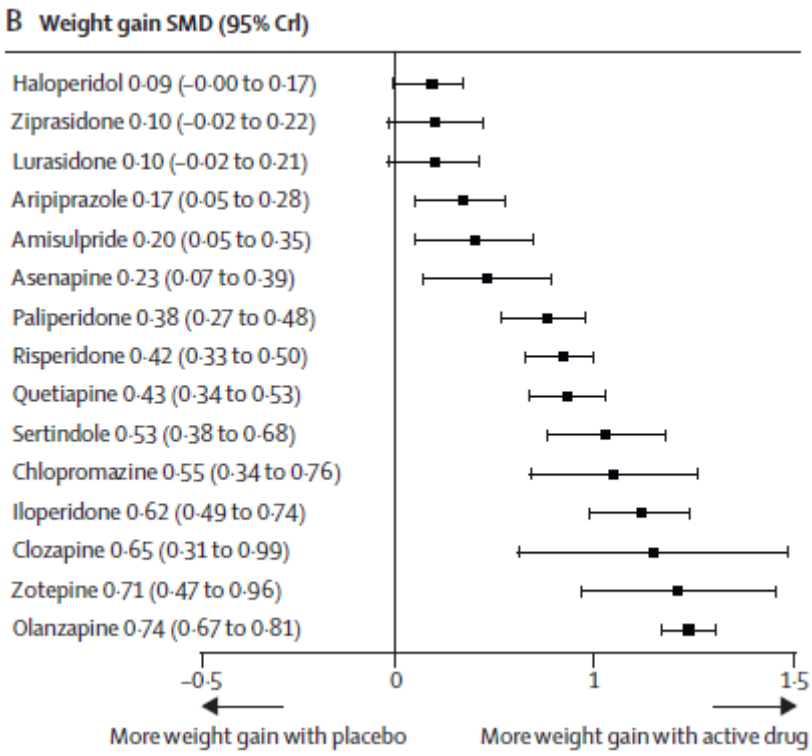

Figure 1.1: Forest plot for weight gain of antipsychotic drug compared with placebo. SMD is the standardised mean difference. CrI is the credible interval. Adapted from Leucht S. *et al.* [11] Of note, the correct scale is -0.5,0,0.5,1

The difference in potency of antipsychotics for inducing weight gain may be explained by the different receptor binding affinities, *i.e* the log of the inhibitory constant (K_i) of each antipsychotic. The physiological mechanism underlying the metabolic disturbances and subsequent weight gain with the use of atypical antipsychotics is only partially understood,[12] however some pathways have been identified. A network meta-regression used the standardized mean difference values for weight gain obtained in the meta-analysis from Leucht et al.[11] and the receptor affinities of different antipsychotics to examine the association between magnitude of weight gain and antipsychotic receptor binding profiles.[13] Weight gain was associated with increased affinity for serotoninergic 5-HT_{2C} receptor, histaminergic H_1 receptor and muscarinic M_1 and M_3 receptors. Evidence suggests that histaminergic transmission is controlling and regulating food intake with antagonism increasing food intake.[14, 15] Also

serotoninergic neurons have been recognized to have an important effect on regulating feeding behavior and satiety signaling.[15] Finally, amisulpride and haloperidol interacts mostly with dopamine D_2/D_3 receptors suggesting that D_2 blockade is probably a common factor of weight gain.[15]

Weight gain may induce cardiometabolic complications, decrease quality of life, negatively impact self-esteem and eventually may alter the adherence to pharmacological treatment.[16] To prevent these consequences induced by the psychiatric disease itself, by the antipsychotics and/or other psychotropic drugs at risk (*i.e*. some antidepressants such as mirtazapine and tricyclics and some mood stabilizers) the monitoring of metabolic parameters in the psychiatric population is critical.

I.3 Smoking cessation treatment: effective therapy for each smoker

The proportion of smokers in the psychiatric population varies between 50% and 87%.[17] Interventions for the management of smoking is necessary since it is an important risk factor for cardiometabolic disorders. According to the 2018 ACC Expert Consensus Decision Pathway on Tobacco Cessation Treatment, first line treatments for smoking cessation are nicotine replacement therapy (NRT) (gum, inhaler, lozenge, nasal spray and patch), varenicline tablets and bupropion sustained release.[18] These medications have shown their efficacy in long-term abstinence, yet a combination of counseling and medication is more effective than the drug alone.

The aim of the nicotine replacement therapy is to reduce the nicotine withdrawal symptoms and the motivation to smoke by temporarily replacing nicotine from cigarettes. After treatment by NRT combination (patch plus gum or lozenge), the 6-month abstinence was 37% while it was 14% in patients taking placebo in a meta-analysis of 83 randomized trials.[19]

Varenicline is a very selective partial agonist of the $\alpha_4\beta_2$ nicotinic acetylcholine receptor (nAchR)[20] which confers a dual action. During abstinence, a nicotine-like effect maintains moderate levels of dopamine and relieves craving and withdrawal symptoms (agonist effect). When subjects are smoking, the higher affinity of varenicline as compared to nicotine for the $\alpha_4\beta_2$ nAchR prevents access of nicotine to the receptor and reduces rewarding effects of nicotine (antagonist effect). After treatment by

varenicline, the 6-month abstinence was 33% while it was 14% in patients taking placebo in a meta-analysis of 83 randomized trials.[19] A meta-analysis involving more than 100'000 participants showed that varenicline increases the chance of quitting with an odds-ratio (OR)=2.88 and a credible interval (CrI) = 2.4-3.47 compared to placebo in 6'100 smokers. Varenicline was shown to be more effective than single form of NRT: patchs (OR=1.51, 95%CrI=1.22-1.87) or gums (OR=1.72, 95%CrI=1.38-2.13) or inhalers, spray, tablets and lozenges (OR=1.42, 95%CrI=1.12-1.79), but was not better than a combination of NRT (OR=1.06, 95%CrI=0.75-1.48). Varenicline was superior to bupropion (OR=1.59, 95%CrI=1.29-1.96).[21]

Pharmacological treatments' effectiveness for smoking cessation was proven. However, the effectiveness may be different between smokers depending on sex or on the nicotine metabolism activity of the patient.[22, 23] Giving the most effective treatment to each smoker is a key contribution to maximizing the quit rate and consequently to decreasing the cardiovascular disturbances induced by smoking.

I.4 Variability in drug response: multiple sources to identify

Smoking cessation treatments increase chances of quitting. However smoking cessation rate at the end-of-treatment rarely exceeds 30%, suggesting a high variability in therapeutic response.[23, 24] Also, a high interindividual variability in antipsychotic-drug-induced weight gain is observed, explained partly by gender, low baseline body mass index, young age, nonwhite ethnicities, first-episode psychotic or genetic factors.

The variability in drug response, either efficacy or toxicity, could also be related to the variability in drug exposure meaning that, when several patients receive the same dose, the plasma drug concentrations may be different. Therapeutic drug monitoring (TDM) uses plasma or serum drug concentrations to individualize the dose of the prescribed drug to maximize the probability of therapeutic response while minimizing the risk of toxicity.[25] This assumes a relationship between plasma concentrations and clinical effects, being therapeutic and/or adverse, and a therapeutic

window where effectiveness and safety are maximized. For antipsychotics, the dose-concentration-effect relationship is highly variable within and between subjects. There are multiple reasons: genetic factors of drug-metabolizing enzymes, smoking status, food intake, drug-drug interactions and often a lack of treatment adherence.[26] Consequently, prediction of concentration-time profiles at a given dose is often difficult.

According to the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) consensus guidelines for TDM in psychiatry, it is mandatory to perform dosage adjustment based on concentration measurement for some drugs for safety reasons (ex: lithium which has a narrow therapeutic window), and for drugs with a high level of recommendation to use TDM (ex: amisulpride, haloperidol, olanzapine which overdosing may lead to extrapyramidal symptoms) after initial prescription or after dose change. TDM is recommended also in specific situations: suspected non-adherence, lack or reduction of clinical improvement, presence of adverse effects, genetic particularity concerning drug metabolism, modification of dose or galenic form and in vulnerable population such as patients with comorbidities, elderly individuals, children, adolescents and pregnant women.[27] Interestingly, TDM is now recommended for patients with abnormally high or low body weight, which was not the case eight years ago in the previous consensus guideline of the AGNP.[25] Because TDM assumes a relationship between dose, plasma concentrations and clinical effects, population pharmacokinetic and pharmacodynamic (PK/PD) modeling is used as a tool to describe dose-exposure relationship (PK) as well as exposure-response relationship (PD) and quantify the degree of associated variability. The ultimate aim of PK and PD modeling is to identify potential contributing factors to these variabilities in order to maximize efficacy while minimizing toxicity. This may lead to higher treatment adherence as illustrated below:

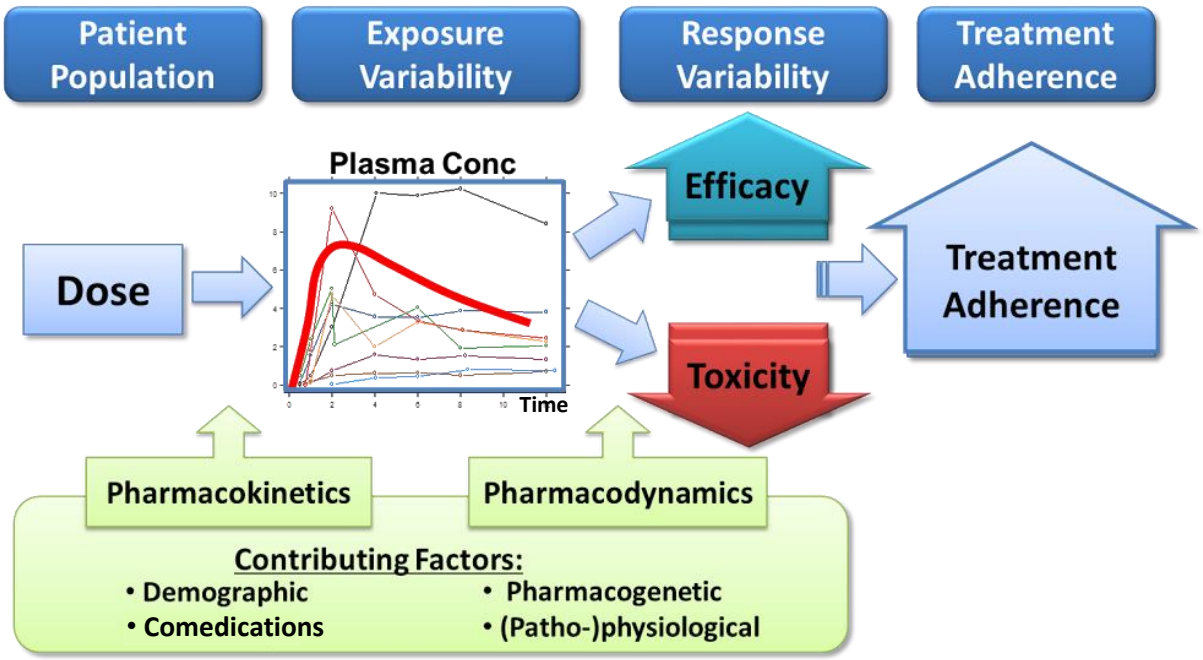

Figure 1.2: Representation of the concept and aims of population pharmacokinetic and pharmacodynamic modeling. Adapted from Woo.[28]

Contributing factors to these variabilities are multifactorial: demographics (e.g. age, body weight), pharmacogenetics (e.g. carrier of a loss-of-function allele of a gene coding for a drug-metabolizing enzyme), patho-physiological alterations (e.g. decrease of renal function as shown by increased creatinine level), and/or comedications (e.g. drugs inhibiting or inducing drug metabolism).

Pharmacogenetics is the study of how genetic variations affect the pharmacokinetics, including their metabolism and transport and the pharmacodynamics of drugs, ultimately affecting their safety and efficacy.[29] Drug-metabolizing enzyme such as cytochromes P450 (CYP), UDP glucuronosyltransferase (UGT), drug transporters like the permeability-glycoprotein transporter (P-gp) and drug receptors exhibit genetic variabilities.[27] The clinical importance of pharmacogenetics is thus well recognized.[30] By definition a genetic polymorphism is a mutation in a gene occurring in at least 1% of the population, mutation which may be responsible for a decrease, complete loss or increased activity of the encoded

protein. Thus, concerning drug-metabolizing enzymes such as CYP or UGT, depending on the number of inactive or active allele an individual can be poor metabolizer (no activity), slow metabolizer (low activity), intermediate, extensive or ultra-rapid metabolizer (highest activity of the CYP). In the case of an active drug with inactive metabolites, unexpected adverse events may appear in poor metabolizers due to increased plasma concentrations while absence of therapeutic response can occur in ultra-rapid metabolizers due to sub-therapeutic plasma concentrations.

Pharmacokinetic modeling provides a mathematical and statistical description of the time course of drug concentrations in the body, as well as its variability. It enables the quantification of the absorption, distribution, metabolism and excretion (ADME) of the drug at any time. Population PK is the study of PK at the population level, in which data from all individuals in a population are evaluated simultaneously. During a population PK analysis, a base model (structural and statistical models) is initially built, and then covariates are tested to explain the observed variability and identify the model describing the data at best (final model). These different steps are briefly discussed in the following paragraphs.

The structural PK model describes the time course of the drug concentration through a system of compartments that represent different abstract region of the body where the drug is homogeneously distributed. These compartments do not represent any particular physiologic part of the body. In the simplest case, *i.e.* intravenous drug administration and one-compartment model with linear elimination, the structural model is expressed by the following equation:[31]

$$C_{pred} = \frac{Dose}{V_{pop}} x\ e^{\frac{-CL_{pop}}{V_{pop}} x\ Time}$$

where C_{pred} is the concentration predicted by the structural model, V_{pop} and CL_{pop} are population parameters representing the volume of distribution and the clearance of the drug, respectively, for the typical individual of the analysis population. Population parameters are also called fixed effects.

One possible expression of the statistical model estimating the interindividual variability of the PK parameter is the following:

$$V_i = V_{pop} + \eta_{1i}$$

$$CL_i = CL_{pop} + \eta_{2i}$$

where V_{pop} and CL_{pop} are the population parameters as previously defined, V_i and CL_i are volume of distribution and clearance, respectively, for the i^{th} individual ; η_{1i} and η_{2i} are the associated interindividual variabilities, two random normally distributed variables with a variance of ω^2_1 and ω^2_2 estimated by the model and a mean of 0.

The covariate model identifies demographic, clinical or genetic factors that affect the concentration of the drug, estimating their effect on the population parameter (V_{pop} or CL_{pop}) while explaining part of the interindividual variability associated to the parameter.

A residual variability also called intraindividual variability quantifies the error between the model predicted and the observed concentrations as follows:

$$C_{obs_{ij}} = C_{ipred_{ij}} + \varepsilon_{ij}$$

where C_{obsij} is the observed concentration, $C_{ipredij}$ is the predicted concentration for the *i*th individual at time *j* by the model and ε_{ij} is the residual variability which has a different value for each observation at time *j* for an individual *i*. The residual variability is normally distributed with a variance σ^2 estimated by the model and a mean of 0. The interindividual variability and the residual variability are also called random effects. The simultaneous estimation of fixed and random effects is possible with nonlinear mixed-effects modeling methods.[32]

The drug concentration can be linked to the drug effect with a pharmacokinetic-pharmacodynamic (PKPD) model which includes a measure of the effect of the drug in the body.[31] Direct effect models, assume rapid equilibrium between the drug circulating in the plasma and that in the site of effect, so that measured concentrations and effect change simultaneously over the time. By contrast, in indirect effect models, the PD response lags behind the drug plasma concentration, because of a delayed equilibrium between the circulating concentration and that in the site of action. Exposure-response model are a class of PKPD model wherein the independent variable is not time but a measure of drug exposure like dose, area under the curve (AUC) or peak plasma concentration (C_{max}).

Once developed, a population PK model allows the adjustment of the first dose to be made *a priori* based on any number of covariates included in the model (demographic, clinical or genetic).[33] The availability of a population model enables also *a posteriori* dose adjustment once a concentration of the drug is measured by estimating the individual PK parameters of the patient using the Bayesian approach. The so built patient-specific PK model allows predicting the expected drug concentration at any time after any given dosage regimen or to calculate the dose needed to achieve a desired target drug concentration.[34] Such prediction takes into account patient specific covariates and concentration measurement error. The first software integrating this process of dosage adjustment process was launched in 1973.[35] The interest for these tools has grown over the last decade, therefore improving the TDM-guided dosage optimization.

I.5 Hypothesis and aims of the thesis work

In the psychiatric population, particularly exposed to cardiometabolic risk factors, the optimization of antipsychotic treatment to choose the right dose and to reduce metabolic side effects, as well as to increase the chance of quitting smoking by giving the right treatment at the right dose to each smoker is critical.

To that extent, the objectives of the present thesis were:

1) To determine factors influencing pharmacokinetic of amisulpride and drug-related adverse events (chapter II), by:
    - Detecting clinical and genetic sources of variability in the pharmacokinetic profile in order to suggest optimal dosing to reach the reference range of trough concentrations in each patient.
    - Studying the relationship between amisulpride exposure and two amisulpride-induced adverse events: hyperprolactinemia and body weight gain.
2) To determine factors influencing pharmacokinetics of quetiapine and body weight gain under quetiapine treatment (chapter III), by:

- Detecting clinical and genetic sources of variability on the drug and its main metabolite pharmacokinetic profile.
- Studying the relationship between quetiapine exposure and body weight gain

3) To assess the influence of clinical and genetic factors on (chapter IV):
    - The risk of smoking relapse when receiving a smoking cessation treatment: nicotine replacement therapy or varenicline.
    - The variability in varenicline pharmacokinetic profile.
    - The relationship between varenicline exposure and smoking abstinence.

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CHAPTER II. AMISULPRIDE, HYPERPROLACTINEMIA AND BODY WEIGHT GAIN

Chapter II in the thesis context

Common adverse effects of amisulpride, in particular at doses > 300 mg/day, are hyperprolactinemia, agitation, insomnia, anxiety, extrapyramidal symptoms, prolonged QTc values, bradycardia and weight gain.[1] In the following study, we chose to work on hyperprolactinemia, the most prevalent adverse event of amisulpride and on body weight gain because of the concern of cardiometabolic risk factors during treatment with antipsychotic drugs.

Hyperprolactinemia decreases gonadotropins secretion, which rapidly induces gonadal dysfunctions in males and females.[2, 3] Moreover, hyperprolactinemia over a long period could cause breast cancer, particularly in postmenopausal women, and loss of bone mineral density, which can lead to osteoporosis and an increased risk of falls in elderly patients.[2, 4] In addition, metabolic disturbances such as insulin resistance, weight gain and cardio-vascular diseases could be induced via a lack of oestrogen during hyperprolactinemia.[2, 5, 6]

A retrospective analysis in 1392 patients showed a statistically significant but low weight gain after 10 weeks of treatment by amisulpride compared to the baseline weight.[7] A multi-center randomized trial was conducted with 380 patients taking olanzapine, which has the highest potential for inducing weight gain among second-generation antipsychotics, or amisulpride at effective doses during 6 months. It showed that the mean weight gain was 3.9± 5.3 kg in the olanzapine group *vs.* 1.6 ±4.9 kg in the amisulpride group. The difference in the amplitude of weight gain between the two treatment groups was significant ($p<0.001$).[8]

Although amisulpride displays a low potential for inducing weight, an evaluation of body weight and metabolic parameters (waist circumference, blood pressure, fasting plasma glucose level and lipid levels) is recommended before, and periodically during the treatment with this drug as for others atypical antipsychotics.[9, 10]

In this context, the department of psychiatry of the Lausanne University Hospital has established a guideline to monitor clinical and cardiometabolic parameters before, and periodically during the first year of psychotropic drugs treatment, and then once per year.[11] If patients accepted a written

informed consent, supplemental analyses of blood samples can be performed and collected data can be used for research such as in the following chapter.

Own contribution

Writing of the research protocol and submission to the ethics committee. Data management after extraction. Population modeling, analysis and interpretation of the data. Writing of the manuscript and submission to the journal.

II.1 Amisulpride: Real-World Evidence of Dose Adaptation and Effect on Prolactin Concentrations and Body Weight Gain by Pharmacokinetic/Pharmacodynamic Analyses

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II.1.1. ABSTRACT

Amisulpride is an antipsychotic used at a wide range of doses. Hyperprolactinemia is the major adverse event and amisulpride might induce body weight gain. The objectives of this work were to characterize the pharmacokinetics of amisulpride in order to suggest optimal dosage regimens to achieve the reference range of trough concentrations at steady-state ($Cmin_{SS}$) and to describe the relationship between drug pharmacokinetics and prolactin and body weight data. The influence of clinical and genetic characteristics on amisulpride pharmacokinetics was quantified (NONMEM®). The final model was used to simulate $Cmin_{SS}$ under several dosage regimens and was combined with a direct E_{max} model to describe the prolactin data. The effect of model-based average amisulpride concentrations over 24h (C_{av}) on weight was estimated using a linear model. A one-compartment model with first order absorption and elimination best fitted the 513 concentrations provided by 242 patients. Amisulpride clearance significantly decreased with age and increased with lean body weight (LBW). $Cmin_{SS}$ were higher than the reference range in 65% of the 60-year individuals receiving 400 mg b.i.d. and in 82% of the >75-year individuals with a LBW of 30 kg receiving 200 mg b.i.d. The pharmacokinetic/pharmacodynamic model included 101 prolactin measurements from 68 patients. E_{max} parameter was 53% lower in males compared to females. Model-predicted prolactin levels are above the normal values for $Cmin_{SS}$ within the reference range. Weight gain did not depend on C_{av}. Amisulpride treatment might be optimized with age and LBW. Hyperprolactinemia and weight gain do not depend on amisulpride concentrations.

II.1.2. INTRODUCTION

Second-generation antipsychotics are the cornerstone of pharmacological treatment for schizophrenia. Among them, amisulpride has been shown to be an effective treatment with a moderate and low propensity for extrapyramidal symptoms and weight gain, respectively.[12-14] In adults and older individuals, a concentration-therapeutic response relationship has been demonstrated leading to the determination of a reference range of amisulpride trough concentrations of 100-320 ng/mL.[13] Although some patients may need amisulpride plasma concentrations above the recommended therapeutic reference range, high plasma levels are associated with increased risks of extra-pyramidal symptoms. Because of all abovementioned reasons, therapeutic drug monitoring of amisulpride is strongly recommended.[15]

Amisulpride has a high propensity to raise prolactin blood levels.[16] Indeed, by D_2 receptor antagonism, amisulpride blocks the dopamine transmission on the lactotroph cells in the anterior pituitary gland which lies outside the blood-brain barrier.[2] Due to the low capacity of the drug to penetrate the blood-brain barrier, amisulpride has a low ratio of central/peripheral concentration, yielding to high dopamine blockade in the pituitary gland and decreases the inhibitory effect of dopamine on prolactin secretion.[4, 16, 17] More than 90% of patients treated by amisulpride have hyperprolactinemia[3, 18], which remains asymptomatic in some cases but may also lead to fast onset of adverse events on gonadal function such as amenorrhea, galactorrhea, infertility, loss of libido, erectile dysfunction and ejaculation deficiency.[2] These clinical manifestations can hamper the adherence of patients to the treatment. To date, a population pharmacokinetic model has been described and a pharmacokinetic/pharmacodynamic (PK/PD) analysis combining this model with prolactin data has been published in elderly patients with Alzheimer's disease.[19, 20] Such information is not yet available in the young and old non-Alzheimer psychiatric population.

While body weight gain is the major adverse effect of second-generation antipsychotics, amisulpride has a low propensity to raise body weight.[7, 14] However, the description of the relationship between

amisulpride concentration and body weight gain is useful as body weight gain can still be observed during treatment with amisulpride.

The objectives of this work were first to characterize the PK profile of amisulpride in an adult and elderly psychiatric population in a real-world clinical setting, and to detect clinical and genetic sources of variability in order to suggest optimal dosing to reach trough concentration reference range in each patient. Secondly, we aimed to describe the relationship between amisulpride concentrations and prolactin levels and body weight data.

II.1.3. MATERIALS AND METHODS

Study population and design

The present study included 242 patients from the Department of Psychiatry of the Lausanne University Hospital who had at least one amisulpride plasma concentration measurement performed between 2007 and 2016. The main diagnosis was schizophrenia and schizotypal disorders **(Table 2.1.1)**. Antipsychotic plasma concentration measurements are requested for therapeutic drug monitoring (TDM) by the treating psychiatrists or by the departmental guideline for metabolic follow-up of patients starting a psychotropic treatment on a routine basis (PsyClin).[11] In the latter case, written informed consent from an ongoing longitudinal clinical and pharmacogenetic study (PsyMetab), as previously described, was obtained from patients.[21] PsyMetab study and retrospective analysis of PsyClin data were both approved by the Ethics Committee of Vaud (CER-VD). Exclusion criteria were undetectable amisulpride plasma concentrations suggestive of non-adherence to treatment, and non-reliable time information about blood sampling or last dose intake. When the blood sampling was not under steady-state conditions (at least 5 days with the same dose), the dosing information history (dose, date and time of administration) were retrieved and added in the dataset.

Table 2.1.1. Characteristics and genetic polymorphism of the study population

Characteristics	Value	% missing data	
Clinical characteristics			
Sex (male), n (%)	132 (55)	0	
Age (years), median (range)	37 (18-91)	0	
Mains diagnosis[1], n (%)		27	
Schizophrenia and schizotypal disorders (F20-F29)	142 (59)	-	
Mood (affective) disorders (F30-F39)	24 (10)	-	
Others (F00-F09, F10-F19, F40-F48, F60-F69, F70-F79)	10 (4)	-	
Body weight (kg), median (range)	75 (43-185)	5.6	
Lean body weight (kg)[2], median (range)	52 (29-98)	14.2	
Body mass index (kg/m^2), median (range)	25 (15-59)	14.2	
Serum creatinine concentration (µmol/L), median (range)	76 (44-167)	36	
CLCRCG[3] (mL/min), median (range)	93 (20-180)	38.4	
Concomitant medications			
P-gp inhibitors[4], n (%)	56 (11)	6	
Lithium, n (%)	42 (8)	45	
Genetic polymorphisms[5]	**Genotype**	**Value**	**Frequencies**
SLC22A1			
rs683369	CC/CG/GG	58/21/6	68/25/7
rs628031	AA/AG/GG	10/32/42	12/38/50
SLC22A2			
rs316003	TT/CT/CC	45/32/6	54/39/7
rs316019	AA/AC/CC	1/19/63	1/23/76
ABCB1			
rs2235048	AA/AG/GG	24/42/19	28/50/22
rs4148738	CC/CT/TT	13/41/31	15/48/37
NR1|2			
rs1523130	CC/CT/TT	38/31/16	45/36/19
rs7643645	AA/AG/GG	32/43/10	38/50/12
rs2461817	AA/AC/CC	16/46/23	19/54/27
NR1|3			
rs2307424	AA/AG/GG	9/42/34	11/49/40
rs4073054	AA/AC/CC	48/28/9	56/33/11

rs2502815	AA/AG/GG	9/36/40	11/42/47
RXRA			
rs3132297	AA/AG/GG	3/19/63	4/22/74
PPARG			
rs3856806	TT/CT/CC	3/18/64	4/21/75
rs2197423	GG/AG	24/61	28/72
rs2920502	GG/GC/CC	44/35/6	52/41/7
PPARGC1A			
rs8192678	TT/TC/CC	7/36/42	8/42/50

1. According to International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) Version for 2010.
2. Lean body weight estimated as described elsewhere.[22]
3. CLCRCG, creatinine clearance was estimated by the Cockcroft-Gault formula if BMI< 25 kg/m^2 and estimated by the Cockcroft-Gault formula integrating lean body weight if BMI≥ 25 kg/m^2.[17]
4. Inhibitors of P-glycoprotein were ritonavir, darunavir, ketoconazole, simvastatin, candesartan, hydrochlorothiazide, omeprazole, esomeprazole, cetirizine, levocetirizine[14]
5. Genetic data were available for n=85 individuals except for rs628031, n=84; rs316003, n=83; rs316019, n=83

P-gp: permeability-glycoprotein

In addition to the accurate time of last drug intake and blood sampling, the following data were recorded at the same time as the blood samples were drawn for PK measurements: sex, age, body weight (BW), height, serum creatinine concentration and concomitant medications with permeability-glycoprotein inhibitors (iPGP)[23] and lithium that might have influenced amisulpride therapy **(Table 2.1.1)**. Lean body weight (LBW) and body mass index (BMI) were calculated using the Janmahasatian[22] and classic formulas, respectively. Creatinine clearance was estimated by the Cockcroft-Gault formula (CLCRCG)[24] using BW if BMI< 25 kg/m^2 and LBW if BMI≥ 25 kg/m^2.[25] For the concentration-prolactin relationship analysis, the following data were also reported: menopause if women were older than 55 years, and concomitant antipsychotics that might have increased prolactin levels such as zuclopenthixol, risperidone, paliperidone, haloperidol and levomepromazine.

Amisulpride and prolactin concentration measurements

All blood samples were collected in EDTA-containing tubes. After centrifugation, plasma samples were stored at -20 °C until routine analysis. Quantification of amisulpride in plasma was performed by high performance liquid chromatography coupled to mass spectrometry until 2012 and then by ultra-high performance liquid chromatography coupled to tandem mass spectrometry.[26] The lower limit of quantification was 1 ng/ml for both methods. Prolactin concentrations were determined by immunoassay on an Abbott Axsym system (Abbott GmbH, Wiesbaden, Germany) before 2014 and by electrochemiluminescence on a Cobas (Roche Diagnostics GmbH, Mannhein, Germany) from 2014. The prolactin concentration equivalence was obtained using the following equation based on internal validation processes: y = 1.05x – 0.56 (y = Abbott AxSym, x = Cobas Roche ; 0 to 268 ng/ml).

Genotyping

Genomic DNA was extracted from EDTA blood sample at the baseline visit using the FlexiGene DNA extraction kit (Qiagen Instruments AG, Hombrechtikon, Switzerland) according to the manufacturer's protocol. The SNPs analysed in this work were obtained using the Cardio-MetaboChip; a custom Illumina iSelect genotyping array designed to test DNA variation of over 200,000 SNPs from regions identified by large scale meta-analyses of genomewide association studies for metabolic and cardiovascular traits: 3642 customized SNPs covering pharmacokinetic genes were added in the Cardio-MetaboChip.[27, 28] The following SNPs of genes coding for the OCT1 and OCT2 transporters, the P-glycoprotein and some nuclear factors were selected based on previously published pharmacogenetic studies and on their minor allele frequency (MAF) in the Caucasian population:[29-41] *SLC22A1* (rs683369), *SLC22A1* (rs628031), *SLC22A2* (rs316003), *SLC22A2* (rs316019), *ABCB1* (rs2235048), *ABCB1* (rs4148738), *NR1|2* (rs1523130), *NR1|2* (rs7643645), *NR1|2* (rs2461817), *NR1|3* (rs2307424), *NR1|3* (rs4073054), *NR1|3* (rs2502815), *RXRA* (rs3132297), *PPARG* (rs3856806), *PPARG*

(rs2197423), *PPARG* (rs2920502), *PPARGC1A* (rs8192678). Quality control excluded samples from the analysis if sex was inconsistent with genetic data from X-linked markers, genotype call rate less than 0.96 or Gene Call score less than 0.15. All these SNPs were in Hardy Weinberg Equilibrium ($p>0.05$). GenomeStudio Data Analysis Software was used to export results generated by Illumina Cardio-MetaboChip.

PK/PD analysis

Non-linear mixed effect modelling was performed using NONMEM® version 7.4.1 program[42] with the PsN-Toolkit (version 4.2.0).[43] Statistical analyses and figures were performed using R (v. 3.3.3, http://www.r-project.org).

PK base model

Log-transformed concentrations were used in the model. A stepwise procedure was used to identify the PK models that best fitted the data. Multi-compartment models with first or zero-order absorption and linear elimination were compared to determine the appropriate structural model, which was finally identified as a one-compartment model with first order absorption. Owing to very limited measurements at early time points after drug intake, the first-order absorption rate (k_a) could not be adequately estimated. Thus, k_a values ranging from 0.4 to 1.8 h^{-1} were tested in the model to achieve a T_{max} between 3-4 h. The k_a value was eventually fixed to 0.9 h^{-1}, value comparable to previously published estimates.[19, 20] Since amisulpride was administered orally, clearance (CL) and volume of distribution (V) were estimated with F, the absolute oral bioavailability, fixed to 0.48.[44] Exponential errors following a log-normal distribution were assumed for the description of inter-individual variability of the parameters. An additive error model on the log scale was used to describe the residual variability.

PK-Prolactin model

The final PK model was combined with a direct E_{max} model[20] to describe the prolactin data as follows:

$$PRL = PRL_{base} + (E_{max} \times CP)/(EC_{50} + CP) \quad (1)$$

where PRL is the prolactin level; PRL_{base}, the prolactin level measured at baseline; CP, the amisulpride concentration predicted by the PK model; EC_{50}, the amisulpride concentration at which the prolactin level reaches 50% of the maximal achievable value (E_{max}).

As prolactin levels are markedly different in men and women[16], the sex effect on Emax was included from the beginning of the structural model development as follows:

$$E_{max} = a \times (1 - b \times MALE) \quad (2)$$

where MALE=1 if male patients and 0 if female patients.

Exponential errors following a log-normal distribution were assumed for the description of inter-individual variability of the three parameters (PRL_{base}, E_{max} and EC_{50}). Individual final PK/PD parameter estimates were used to calculate prolactin and amisulpride concentrations at time of amisulpride trough concentrations, *i.e* 12h and 24h after last dose intake for two or one administration per day, respectively. This allowed comparing the prolactin levels variation across the recommended reference range of amisulpride trough concentrations.[15]

Exposure-Body Weight model

Average amisulpride concentration over 24 hours (C_{av}) were derived from the final PK model using:

$$C_{av} = AUC_{0\text{-}24}/24 \quad (3)$$

where $AUC_{0\text{-}24}$ were computed by integration in NONMEM® based on the individual dose history and PK parameters. Linear mixed-effects models were fitted on the longitudinal body weight values to estimate the effect of time under treatment and amisulpride C_{av} using the nlme package in R.

Covariate analysis

Empirical Bayesian estimates (EBEs) of the PK parameters were derived and plotted against the available subject characteristics (age, sex, BW, LBW, height, BMI, CLCRCG, iPGP and several genetic polymorphisms **(Table 2.1.1)**). Due to substantial eta-shrinkage on V (68%) in the base model, the graphic exploration was interpreted cautiously between EBEs of V and covariates.[45] For the PK-prolactin model, EBEs of E_{max} parameter were derived and plotted against relevant factors (age, menopause, BW, LBW, season of blood sampling and concomitant antipsychotics likely increasing prolactin level. Potentially influencing covariates were then incorporated sequentially in the model and tested for significance on the parameters. The covariate analysis was performed using a stepwise insertion/deletion approach testing linear or non-linear functions as appropriate (categorical covariates coded as 0 and 1, continuous covariates centered on their median value). Missing values for BW, LBW, BMI and CLCRCG were imputed to the population median value. Parameter values were estimated for each genotypic group (rich model), defined as the reference allele group (Ref) and alternative allele groups: heterozygote alternative (Het-Alt) and homozygous alternative (Hom-Alt), or for further regrouped (reduced model) subpopulations.

Parameter estimation and model selection

The log likelihood ratio test, based on changes in the objective function value (ΔOFV), was employed to discriminate between hierarchical models. Since a ΔOFV between any two models approximates a χ^2 distribution, a 3.8- (p=0.05) point change of OFV was considered statistically significant for one additional parameter in model building. To account for multiple testing in the covariate model the p-value was corrected by the number of tests. A change in OFV > 6.6 (p= 0.05/4= 0.0125) was considered statistically significant for one additional parameter during backward deletion steps in the PK model. The Akaike's information criterion (AIC) was instead employed to choose between non-hierarchical models. Diagnostic goodness-of-fit plots, precision and plausibility of the model parameters were also

used to assess the reliability of the results. All models were fitted using the first-order conditional estimation method with interaction (FOCEI) with the subroutines ADVAN2 TRANS2 for the pharmacokinetic model and the subroutine ADVAN6 for the PK-prolactin model.

Model evaluation

The final PK and PK-prolactin models stability was assessed by non-parametric bootstrap method implemented in PsN, generating 2000 datasets by re-sampling from the original dataset. Median parameters values with their 95%CI were thus derived and compared with the final model estimates. Visual predictive checks (VPCs) of final PK and PK-prolactin models were performed with PsN toolkit by simulations (n=1000).

Simulations of dosage regimens

Trough concentrations were simulated in 500 individuals per strata of age and LBW values with the final PK model including inter- and intra-individual variability after administration of several doses recommended in guidelines: 300 mg q.d, 200 mg b.i.d and 400 mg b.i.d in adults (4 combinations of age (20 and 60 years) and LBW (40 and 85 kg)); 50 mg q.d, 200 mg q.d. and 200 mg b.i.d.[46] in elderly individuals (4 combinations of age (75 and 85 years) and LBW (30 and 60 kg)). The distribution of the through concentrations ($Cmin_{SS}$) per strata were plotted with the recommended reference range of trough concentrations (100-320 ng/mL).[13, 15]

II.1.4. RESULTS

Study population and data

The 242 patients provided a total of 513 amisulpride concentrations for the PK analysis. Subject's characteristics are presented in **Table 2.1.1**. Blood samples for pharmacokinetic measurements were collected at a median time of 13.3 h (range, 0.05 h-58 h) after last dose intake. A median of two samples (range, 1–12) of amisulpride was collected per patient. Amisulpride plasma concentrations ranged from 5 to 1514 ng/mL across a range of daily doses from 50 to 2000 mg (median = 600 mg). For the PK-prolactin model, a total of 101 prolactin plasma concentrations from 68 patients were available (median = 73 ng/mL, range = 4 - 311 ng/mL) and plotted against the observed amisulpride concentrations in **Figure 2.1.1**. Nineteen prolactin concentrations were available before the beginning of amisulpride treatment (from - 40 days to the starting treatment day). Among the 82 prolactin measurements under treatment, only 3 were below the threshold for hyperprolactinemia set at 20 and 25 ng/mL for males and females, respectively.[47] For the exposure-body weight analysis, 284 body weight values (median = 73 kg, range = 45-114 kg) from 113 patients were available for analysis. Data until 101 days of treatment were included in analysis. Ninety patients had a baseline body weight value measured from 15 days before treatment beginning.

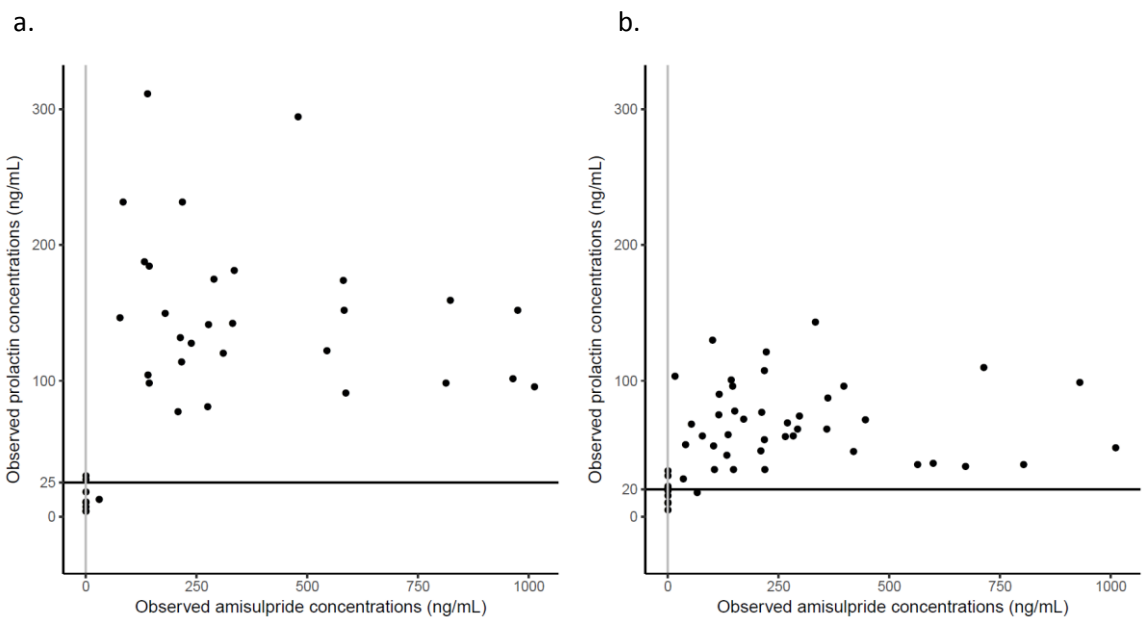


Figure 2.1.1: Observed prolactin concentrations versus amisulpride concentrations in females (a.) and males (b.). Vertical grey line represents the beginning of amisulpride treatment. Horizontal line represents threshold for hyperprolactinemia defined over 25 ng/mL in females and 20 ng/mL in males.[47]

PK-PD analysis

PK analysis

A one-compartment model with first-order absorption and elimination described adequately the data. No improvement to the fit was observed using a two-compartment model (ΔOFV = -2.8). As previously discussed, k_a was fixed to 0.9 h^{-1}. The estimates and the variability (CV%) of the base PK model were a CL of 39.6 L/h (47%) and a V of 954 L (53%).

Univariate analyses showed that the effect of age (ΔOFV = -71.7, $p < 0.001$), CLCRCG (ΔOFV = -50.3, $p < 0.001$), BW (ΔOFV = -19.1, $p < 0.001$), LBW (ΔOFV = -34.0, $p < 0.001$) and sex (ΔOFV = -21.6, $p < 0.001$) on CL improved significantly the description of the data. According to AIC, the LBW effect was chosen upon BW effect (AIC = 133.2 and AIC = 148.0, respectively) on CL. In contrast, BMI, iPGP and lithium

were not associated with CL (ΔOFV = -2.2 for 1 additional parameter, ΔOFV ≥ -5.4 for 2 additional parameters, $p > 0.07$), neither the genetic covariates (ΔOFV = -1.6 for 2 additional parameters, ΔOFV ≥ -5.7 for 3 additional parameters, $p > 0.4$). No covariates tested showed any significant influence on V (ΔOFV = -3.36, $p = 0.07$).

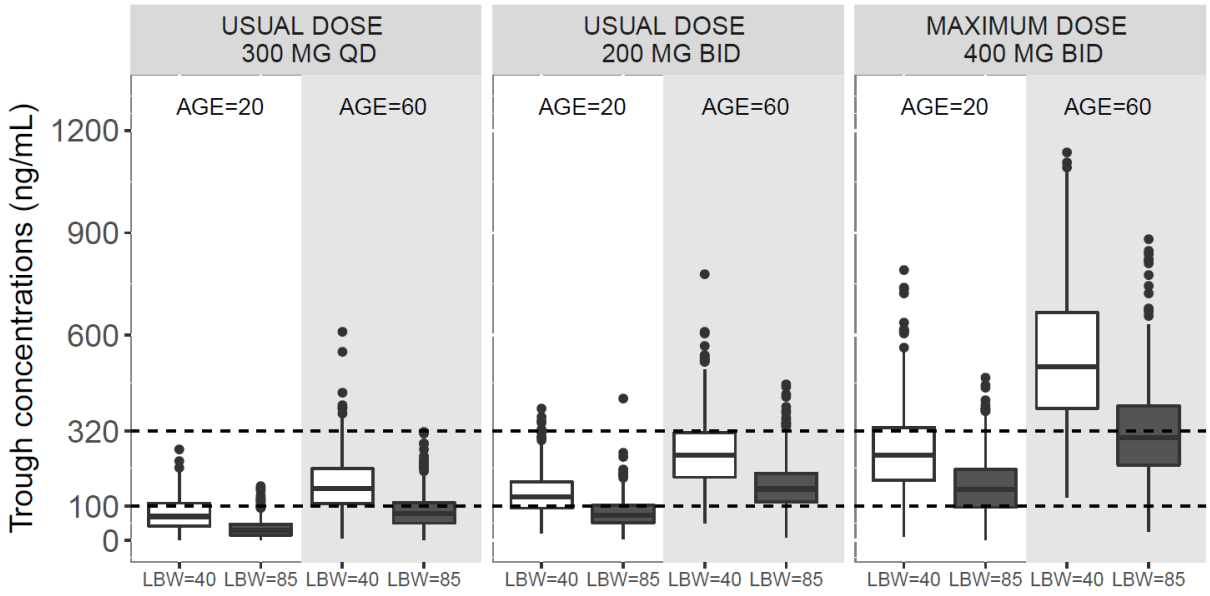
In multivariate analyses, age, LBW and CLCRCG remained as significant covariates in the forward insertion step (ΔOFV ≤ -5.8, $p \leq 0.01$). CLCRCG did not remain statistically significant after the backward deletion (ΔOFV = 2.7, $p = 0.1$). Our final results suggest that in an individual of 40 years and a LBW of 50 kg CL is decreased by 1.5 compared to the same individual with a LBW of 100 kg and increased by 0.5 compared to an 80-year individual. Age and LBW explained 42% and 22% of the variability in amisulpride clearance, respectively.

Structural, final PK model and bootstrap results are summarized in **Supplementary Table S2.1.1.** The model was considered reliable since the parameter estimates differed less than 10% from the bootstrap medians. The prediction-corrected VPC indicates that the final model described the data adequately **(Supplementary Figure S2.1.1).**

Simulations of dosage regimens

In adults, simulations of doses of 300 mg q.d., 200 mg b.i.d. and 400 mg b.i.d. indicated that 64%, 31% and 9% of $Cmin_{SS}$ were below the therapeutic reference range, 35%, 62% and 50% were in the reference range and 1%, 7% and 41% were over the reference range, respectively. Especially, 65% were over the reference range if age was 60 years or more with the maximum dose **(Figure 2.1.2.a.)**. Concerning elderly individuals, simulations of doses of 50 mg q.d., 200 mg q.d. and 200 mg b.i.d. showed that 97%, 16% and 1% were below the reference range, 3%, 74% and 33% were in the reference range and 0%, 10% and 66% were over the reference range. In particular, 82% were over the reference range if LBW was 30 kg at 200 mg b.i.d. **(Figure 2.1.2.b.)**.

a.



b.

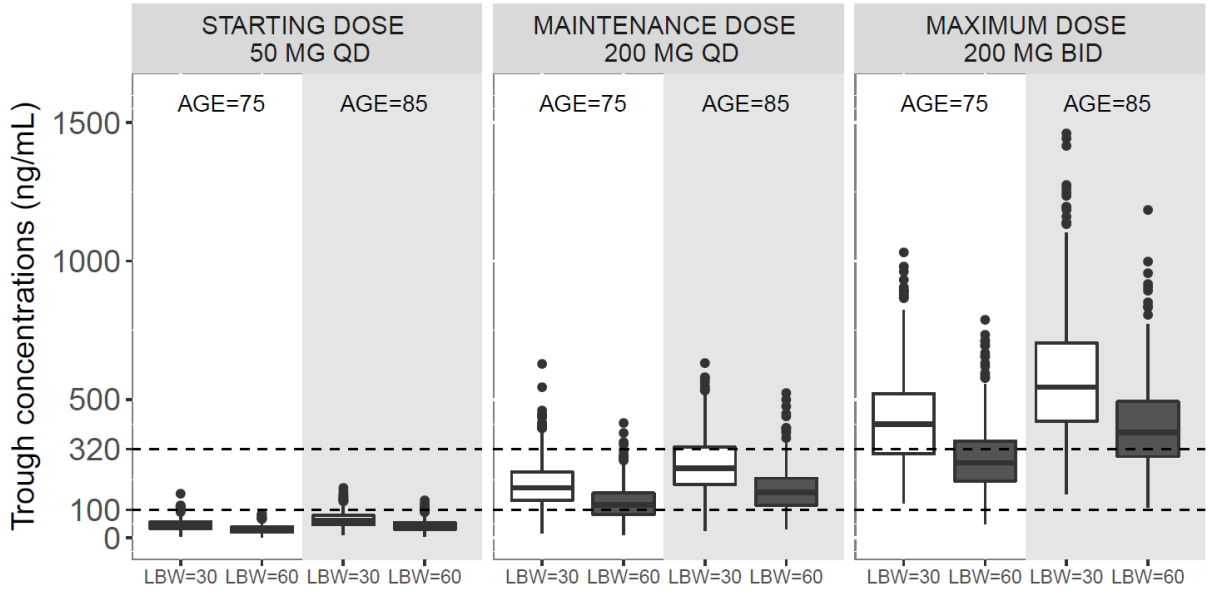


Figure 2.1.2: Distribution of the simulated amisulpride trough concentrations after administration of amisulpride dose at steady state in 500 adult (a.) and elderly (b.) individuals in each of the following strata of age and lean body weight: a. 20 or 60 years old (white and grey background, respectively) and 40 or 85 kg (white and grey fill, respectively) ; b. 75 or 85 years old (white and grey background,

respectively) and 30 or 60 kg (white and grey fill, respectively). Boxes represent 25th, 50th and 75th percentile of trough concentrations; data higher than 75th percentile plus (1.5 x IQR) and lower than 25th percentile minus (1.5 x IQR) are plotted individually; IQR is the interquartile range defined by 75th minus 25th percentile. Dashed lines represent the reference range of trough concentrations.[13, 15]

PK-Prolactin model

Univariate analyses showed that none of the factors had a significant effect on E_{max} parameter ($p>0.06$), besides the already included sex effect. Final PK-prolactin model parameters and the bootstrap results are presented in **Table 2.1.2**. All parameters were estimated with good precision (RSE ≤ 36%). The model was considered reliable since the parameter estimates differed less than 10% from the bootstrap. The prediction-corrected VPC indicates that the final model described the data adequately **(Supplementary Figure S2.2.2)**. E_{max} parameter was estimated to decrease by 53% in males compared to females. Prolactin values predicted by the final PK-Prolactin model at time of amisulpride trough concentrations were plotted against amisulpride trough concentrations in **Figure 2.1.3**. This showed that when amisulpride trough concentrations are in the therapeutic reference range, prolactin levels are over the normal values in males (model-predicted median= 71 ng/mL, range= 41-135 ng/mL), and in pre- (147 ng/mL, 78-309 ng/mL) and postmenopausal (128 ng/mL, 75-186 ng/mL) women.

Table 2.1.2 Parameter estimates of the final PK-prolactin model with bootstrap results.

Parameter	Final population parameters		Bootstrap evaluation (n=2000 samples)	
	Estimate	RSE[a] (%)	Median	$CI_{95\%}$
PK				
CL (L/h)	44	4	44	41 ; 48
V (L)	956	11	961	705 ; 1258
k_a (h^{-1})	0.9 fixed	-	-	-
θ_{AGE_CL}	-0.46	10	-0.47	-0.54 ; -0.36
θ_{LBW_CL}	0.54	36	0.54	0.27 ; 0.85
IIV_{CL} (CV%)[b]	33	12	33	24 ; 42
IIV_V (CV%)[b]	65	24	63	35 ; 92
Proportional residual error (%)[c]	53	4	53	46 ; 60
PRL E_{max}				
PRL_{base} (ng/mL)	16	35	16	11 ; 20
E_{max} (ng/mL)	141	13	142	116 ; 182
EC_{50} (ng/mL)	42	34	43	4.6 ; 110
θ_{MALE_EMAX}	-0.53	13	-0.54	-0.65 ; -0.38
IIV_{Emax} (CV%)[b]	50	16	49	36 ; 62
Additive residual error (ng/mL)	15	13	15	10 ; 20

CL, amisulpride clearance; V, amisulpride volume of distribution; k_a, first-order absorption rate; θ_{AGE_CL}, age effect on CL; θ_{LBW_CL}, LBW effect on CL; IIV_{CL}, inter-individual variability of CL; IIV_V, inter-individual variability of V; PRL_{base}, prolactin level measured at baseline; E_{max}, maximal achievable value of prolactin; EC_{50}, amisulpride concentration at which the prolactin level reaches 50% of the E_{max}; θ_{MALE_EMAX}, effect of male on E_{max}; IIV_{Emax}, inter-individual variability of E_{max}.

a. Relative standard errors of the estimates (SE) defined as SE/estimate directly retrieved from NONMEM®.

b. Interindividual variability defined as CVs (%).

c. Proportional residual error as percentage on the linear scale derived from the additive residual error on the log scale

Final model:

CL (L) = 44 x (1 - 0.46 x ((age - median age)/median age)) x (1 + 0.54 x ((lean body weight – median lean body weight)/median lean body weight)

E_{max} (ng/mL) = 141 x (1-0.53*MALE) with MALE=1 if male patients and MALE=0 if female patients.

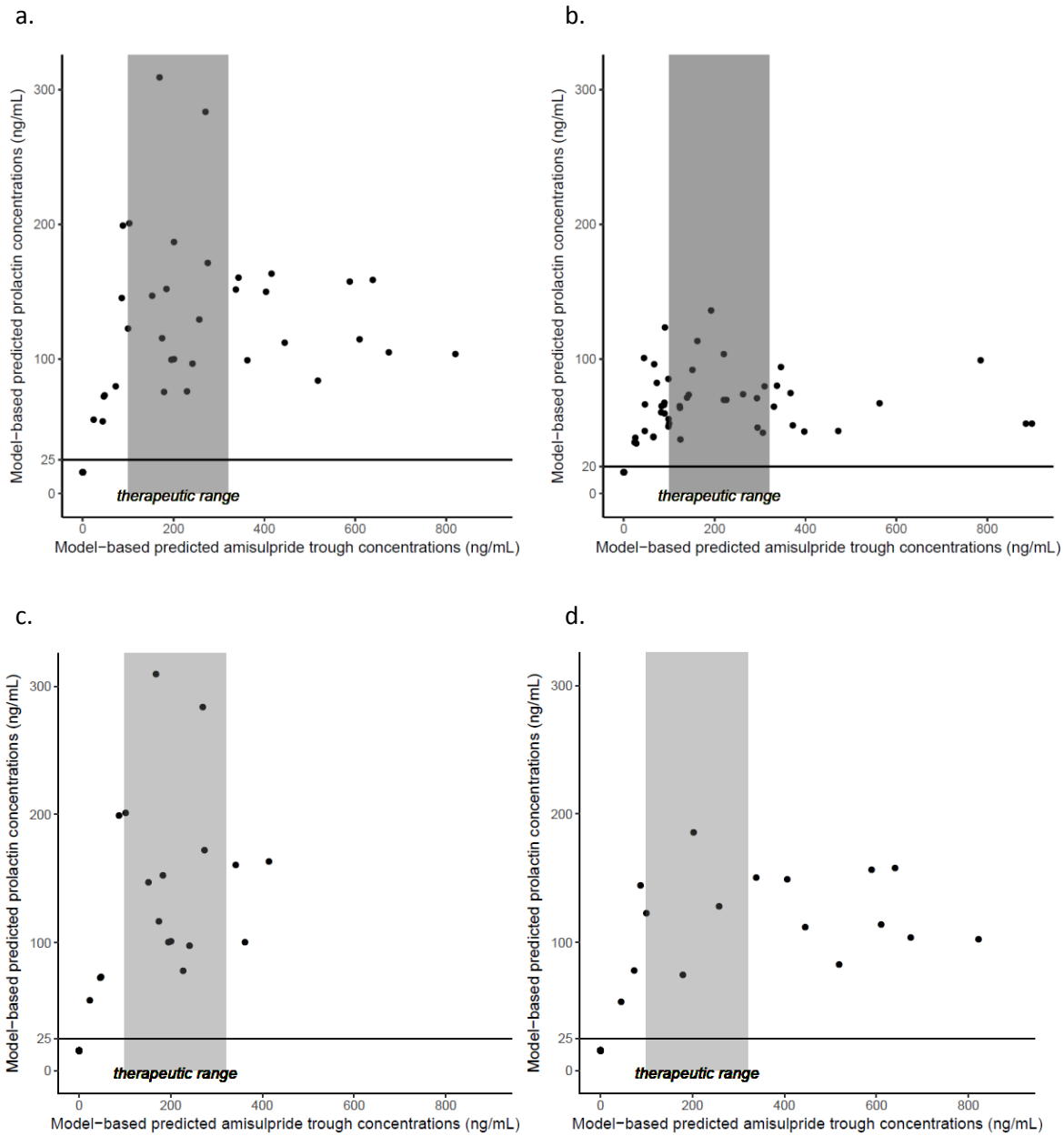


Figure 2.1.3: Model-based predicted prolactin plasma levels at time of amisulpride trough concentrations in females (a.), males (b.), premenopausal females (c.) and postmenopausal females (d.). Prolactin and amisulpride concentrations were predicted with the final PK-Prolactin model. Horizontal line represents threshold for hyperprolactinemia defined over 25 ng/mL in females and 20 ng/mL in males.[47]

Exposure-Body Weight analysis

Univariate models showed that time under treatment and C_{av} had a significant effect on body weight measures. Body weight increased by 0.16 kg for 10 additional days of treatment (p=0.008) and by 0.2 kg for 100 additional ng/mL of amisulpride plasma concentrations (p=0.04). In multivariate analysis, none of these two variables were significant (p ≥ 0.09, **Table 2.1.3**).

Table 2.1.3: Effect estimates of time since beginning of amisulpride treatment and amisulpride average concentration on body weight.

	Estimate	**95%CI**	**p-value**
Univariate model			
Time (days)	0.016	0.004; 0.027	0.008
Univariate model			
C_{av} (ng/mL)	0.002	0.0001; 0.004	0.04
Multivariate model			
Time (days)	0.013	-0.002; 0.028	0.09
C_{av} (ng/mL)	0.001	-0.002; 0.003	0.64

95%CI, 95% of Confidence Interval; C_{av}, amisulpride average concentrations

II.1.5. DISCUSSION

A guide for optimizing amisulpride dose adjustments with regard to therapeutic response and adverse events based on PK/PD analysis has been previously proposed in a particular population of elderly patients with Alzheimer's disease. This population approach was not used so far in adult and older psychiatric patients. The present study provides a description of a PK profile of amisulpride which, combined with prolactin and body weight data, could be used for amisulpride treatment optimization.

The PK analysis showed that amisulpride concentrations were well described by a one-compartment model with parameters consistent with previously reported PK model parameters.[19] Amisulpride clearance was close to the reported value of 54.3 L/h. The volume of distribution estimated was concordant with the steady-state volume of distribution published for a two-compartment model (V_{ss} = 1191 L).[19] Since 95% of the drug concentrations available in our study population were collected less than 24 hours after dose administration, the characterization of the second compartment could not be done. Moreover, determination of the initial amisulpride elimination phase is not always accurately determined in PK modelling due to the complexity of the absorption phase characterized by two plasma concentration peaks.[48] In the present study, the k_a value was fixed to 0.9 h^{-1} in order to obtain a calculated T_{max} of 3.6 h. This T_{max} value corresponds to the second peak of absorption, which lies between 3 and 4 h.[44] The first peak at 1 h can be estimated only in cases of intensive sampling immediately after drug intake. Age and body weight also significantly contributed to amisulpride clearance variability in the previously published PK model while creatinine clearance effect was not present in the final model.[19] Of note, in a study including 85 patients, the dose-corrected plasma concentrations of amisulpride were higher in patients taking lithium.[44] In our work, a 6% decrease (p=0.08) of amisulpride clearance was observed in the presence of lithium possibly due to a competition of both compounds for renal elimination.

Simulations highlighted a high variability in plasma concentrations of patients receiving the same dose. In elderly patients, the maximum recommended dose of 200 mg b.i.d. leads to concentrations over the

reference range especially in the oldest patients and should thus be cautiously used considering the potential occurrence of adverse effects such as hypotension and/or sedation.[15, 49] In this vulnerable population amisulpride should be prescribed using slow titration starting from the 50 mg q.d. In some cases, the therapeutic doses of 100 or 200 mg q.d. could result in plasma levels below the reference range of concentrations. In elderly patients, the threshold of striatal dopamine $D_{2/3}$ receptor occupancy to obtain a therapeutic effect has been shown to be lower compared to younger patients (50-60% and 65-80%, respectively).[50] Thus, lower doses are often sufficient to reach therapeutic effect in elderly patients. In adults, the maximum dose of 800 mg/d recommended in Switzerland (up to 1200 mg/d in other countries[46]) can lead to plasma concentrations largely over the reference range especially when age increases and/or for low body weight patients. On the other hand, a dose of 300 mg q.d, which is considered therapeutic[46], can fall below the reference range especially in very young adults or patients with high lean body weight. In the study by Muller *et al.*, the reference range of 100-320 ng/mL was determined based on the best predictive probabilities of avoiding non-response with minimal extra-pyramidal symptoms. However, inherently to statistical methods and to the variability of clinical response, some patients are non-responders with plasma concentrations in this range (9% in the study population of Muller *et al.*) and need amisulpride concentrations higher than 320 ng/mL to obtain a therapeutic response.[13, 15] Thus, therapeutic drug monitoring appears to be very useful especially when adverse effects are more likely (*i.e* in elderly individuals or in low body weight patients) and when clinical response is poor in order to discriminate a lack of adherence or a non-response with recommended doses.

The PK-prolactin model was in accordance with a previously reported model in elderly individuals.[20] In males and in females, our E_{max} estimates were close to the reported values of 52 and 124 ng/mL, respectively. EC_{50} value (*i.e.* 42 ng/ml) was slightly higher than the reported value of 18 ng/mL but of the same order of magnitude compared to amisulpride therapeutic concentrations (> 100 ng/mL). Sex effect was introduced from the beginning of the structural model development as its effect on prolactin plasma levels is very important. Oestrogen is involved in the regulation of prolactin secretion

and females are very sensitive to prolactin increase due to the action of oestrogens at central and peripheral levels. It has been recommended to monitor prolactin, based on sex but also on menopausal status, especially in premenopausal patients in whom antipsychotic-induced hyperprolactinemia can induce early menopause.[5] Thus, we investigated the effect of menopause on E_{max} parameter in the model. A decrease of E_{max} parameters was found in postmenopausal women and in males compared to premenopausal women which was however non-significant (21% and 58%, respectively, ΔOFV= -1.6, p= 0.2).

Prediction of prolactin levels at the time of trough concentrations showed that an amisulpride exposure at therapeutic concentrations results in prolactin levels over the normal values **(see Figure 2.1.3)**. This is in accordance with previous studies showing that amisulpride is a prolactin-raising antipsychotic at relatively low dose and that there is no dose-effect within the ranges of doses necessary for obtaining an antipsychotic effect.[4, 16] This is also supported by studies on dopamine receptor occupancy in adults showing that therapeutic response to antipsychotics occurs at a range of striatal D_2 receptor occupancy of 65-80%, while hyperprolactinemia is already observed at 73% of occupancy of the same receptor.[51-53] Thus, the threshold for receptor occupancy leading to hyperprolactinemia lies in the same range of receptor occupancy for therapeutic effects.

This present study and previous studies[3, 18] suggest that patients receiving amisulpride will have hyperprolactinemia. Thus, taking into account an amisulpride-induced hyperprolactinemia in the clinical evaluation will be beneficial for patients without early clinical symptoms in order to avoid longer term adverse events. Some recommendations have been already proposed and should be considered given that amisulpride has been shown to be one of the most efficacious antipsychotics with a low potential to induce weight gain.[14, 54-57]

In the present study, patients gained approximately 1.5 kg following a 3-month treatment period, and weight increase was not dependent on amisulpride concentrations. The low body weight gain found

in the present study is comparable to the value reported after a 100-day treatment period in a meta-analysis of randomized studies conducted by the manufacturer.[7]

The present study has to be interpreted considering some limitations. The sparse sampling in the PK analysis prevented to estimate inter-occasion variability in amisulpride clearance, which may have contributed to the relatively high residual variability (53%) in the final model. Oestrogen-based contraceptives increase prolactin levels, but data on contraceptives intake was not available in the medical records and was thus not taken into account in the PK-prolactin analysis. Furthermore, physiological pulsatile secretion of prolactin varies notably within waking hours, depending also on meals and menstrual cycle[58, 59] and such fluctuations were not taken into account in the present work. However, to our knowledge, it is not known whether, and in such cases, to what extent, the physiological pulsatile secretion of prolactin is maintained during antipsychotic treatment due to the permanent reduction of the inhibitory effect of dopamine on prolactin secretion.

In conclusion, the results of the present study support the importance of therapeutic drug monitoring of amisulpride and dose adjustment based on age and body weight or lean body weight in overweight and obese patients. Hyperprolactinemia was not dependent on amisulpride concentrations and thus amisulpride dose reduction would not be appropriate when aiming to reduce prolactin levels. Further studies are needed to evaluate the best solutions to avoid short and long-term adverse consequences of antipsychotic-induced hyperprolactinemia.

II.1.6. SUPPLEMENTARY MATERIAL

Table S2.1.1 Parameter estimates of the structural and final PK models with bootstrap results of the final model.

Parameter	Structural population parameters		Final population parameters		Bootstrap evaluation (n=2000 samples)	
	Estimate	RSE[a] (%)	Estimate	RSE[a] (%)	Median	$CI_{95\%}$
CL (L/h)	39.6	4	43.9	4	43.8	39.9 ; 48.1
V (L)	954	11	926	10	923	695 ; 1249
k_a (h^{-1})	0.9 fixed	-	0.9 fixed	-	-	-
θ_{AGE_CL}	-	-	-0.47	10	-0.46	-0.54 ; -0.35
θ_{LBW_CL}	-	-	0.53	37	0.53	0.25 ; 0.83
IIV_{CL} (CV%)[b]	47	8	34	11	33	24 ; 42
IIV_V (CV%)[b]	53	40	58	28	57	32 ; 84
Proportional residual error (%)[c]	54	4	53	3	53	46 ; 60

CL, amisulpride clearance; V, amisulpride volume of distribution; k_a, first-order absorption rate; θ_{AGE_CL}, age effect on CL; θ_{LBW_CL}, LBW effect on CL; IIV_{CL}, inter-individual variability of CL; IIV_V, inter-individual variability of V.

a. Relative standard errors of the estimates (SE) defined as SE/estimate directly retrieved from NONMEM®.

b. Interindividual variability defined as CVs (%).

c. Proportional residual error as percentage on the linear scale derived from the additive residual error on the log scale

Final model: CL (L) = 43.9 x (1 - 0.47 x ((age - median age)/median age)) x (1 + 0.53 x ((lean body weight – median lean body weight)/median lean body weight)

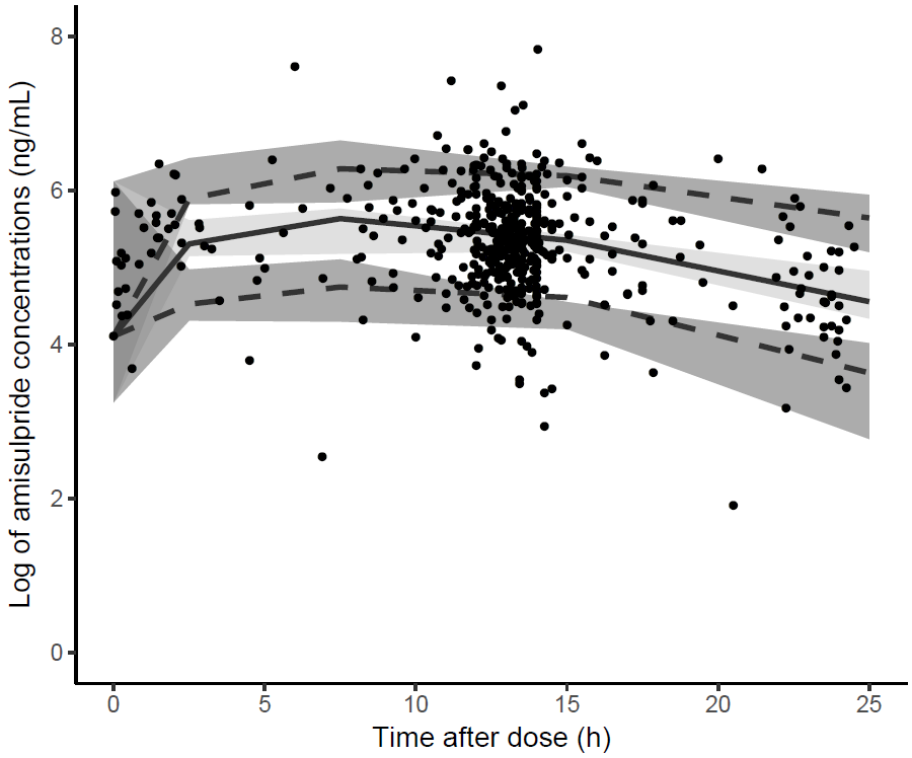


Figure S2.1.1: Prediction-corrected visual predictive check of the PK final model (n=513 amisulpride concentrations). Circles represent amisulpride plasma concentrations. The continuous line represents the median observed plasma concentration and the dashed lines represent the observed 5% and 95% percentiles. Shaded areas represent a simulation-based 95% confidence interval for the median, the 5% and 95% percentiles.

a. b.

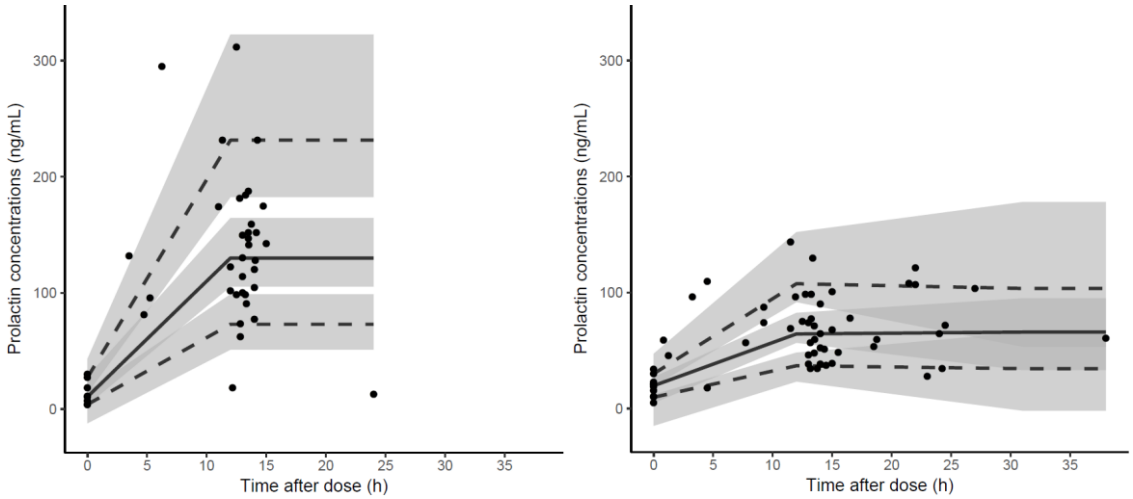


Figure S2.1.2: Visual predictive check of the final PK-Prolactin model (n=101 prolactin plasma levels) in a. females and b. males. Black dots represent prolactin plasma concentrations. The continuous line represents the median observed plasma concentration and the dashed lines represent the observed 5% and 95% percentiles. Shaded areas represent a simulation-based 95% confidence interval for the median, the 5% and 95% percentiles.

Annex 2.1.1 NONMEM code for the final PKPD model

```
$PROBLEM PK AMISULPRIDE

$INPUT ID CODE=DROP DAT1=DROP TIME OCC CONCDAY DOSE AMT CMT SS II DV MDV AMICONC
MALE AGE ETHN HEIT BW LBW BMI SMOKE CLCR1b AJEUNPRL DATMONTH SPRINGSUMMER
MENOP55 OPIA_COC ALCOOL PATHO APPRL1 APPRL2 APPRL APPRLb RISP_PALI RISP_PALI_HALDOL
APBEFORE2 APBEFORE3 HRPRL TYPE2 ADM_3_PER_DAY

$DATA ami_pkpd4_cmt_prl2.csv IGNORE=#

$SUBROUTINES ADVAN6 TRANS=1 TOL=3

$MODEL NCOMP=3
COMP=(GUT)
COMP=(CENTRAL)
COMP=(EFFECT)

$PK
IF (AMT.GT.0) THEN
 TDOS=TIME
 TAD=0.0
 ENDIF
 IF (AMT.EQ.0) TAD=TIME-TDOS

MAGE=37
FAGE=(AGE-MAGE)/MAGE
MLBW1=52
LBW1=LBW
IF (LBW.EQ.-99) LBW1= MLBW1
FLBW1= (LBW1-MLBW1)/MLBW1
TVCL=THETA(1)*(1+THETA(5)*FAGE)*(1+THETA(6)*FLBW1)
CL = TVCL * EXP(ETA(1))
TVV = THETA(2)
V  = TVV * EXP(ETA(2))
TVKA = THETA(3)
KA = TVKA * EXP(ETA(3))
KE = CL/V
S2 = V/1000
F1 = 0.48
TVEMAX = THETA(7)*(1+THETA(11)*MALE)
EMAX = TVEMAX*EXP(ETA(4))
EC50 = THETA(8)*EXP(ETA(5))
E0 = THETA(10)*EXP(ETA(6))

$DES
DADT(1) = -KA*A(1) ; GUT
DADT(2) = KA*A(1)-KE*A(2)

$ERROR
CP = A(2)/S2  ; RECUPERATION CONC PLASMATIQUE ; A(2) sur échelle normale (pas en log)
```

```
E = E0 + (EMAX*CP/(EC50+CP))
TYPE = 0 ; CMT3 PRL
IF (CMT.EQ.2) TYPE=1 ; CMT2 AMISULPRIDE CONC
IPRED = LOG(F+0.0001)*TYPE + (E+0.0001)*(1-TYPE)    ; IPRED = LOG(F) donc IPRED ressort en
LOG pour pouvoir être comparé aux DV.
IRES = DV-IPRED
IF (F.GT.0) W = THETA(4)*TYPE    ; RE PK ADD if log scale > proportional in natural scale
IF (F.GT.0) IWRES = (IRES/W)*TYPE ; PK
YP = (IPRED + W*EPS(1))*TYPE ; PK
W = (SQRT(THETA(9)**2))*(1-TYPE)  ; RE PD ADD
YE = (IPRED + W*EPS(2))*(1-TYPE) ; PD
IWRES = (IRES/W)*(1-TYPE)
Y = YP*TYPE + YE*(1-TYPE)

$THETA
43.9 FIX ; CL
926 FIX ; V
0.9 FIX  ;KA
0.529 FIX ; RE PK
-0.467 FIX     ;AGECL
0.528 FIX   ;LBWCL
(10,125,400) ; EMAX
(1,40) ; EC50 (IN Reeves)
10 ; RE PRL
20 ; E0
-0.4 ; EMAX MALE COEF

$OMEGA
0.107 FIX ; IIV CL
0.292 FIX ; IIV V
0 FIX  ; IIV KA
0.1 ; IIV EMAX
0 FIX ; IIV EC50
0 FIX ; IIV E0

$SIGMA ; PK
1 FIX ;

$SIGMA ; PD
1 FIX

$EST METHOD=1 INTER MAXEVAL=9999 NOABORT PRINT=5 POSTHOC NSIG=3 MSFO=run3i

$COVARIANCE PRINT=E MATRIX=S

$TABLE ID OCC TAD TIME MDV AMT DOSE IPRED IWRES CWRES E CMT DV PRED NPDE
ADM_3_PER_DAY NOAPPEND ONEHEADER NOPRINT FILE=sdtab3i
$TABLE ID OCC CL V KA TVCL EMAX EC50 E0 ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 MDV CMT NOAPPEND
ONEHEADER NOPRINT FILE=patab3i
$TABLE ID OCC MDV AGE LBW BW BMI NOAPPEND ONEHEADER NOPRINT FILE=cotab3i
```

```
$TABLE ID OCC MALE MENOP55 OPIA_COC ALCOOL PATHO APPRL1 APPRL2 APPRL APPRLb RISP_PALI
RISP_PALI_HALDOL APBEFORE2 APBEFORE3 AJEUNPRL SPRINGSUMMER NOAPPEND ONEHEADER
NOPRINT FILE=catab3i
```

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CHAPTER III. QUETIAPINE AND BODY WEIGHT GAIN

Chapter III in the thesis context

Quetiapine is one of the most prescribed second-generation antipsychotic.[1] This drug is well tolerated with a low propensity of inducing extra-pyramidal symptoms (EPS), but can induce significant weight gain and other metabolic effects.[2, 3]

Adverse-effects burden of the antipsychotic treatment comprises not only physical deterioration but also alteration of medication adherence leading to higher likelihood of symptom relapse and rehospitalization.[4] Adverse effects are often evaluated during randomized clinical trials (RCTs) on selected patients with a limited treatment duration. In a large naturalistic study including more than 1'000 patients aged from 18 to 65 years old from hospitals in Oslo, 35 % and 42% of patients treated with monotherapy (mainly olanzapine, quetiapine, aripiprazole and risperidone) and polytherapy (one antipsychotic and other psychotropic drugs) reported weight gain. Only 11.5% of untreated patients reported weight gain. This side effect was positively associated with dose (OR=1.24, 95%CI=1.03-1.50) and inversely associated with duration of treatment (OR=0.99, 95%CI=0.98-0.99). In this study, weight gain was the fourth most frequent adverse event and was reported as the most distressing one especially for women in another study.[5]

As in the previous chapter, the following work was conducted on data from the follow-up of the clinical and cardiometabolic parameters during antipsychotic treatment.

Own contribution

Writing of the research protocol and submission to the ethics committee. Data management after extraction. Population modeling, analysis and interpretation of the data. Writing of the manuscript.

III.1. Quetiapine: Real-World Evidence of Dose Adaptation and Effect on Body Weight Gain

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In preparation

III.1.1 INTRODUCTION

Quetiapine, a second-generation antipsychotic is widely prescribed for treating schizophrenia and bipolar disorders in adult and elderly patients.[6] The immediate release (IR) and extended-release (XR) forms (administration once per day) were approved by the Food and Drug Administration (FDA) in 1997 and 2007, respectively.[7, 8] There is a linear dose-concentration relationship with high variability in concentrations after administration of the same dose.[7, 9, 10] Quetiapine recommended doses range from 150 mg up to 800 mg/day.[6] Quetiapine is also used off-label at low doses (25-100 mg/day) for sedation or anxiety, a criticized but important and growing practice in many countries.[11] A few studies examined the relationship between quetiapine concentrations and therapeutic response and failed to determine an optimal therapeutic range for quetiapine.[3] However, a reference range of 100-500 ng/mL corresponding to the doses within the therapeutic ranges was suggested for quetiapine therapeutic drug monitoring (TDM).[3, 12] TDM of quetiapine is recommended to check whether the observed concentrations are expected under the administered dose and to adapt the dose in case of non-response or toxicity.

Quetiapine is extensively metabolized (98.9% recovered radioactivity as metabolites)[13] prior to excretion mostly in urine. The main active metabolite is N-desalkylquetiapine, *i.e* norquetiapine, largely formed by cytochrome P450 3A4 (CYP3A4) and to a lesser extent by CYP3A5 and CYP2D6.[14, 15] Norquetiapine is metabolized by CYP2D6 mainly and CYP3A4. [16] Drugs that are CYP3A4 inducers or inhibitors may account for quetiapine concentrations variability and may increase or decrease norquetiapine production.[17] In a clinical study, ketoconazole and carbamazepine, inhibitor and inducer of CYP3A4 respectively, both affected quetiapine pharmacokinetics. The mean quetiapine area under the curve between two administrations (AUC_τ) and clearance was increased by 522% and decreased by 84%, respectively, in 12 healthy men coadministered with ketoconazole. In 14 patients receiving quetiapine and carbamazepine, the mean AUC_τ was decreased by 87% and clearance increased by 7 times.[14] Genetic polymorphism may also alter quetiapine metabolism such as the *CYP3A4**22 polymorphism which has been recently identified[18] and shown to decrease enzyme activity. Dose-

corrected quetiapine trough concentrations were 2.5-fold higher in *CYP3A4**22 carriers patients (n=31) as compared to wild-type patients (n=207).[19]

Regarding the safety profile, body weight gain is the major adverse effect of second-generation antipsychotics[20, 21] and among them quetiapine has an intermediate propensity for weight gain.[2, 20, 22, 23] In a study involving 300 patients treated by quetiapine monotherapy, the mean weight gain was 3.2 kg (95% CI= 2.3-4.1 kg), and 39% of patients gained at least 7% of the baseline body weight after 52 weeks of follow-up.[24] The weight gain was more important in the first 12 weeks of treatment than after 6 months and one year of treatment. Relationship between quetiapine dose and weight gain is reported with controversial results. A double-blind randomized controlled trial including 268 individuals reported 25% of patients gaining at least 7% of body weight at baseline after 6 weeks of high dose treatment (>750 mg)[25] versus 16% of patients gaining at least 7% of baseline body weight in the low dose group (<250 mg). However, an open-label extension phase of a clinical trial (n=178, 80 weeks)[26] and two retrospective analyses of one year of treatment found no association between dose and body weight gain.[27]

To date, no investigation has been performed on the relationship between weight gain and quetiapine concentrations especially at very low doses (25 mg/day). The objectives of this work were first to characterize the pharmacokinetic profile of quetiapine and its active metabolite norquetiapine, and to detect sources of variability in an adult and elderly psychiatric population of a real-world clinical setting. Secondly, we aimed at describing the relationship between quetiapine exposure and body weight data.

III.1.2. MATERIALS AND METHODS

Study population and design

The present study included Lausanne University Hospital inpatients who had at least one quetiapine plasma concentration measurement performed between 2007 and 2017. Antipsychotic plasma concentration measurements are requested for TDM by the treating psychiatrist or by the department guideline for metabolic follow-up of patients starting a psychotropic treatment on a routine basis (PsyClin).[28] In the latter case, written informed consent from an ongoing longitudinal clinical and pharmacogenetic study (PsyMetab) was obtained from patients, as described elsewhere.[29] PsyMetab study and retrospective analysis of PsyClin data were both approved by the Ethics Committee of Vaud (CER-VD). Exclusion criteria were undetectable quetiapine plasma concentrations suggestive of non-adherence to treatment and absence or non-reliable time information about blood sampling or last dose intake.

In addition to the accurate time of last drug intake and blood sampling, the following data were recorded at the same time as the blood samples were drawn for pharmacokinetic measurements: drug form administered (IR/XR), sex, age, body weight (BW), plasma albumin concentration, intake of grapefruit juice, and concomitant medications with Permeability-GlycoProtein inhibitors (iPGP) and CYP3A4 inducers/inhibitors according to guidelines[30-32] that might have influenced quetiapine therapy. Dosing information history (dose, date and time of administration) up to 5 days before the concentration measurement was retrieved from patients' clinical records and added in the dataset.

Quetiapine and norquetiapine concentration measurements

All blood samples were collected in EDTA-containing tubes. After centrifugation, plasma samples were stored at -20 °C until routine analysis. Quantification of quetiapine in plasma was performed by high performance liquid chromatography coupled to mass spectrometry until 2012 (detailed method

available on request), and then by ultra-high performance liquid chromatography coupled to tandem mass spectrometry, with inclusion of norquetiapine in the new method.[33] The lower limit of quantification was 1 ng/ml for both analytes and methods.

Genotyping

Genomic DNA was extracted from EDTA blood samples using the FlexiGene DNA extraction kit (Qiagen Instruments AG, Hombrechtikon, Switzerland) until 2011 and subsequently using the QIAamp DNA Blood Mini Kit with the QIAcube automate (QIagen Instruments AG, Hombrechtikon, Switzerland), according to the protocol outlined by the manufacturers. The SNPs analysed in this work were obtained using the Infinium™ Global Screening Array MD-24 v2.0 BeadChip (illumina®, San Diego, United States) which includes SNPs from pharmacokinetic genes selected by the Clinical Pharmacogenetics Implementation Consortium (CPIC). The following SNPs located at genes encoding CYP3A4, CYP3A5, P-gp and POR were selected based on previously published pharmacogenetic studies: *CYP3A4* (rs4646437, rs2740574, rs35599367 *i.e CYP3A4*22*), *CYP3A5* (rs776746 *i.e CYP3A5*3*), *ABCB1* (rs1128503, rs9282564) and *POR* (rs1057868).

Pharmacokinetic analysis

Non-linear mixed effect modelling was performed using NONMEM® version 7.4.3 program[34] with the PsN-Toolkit (version 4.8.0).[35] Statistical analyses and figures were performed using R (v. 3.3.3, http://www.r-project.org).

Pharmacokinetic base model: structural and error model

First, the pharmacokinetic analysis was conducted on the subset of patients receiving only the XR quetiapine form during the entire study period. A stepwise procedure was used to identify models that best fitted the data. Multi-compartment models with linear elimination were compared to determine the appropriate structural model, which was finally identified as a two-compartment model with first-order absorption (KA_{XR}) and elimination. Secondly, patients taking only the IR form were added in the dataset. This allowed estimating the ratio (KA_{IR_rel}) between the first-order absorption rate of IR data (KA_{IR}) and KA_{XR}. Thirdly, patients treated with both the XR and IR forms were included in the dataset, fixing KA_{XR} and KA_{IR_rel} to the previously determined values while estimating the other quetiapine PK parameters. Finally, a joint parent/metabolite PK model was build using molar units for both compounds and assuming a linear conversion from quetiapine to norquetiapine. Multi-compartment models with linear elimination were fitted to the data to determine the appropriate structural model for norquetiapine, which was finally identified as a one-compartment model. Owing to identifiability problems, parent and metabolite were assumed to have the same apparent central volume of distribution (V2=V3) **(Figure 3.1.1)**. Since quetiapine was administered orally, parent and metabolite PK parameters represent apparent values.

Exponential errors following a log-normal distribution were assumed for the description of inter-individual variability of the parameters. Correlations between pharmacokinetic parameters were investigated. Proportional, additive and combined proportional-additive error models were finally compared to describe the residual variability. The correlation between quetiapine and norquetiapine concentration measurements was tested using the L2 function in NONMEM®.

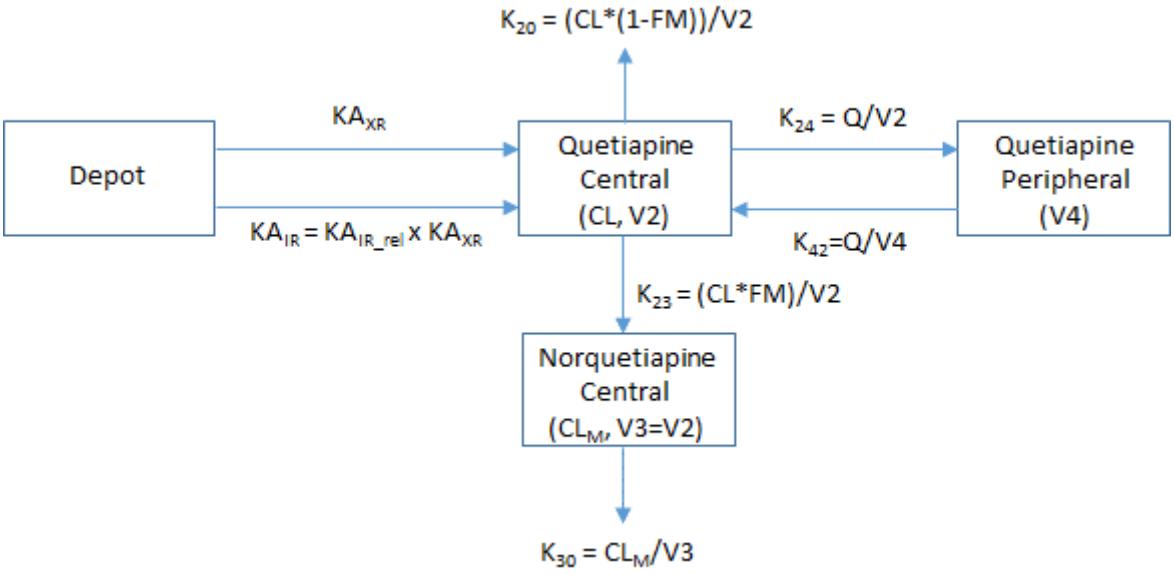

Figure 3.1.1: Compartmental model used to describe quetiapine and norquetiapine plasma-concentration-time profiles. KA_{XR} first-order absorption rate constant from depot to quetiapine central compartment if extended-release form administered; KA_{IR} first-order absorption rate constant from depot to quetiapine central compartment if immediate release form administered; KA_{IR_rel} ratio between KA_{IR} and KA_{XR}; K_{23} metabolic rate constant; FM fraction of quetiapine metabolized in norquetiapine; CL apparent quetiapine clearance; CL_M apparent norquetiapine clearance; K_{20} quetiapine elimination rate constant; K_{30} norquetiapine elimination rate constant; Q intercompartment clearance; K_{24} and K_{42} the intercompartment rate constants; V2 and V3 apparent central volume of distribution of quetiapine and norquetiapine, respectively; V4 apparent peripheral volume of distribution of quetiapine.

Covariate analysis

After visual exploration of the relationships between the PK parameters and the available clinically relevant factors, potentially influencing covariates were incorporated sequentially in the model and tested for significance on the parameters. The covariate analysis was performed using a stepwise insertion/deletion approach testing power function as recommended in a recent guideline[36] for

categorical covariates (coded as 0 and 1) and linear function for continuous covariates (centered on their median value). Missing values for albuminemia were imputed to the population median value. Parameter values were estimated for each genotypic group (rich model), defined as the reference allele group (Ref) and alternative allele groups: heterozygote alternative (Het-Alt) and homozygous alternative (Hom-Alt) or for further regrouped (reduced model) subpopulations. Parameter values were estimated for each phenotypic groups (rich model), defined as poor (PM), intermediate (IM) and extensive (EM) metabolizers.

Parameter estimation and model selection

All models were fitted using the first-order conditional estimation method with interaction (FOCEI) with the subroutines ADVAN4 TRANS4 for the pharmacokinetic model on quetiapine data, the subroutine ADVAN5 for the parent-metabolite PK model. The log-likelihood ratio test, based on changes in the objective function value (ΔOFV) was employed to discriminate between hierarchical models. Since a ΔOFV between any two models approximates a χ^2 distribution, a 3.8- (p=0.05) point change of OFV was considered statistically significant for one additional parameter in model building. To account for multiple testing in the covariate model the p-value was corrected by the number of tests. A change in OFV > 7.0 (p= 0.008) was considered statistically significant for one additional parameter during backward deletion steps in the pharmacokinetic model. The Akaike's information criterion (AIC) was instead employed to choose between non-hierarchical models. Diagnostic goodness-of-fit plots, precision and plausibility of the model parameters were also used to assess the reliability of the results.

Model evaluation

Prediction-corrected Visual predictive check (pcVPC) was additionally performed with PsN toolkit by simulations (n=1000) based on the final pharmacokinetic model. An independent dataset of patients

with samples collected exclusively between 2015 and 2017 was used for final model external validation. Quetiapine and norquetiapine concentrations were predicted based on the final PK model using the MAXEVAL=0 option in NONMEM®. The predictive performance of the model was assessed by the bias expressed as the mean prediction error (MPE) and by the precision expressed as root mean square prediction error (RMSE) using log-transformed concentrations with the associated 95% CIs using the following equations:

$$MPE = e^{mean(\ln(C_{pred})-\ln(C_{obs}))} - 1$$

$$RMSE = e^{\sqrt{mean(\ln(C_{pred})-\ln(C_{obs}))^2}} - 1$$

where C_{pred} are individual predictions, C_{obs} are the observed concentrations of the validation group.[37]

Quetiapine-body weight analysis

An exploration of the relationship between quetiapine exposure, defined by the 24-hour area under the concentration-time curve (AUC_{0-24}) and body weight values was conducted. AUC_{0-24} were computed assuming steady-state by the following equation:

AUC_{0-24} = DOSE/CL

where DOSE is the total daily dose and CL is the individual parameter of quetiapine clearance. The norquetiapine exposure was excluded from analysis.

The following four models were compared to describe the body weight values as a function of treatment duration, quetiapine exposure or both variables:

Model 1: BW = BBW+SLOPE*TIME (1)

Model 2: BW = BBW+SLOPE2*(AUC_{0_24}/MAUC) (2)

Model 3: $BW = BBW+SLOPE*TIME+SLOPE2*(AUC_{0\_24}/MAUC)$ (3)

Model 4: $BW = BBW+SLOPE*TIME+SLOPE2*(AUC_{0\_24}/MAUC)+SLOPE3*TIME*(AUC_{0\_24}/MAUC)$ (4)

where BW is the body weight value; BBW, the baseline body weight value; SLOPE and SLOPE2, the slope values for treatment duration and quetiapine AUC_{0_24} effects, respectively; MAUC, the median of AUC_{0_24} in the analysis population; and SLOPE3, the slope value characterizing the interaction between time and AUC_{0_24}.

Additive error or exponential errors following a log-normal distribution were tested for the description of inter-individual variability of the parameters. Proportional, additive and combined proportional-additive error models were finally compared to describe the residual variability.

All models were fitted using the first-order estimation method (FOCE) with $PRED in NONMEM®.

III.1.3. RESULTS

Study population and data

Among the 819 patients included, 710 providing a total of 1494 and 473 quetiapine and norquetiapine concentrations, respectively, which were used for population pharmacokinetic model building. Blood samples were collected at a median time of 12.5 h (range, 0.02 h-29 h) after last dose intake. A median of 1 (range, 1-19) and of 1 (range, 1-12) sample per patient of quetiapine and norquetiapine, respectively, was collected. Quetiapine and norquetiapine plasma concentrations ranged from 1 to 1680 ng/mL and from 1 to 882 ng/mL across a range of daily doses from 12.5 to 4000 mg (median = 500 mg). Subject's characteristics are presented in **Table 3.1.1**. For the exposure-body weight analysis, 2282 body weight values (median = 71 kg, range = 34-150 kg) from 696 patients were available for analysis. One hundred and twenty-five patients had a body weight value at baseline.

Table 3.1.1: Characteristics and genetic polymorphism of the study populations

Characteristics	**Model building (n=710)**	**Model validation (n=116)**
Sex (male), n (%)	318 (45)	64 (55)
Age (years), median (range)	47 (18-98)	41 (18-87)
Body weight (kg)		
median (range)	71 (34-149)	68 (41-200)
missing data, (%)	7	1
Albuminemia (g/L)		
median (range)	42 (22-55)	41 (22-48)
missing data, (%)	94	88
CYP3A4 inhibitors, n(%)[1]	17 (1)	0
Strong inhibitors[2]	4 (0.3)	0
Moderate inhibitors[3]	7 (0.5)	0
Weak inhibitors[4]	2 (0.07)	0
Grapefruit juice, n(%)[1]	23 (1.5)	0
CYP3A4 inducers, n(%)[1]	11 (0.7)	0
Strong inducers[5]	9 (0.6)	0
Moderate inducers[6]	2 (0.1)	0
P-gp inhibitors[7], n(%)[1]	85 (5)	14 (7)
P-gp inducers[8], n(%)[1]	3 (0.2)	0
Genetic polymorphisms	**Genotype[9]**	**Genotype[10]**
CYP3A4		
rs4646437	GG/GA/AA	GG/GA/AA
n	215/60/13	24/14/2
%	75/21/4	60/35/5
rs2740574	TT/TC/CC	TT/TC/CC

n	258/30/4	28/10/2
%	89/10/1	70/25/5
rs35599367	GG/GA/AA	GG/GA/AA
n	265/26/1	39/1/0
%	90.7/9/0.3	98/2/0
CYP3A5		
rs776746	CC/CT/TT	CC/CT/TT
n	238/46/8	27/11/2
%	82/16/2	68/27/5
ABCB1		
rs1128503	GG/GA/AA	GG/GA/AA
n	101/143/48	18/18/4
%	35/49/16	45/45/10
rs9282564	TT/TC/CC	TT/TC/CC
n	246/43/3	37/2/1
%	84/15/1	93/5/2
POR		
rs1057868	CC/CT/TT	CC/CT/TT
n	148/125/19	22/13/5
%	51/43/6	55/33/12

1. Values reported as number and percentage of concentrations with a co-medication.
2. Strong CYP3A4 inhibitors were amiodarone, atazanavir, darunavir, diltiazem and ritonavir.
3. Moderate CYP3A4 inhibitors were desogestrel, clobazam, fluoxetine and nifedipine.
4. Weak CYP3A4 inhibitors was ranitidine.
5. Strong CYP3A4 inducers were phenytoin, metamizole and carbamazepine.
6. Moderate CYP3A4 inducers were oxcarbazepine, bosentan and etravirine.
7. P-gp inhibitors were amiodarone, atazanavir, carvedilol, diltiazem, duloxetine, fusidic acid, haloperidol, irbesartan, ketoconazole, methadone, paroxetine, posaconazole and ritonavir.
8. P-gp inducers were carbamazepine and phenytoin.
9. Genetic data available for n=292 individuals except for rs4646437, n=288.
10. Genetic data available for n=40 individuals.

P-gp: Permeability-GlycoProtein; CYP3A4: cytochrome P450 3A4; CYP3A5: cytochrome P450 3A5; *ABCB1*: gene coding for P-gp; *POR*: gene coding for cytochrome P450 oxidoreductase.

Pharmacokinetic analysis

Pharmacokinetic analysis

The model depicted in **Figure 3.1.1** best described the quetiapine and norquetiapine data. A two-compartment model with first-order absorption and elimination described adequately all the quetiapine data. Improvement to the fit was observed using a two-compartment model (ΔOFV = -14) for two additional parameters. KA_{XR} and KA_{IR} were estimated at 0.12 h^{-1} and 0.91 h^{-1}, respectively, in XR and IR data sub-analyses, and fixed to such values for the pharmacokinetic model development on all the quetiapine data. As expected, very close values for the other population PK parameters were estimated using data of the XR and IR forms alone. Residual variability was described by a proportional error for quetiapine and norquetiapine data. The estimates and the variability (CV%) of the joint parent/metabolite base pharmacokinetic model are reported in **Table 3.1.2**.

Table 3.1.2 Parameter estimates of the base and final pharmacokinetic models of quetiapine and norquetiapine of the final model.

Parameter	Base population parameters		Final population parameters	
	Estimate	RSE[a] (%)	Estimate	RSE[a] (%)
CL (L/h)	104	3	106	3
V2, V3 (L)	40	6	36	7
Q (L/h)	189	19	190	6
V4 (L)	576	8	594	5
KA_{XR} (h^{-1})	0.12 fixed	-	0.12 fixed	-
KA_{IR_rel} (h^{-1})	7.6 fixed	-	7.6 fixed	-
FM (%)	16	15	15	6
CL_M (L/h)	14	20	13	5
$\theta_{3A4INHWM\_CL}$	-	-	0.75	43
$\theta_{3A4INHS\_CL}$	-	-	0.07	75
θ_{3A4IND_CL}	-	-	3.8	26
$\theta_{CYP3A4HetAlt\_CL}$	-	-	0.68	14
$\theta_{CYP3A4HomAlt\_CL}$	-	-	0.49	65
IIV_{CL} (CV%)[b]	69	4	66	4
$IIV_{KAXR, KAIR\_rel}$ (CV%)[b]	86	15	75	11
IIV_{FM} (CV%)[b]	32	29	29	48
IIV_{CLM} (CV%)[b]	35	20	37	23
Quetiapine proportional RE (%)	53	6	54	5
Norquetiapine proportional RE (%)	38	8	38	10

CL apparent quetiapine clearance; V2 and V3 apparent central volume of distribution of quetiapine and norquetiapine; Q intercompartment quetiapine clearance; V4 apparent peripheral volume of distribution of quetiapine; KA_{XR} first-order absorption rate constant if extended-release form administered; KA_{IR} first-order absorption rate constant if immediate release form administered; KA_{IR_rel} ratio between KA_{IR} and KA_{XR}; FM fraction of quetiapine metabolized in norquetiapine; CL_M apparent norquetiapine clearance; $\theta_{3A4INHWM\_CL}$ effect of weak and moderate inhibitors of CYP3A4 on CL; $\theta_{3A4INHS\_CL}$ effect of strong inhibitors of CYP3A4 on CL; θ_{3A4IND_CL} effect of inducers of CYP3A4 on CL; $\theta_{CYP3A4HetAlt\_CL}$ effect of CYP3A4*1/*22 on CL; $\theta_{CYP3A4HomAlt\_CL}$ effect of CYP3A4*22/*22 on CL; IIV_{CL} inter-individual variability of CL; $IIV_{KAXR, KAIR\_rel}$ inter-individual variability of KA_{XR} and KA_{IR_rel}; IIV_{FM} inter-individual variability of FM; IIV_{CLM} inter-individual variability of CL_M; RE residual error

a. Relative standard errors of the estimates (SE) defined as SE/estimate directly retrieved from NONMEM®.

b. Interindividual variability defined as CVs (%).

Final model:
CL (L/h) = 106 x $0.75^{3A4INHWM}$ x $0.07^{3A4INHS}$ x 3.8^{3A4IND} x $0.68^{CYP3A4HetAlt}$ x $0.49^{CYP3A4HomAlt}$ with 3A4INHWM =1 or 0 if comedication with drug inhibiting weakly or moderately CYP3A4 or not, respectively; with 3A4INHS =1 or 0 if comedication with drug inhibiting strongly CYP3A4 or not, respectively; with 3A4IND=1 or 0 if comedication with drug inducing CYP3A4 or not, respectively; with CYP3A4HetAlt=1 or 0 if patient is CYP3A4*1/*22 or not, respectively; with CYP3A4HomAlt=1 or 0 if patient is CYP3A4*22/*22 or not, respectively.
KA_{IR} (h^{-1}) = 0.12 x 7.6

Univariate analyses showed that the effect of albuminemia (ΔOFV=-6.4, p=0.01), CYP3A4 inhibitors (ΔOFV=-16.7, p<0.001), CYP3A4 inducers (ΔOFV=-21.4, p<0.001) and *CYP3A4* rs35599367 (ΔOFV=-10.5, p=0.001) on CL improved significantly the description of the data. The effect of age (ΔOFV=-4.9, p=0.03) and male (ΔOFV=-7.4, p=0.007) were significant on the CLM parameter. Age has no significant effect on CL (p=0.2). The effect of age, a time-varying covariate, was also tested with BAGE, the age at the time of the first concentration, and DAGE, the age difference from the first concentration to each concentration, as previously proposed[38], and was significant on CL but with poor precision of parameter estimates (RSE > 200%) and was thus not retained.

In multivariate analyses, in the forward insertion step, albuminemia, CYP3A4 inhibitors, CYP3A4 inducers and *CYP3A4* rs35599367 remained as significant covariate on CL. Male remained as significant covariate on CLM. However, albuminemia on CL and MALE on CLM were discarded during the backward deletion step (ΔOFV < 7.0, p < 0.008). Our results suggest that CL is decreased by 0.25 in the presence of a drug weakly or moderately inhibiting CYP3A4, decreased by 0.93 in the presence of a drug strongly inhibiting CYP3A4, increased by 3.8 in presence of drugs that are inducers of CYP3A4, decreased by 0.32 if patients are CYP3A4*1/*22 and by 0.51 if patients are CYP3A4*22/*22.

Final pharmacokinetic model are summarized in **Table 3.1.2.** The prediction-corrected Visual Predictive Check (pcVPC) indicates that the final model described the quetiapine and norquetiapine data adequately **(Figure 3.1.2)**.

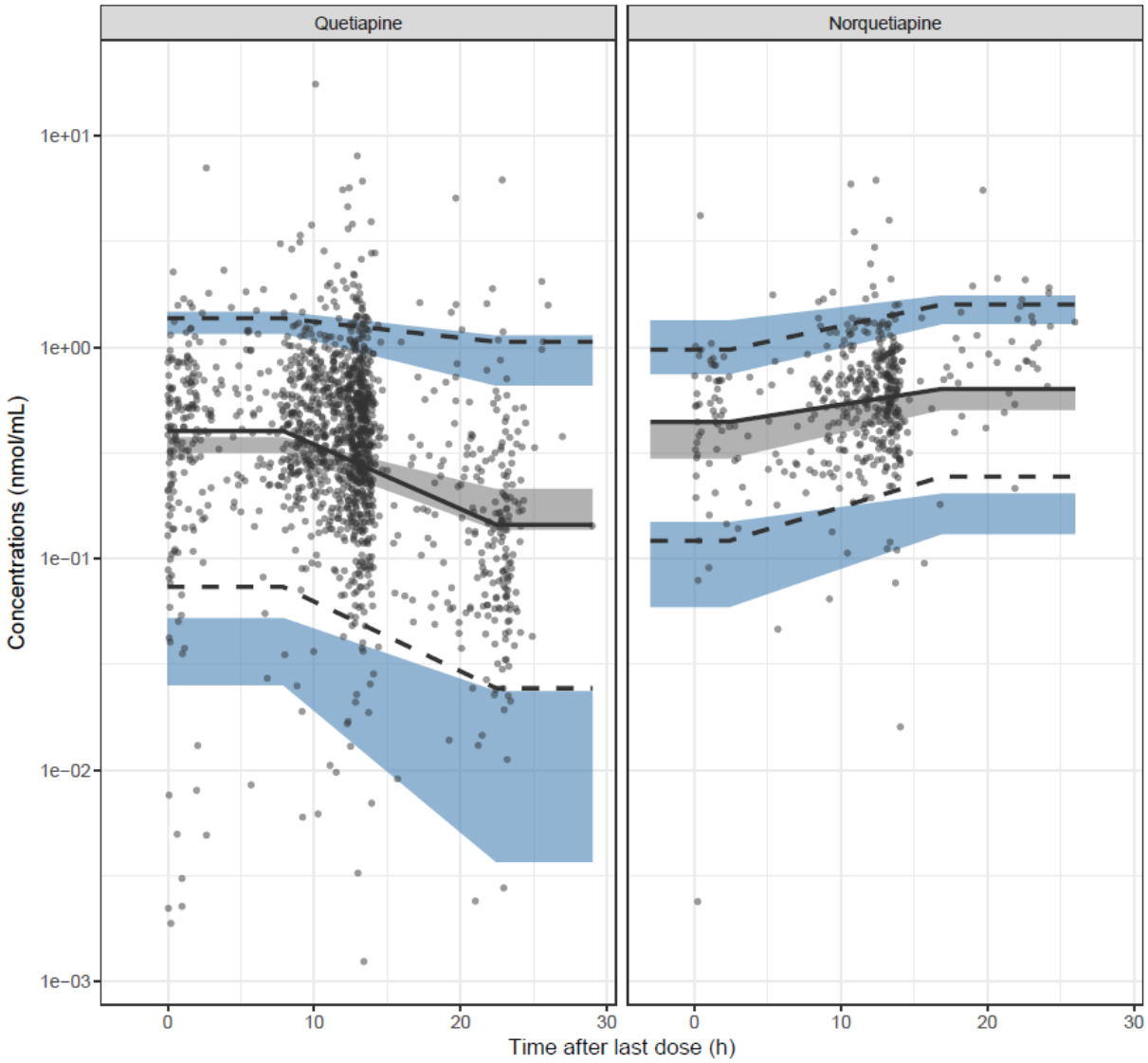

Figure 3.1.2: prediction-corrected Visual Predictive Check of the pharmacokinetic final model (n=1494 quetiapine and n=473 norquetiapine concentrations). Dots represent quetiapine (a.) and norquetiapine (b.) plasma concentrations. The continuous line represents the median observed plasma concentration and the dashed lines represent the observed 5% and 95% percentiles. Blue shaded areas represent the simulation-based 95% confidence interval for the 5% and 95% percentiles. Grey shaded area represent the simulation-based 95% confidence interval for the median.

A total of 187 quetiapine and norquetiapine concentrations from 116 patients were available in the confirmatory dataset for external validation **(Table 3.1.1)**. The external validation analysis showed a

small bias of -8% (95%CI= -13% to -3%) and of -4% (95%CI= -8% to -1%) with a precision of 50% and 31% for quetiapine and norquetiapine individual predictions, similar to the proportional part of the model residual error.

Quetiapine-body weight analysis

The body weight values were plotted against the treatment duration and the model-based quetiapine AUC_{0_24} in **Figure 3.1.3**.

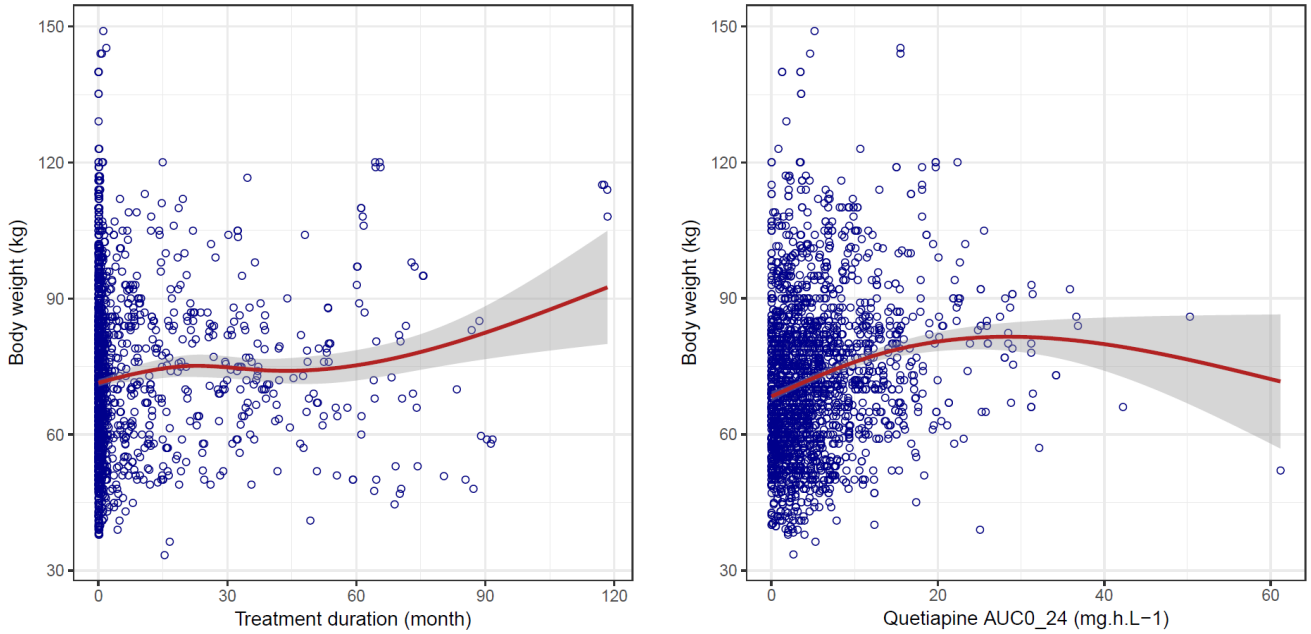

Figure 3.1.3: Scatter plot of observed body weight values versus treatment duration (a.) and quetiapine $AUC_{0\text{-}24}$ (b.). Red curve represents the non-parametric weighted smoothing of the data with its standard error represented by the shaded area.

The AIC values of the four models are reported in **Table 3.1.3**.

Table 3.1.3 Comparison of linear models based on AIC value

Model number	Equation	AIC
1	BW = BBW+SLOPE*TIME	10679
2	BW = BBW+ SLOPE2*(AUC_{0_24}/MAUC)	11085
3	BW = BBW+SLOPE*TIME+SLOPE2*(AUC_{0_24}/MAUC)	10661
4	BW = BBW+SLOPE*TIME+SLOPE2*(AUC_{0_24}/MAUC)+SLOPE3*TIME*(AUC_{0_24}/MAUC)	10664

AIC: Akaike's information criterion; BW body weight; BBW baseline body weight; SLOPE and SLOPE2 the slope values for treatment duration and quetiapine AUC_{0_24} effects, respectively; MAUC the median of AUC_{0_24} in the analysis population; and SLOPE3 the slope value characterizing the interaction between time and AUC_{0_24}.

Model 3 was the best describing the data and the model parameters are presented in **Table 3.1.4.**

Table 3.1.4 Parameter estimates of the quetiapine-body weight model

Parameter	Estimate	RSE[a] (%)	95%CI[b]	Shrinkage (%)
BBW (kg)	69	1	68-70	-
SLOPE ($kg.month^{-1}$)	0.42	128	-0.63 – 1.46	-
SLOPE2 (kg)	0.42	39	0.10 – 0.74	-
IIV_{BBW} (CV%)[c]	23	3	-	1
IIV_{SLOPE} (CV%)[c]	94	83	-	53
IIV_{SLOPE2} (CV%)[c]	101	78	-	73
Proportional RE (%)	3.8	23	2.1 – 5.5	-

BBW baseline body weight; SLOPE and SLOPE2 the slope values for treatment duration and quetiapine AUC_{0_24} effects, respectively; IIV_{BBW} inter-individual variability of BBW; IIV_{SLOPE} inter-individual variability of SLOPE; IIV_{SLOPE2} inter-individual variability of SLOPE2; RE residual error

a. Relative standard errors of the estimates (SE) defined as SE/estimate directly retrieved from NONMEM®.

b. Parametric 95% confidence interval of the estimate

c. Interindividual variability defined as CVs (%).

The SLOPE parameter was 0.42 $kg.month^{-1}$ meaning a body weight increase of 0.42 kg per month of quetiapine treatment. SLOPE2 was 0.42 kg meaning an increase of 2.8 kg when AUC_{0_24} changes from 10 to 40 $mg.h.L^{-1}$. Residual variability was described by a proportional error model and estimated to

be 3.8 %. The inter-individual variability of SLOPE (IIV_{SLOPE}) and SLOPE2 (IIV_{SLOPE2}) was 94% and 101%. Parameters SLOPE, IIV_{SLOPE} and IIV_{SLOPE2} were poorly estimated (RSE ≥ 78%) while the others parameters were correctly estimated (RSE ≤ 39%). Shrinkage was high (≥ 53%) for the IIV_{SLOPE} and IIV_{SLOPE2} parameters.

III.1.4. DISCUSSION AND PERSPECTIVES

A two-compartment model best described the quetiapine concentrations in our psychiatric population. Quetiapine clearance (106 L/h, 95%CI=100-111 L/h) was slightly higher than the reported value of 69 L/h (95%CI=56-84 L/h) of a previously reported one-compartment pharmacokinetic model developed in Chinese.[39] The steady-state volume of distribution estimate (V_{ss} = 630 L, 95%CI=200-695 L) was concordant with the volume of distribution published for the one-compartment model (V = 574 L, 95%CI=510-643 L). The KA_{XR} value and the relative parameter KA_{IR_rel} were similar to the reported values (KA_{XR}= 0.1 h^{-1} and KA_{IR_rel} =15, respectively). The CYP3A4 inhibitors and inducers increased significantly the quetiapine clearance in the present work as expected.[14] High fat meal increases quetiapine bioavailability.[40] However, meal composition was not available and could thus limit the absorption parameters estimation.

A basic model of the relationship between quetiapine exposure and body weight was developed. Weight gain seemed to depend on quetiapine exposure. Therefore, decreasing the quetiapine exposure might limit the weight gain. However, when considering the antimaniac and antipsychotic effects, because quetiapine is a weak antagonist of D_2 receptor, such dose decrease would decrease the efficacy of quetiapine. In order to improve the body weight prediction during quetiapine treatment a more sophisticated model will be developed. First, a piece-wise linear model with two or more slopes as a function of treatment duration will be fitted to the data to investigate whether the weight gain is more important at the beginning of the treatment compared to long-term treatment. For that purpose, it is necessary to report the date of treatment start in the dataset. Secondly, comedication with antipsychotic drugs inducing weight gain as well as other contributors of body weight gain (age, gender, BMI at baseline) will be included in the covariate analysis. In this preliminary analysis, norquetiapine exposure was not taken into account in the exposure-body weight model. Norquetiapine (like quetiapine) binds to the serotoninergic 5-HT_2 and histaminergic receptors, which might be associated with weight gain.[20, 41] Therefore, one can assume that norquetiapine exposure may participate to the weight gain. Exposure to the active moiety, *i.e* the sum of the quetiapine and

norquetiapine exposures could be more informative than that to quetiapine alone. We could predict the norquetiapine AUC with the final pharmacokinetic model to calculate the exposure to the active moiety for all the patients in the dataset, and then develop a model to depict the relationship between body weight gain and active moiety exposure.

Eventually, body weight gain will be simulated after administration of common three ranges of doses such as 25 to 150 mg/day (anxiolytic and antidepressive effects), 150 to 300 mg/day (for antidepressive effects), 300 to 800 mg/day (used mainly for antimaniac and antipsychotic effect).

Annex 3.1.1 NONMEM® code for the final covariate joint parent-metabolite PK model

```
$PROBLEM PK QUETIAPINE + NORQUETIAPINE IR+XR+BOTH

$INPUT ID OCC DAT1=DROP TIME RELEASE DRUGSADM DOSE AMT CMT SS NBADMDAY TADR DV
EVID MALE AGE BAGE DAGE BW HEIGHT LBW BMI INH3A4W INH3A4M INH3A4S INDU3A4 SNP6
OUTL BUILD L2

$DATA  Quet_data_pk_nm.csv IGNORE=# IGNORE=(BUILD.EQ.0) IGNORE=(TADR.GT.30)

$SUBROUTINES ADVAN5

$MODEL NCOMP=4
COMP=(DEPOT)
COMP=(DRUG)
COMP=(MET)
COMP=(PERIPH)

$PK
IF (AMT.GT.0) THEN
 TDOS=TIME
 TAD=0.0
 ENDIF
 IF (AMT.EQ.0) TAD=TIME-TDOS



P1=0
IF (INH3A4W.EQ.1) P1=1
IF (INH3A4M.EQ.1) P1=1
P2=0
IF (INH3A4S.EQ.1) P2=1
IF (RITO.EQ.1) P2=1

IF (SNP6.EQ.0) TVCL=THETA(1)*(THETA(9)**P1)*(THETA(10)**P2)*(THETA(11)**INDU3A4)
IF (SNP6.EQ.1)
TVCL=THETA(1)*(THETA(9)**P1)*(THETA(10)**P2)*(THETA(11)**INDU3A4)*THETA(12)
IF (SNP6.EQ.2)
TVCL=THETA(1)*(THETA(9)**P1)*(THETA(10)**P2)*(THETA(11)**INDU3A4)*THETA(13)
IF (SNP6.EQ.-99) TVCL=THETA(1)*(THETA(9)**P1)*(THETA(10)**P2)*(THETA(11)**INDU3A4)


CL = TVCL * EXP(ETA(1))

TVV2 = THETA(2)   ; CENTRAL V PARENT+METABOLITE
V2  = TVV2 * EXP(ETA(2))

LOGIT=THETA(3)
TVFM  = EXP(LOGIT) / (1 + EXP(LOGIT))
FM = TVFM*EXP(ETA(3))

V3 = V2
```

```
TVQ = THETA(4)
Q = TVQ * EXP(ETA(4))

TVV4 = THETA(5)   ; PERIPH V PARENT
V4  = TVV4 * EXP(ETA(5))

TVCLM = THETA(6)
CLM = TVCLM * EXP(ETA(6))

K24 = Q/V2
K42 = Q/V4
K20 = CL/V2 - K23
K30 = CLM/V3
K23 = (CL*FM)/V2


TVKA = THETA(7)
IF(RELEASE.EQ.1) TVKA = THETA(7)*THETA(8)
KA = TVKA * EXP(ETA(7))
K12 = KA

S2 = V2 ; QUET AMT en micromol, conc en nmol/mL = micromol/L
S3 = V3 ; NORQUET

$ERROR
Q2 = 0
Q3 = 0
IF (CMT.EQ.2) Q2=1
IF (CMT.EQ.3) Q3=1
IPRED2  = A(2)/S2
IPRED3  = A(3)/S3
IPRED   = IPRED2*Q2+IPRED3*Q3
IRES  = IPRED-DV

SD1=SQRT(SIGMA(1,1))
SD2=SQRT(SIGMA(2,2))


W2=SQRT((SD1*IPRED2)**2)
W3=SQRT((SD2*IPRED3)**2)

Y2 = IPRED2 *(1+ERR(1))
IWRES2 = IRES/W2
Y3 = IPRED3*(1+ERR(2))
IWRES3 = IRES/W3
Y          = Q2*Y2+Q3*Y3
IWRES   = Q2*IWRES2+Q3*IWRES3

$THETA ;
(0,90)  ; CL
(0,38)  ; V2 V3
(-1.5) ; FM
(0,170)  ; Q
```

```
(0,479)  ; V4
(0,13) ; CL MET
0.123 FIX ; KA
7.6 FIX ; KA IR REL
0.8 ; INH3A4WM ON CL
0.1 ; INH3A4S/R ON CL
3.2 ; INDU3A4 ON CL
0.7 ; SNP6=1
0.5 ; SNP6=2

$OMEGA
0.3 ; IIV CL
0 FIX ; IIV V2/V3

$OMEGA
0.1 ; IIV FM

$OMEGA
0 FIX ; IIV Q
0 FIX ; IIV V4

$OMEGA
0.3 ; IIV CLM

$OMEGA
0.65 ; IIV KA

$SIGMA BLOCK(2)
0.251 ; QUET PROP
0.2 0.2 ; NORQ PROP


$EST METHOD=1 INTER MAXEVAL=9999 NOABORT SIG=1 PRINT=5 POSTHOC MSFO=run297

$COVARIANCE PRINT=E MATRIX=R

$TABLE ID OCC CMT TAD TADR TIME EVID AMT DOSE IPRED IWRES CWRES DV PRED NPDE
NOAPPEND ONEHEADER NOPRINT FILE=sdtab297
$TABLE ID MALE CL V2 Q K23 K20 K30 V3 V4 CLM KA ETA1 ETA2 ETA3 ETA4 ETA5 ETA6 ETA7 EVID
NOAPPEND ONEHEADER NOPRINT FILE=patab297
```

Annex 3.1.2 NONMEM® code for the structural quetiapine-body weight model

```
$PROBLEM  PKPD QUETIAPINE BW

$INPUT ID OCC DAT1=DROP TIME RELEASE DRUGSADM DOSE AMT AMTMOL CMT SS DVOLD
NBADMDAY TADR CONC LCONC CONCMOL LCONCMOL EVID BW=DV OUTL BUILD ICL IV2 IQ IV4 IKA

$DATA  Quet_data_pkpd_ipa_nm.csv IGNORE=# IGNORE=(BUILD.EQ.0) IGNORE(CMT.LE.2)
TRANSLATE=(TIME/730) ; time in month

$PRED
CL = ICL
AUC = DOSE/CL
MAUC=4.6
;------ baseline BW --------
BBW = THETA(1)*EXP(ETA(1))
;------ slopes --------
TVSLOPE = THETA(2)
SLOPE = TVSLOPE+ETA(2)
TVSLOPE2 = THETA(4)
SLOPE2 = TVSLOPE2+ETA(3)
;------ model equation --------
BWT = BBW+SLOPE*TIME+SLOPE2*(AUC/MAUC)

IPRED = BWT
IRES = DV - IPRED
W=SQRT((THETA(3)*IPRED)**2)
IWRES=IRES/W
Y=IPRED+W*ERR(1)

$THETA ;
(60,65) ; BBW
(0.45) ; SL TIME
(0,0.06) ;RE PROP
(0,0.36) ; SL AUC

$OMEGA
0.1 ; IIV BBW
0.1 ; IIV SL TIME
0.1 ; IIV SL AUC

$SIGMA
1 FIX ;

$EST METHOD=1 MAXEVAL=9999 PRINT=5 POSTHOC MSFO=run94

$COVARIANCE PRINT=E


$TABLE ID OCC CMT TADR TIME AUC EVID AMT DOSE IPRED IWRES CWRES DV PRED NPDE
NOAPPEND ONEHEADER NOPRINT FILE=sdtab94
$TABLE ID BBW SLOPE ETA1 ETA2 ETA3 EVID NOAPPEND ONEHEADER NOPRINT FILE=patab94
$TABLE ID CMT TIME AUC EVID NOAPPEND ONEHEADER NOPRINT FILE=mytab94
```

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CHAPTER IV. TAILORING SMOKING CESSATION STRATEGY

Chapter IV in the thesis context

Smoking cessation treatments are useful for helping people to stop smoking and therefore to reduce cardiovascular risk factors. Smoking and smoking cessation are also important to consider in the treatment of psychiatric diseases, as they influence treatments with psychotropic drugs metabolized by the cytochrome P450 1A2 (CYP1A2). This cytochrome metabolizes some antipsychotics such as clozapine and olanzapine, and induction of CYP1A2 by smoking has been shown to decrease their plasma concentrations.[1] Lower drug concentrations may result in reduced drug efficacy and impel clinicians to increase the dose administered. A modification of the tobacco consumption in patients may trigger potential risks. Indeed, the induction of CYP1A2 ceases as patients quit smoking, leading to higher drug plasma concentrations and consequently to potential side effects.

In order to raise the level of understanding of the impact of smoking or smoking cessation on CYP1A2 activity, the Unit of Pharmacogenetics and Clinical Psychopharmacology of the Department of Psychiatry and the University Outpatient Clinic of Lausanne conducted a prospective study between 2008 and 2010. Smokers from the general population willing to stop smoking were recruited for a smoking cessation program composed of counselling and pharmacological treatments: varenicline or NRT.[2] This study's secondary objectives were to identify the clinical and genetic factors influencing the success of smoking cessation as well as the PK of varenicline. Both secondary objectives are the purpose of the following chapter.

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Own contribution

Part I

Data management after extraction. Statistical analysis and interpretation of the data. Writing of the manuscript, submission to the journal and publication process.

Part II

Data management after extraction. Population modeling, analysis and interpretation of the data. Writing of the manuscript, submission to the journal and publication process.

IV.1. Association of nicotine metabolism and sex with relapse following varenicline and nicotine replacement therapy

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IV.1.1. ABSTRACT

Nicotine is metabolized into cotinine and then into trans-3'-hydroxycotinine, mainly by cytochrome P450 2A6 (CYP2A6). Recent studies reported better effectiveness of varenicline in women and in nicotine normal metabolizers phenotypically determined by nicotine-metabolite ratio. Our objective was to study the influence of nicotine-metabolite ratio, CYP2A6 genotype and sex on the response to nicotine replacement therapy and varenicline. Data were extracted from a longitudinal study which included smokers participating in a smoking cessation program. Response to treatment was defined by the absence of relapse when a set threshold of reduction in cigarettes per day relative to the week before the study was no more reached. The analysis considered total and partial reduction defined by a diminution of 100% and of 90% in cigarettes per day, respectively. The hazard ratio of relapsing was estimated in multivariate Cox regression models including the sex and the nicotine metabolism determined by the phenotype or by CYP2A6 genotyping (rs1801272 and rs28399433). In the normal metabolizers determined by phenotyping and in women, the hazard ratio for relapsing was significantly lower with varenicline for a partial decrease (HR=0.33, 95%CI=[0.12-0.89] and HR=0.20, 95%CI=[0.04-0.91], respectively) and non-significantly lower for a total cessation (HR=0.45, 95%CI=[0.20-1.0] and HR=0.38, 95%CI=[0.14-1.0]). When compared to the normal metabolizers determined by phenotyping, the hazard ratio for a partial decrease was similar in the normal metabolizers determined by genotyping (HR=0.42, 95%CI=[0.18-0.94]) while it was significantly lower with varenicline for a total cessation (HR=0.50, 95%CI=[0.26-0.98]). Women and normal nicotine metabolizers may benefit more from varenicline over nicotine replacement therapy.

IV.1.2. INTRODUCTION

Smokers willing to stop smoking may be helped by a pharmacotherapy such as nicotine replacement therapy, bupropion or varenicline. These three medications are used worldwide and several head-to-head effectiveness comparisons have been performed with mixed results. Varenicline has been associated with greater continuous abstinence rates than NRT after 52 weeks of follow-up.[1, 2] A multicenter study including 47 smoking cessation services concluded in a small advantage of varenicline over NRT combinations (*i.e*. any formulations such as gums, spray and/or patches) although variations in population characteristics or clinical practice appear to influence the effectiveness of both therapies.[3] A randomized parallel clinical trial in 272 subjects reported equivalent success between varenicline and nicotine patches at 1, 6 and 12 months of follow-up[4], while a meta-analysis concluded that varenicline and combined NRT were equally effective in continuous or prolonged abstinence during at least 6 months.[5]

In humans, nicotine is metabolized into cotinine and then into trans-3'-hydroxycotinine, mainly by cytochrome P450 2A6 (CYP2A6).[6, 7] The nicotine-metabolite ratio refers to the 3'-hydroxycotinine / cotinine ratio during smoking ad libitum, and is a marker of CYP2A6 activity. Interestingly, nicotine-metabolite ratio seems to be associated with 1-week abstinence, with nicotine slow metabolizers being more likely to achieve abstinence than normal metabolizers (odds ratio [OR] = 1.32, 95% confidence interval [CI] = 1.05, 1.67; P = .019).[8] Furthermore, a recent double-blind placebo-controlled trial reported that varenicline had greater effectiveness than NRT in nicotine normal metabolizers as determined by the nicotine-metabolite ratio.[9]

Compared to CYP2A6 genotyping, nicotine-metabolite ratio has the advantage of taking into account both genetic and environmental (eg. oestrogen) effects on CYP2A6 activity.[9-12] CYP2A6 activity is indeed induced by oestradiol, leading to increased nicotine metabolism[13] and women are more likely to be normal metabolizers than slow metabolizers[9, 14], especially when receiving oestrogen-based hormonal therapy.[15] The influence of sex on the effectiveness of smoking cessation treatment has been

largely described. A meta-analysis of 14 studies comparing smoking cessation rate in men and women receiving nicotine patches reported a significantly lower rate of abstinence in women.[16] Men were also found to have a significantly better abstinence rate with the combination varenicline-bupropion than with varenicline alone, whereas women had a similar response to both treatments.[17, 18] It has therefore been suggested that women benefit more than men from varenicline alone and thus the addition of bupropion to varenicline would not improve the response in women.[19, 20] Interestingly, varenicline compared to nicotine patches doubled the odds of abstinence at the end of a 4-week treatment in an exploratory short-term double-blind randomized trial among women smokers.[21] In a longer term, at 12 weeks, women had greater quit rates when receiving varenicline compared to NRT in a study involving almost 7000 smokers.[22] Very recently a meta-analysis of 32 studies representing more than 14' 000 smokers reported a greater efficacy in women taking varenicline compared to transdermal nicotine or bupropion. In men, no difference was shown between the three treatments.[23]

Several single nucleotide polymorphisms (SNP) in the CYP2A6 gene that affect enzyme activity have been characterized.[24, 25] One of the most studied allele is the rs1801272 (479T>A, Leu160His, CYP2A6*2).[26, 27] It leads to a predicted CYP2A6 activity between 40 and 50% of the normal activity, or less than 40% if two mutated alleles A are present.[11] Thus, individuals carrying one or two CYP2A6*2 alleles are considered slow nicotine metabolizers.[28] Another common polymorphism, the rs28399433 (CYP2A6*9, -48T>G) located in the TATA box of the 5' flanking region of the CYP2A6 gene has been identified. The activity of the mutated TATA was reduced by 55% when compared to the wild allele and was shown to reduce mRNA expression and enzyme activity.[29, 30]

The primary objective of our post-hoc analysis of data from usual clinical care was to explore the influence of nicotine metabolism determined by the nicotine-metabolite ratio and of sex on the response to NRT and varenicline. Based on previous clinical trials[9, 16, 21], we hypothesized that nicotine normal metabolizers and women would benefit more from varenicline. The second objective was to compare the influence of nicotine metabolism determined phenotypically by the nicotine-metabolite

ratio and genetically by the CYP2A6 rs1801272 and rs28399433 polymorphisms on the response to NRT and varenicline.

IV.1.3. MATERIALS AND METHODS

Study design and participants

The original aim of this clinical and pharmacogenetic study was to examine the influence of smoking cessation on the activity of CYP1A2 isoform.[31] For this purpose it included smokers from the general population wishing to participate in a smoking cessation program. This program offered a 3-month study period (5 visits every week from week 0 to week 4, 4 visits every 2 weeks from week 4 to week 12) comprising smoking-cessation counseling and pharmacological treatment prescription (combined nicotine replacement therapies: patches, gums and/or inhaler or varenicline) and a 6-month concluding visit. Details of the inclusion and exclusion criteria as well as clinical measures have been previously described.[31] Blood sampling performed before the quit date was used to measure the nicotine-metabolite ratio. Abstinence was assessed during the follow-up by self-declaration and by measuring expired CO levels (Micro Smokerlyzer; Bedfont Scientific, Rochester, England). Abstinence was confirmed if CO level was less than 10 parts per million (ppm). The number of cigarettes smoked between two visits was also recorded. The study was approved by the ethics committee of the Lausanne University Medical School and by the Swiss Agency for Therapeutic Products (Swissmedic, Bern, Switzerland). Written informed consent was obtained from all participants.

Treatment

After a counseling session with a clinician, participants chose to receive either varenicline or combined nicotine replacement therapies (patches, gums and/or inhaler) in agreement with the clinician. NRT and varenicline were prescribed according to the manufacturers' information, to guidelines for smoking cessation[32], and to patients' preferences. Main counter-indications included cardiovascular diseases (unstable angina pectoris, recent myocardial infarction) or skin disorders (eg. psoriasis, chronic dermatitis, urticaria) for NRT, and depression, past antidepressant treatments or other psychiatric diseases for varenicline. NRT formulations and dosing were chosen according to the

nicotine dependence score measured by the Fagerström test for nicotine dependence (FTND) ranging from 0 to 10.[33] For instance, patients with a high dependence score (8 to 10) were prescribed with high concentration patches, combined with other NRT formulations (gums and/or inhaler). In patients with low dependence scores (0 to 3), one NRT formulation could be sufficient (low dose patches or gums/inhaler). NRT dosing was gradually decreased each month, for total treatment duration of 12 weeks. Varenicline was prescribed starting from one week before the quit date (0.5 mg once daily on days 1-3 and 0.5 mg twice daily on days 4-7), then continued with 1 mg twice daily for a total of 12 weeks. The pharmacological treatment was proposed for 12 weeks free-of-charge and treatments were delivered at each visit. Varenicline treatment but not combined NRT could be proposed for 12 supplementary weeks as recommended in the manufacturer's information.

If needed and with the approval of a clinician, a switch of treatment was allowed during the study. As the aim of the present study was to compare the effectiveness on smoking cessation of these two treatments, data from subjects who switched from one treatment group to another were excluded from the analysis **(Figure 4.1.1)**.

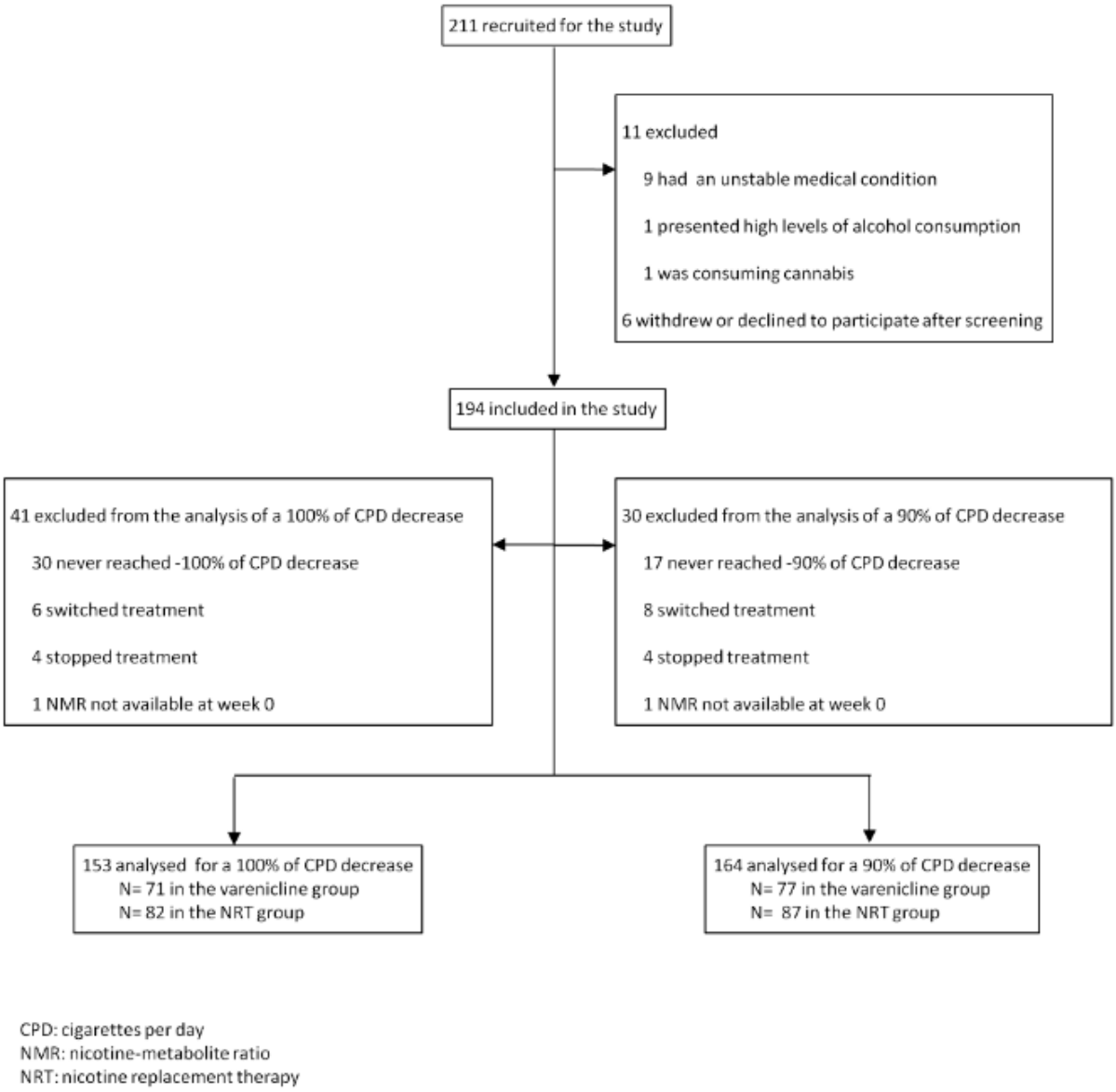


Figure 4.1.1: Flow chart for selection of patients

Nicotine-metabolite ratio

Cotinine and 3'-hydroxycotinine plasma levels were simultaneously measured by an ultra performance liquid chromatography-tandem mass spectrometry method while participants were smoking ad libitum at the beginning of the study.[34] Subjects were phenotypically considered slow metabolizers if nicotine-metabolite ratio was inferior to 0.26 and normal metabolizers if nicotine-metabolite ratio was

higher or equal to 0.26 as previously described.[12] The cut-off was set to the originally determined value of 0.26 reported in Schnoll *et al*.[12]

Genotyping

CYP2A6 SNPs were obtained using the CardioMetaboChip, a custom Illumina iSelect genotyping array designed to test DNA variation of over 200,000 SNPs from regions identified by large scale meta-analyses of genomewide association studies for metabolic and cardiovascular traits. 3642 customized SNPs covering pharmacokinetic genes were added in the Cardiometabochip[35, 36], among which 8 SNPs from the CYP2A6 gene (rs5031016, rs8192730, rs1809810, rs1801272, rs28399453, rs28399454, rs28399433, rs2892625). Four SNPs were excluded from analysis due to a very low minor allele frequencies (MAF<0.005) as reported in European population (rs5031016, rs8192730, rs1809810, rs28399454).[37] The rs28399453 polymorphism is not reported to influence CYP2A6 activity and was thus excluded from analysis. Quality control excluded samples from the analysis if sex was inconsistent with genetic data from X-linked markers, genotype call rate less than 0.96 or Gene Call score less than 0.15. The SNP rs2892625 was excluded from analysis due to a Gene Call score < 0.15. The two polymorphisms rs1801272 and rs28399433 were in Hardy Weinberg Equilibrium (p=0.68 and p=0.65, respectively) and were used for determining CYP2A6 genotype. In the sample analysis, individuals were homozygous non-mutated (TT) or heterozygous (TA) for the SNP rs1801272 and individuals were homozygous non-mutated (TT) or heterozygous (TG) for the SNP rs28399433. There were no homozygous mutated for any of the SNPs in the sample analysis. Subjects were classified as slow metabolizers if they were heterozygous for the SNPs rs1801272 or rs28399433, or for both SNPs according to results previously published.[28-30] The homozygous non-mutated for both SNPs were defined as normal metabolizers. GenomeStudio Data Analysis Software was used to export results generated by Illumina CardioMetaboChip.

Statistical analysis

In this work, total smoking cessation and partial smoking reduction is used to evaluate response to nicotine replacement therapy or varenicline. Response to treatment was defined by the absence of relapse (*i.e.* when the reduction in cigarettes per day (CPD) consumption relative to the week before the study was not reached anymore).

Exploratory analysis for selection of thresholds

An exploratory analysis was first conducted to find the most appropriate threshold in CPD reduction defining partial smoking reduction. The week before the study and at each visit, participants self-reported the number of cigarettes per day or over the period between two visits. For non-daily smokers, the number of cigarettes smoked over the period between 2 visits was divided by the number of days of the period to calculate CPD. Overall, there was a concordance between the CPD and the CO levels recorded ($r2=0.49$, $p < 0.01$). For each subject and each visit the reduction in the number of CPD relative to the number of CPD the week before the study was calculated. A binary variable was defined as follows: status=0 if the threshold of CPD reduction was reached, status=1 if the threshold was not reached anymore (*i.e* relapse). By varying the potential thresholds between 50% and 100% in the reduction of CPD, 51 variables status were coded for each subject at each visit. Then, for each threshold of reduction and each subject, a time-to-event variable was determined as the duration from the first visit when the threshold was reached to the time when the threshold was not reached anymore, or to the end of follow-up. Consequently, data from the visits during which the threshold was not already reached and participants who never reached the threshold were excluded from analysis **(Figure 4.1.1)**. Fifty-one hazard-ratios (HR) representing the risk of relapse (for each one of 51 potential thresholds) were estimated to assess the effect of treatment (varenicline vs. nicotine replacement therapy) by adjusting for nicotine-metabolite ratio (slow metabolizers vs. normal metabolizers) and sex (men vs. women) in fitting a Cox proportional hazard regression model.

HR and the 95%CI are presented in **Supplementary Figure S4.1.1**. With increasing threshold, the number of subjects included in each analysis decreased and the number of relapses increased. From 90% to 100% of CPD decrease, the 95%CI of the HR was the smallest, stable, and mostly below the value of 1. Two thresholds of reduction: the first one considering total abstinence: diminution of 100%, and the second one considering a partial reduction of smoking: diminution of 90%, were therefore selected for further analysis.

Relapse analysis for a total cessation and a partial reduction of CPD

Participants were considered as relapser if their reduction in CPD consumption became lower than the predefined threshold and as no relapser if they maintained the predefined threshold of CPD reduction during the overall period of follow-up. In the overall sample, Chi-squared tests evaluated the distributions of treatment group, nicotine metabolism (phenotypically determined by the nicotine-metabolite ratio) and sex among the relapsers and non relapsers as well as the distribution of the normal metabolizers and slow metabolizers among men and women. The cumulative probability of maintaining the selected threshold of CPD reduction by treatment group in the overall sample analysis was assessed using Kaplan-Meier survival analyses with subsequent Log-Rank tests.

Multivariate Cox proportional hazards regression models were carried out to obtain the HR of relapse and its 95%CI for the treatment (varenicline vs. NRT) in the overall sample and in subgroups: normal metabolizers, slow metabolizers, men and women. The analyses were adjusted for nicotine-metabolite ratio, nicotine-metabolite ratio-by-treatment interaction, sex, age of onset for smoking and the number of CPD usually smoked in the overall sample. In normal and slow metabolizers, the analyses were adjusted for sex, age of onset for smoking and the number of CPD usually smoked. In men and women, the adjustment was made by the nicotine-metabolite ratio, the nicotine-metabolite ratio-by-treatment interaction, the age of onset for smoking and the number of CPD usually smoked. All statistical analyses were performed using the R software (v. 3.1.2, http://www.r-project.org). Results

were considered statistically significant if $p \leq 0.05$ (two-tailed). Finally, with regards to the Cox regression, proportional hazard assumption was verified using the Grambsch Therneau test and results were satisfactory.[38]

Influence of the genetically determined nicotine metabolism on the treatment response

To investigate the effect of nicotine metabolisation effect on risk of relapse, the association of nicotine metabolism genetically determined (normal and slow metabolizers) versus the relapse status (relapser and no relapser) was first assessed using Chi-squared test. Then, the sample was stratified between normal metabolizers and slow metabolizers to obtain the HR of relapse and its 95% CI for the treatment (varenicline vs. NRT) in two subgroups using Cox proportional hazards regression models by adjusting for sex, age of onset for smoking and the number of CPD usually smoked.

IV.1.4. RESULTS

Study participants and analysed sample

211 smokers were recruited for a smoking cessation study and 194 subjects fulfilled the inclusion criteria. 153 and 164 participants were analyzed for the 100% (total cessation) and for the 90% (partial decrease) of CPD decrease, respectively **(Figure 4.1.1)**.

Participants did not differ in any of the examined variables except in the age of onset for smoking with a lower age in the varenicline compared to the NRT group **(Table 4.1.1)**. In addition, in the analysis of a 90% of CPD decrease, the number of usually smoked CPD was significantly smaller in the NRT group than in the varenicline group (n=20 CPD and n=24 CPD respectively, p=0.03), but there was no significant difference in the number of smoked CPD the week before the study. Separate data between male and female are shown in **Supplementary Table S4.1.1**. Of note, the number of usually smoked cigarettes per day is significantly different between NRT and varenicline in males but not in females, which was taken into account in the statistical analysis. Although compliance cannot be ascertained, all participants were asked about the actual intake of varenicline or nicotine patches, gums or inhalers at each visit.

Table 4.1.1: Description of the population included in the analysis of a 100% and a 90% of CPD decrease

Characteristic	Analysis for a 100% of CPD decrease					Analysis for a 90% of CPD decrease				
	NRT (N=82)		Varenicline (N=71)		p	NRT (N=87)		Varenicline (N=77)		p
	N	%	N	%		N	%	N	%	
Men	43	52	33	46	0.57[2]	43	49	37	48	0.9[2]
Nicotine NM based on NMR	52	63	43	61	0.8[2]	56	64	47	61	0.8[2]
Nicotine NM based on genotyping	77	94	66	93	1[2]	76	87	65	84	0.75[2]
Treatment duration[4]					0.27[2]					0.21[2]
Less than or equal to 2 months	36	44	24	34		40	46	27	35	
More than 2 months	45	55	46	65		46	53	49	64	
Subjects with smokers at home	20	24	19	27	0.9[2]	20	23	20	26	0.8[2]
Ethnicities					0.9[3]					0.9[3]
Caucasian	79	97	68	97		84	97	74	97	
African/African-American	2	2	1	1		2	2	1	1	
Arabic	0	0	1	1		0	0	1	1	
Others	1	1	1	1		1	1	1	1	
	Mean	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**	
Age, years	41	11	38	10	0.1[1]	41	11	39	11	0.2[1]
Cotinine before the quit date, ng/ml	271	140	264	127	0.95[1]	269	138	262	124	0.96[1]

3'-hydroxycotinine before the quit date, ng/ml	87	50	89	51	0.69[1]	87	50	88	50	0.76[1]
Age of onset for smoking, years	19	7	16	3	0.009[1]	19	7	17	4	0.02[1]
Number of usually smoked CPD	20	8	22	10	0.07[1]	20	8	24	11	0.03[1]
Number of smoked CPD during the week before the study	19	8	20	10	0.63[1]	19	8	21	11	0.37[1]
Number of previous quit attempts	1.6	0.9	1.7	0.8	0.6[1]	1.6	0.9	1.7	0.8	0.7[1]
FTND[6]	5	2.1	5[5]	2.0[5]	0.8[1]	5	2.2	5[5]	2.0[5]	0.7[1]

CPD: Cigarettes Per Day, NRT: Nicotine Replacement Therapy, NM: Normal Metabolizer, NMR: Nicotine-metabolite Ratio, FTND: Fagerström Test for Nicotine Dependence

[1]: Wilcoxon rank sum test

[2]: Chi-squared test

[3]: Fisher exact test

[4]: For both analyses, the treatment duration was unknown for one subject in the NRT group and one subject in the varenicline group.

[5]: Missing data for one individual

[6]: Fagerström Test for Nicotine Dependence score ranged from 0 to 10.

When phenotypically determined, women were more likely to be normal metabolizers than slow metabolizers in the analyses for a 100% and a 90% of CPD decrease (**Table 4.1.2**, p=0.03 and p=0.01, respectively).

Table 4.1.2: Distribution between nicotine metabolism and sex

	Analysis for a 100% of CPD decrease			Analysis for a 90% of CPD decrease		
Sex	**NM[1]**	**SM[1]**	**p[2]**	**NM[1]**	**SM[1]**	**p[2]**
Men	40	36	0.03	42	38	0.01
Women	55	22		61	23	

CPD: Cigarettes Per Day, NM: Normal Metabolizer, SM: Slow Metabolizer

[1] phenotypically determined by the nicotine-metabolite ratio

[2] Chi-squared test

Relapse analysis for a total cessation and a partial reduction of CPD

For the decrease of 100% and 90%, relapse was significantly associated with the treatment (**Table 4.1.3**, p=0.04 and p=0.02, respectively), NRT associated with more cases of relapse.

Table 4.1.3: Distribution of study participants according to the binary variable: no relapse (status = 0) and relapse (status = 1), treatment, sex, nicotine metabolism phenotypically determined and nicotine metabolism genetically determined.

Variables	Analysis for a 100% of CPD decrease					Analysis for a 90% of CPD decrease				
	No relapse		Relapse		p	No relapse		Relapse		p
	N	%	N	%		N	%	N	%	
Treatment					0.04[1]					0.02[1]
NRT	47	57	35	43		61	70	26	30	
Varenicline	53	75	18	25		67	87	10	13	
Sex					0.34[1]					0.69[1]
Men	53	70	23	30		64	80	16	20	
Women	47	61	30	39		64	76	20	24	
Nicotine metabolism based on phenotype[2]					0.84[1]					0.46[1]
NM	61	64	34	36		78	76	25	24	
SM	39	67	19	33		50	82	11	18	
Nicotine metabolism based on genotype[3]					1[4]					1[1]
NM	86	66	45	34		110	78	31	22	
SM	14	64	8	36		18	78	5	22	

CPD: Cigarettes Per Day, NRT: Nicotine Replacement Therapy, NM: Normal Metabolizer, SM: Slow Metabolizer

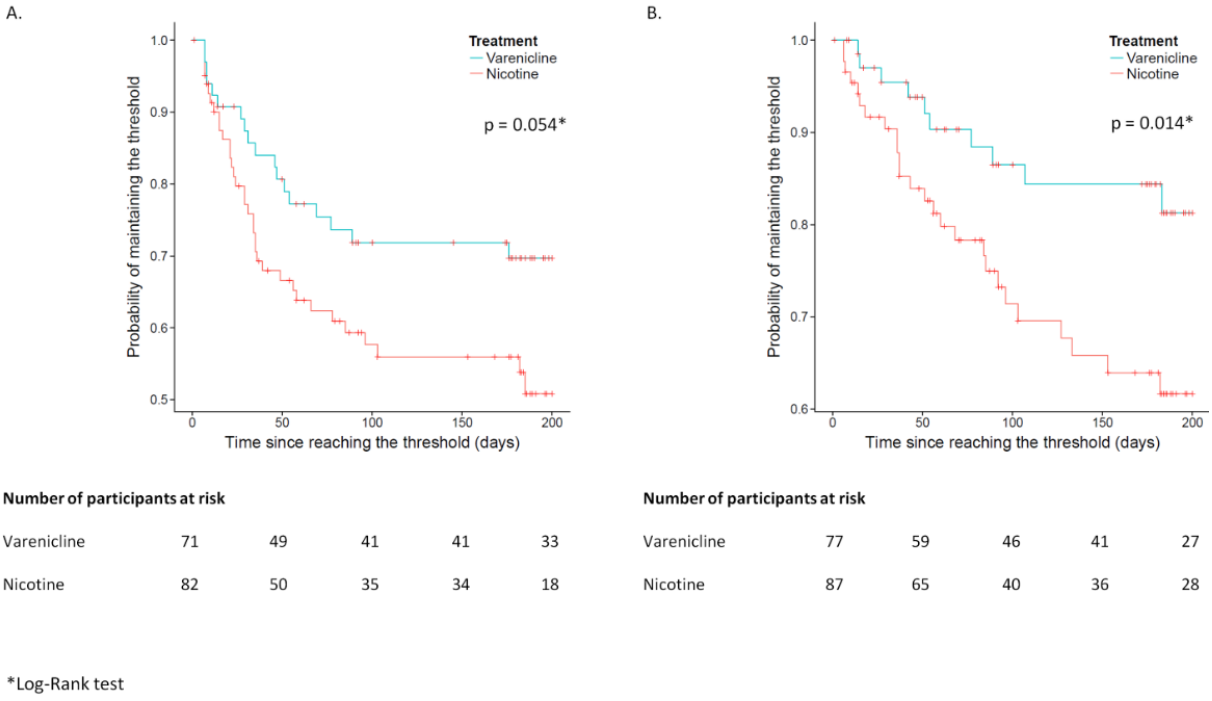
[1] Chi-squared test

[2] Phenotypically determined by the nicotine metabolic ratio (slow metabolizer if nicotine-metabolite ratio < 0.26 and normal metabolizer if nicotine-metabolite ratio >=0.26)

[3] Genetically determined by the genotyping of *CYP2A6* rs1801272 and rs28399433.

[4] Fisher exact test

The Kaplan-Meier estimate of the probability of maintaining the threshold was significantly higher for varenicline when compared to NRT for a 90% of CPD decrease (**Figure 4.1.2B**, p=0.014). The estimate was also higher for varenicline but not significant for a 100% of CPD decrease (**Figure 4.1.2A**, p=0.054).



Number of participants at risk

Varenicline	71	49	41	41	33
Nicotine	82	50	35	34	18

Number of participants at risk

Varenicline	77	59	46	41	27
Nicotine	87	65	40	36	28

*Log-Rank test

Figure 4.1.2: Kaplan-Meier estimates of probability of maintaining the threshold of -100% (A), and of -90% (B) in the overall analysis sample. Each cross on the curves represents a lost to follow-up.

The multivariate Cox regression predicted in the overall sample that participants treated with varenicline had a significant lower risk of relapse for a 90% of CPD decrease (**Table 4.1.4**, HR=0.34, 95%CI=[0.13-0.90]) and a non-significant lower risk of relapse for a 100% of CPD decrease (HR=0.49, 95%CI=[0.22-1.07]). In the nicotine normal metabolizers determined by phenotyping, varenicline was significantly better for a partial decrease (HR=0.33, 95%CI=[0.12-0.89]) and non-significantly better (HR=0.45, 95%CI=[0.20-1.0]) for a total cessation. In women, the risk of relapse with varenicline was

significantly lower for a partial decrease (HR=0.20, 95%CI=[0.04-0.91]) but non-significantly lower for a total cessation (HR=0.38, 95%CI=[0.14-1.0]). No treatment was found significantly better in slow metabolizers determined by phenotyping for a total cessation and a partial decrease (HR=0.70, 95%CI=[0.28-1.75] and HR=0.48, 95%CI=[0.14-1.7], respectively) and in men, for a total cessation and a partial decrease (HR=0.70, 95%CI=[0.20-2.5] and HR=0.55, 95%CI=[0.15-2.0], respectively).

Table 4.1.4: Adjusted hazard ratios of relapse for a 100% and a 90% of CPD decrease in the overall sample and in subgroups: normal metabolizers, slow metabolizers, men and women.

	Analysis for a 100% of CPD decrease			Analysis for a 90% of CPD decrease		
Analysis groups	**N**	**Hazard Ratio**	**95%CI**	**N**	**Hazard Ratio**	**95%CI**
In overall sample						
Treatment (V vs. NRT)	71 vs. 82	0.49	0.22-1.07	77 vs. 87	0.34	0.13-0.90
In NM (phenotyping)[1]						
Treatment (V vs. NRT)	43 vs. 52	0.45	0.20-1.0	47 vs. 56	0.33	0.12-0.89
In SM (phenotyping)[1]						
Treatment (V vs. NRT)	28 vs. 30	0.70	0.28-1.75	30 vs. 31	0.48	0.14-1.7
In NM (genotyping)[2]						
Treatment (V vs. NRT)	59 vs. 72	0.50	0.26-0.98	65 vs. 76	0.42	0.18-0.94
In SM (genotyping)[2]						
Treatment (V vs. NRT)	12 vs. 10	1.05	0.22-5.1	12 vs. 11	0.81	0.06-10.2
In men						
Treatment (V vs. NRT)	33 vs. 43	0.70	0.20-2.5	37 vs. 43	0.55	0.15-2.0
In women						
Treatment (V vs. NRT)	38 vs. 39	0.38	0.14-1.0	40 vs. 44	0.20	0.04-0.91

CPD: Cigarettes Per Day, V: Varenicline, NRT: Nicotine Replacement Therapy, SM: Slow Metabolizer, NM: Normal Metabolizer

1. Phenotypically determined by the nicotine-metabolite ratio (NMR) (SM if NMR < 0.26 and NM if NMR >=0.26)

2. Genetically determined by the CYP2A6 rs1801272 andrs28399433 genotyping.

Comparison of the influence of the nicotine metabolism determined either phenotypically or genetically, on treatment response

The frequency of slow metabolizers was found to be much lower than the normal metabolizers when classifying participants according to CYP2A6 genotype as compared to the classification based on phenotype **(Table 4.1.1)**.These differences could probably be explained by the fact that classification based on phenotyping includes environmental factors, while genotyping was based on two SNPs only, whereas other mutations are possibly contributing to a slow metabolizer phenotype.

For a partial decrease, the multivariate Cox regression predicted a similar significant lower risk of relapse with varenicline in the normal metabolizers determined by genotyping when compared to the normal metabolizers determined by phenotyping (**Table 4.1.4**, HR=0.42, 95%CI=[0.18-0.94] and HR=0.33, 95%CI=[0.12-0.89], respectively). But for a total cessation, the lower risk of relapse with varenicline in the normal metabolizers determined by genotyping was significant (HR=0.50, 95%CI=[0.26-0.98]) however it was non-significant in the normal metabolizers determined by phenotyping (HR=0.45, 95%CI=[0.20-1.0]). Concerning the slow metabolisers no treatment was found significantly better in the case of a genotype-based and phenotype-based determination (HR=0.81, 95%CI=[0.06-10.2] and HR=0.48, 95%CI=[0.14-1.7], respectively) for a partial decrease and a total cessation (HR=1.05, 95%CI=[0.22-5.1] and HR=0.70, 95%CI=[0.28-1.75], respectively).

IV.1.5. DISCUSSION

It has recently been shown that nicotine normal metabolizers (phenotypically determined by the nicotine-metabolite ratio) have better quit rates with varenicline compared to nicotine patches.[9] To our knowledge, the present study is the first to replicate this finding in usual clinical care data, using both phenotyping and genotyping tests. Varenicline response was superior to NRT in phenotype-based normal metabolizers in the case of a partial reduction. The finding that women smokers have higher response with varenicline over NRT for a partial reduction are in accordance with other recent findings.[19-21] The influence of sex in the effectiveness of the smoking cessation agent is now well acknowledged.[39, 40] Because CYP2A6 mRNA is induced by oestradiol[13], women and especially premenopausal women, may metabolize nicotine and cotinine faster than men.[15] Supposedly, with nicotine being rapidly metabolized in women, the pharmacological effect of NRT on withdrawal symptoms is lower, which is in agreement with the reported lower success rate with nicotine patches.[16] No difference was observed in treatment success in slow metabolizers and in men.

Of note, in normal metabolizers genetically determined by the rs1801272 and rs28399433 mutations, the multivariate Cox regression model showed similar response to treatment for a partial CPD decrease compared to the same subgroup phenotypically determined. For a total abstinence, response to treatment was similar in normal metabolizers genetically or phenotypically determined, except a lack of statistical power in the analysis to observe a significant result in the phenotype-based normal metabolizers. The similarity of the result could be explained, at least in part, by the demonstrated influence of these mutations on CYP2A6 activity.[11, 29, 30] On the other hand, it can be assumed that the prediction of CYP2A6 activity based on nicotine-metabolite ratio is more accurate, as it integrates both genetic factors (taking into account all genetic variations and not only one mutation) and environmental factors (e.g. induction by oestrogens).

In the overall sample analyzed, the probability of maintaining a diminution of 90% of smoked cigarettes per day over 6 months was higher in the varenicline group than in the NRT group, while a trend was

found when using a total abstinence as threshold. These results are not in agreement with the equivalent efficacy of varenicline and combined NRT reported in a meta-analysis[5] but agreed in a more recent meta-analysis comparing both treatments.[41] It has to be stressed that the sample size in the present study was limited and the results should therefore be replicated. However, a special emphasis should be put on the selection of outcomes, which differ between the present and the meta-analysis:[5] time during which a threshold of total cessation or partial reduction in CPD is maintained vs. continuous or prolonged abstinence at least 6 months from the start of the treatment, respectively.

In the present study, the analyses considered an absolute abstinence (reduction of 100%) as well as a partial reduction in CPD (90% reduction). Both thresholds gave essentially similar results. It should be mentioned that reduction in CPD consumption (instead of cessation) does not cancel health risks: a significantly higher risk of dying, especially from ischaemic heart disease and lung cancer, was observed in men and women smoking 1 to 4 cigarettes per day compared to never-smokers.[42] Thus, changes in CO and cotinine plasma levels consistently showed smaller reduction than CPD[43] due to the phenomenon of oversmoking: smokers involuntarily increase the number and depth of inhalations from the remaining cigarettes to obtain the necessary nicotine quantity.[44] This phenomenon is also observed in the present study: for the analysis of 90% of CPD decrease when compared to the NRT group, the number of usually smoked CPD is significantly lower in the varenicline group while there is no difference in cotinine and 3'-hydroxycotinine plasma levels. On the other hand, consumption reduction seems not to discourage smokers unmotivated to a project of total abstinence and can even encourage them when it is supported by NRT.[43, 45] Gradual reduction before the quit day appears to be associated with equivalent smoking cessation rates compared with abrupt cessation.[46] Sustained reduction was defined as reduction in 50% or more of the baseline reported cigarettes consumption in some industry sponsored trials.[47] In this work, when the reduction in cigarette consumption was below 90% of baseline, more subjects reached this objective, the number of relapses was lower and Cox regressions provided a very large HR 95%CI. We hypothesize that decreasing the cigarettes

consumption by less than 90% of baseline value is a more feasible objective, leading to the difference between treatment responses being smaller with a large HR 95%CI.

Several limitations of the present study must be discussed. Firstly, this clinical and pharmacogenetic study was not designed for the purpose of the present evaluation. However the valuable comparison of varenicline and NRT effectiveness by nicotine metabolism and sex obtained with clinical trials previously published deserved an investigation in the natural context of a smoking cessation program as proposed in tobacco consultation. Secondly the analysis used a unique longitudinal dataset with rather small sample size per treatment group and no corrections for multiple testing were performed. Analyses should therefore be repeated in a larger cohort to confirm the influence of sex and nicotine metabolism on the response to varenicline and nicotine replacement therapy. However, the present findings are in agreement with a recently published study[9], and it is remarkable that similar results were obtained when examining the influence of CYP2A6 activity based both on phenotyping and genotyping methods. Thirdly, this study was not randomized and it is not known whether the choice of the treatment by the participant could have an influence on the results. However the significant difference in age of onset for smoking and in number of usually smoked cigarettes per day between the varenicline and the NRT group has been taking into account in the multivariate analysis by the correction with these two variables.

In summary, treatment of nicotine dependence is very challenging and can be hampered by the lack of motivation, environmental factors or ineffective pharmacological treatment.[48] In the present work, we showed that nicotine normal metabolizers are more likely to benefit from varenicline over NRT in usual clinical care data. Women who are reported to achieve a lower abstinence rate with NRT than men may have better success in smoking cessation with varenicline treatment. Future studies should also address the question on whether normal metabolizers and women would equally benefit, instead of varenicline, from higher nicotine doses. Because of the scarcity of existing data, our results contribute valuably to the extensive process of tailoring smoking cessation strategy.

IV.1.6. SUPPLEMENTARY MATERIAL

Table S4.1.1: Description of the population included in the analysis of a 100% and a 90% of CPD decrease by sex

Characteristic	Analysis for a 100% of CPD decrease In males			Analysis for a 90% of CPD decrease In males			Analysis for a 100% of CPD decrease In females			Analysis for a 90% of CPD decrease In females		
	NRT (N=43) N	Varenicline (N=33) N	p	NRT (N=43) N	Varenicline (N=37) N	p	NRT (N=39) N	Varenicline (N=38) N	p	NRT (N=44) N	Varenicline (N=40) N	p
Nicotine NM based on NMR	23	17	1[2]	23	19	1[2]	29	26	0.7[2]	33	28	0.8[2]
Nicotine NM based on genotyping	40	30	1[3]	40	34	1[3]	32	29	0.7[2]	36	31	0.8[2]
Treatment duration[4]			0.6[2]			0.7[2]			0.4[2]			0.3[2]
Less than or equal to 2 months	17	10		17	12		19	14		23	15	
More than 2 months	25	22		25	24		20	24		21	25	
Subjects with smokers at home	9	9	0.7[2]	9	10	0.7[2]	11	10	1[2]	11	10	1[2]
Ethnicities			0.7[3]			0.7[3]			1[3]			0.8[3]
Caucasian	42	31		42	35		37	37		42	39	
African/African-American	1	1		1	1		1	0		1	0	
Arabic	0	1		0	1		0	0		0	0	
Others	0	0		0	0		1	1		1	2	

	Mean (sd)	Mean (sd)		Mean (sd)	Mean (sd)		Mean (sd)	Mean (sd)		Mean (sd)	Mean (sd)	
Age, years	41 (11)	38 (9)	0.2[1]	42 (11)	39 (10)	0.3[1]	40 (12)	38 (11)	0.4[1]	40 (11)	39 (12)	0.6[1]
Cotinine before the quit date, ng/ml	288 (162)	288 (124)	0.6[1]	288 (162)	288 (120)	0.6[1]	252 (108)	242 (126)	0.7[1]	251 (108)	238 (125)	0.5[1]
3'-hydroxycotinine before the quit date, ng/ml	85 (53)	87 (56)	0.9[1]	85 (53)	87 (56)	0.9[1]	89 (48)	90 (46)	0.8[1]	89 (47)	89 (45)	0.8[1]
Age of onset for smoking, years	18 (6)	17 (4)	0.06[1]	18 (6)	17 (4)	0.05[1]	19 (8)	16 (3)	0.09[1]	19 (8)	17 (4)	0.2[1]
Number of usually smoked CPD	21 (9)	25 (10)	0.03[1]	21 (9)	26 (10)	0.02[1]	19 (7)	21 (11)	0.7[1]	20 (7)	22 (12)	0.5[1]
Number of smoked CPD during the week before the study	20 (9)	22 (10)	0.2[1]	20 (9)	23 (10)	0.2[1]	19 (7)	19 (11)	0.7[1]	19 (8)	20 (12)	1[1]
Number of previous quit attempts	1.7 (0.9)	1.7 (0.7)	0.8[1]	1.7 (0.9)	1.6 (0.7)	0.7[1]	1.6 (0.9)	1.8 (0.9)	0.3[1]	1.5 (0.9)	1.8 (0.9)	0.3[1]
FTND[6]	4.7 (2.2)	5.1 (2.1)[5]	0.3[1]	4.7 (2.2)	5.2 (2.2)	0.2[1]	5.4 (2.0)	5.0 (2.1)	0.4[1]	5.4 (2.1)	5.0 (2.1)	0.5[1]

CPD: Cigarettes Per Day, NRT: Nicotine Replacement Therapy, NM: Normal Metabolizer, NMR: Nicotine-metabolite Ratio, FTND: Fagerström Test for Nicotine Dependence

[1]: Wilcoxon rank sum test

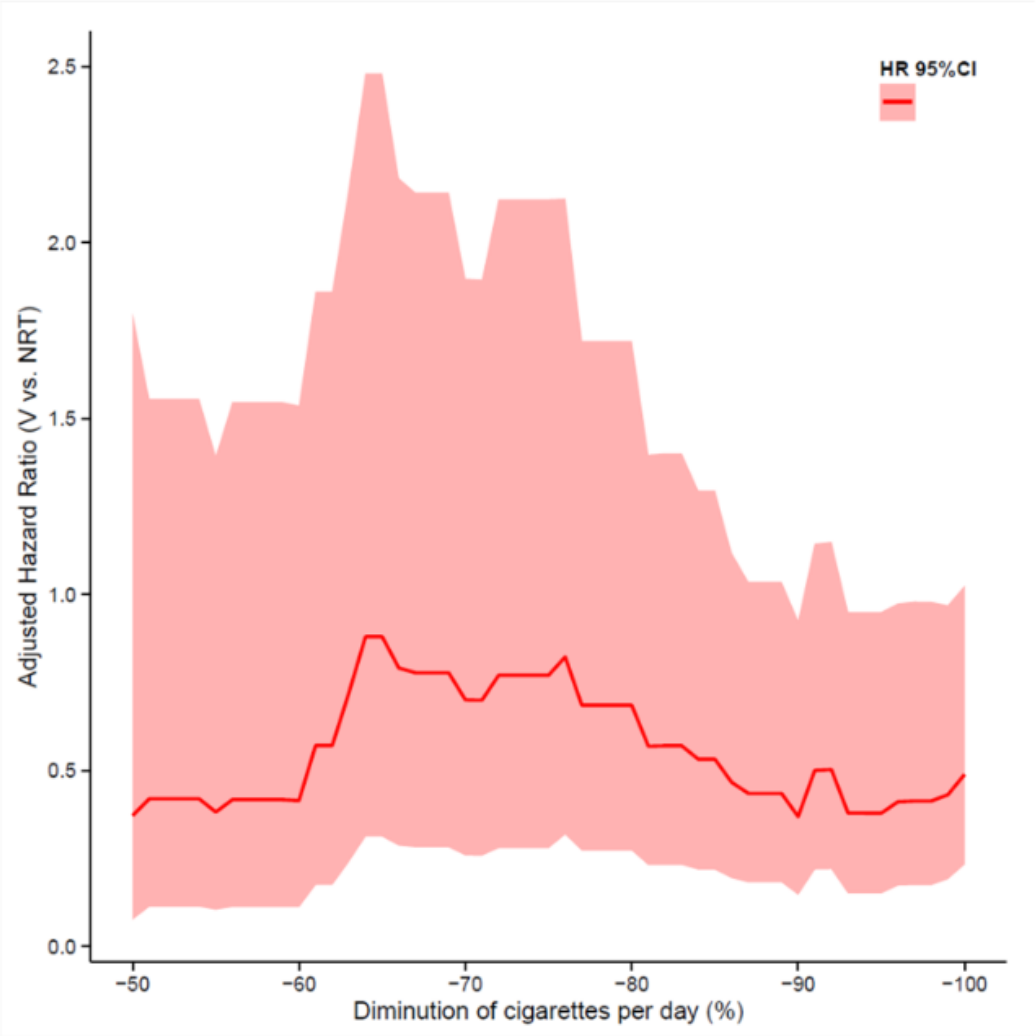
[2]: Chi-squared test

[3]: Fisher exact test

[4]: For both analyses in males, the treatment duration was unknown for one subject in the NRT group and one subject in the varenicline group.

[5]: Missing data for one individual

[6]: Fagerström Test for Nicotine Dependence score ranged from 0 to 10



n subjects	170	168	169	166	164	153
n relapses	13	18	24	27	36	53

V: Varenicline, NRT: Nicotine Replacement Therapy

Figure S4.1.1: Hazard ratios (HR) depending on the threshold of cigarettes per day decrease used in each analysis. The line represents the hazard ratio; the area represents the 95% confidence interval (95% CI) of the hazard ratio. The sample size (n subjects) and the number of relapse are reported for each analysis with a rounding threshold (-50%, -60%, -70%, -80%, -90%, -100%).

IV.1.7. REFERENCES

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IV.2. Influence of body weight and *UGT2B7* polymorphism on varenicline exposure in a cohort of smokers from the general population

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IV.2.1 ABSTRACT

The abstinence rate to tobacco after varenicline treatment is moderate and might be partially affected by variability in varenicline concentrations. This study aimed at characterizing the sources of variability in varenicline pharmacokinetics and to relate varenicline exposure to abstinence. The population pharmacokinetic analysis (NONMEM®) included 121 varenicline concentrations from 82 individuals and tested the influence of genetic and non-genetic characteristics on apparent clearance (CL/F) and volume of distribution (V/F). Model-based average concentrations over 24 h (C_{av}) were used to test the impact of varenicline exposure on the input rate (Kin) expressed as a function of the number of cigarettes per day in a turnover model of 373 expired carbon monoxide levels. A one-compartment model with first order absorption and elimination appropriately described varenicline concentrations. CL/F was 8.5 L/h (coefficient of variation, 26%), V/F was 228 L and the absorption rate (k_a) was fixed to 0.98 h^{-1}. CL/F increased by 46% in 100-kg individuals compared to 60-kg individuals and was found to be 21% higher in *UGT2B7* rs7439366 TT individuals. These covariates explained 14% and 9% of the inter-individual variability in CL/F, respectively. No influence of varenicline C_{av} was found on Kin in addition to the number of cigarettes. Body weight mostly and to a smaller extent genetic polymorphisms of *UGT2B7* can influence varenicline exposure. Dose adjustment based on body weight and, if available, on *UGT2B7* genotype might be useful to improve clinical efficacy and tolerability of varenicline.

IV.2.2 INTRODUCTION

Varenicline, a selective partial agonist of the $\alpha_4\beta_2$ nicotinic acetylcholine receptor, indicated as an aid to smoking cessation, has a moderate efficacy on smoking abstinence at best. In more than 160,000 treatment episodes of smokers from smoking cessation clinic, the reported 4-week success rate was 44%.[1] The continuous abstinence rate was 44% during the treatment period of week 9 to 12 and decreased to 30% between the week 9 to 24 in a randomized trial including 1000 smokers.[2] Based on dose-ranging studies, the manufacturer established an exposure-response relationship characterized by an increase of the probability of continuous abstinence during the treatment period from the week 9 to 12 (9-12 CAR) with increasing varenicline exposure. The probability of 9-12 CAR was estimated from 0.3 to 0.7 over the range of varenicline $AUC_{0\text{-}24,SS}$ from 60 to 450 ng.h/mL in individuals receiving the recommended dose 1 mg b.i.d.[3]

The drug is mainly eliminated in the form of the unchanged molecule (>90%) and in two pharmacologically inactive metabolites (<10%) of which one is obtained by glucuronidation via the UDP-glucuronosyltransferase 2B7 isoform (UGT2B7).[4, 5] Varenicline is excreted unchanged in urine mainly via glomerular filtration but active tubular secretion also occurs trough organic cation transporter 2 (OCT2).[5] A variety of genetic polymorphisms of the *SLC22A2* gene coding for the OCT2 transporter and of the *UGT2B7* gene might have an influence on varenicline exposure. Expression of the genes coding for phase I and phase II drug-metabolizing enzymes and transporters, such as UGT2B7 and OCT2 are regulated by transcription factors such as: the constitutive androstane receptor (CAR, encoded by the *NR1*|*3* gene), the pregnane X receptor (PXR, encoded by the *NR1*|*2* gene), the estrogen receptor 1 (encoded by the *ESR1* gene), the farnesoid X receptor (FXR, encoded by the *NR1H4* gene), the peroxisome proliferator activated receptor gamma (PPARγ encoded by the *NR1C2* gene) and its coactivator PPAR coactivator 1-alpha (PPARGC1A) which all have genetic polymorphisms.[6-14] Finally, genetic variability in the $\alpha_4\beta_2$ nicotinic acetylcholine receptor (*CHRNA4* gene) where varenicline binds might also explain variability in the effectiveness of the drug. To date, a published population

pharmacokinetic analysis of varenicline showed the influence of demographic and clinical variation on drug exposure but genetic characteristics were not studied.[15]

The objectives of this study were to develop a population pharmacokinetic model to assess the influence of clinical and genetics factors on varenicline exposure, and to further explore the relationships between varenicline blood levels and treatment success measured by the expired carbon monoxide (CO) during a 3-month treatment period.

IV.2.3 MATERIALS AND METHODS

Study population and design

Smokers from the general population wishing to participate in a smoking cessation program were recruited in the clinical and pharmacogenetic study. The clinical and pharmacogenetic study consisted of a 3-month study period (5 visits every week from week 0 to week 4, 4 visits every 2 weeks from week 4 to week 12) comprising smoking cessation counselling and pharmacological treatment prescription (combined nicotine replacement therapies: patches, gums and/or inhaler or varenicline), and a 6-month concluding visit. Details of the inclusion and exclusion criteria as well as clinical measurements have been previously described.[16] Among the 194 smokers included in the study, 95 subjects chose to receive varenicline. Two blood samplings performed at the beginning and after one month of treatment were used to determine varenicline and cotinine plasma concentrations. Cotinine is the major metabolite of nicotine with a half-life of 16-17 hours. Subjects are biochemically confirmed abstinent from smoking if plasma cotinine ≤ 15 ng/Ml.[17-19] At each visit, abstinence from smoking was assessed by self-declaration and by expired CO levels (Micro Smokerlyzer; Bedfont Scientific, Rochester, England) lower than 10 ppm.[19, 20] Smoking withdrawal symptoms were also recorded by a self-administered questionnaire.[21] Each symptom was assessed by 3 or 4 questions and summarized with a score from a minimal to a maximal grade as follow: depression (min: 4 - max: 20), craving (4-20), irritation (4-20), concentration (3-15), appetite and weight gain (3-15), and insomnia (3-15). Co-medications were also registered. The study was approved by the Ethics Committee of the Lausanne University Medical School and by the Swiss Agency for Therapeutic Products (Swissmedic, Bern, Switzerland). Written informed consent was obtained from all participants.

Varenicline and cotinine concentration measurements

Briefly, all blood samples were collected in EDTA-containing tubes. After centrifugation, plasma samples were stored at -20°C until analysis. Varenicline and cotinine plasma concentrations were determined by ultra performance liquid chromatography coupled with tandem mass spectrometry as previously described.[22] The analytical method was validated according to Food and Drug Administration guidelines and to Société Française des Sciences et Techniques Pharmaceutiques with the following assessments and results: accuracy (96.1–108.2%) and (90.6-97.8%), repeatability (4.6–12.3%) and (5.0-8.8%) and intermediate precision (4.6–15.9%) and (5.0-8.8%) for varenicline and cotinine, respectively. The limit of quantification (LOQ) for both compounds was 1 ng/mL.

Genotyping

Genomic DNA was extracted from EDTA blood sample at the baseline visit using the FlexiGene DNA extraction kit (Qiagen Instruments AG, Hombrechtikon, Switzerland) according to the manufacturer's protocol. The following SNPs were selected based on previously published pharmacogenetic studies and on their minor allele frequency (MAF) in the Caucasian population:[7, 8, 23-37] *SLC22A2* (rs316003), *SLC22A2* (rs316019), *SLC22A2* (rs2279463), *UGT2B7* 802T>C (rs7439366), *NR1*|*2* (rs1523130), *NR1*|*2* (rs2472677), *NR1*|*2* (rs7643645), *NR1*|*3* (rs2307424), *NR1*|*3* (rs4073054), *NR1*|*3* (rs2502815), *RXRA* (rs3132297), *RXRA* (rs3818740), *PPARG* (rs3856806), *PPARG* (rs1801282), *PPARGC1A* (rs8192678), *ESR1* (rs6902771), *ESR1* (rs9322336), *FXR* (rs4764980), *HNF1* (rs1169288), *CHRNA4* (rs1044396). The single nucleotide polymorphisms (SNP) tested in the pharmacokinetic model were genotyped by a real-time-PCR with 5'-nuclease allele discrimination assays (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland). The *CHRNA4* (rs1044396) polymorphism tested in the exposure-response analysis was obtained using the Cardio-MetaboChip, a custom Illumina iSelect genotyping array designed to test DNA variation of over 200,000 SNPs. GenomeStudio Data Analysis Software was used to export results generated by Illumina Cardio-MetaboChip. Quality control

excluded samples from the analysis if sex was inconsistent with genetic data from X-linked markers, genotype call rate less than 0.96 or Gene Call score less than 0.15. All these SNPs were in Hardy Weinberg Equilibrium ($p>0.05$).

Pharmacokinetic-pharmacodynamic analysis

Non-linear mixed effect modelling was performed using NONMEM® version 7.3 program[38] with the PsN-Toolkit (version 3.2.4).[39]

Base model

A stepwise procedure was used to identify models that best fitted the data. Multi-compartment models with linear elimination were first fitted to the data to determine the appropriate structural model, which was finally identified as a one-compartment model. Since varenicline was administered orally, apparent clearance (CL/F) and volume of distribution (V/F) were estimated (where F is the absolute oral bioavailability). Exponential errors following a log-normal distribution were assumed for the description of inter-individual variability of the parameters. Proportional, additive and combined proportional-additive error models were finally compared to describe the residual variability.

Exposure-response analysis

Average varenicline concentration over 24 hours (C_{av}) at each visit day assuming a complete adherence to treatment were derived using:

$$C_{av} = AUC_{0\text{-}24}/24 \quad (1)$$

where AUC_{0-24} were computed analytically in NONMEM® based on the individual dose history and pharmacokinetic parameters.

Only data from visits during the period of effective dose (1 mg twice daily) were included. Whenever a cotinine measurement was available, cotinine and CO levels were compared based on the definition of abstinence (≤ 15 ng/mL and < 10 ppm, respectively). If they were discordant, data of the visit were excluded from further analyses.

CO observations were used as a clinical marker of response to varenicline and were described by an indirect response model using the following equations describing a basal turnover:

$$dCO/dt = Kin - Kout * CO$$

$$\text{initial condition: } CO(0) = Kin / Kout \quad (2)$$

$$Kin = A + EFFCPD * CPD \quad (3)$$

where Kin is a zero order input rate and Kout a first-order degradation rate. The effect (EFFCPD) of the number of cigarettes per day (CPD) on Kin was directly integrated in the model as a surrogate marker of varenicline therapy. Finally, A represents the contribution of all other sources of CO production (environmental sources and endogenous metabolism) to Kin.[40]

Covariate analysis

Bayesian estimates of the pharmacokinetic parameters were derived and plotted against the available subject characteristics (body weight (BW), age, ethnicity, sex, height, glomerular filtration rate (GFR)

estimated with the Modification of Diet in Renal Disease (MDRD) formula[41], smoking status, co-medications and several genetic polymorphisms (see **Table 4.2.1**)). Potentially influencing covariates were then incorporated sequentially in the model and tested for significance on the pharmacokinetic parameters. The covariate analysis was performed using a stepwise insertion/deletion approach testing linear or non-linear functions as appropriate (categorical covariates coded as 0 and 1, continuous covariates centered on their median value). Parameter values were estimated for each genotypic group (rich model), defined as the reference allele group (Ref) and alternative allele groups: heterozygote alternative (Het-Alt) and homozygous alternative (Hom-Alt) or for further regrouped (reduced model) subpopulations.

In the exposure-response analysis, several covariates (continuous: C_{av}, nicotine metabolite ratio, *i.e.* 3-hydroxycotinine/cotinine ratio during smoking ad libitum, BW, age, study duration and recorded scores of smoking withdrawal symptoms; categorical: sex, *UGT2B7* 802T>C (rs7439366) and *CHRNA4* (rs1044396) polymorphisms) were tested on EFFCPD. Influence of sex, age and BW on A parameter was also investigated.

Table 4.2.2: Baseline characteristics and genetic polymorphisms of the populations evaluated in the pharmacokinetics and the exposure-response analyses of varenicline.

Baseline characteristics	Pharmacokinetic analysis subjects		Exposure-response analysis subjects	
	Value	% or range	Value	% or range
Demographic characteristics				
Median age (yr)	39	19-64	38	19-64
Median BW (kg)	77	45-131	76	50-131
Median height (cm)	172	155-197	172	155-197
Sex (men/women) (no.)	41/41	50/50	37/39	51/49
Ethnicity (no.)				
Caucasian	78	95	72	95
Asian	1	1	1	1
African-Americans	1	1	1	1
Others	2	3	2	3
Clinical characteristics				
Median GFR [a] (mL/min)	117	77-180	115	77-180
Median NMR	0.32	0.03-0.84	0.32	0.03-0.84
Concomitant medications (no/yes)				
OCT Substrates/Inhibitors[b]	78/5	95/5	73/3	96/4
PXR agonists[c]	79/3	96/4	74/2	97/3
Genetic polymorphisms	**Genotypes**	**Value**	**Frequencies**	**Value**	**Frequencies**
SLC22A2					
rs316019	GG/GT/TT	65/16/1	79/20/1	-	-
rs316003	TT/TC/CC	43/35/4	52/43/5	-	-
rs2279463	AA/AG/GG	63/16/3	77/19/4	-	-
UGT2B7					
rs7439366	TT/TC/CC	20/41/21	24/50/26	17/39/20	23/51/26
NR1|2					
rs1523130	CC/CT/TT	36/39/7	44/48/8	-	-
rs2472677	TT/TC/CC	30/39/13	36/48/16	-	-
rs7643645	AA/AG/GG	31/36/15	38/44/18	-	-
NR1|3					
rs2307424	CC/CT/TT	35/33/14	43/40/17	-	-

rs4073054	TT/TG/GG	36/34/12	44/41/15	-	-
rs2502815	CC/CT/TT	47/30/5	57/37/6	-	-
RXRA					
rs3132297	CC/CT/TT	54/25/3	66/30/4	-	-
rs3818740	TT/TC/CC	39/33/10	48/40/12	-	-
PPARG					
rs1801282	CC/CG/GG	66/16/0	80/20/0	-	-
rs3856806	CC/CT/TT	70/12/0	85/15/0	-	-
PPARGC1A					
rs8192678	CC/CT/TT	40/37/5	49/45/6	-	-
ESR1					
rs6902771	CC/CT/TT	31/33/18	38/40/22	-	-
rs9322336	CC/CT/TT	2/17/63	2/21/77	-	-
FXR					
rs4764980	AA/AG/GG	26/34/22	32/41/27	-	-
HNF1					
rs1169288	AA/AC/CC	34/37/11	42/45/13	-	-
CHRNA4					
rs1044396	AA/AG/GG	19/47/16	23/57/20	18/44/14	24/58/18

a. GFR, glomerular filtration rate estimated by the Modification of Diet in Renal Disease equation.[41]

b. OCT substrates/inhibitors were amlodipine, candesartan, carvedilol, flupenthixol, hydroclorothiazide, metformin and pantoprazole.[42]

c. PXR agonists were atorvastatin, simvastatin, omeprazole.[43, 44]

BW: Body weight

OCT: Organic Cation Transporter

PXR: Pregnane X receptor

NMR: Nicotine Metabolite Ratio

Parameter estimation and model selection

All models were fitted using the first-order conditional estimation method with interaction (FOCEI) with the subroutines ADVAN2 TRANS2 for the pharmacokinetic model and the subroutine ADVAN13 for the exposure-response analysis. The log likelihood ratio test, based on changes in the objective

function value (ΔOFV) was employed to discriminate between hierarchical models. Since a ΔOFV between any two models approximates a χ^2 distribution, a 3.8- (p=0.05) and 6.4- (p=0.01) point change of OFV was considered statistically significant for one additional parameter in model building and backward deletion steps, respectively. The Akaike's information criterion (AIC) was instead employed to choose between non-hierarchical models. Diagnostic goodness-of-fit plots, precision and plausibility of the model parameters were also used to assess the reliability of the results.

Model evaluation and simulation

For pharmacokinetic and exposure-response analyses, the final model stability was assessed by medians of the non-parametric bootstrap method implemented in PsN[39], generating 2000 datasets by re-sampling from the original dataset. Median parameters values with their 95%CI were thus derived and compared with the final model estimates. The predictive performance of the pharmacokinetic models was evaluated by calculation of the normalized prediction distribution errors (NPDEs)[45]. Visual predictive check (VPC) was finally performed with NONMEM® by simulations based on the final pharmacokinetic model with interpatient variability using 1000 *UGT2B7* TC (Het-Alt) or CC (Hom-Alt) individuals of 77 kg (median population BW) to calculate the average concentration time profile with 95% prediction intervals (95%PI).

The trough concentrations at steady-state ($Cmin_{SS}$) after administration of 1 mg b.i.d were calculated based on the final model in 500 individuals per strata of *UGT2B7* rs7439366 genotypes and BW values and then plotted with the trough concentrations range expected for the recommended dose of 1 mg b.i.d (4-5 ng/mL) reported in the clinical trials supporting the development of varenicline for comparison.[46] Figures were generated with GraphPad Prism® (Version 5.00 for Windows, GraphPad Software, San Diego California USA, http://www.graphpad.com/) and with R (v. 3.1.2, http://www.r-project.org).

IV.2.4 RESULTS

Study population and data

Among the 95 subjects enrolled in the study and receiving varenicline, 8 subjects had early treatment discontinuation and 5 subjects had no detectable varenicline levels suggesting a poor treatment adherence. The remaining 82 subjects provided a total of 121 concentrations for the pharmacokinetic analysis: 70 samples were collected at treatment initiation (from days 1 to 10) and 51 samples were collected after one month of treatment (from days 35 to 88). Subjects received varenicline according to the manufacturers' recommended dosage regimen except for 8 subjects who took 1 mg q.d. after the first week.[47] Varenicline steady-state plasma concentrations ranged from 1.5 to 3.5 ng/mL at dose 0.5 mg q.d. (during days 1-3), 1.6 to 8.2 ng/mL at dose 0.5 mg b.i.d. (during days 4-7) and 2.4 to 19.9 ng/mL at dose 1 mg b.i.d. (from day 8) and the time after dose ranged from 0.33 h to 55.5 h (median = 6.05 h). Subject's characteristics are presented in **Table 4.2.1**. For the exposure-response analysis, a total of 373 CO measurements from 76 patients were available (median= 2 ppm, range= 0-45 ppm). Varenicline C_{av} ranged from 2.7 to 12.9 ng/mL (8.5 ng/mL), number of cigarettes per day ranged from 0 to 40 (median=0), nicotine metabolite ratio ranged from 0.03 to 0.84 (median=0.32), study duration ranged from 11 days to 321 days (median=193 days), score ranged from 4 to 20 (median=5) for craving, from 4 to 20 (median = 4) for depression, from 4 to 20 (median=5) for irritation, from 3 to 15 (median = 3) for concentration, from 3 to 15 (median = 6) for appetite and weight gain and, finally, from 3 to 15 (median = 4) for insomnia.

Pharmacokinetic-pharmacodynamic analysis

Pharmacokinetic analysis

A one-compartment model with first-order absorption and elimination described adequately the data. No improvement to the fit was observed using a two-compartment model (ΔOFV = -0.3). Owing to very

limited measurements at early time points after drug intake, the absorption parameter k_a could not be estimated and was thus fixed to 0.98 h^{-1} to achieve a peak concentration 3-4 hours after drug intake as reported in literature.[15] The association of an inter-individual variability to V/F or k_a in addition to CL/F did not improve the model fit (ΔOFV ≥ -0.3). Finally, residual variability was described by a proportional error model. The estimates of the base pharmacokinetic model were a CL/F of 9.6 L/h and a V/F of 211 L. The inter-individual variability for CL/F was 26% with a shrinkage of 22% which is not misleading for covariate analysis.[48]

Univariate analyses showed that the effect of BW (ΔOFV = -14.5, p<0.001), height (ΔOFV = -11.1, p<0.001) and sex (ΔOFV = -7.4, p=0.007) on CL/F improved significantly the description of the data. In contrast, age, ethnicity, smoking status, co-medications and GFR were not associated to varenicline pharmacokinetics (ΔOFV > -2.2, p>0.14). Both linear and allometric power functions described adequately the relationship between CL/F and BW (ΔOFV ≤ -14.5). According to the AIC, the allometric power function was chosen to depict the BW impact on CL/F (AIC= 242 and AIC= 240 for the allometric and linear relationships, respectively).

Among all tested genetic covariates, only *UGT2B7* rs7439366 (ΔOFV = -9.0, p=0.003) and *NR1*|*2* rs1523130 (ΔOFV = -5.6, p=0.02) showed an influence on varenicline CL/F. No statistical significant differences in CL/F between *UGT2B7* rs7439366 Hom-Alt and Het-Alt individuals as well as between *NR1*|*2* rs1523130 Hom-Alt and Het-Alt individuals were observed compared to the rich model (ΔOFV > -0.03, p>0.87). A CL/F of 8.5 L/h was estimated for *UGT2B7* rs7439366 Hom-Alt and Het-Alt subjects and increased to 10.3 L/h for Ref individuals. *NR1*|*2* rs1523130 Hom-Alt and Het-Alt individuals were found to have a CL/F of 9.8 L/h, which decreased to 7.4 L/h for Ref subjects.

Multivariate analyses revealed that height and sex were correlated to BW, which was thus identified as the only significant non-genetic covariate influencing varenicline elimination. The inclusion of the *UGT2B7* rs7439366 polymorphism on CL/F further improved the description of the data (ΔOFV = -6.4, p=0.01). On the other hand *NR1*|*2* rs1523130 did not remain statistically significant (ΔOFV = 1.5,

p=0.22). Our final results suggest that CL/F is increased by 46% in 100-kg individuals compared to 60-kg individuals and by 21% in *UGT2B7* rs7439366 Ref individuals with respect to Hom-Alt and Het-Alt individuals. BW and the *UGT2B7* rs7439366 polymorphism explained 14% and 9% of the inter-individual variability in CL/F, respectively.

Of 2000 replicates analyzed during the bootstrap analysis of the pharmacokinetic model, 1% failed to minimize successfully and were excluded. The bootstrap and the final model results are presented in **Table 4.2.2**. The model was considered reliable since the parameter estimates differed less than 10% from the bootstrap medians and lied within the bootstrap 95%CI.

Table 4.2.3: Final varenicline population pharmacokinetic parameter estimates and their bootstrap evaluations.

Parameter	Final population parameter		Bootstrap evaluation (n=2000 samples)		Difference (%)[e]
	Estimate	RSE (%)[a]	Median	$CI_{95\%}$	
CL/F (L/h)	$8.5x(BW/70)^{0.75}$	4	$8.5\cdot(BW/70)^{0.75}$	(7.9; 9.2)	-0.3
EFFUGT[b]	0.21	49	21	(0.4; 44)	3.3
V/F (L)	228	8	229	(192; 275)	0.8
k_a (h^{-1})	0.98	--	0.98	--	--
IIV CL/F (CV%)[c]	19	22	18	(9; 26)	-5.3
σ (CV%)[d]	23	18	23	(19; 27)	0

Final model: CL/F (L/h) = $8.5 \times (BW/70)^{0.75} \times (1+Q \times 0.21)$ with Q=1 if *UGT2B7* rs7439366 Ref subjects and Q=0 if *UGT2B7* rs7439366 Hom-Alt/Het-Alt subjects.

CL/F, typical apparent clearance for *UGT2B7* rs7439366 Hom-Alt/Het-Alt subjects, function of subjects' body weight (BW); V/F, typical apparent volume of distribution; k_a, typical absorption rate constant.

a. Relative standard errors of the estimates (SE) defined as SE/estimate directly retrieved from NONMEM®.

b. Relative deviation of CL/F due to *UGT2B7* rs7439366 Ref subjects.

c. Intersubject variability defined as CVs (%).

d. Residual intrasubject variability expressed as CVs (%).

e. Difference (%) = (bootstrap median value – typical value from final model)/ typical value from final model x 100.

NPDE distribution was not found to be significantly different from a normal distribution and the goodness-of-fit plots were satisfactory meaning that the model adequately described the observed data **(Supplementary Figures S4.2.1 and S4.2.2)**. The VPC of the observed dose-normalized concentrations versus time from the beginning of the treatment is shown in **Figure 4.2.1** and the prediction- and variability-corrected VPC of the concentrations versus time after dose is shown in **Supplementary Figure S4.2.3**.

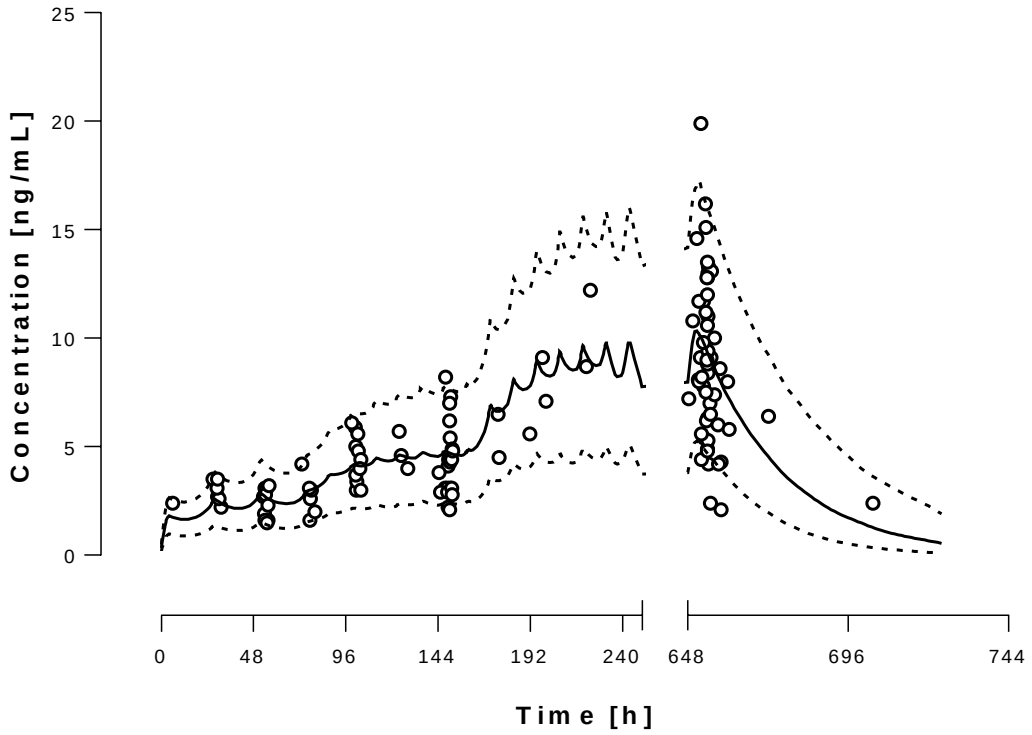

Figure 4.2.1: Observed dose-normalized varenicline plasma concentrations and simulated dose-normalized varenicline plasma concentrations after administration of 0.5 mg q.d. during days 1-3, 0.5 mg b.i.d. during days 4-7 and 1 mg b.i.d. from day 8 to the end of the therapy, with mean population prediction (solid lines) and 95% prediction intervals (dotted lines) computed for 1000 UGT2B7 rs7439366 TC or CC carriers weighting 77 kg, the mean population body weight.

Major differences in varenicline exposure were observed with regard to BW and *UGT2B7* rs7439366 polymorphism in **Figure 4.2.2**. In patients with high BW, 31% and 72% of Ref and Hom-Alt/Het-Alt individuals, respectively are under the targeted range. Patients with very low BW and especially Hom-Alt/Het-Alt individuals are over the targeted range.

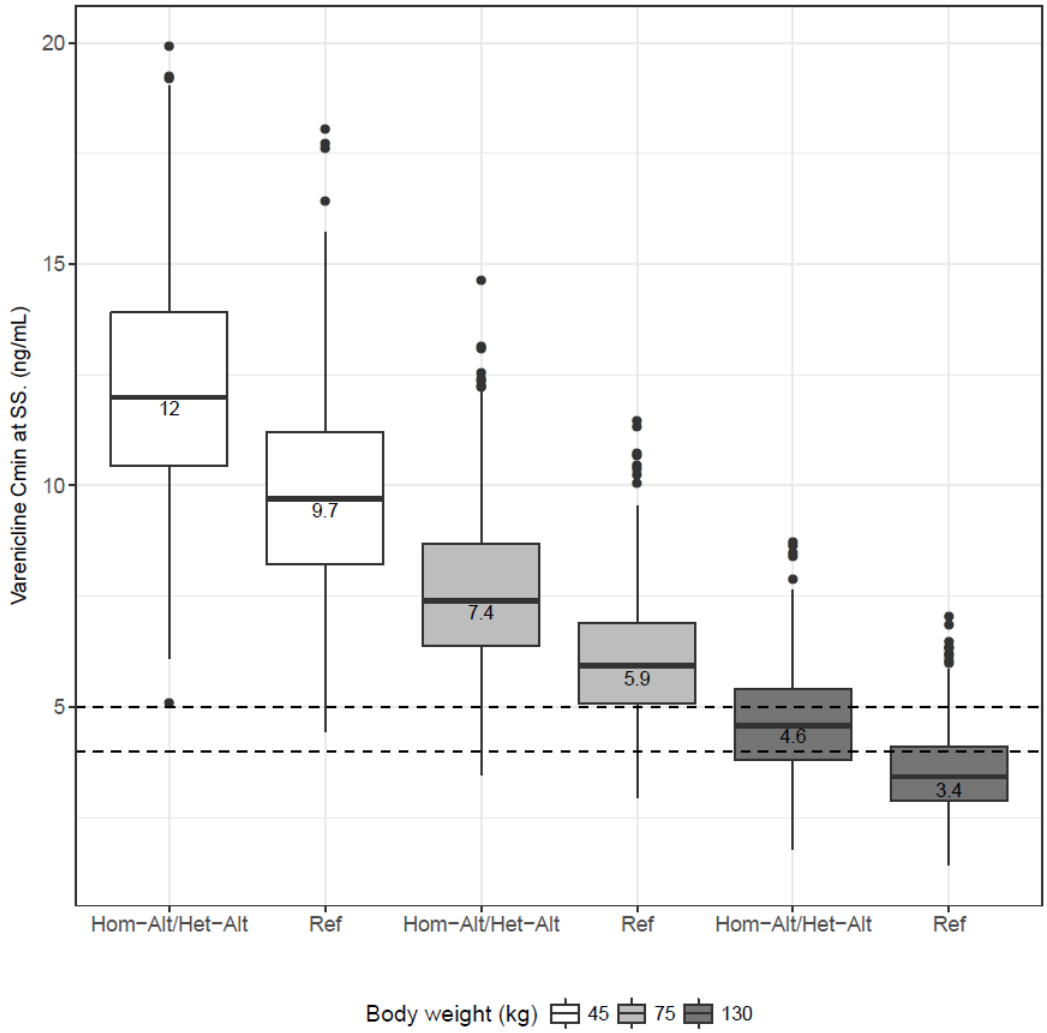

Figure 4.2.2: Distribution of the predicted varenicline through concentration (Cmin) simulated in 500 subjects per strata of body weight value and UGT2B7 802T>C genotype after administration of varenicline 1 mg b.i.d at steady-state (SS). Dashed lines represent through concentrations range

expected for the recommended dose of 1 mg b.i.d (4-5 ng/mL) previously reported.[46] The median values are displayed.

Exposure-response analysis

The basal turnover model fitted the data correctly. The A parameter estimate was 5.8 ppm/d and the EFFCPD parameter 1.9 ppm/d, meaning that for one more cigarette smoked during the day, Kin is increased by 1.9 ppm. Kout could not be estimated and was thus fixed to 2.77 day^{-1} to achieve a CO mean half-life of 6 hours.[49] Residual variability was described by an additive error model and estimated to 2.3 ppm. The inter-individual variability of the input rate Kin (IIV Kin) was 46%. All the parameters were correctly estimated (relative standard error, RSE< 15%).

Univariate analysis showed that sex (ΔOFV = -11.3, p<0.001), score of insomnia (ΔOFV=-15.7, p<0.001), score of depression (ΔOFV=-6.1, p=0.01) and score of irritation (ΔOFV=-5.4, p=0.02) had a significant impact on the EFFCPD parameter. All remaining covariates were not improving the basal model (ΔOFV > -3.70, p=0.054).

Multivariate analysis did not discard any of the identified influential covariates on EFFCPD (ΔOFV > 7.7, p=0.006). The model suggested a decrease in EFFCPD of 63% in female compared to male individuals, an increase in EFFCPD of 154% in individuals with the highest score on insomnia (15) compared to the lowest score (3), an increase of 96% in individuals with the highest score of depression (20) compared to the lowest score (4) and a decrease of 62% in individuals with the highest score of irritation (20) compared to the lowest score (4) **(see Supplementary Table S4.2.1)**. The effects of sex, score of insomnia, depression and irritation were correctly estimated (RSE = 8%, 24%, 39% and 12%, respectively). The final model results are presented in **Supplementary Table S4.2.1**.

Of 2000 replicates analyzed during the bootstrap analysis of the exposure-response model, 1% failed to minimize successfully and were excluded. The parameter estimates of A, IIV Kin and the residual

error differed less than 10% from the bootstrap medians and lied within the bootstrap 95%CI. However, the estimates of EFFCPD and of the effects of the sex, score of insomnia, depression and irritation differed more than 16% from the bootstrap medians. 95% CI of the bootstrap medians for the effect of the sex and the three scores were not significant.

IV.2.5 DISCUSSION

This work presents the exposure to varenicline in a real setting of patients enrolled in a clinical and pharmacogenetic study. The pharmacokinetics of varenicline are in good accordance with reported values. The CL/F estimate for a 70-kg individual with normal creatinine clearance was 8.5 L/h (95%CI=7.9-9.2) in Hom-Alt/Het-Alt individuals and increased by 21% in Ref individuals (10.3 L/h, 95%CI=8.5-12.2). This range is in accordance with the clearance reported for a 70-kg individual with a creatinine clearance of 100 mL/min (10.4 L/h, 95%CI=10.2-10.6).[15] The V/F estimated in the present study (228 L, 95%CI=192-275) was significantly lower than the steady-state volume of distribution published for a two-compartment model (Vss=415 L, 95%CI=371-463) and so was our estimation of the elimination half-life (19h vs 24h). Since 95% of the drug concentrations available in our study population were collected less than 12 hours after dose administration, the characterization of the profound compartment could not be done. Nevertheless, it is in agreement with the central volume estimated in adolescents by a one-compartment model (V =215 L, 95%CI=204-238).[50] Only 10% of the study population had a mildly reduced kidney function (defined as GFR estimated with MDRD formula < 90 mL/min). The absence of subjects with severe impaired renal function might explain the absence of effect of this variable on varenicline elimination, previously described in adults.[15] The IIV CL/F (26%) estimated in the base one-compartment model was found to be explained in part by the BW and the genetic polymorphism of *UGT2B7* rs7439366 resulting in a decreased of IIV CL/F to 19% close to the reported value of 25%.[15]

The originality of our work is in the analysis of the influence of genetic factors (*i.e*. *OCT2*, *UGT2B7* and nuclear factors) on varenicline clearance. *UGT2B7* rs7439366 Hom-Alt/Het-Alt individuals had a decrease of varenicline clearance compared to Ref subjects. This is in accordance with a reported reduced morphine-6-glucuronide/morphine ratio in Hom-Alt individuals compared to Ref individuals in a study involving 86 patients[51] although contradictory results have been published.[52, 53] Of note, it

has been showed that PXR is not involved in *UGT2B7* expression[54, 55] but could regulate the OCT2 expression as suggested in rats.[56]

Despite the weak proportion of variability in the varenicline concentrations separately explained by BW (14%) or *UGT2B7* rs7439366 polymorphism (9%), **Figure 4.2.2** highlighted a 4-times variation in varenicline exposure between the two extreme groups of BW and *UGT2B7* rs7439366 polymorphism ($Cmin_{SS}$ = 12 ng/mL and 3.4 ng/mL in the group of 45 kg/Hom-Alt/Het-Alt and 130 kg/Ref individuals, respectively). The variation in varenicline exposure decreased to 2.6 if only the two extreme groups of BW are considered ($Cmin_{SS}$ = 12 ng/mL and 4.6 ng/mL in the group of 45 kg and 130 kg with the same *UGT2B7* rs7439366 genotype, respectively). Moreover, a positive association between varenicline exposure and probability of abstinence or nausea incidence has been shown at the dose 1 mg b.i.d.[3] Subjects with very low BW and especially the Hom-Alt/Het-Alt subjects are overexposed to varenicline and nausea could be more frequent and could affect the patient adherence to the treatment. In contrary, overweight smokers and Ref individuals in particular might have a lower probability of abstinence at this dose due to underexposition to the drug. Thus, dose adjustment according to BW values and *UGT2B7* genotype if known should be considered to maximize the probability of abstinence while limiting the apparition of nausea, the most prevalent side-effect of varenicline. For overweight subjects and especially *UGT2B7* Ref individuals, this work suggests to increase the dose as it has been already proposed for nonresponders to the standard dose in a clinical experience.[57] We acknowledge that in the present study the effect of *UGT2B7* rs7439366 polymorphism observed on varenicline concentrations is more important than expected based on the data published by Obach *et al.*[4] It is indeed reported that varenicline N-carbamoylglucuronide represent in excreta 3.6% of the dose but the study included only 6 subjects with unknown metabolizer status for UGT2B7. In the present study, the genotypes *UGT2B7* rs7439366 CC/TC corresponded to the majority of the study sample (77%, **Table 4.2.1**) which is in good agreement with the reported genotype distribution in the European population and excluded a selection bias.[58] In the present study, the effect of *UGT2B7* rs7439366 polymorphism

on varenicline pharmacokinetics is of limited clinical relevance and however lower than the body weight effect.

In this study, the exposure-response analysis did not show any influence of varenicline concentrations on CO levels contrary to the study from Ravva *et al.*[3] Here, most of the patients (92%) were abstinent (expired CO measurements below 10 ppm) thus preventing the estimation of any positive relationship between varenicline exposure and abstinence. In contrast in the study from Ravva *et al.* positive relationship was characterized in a study population with almost 50% of abstinent individuals. The bootstrap analysis revealed that the effect of the covariates were not statistically significant possibly due to the right-skewed distribution of the covariates in our analyzed population.

Limitations of the present work should be noted. First, the limited amount of pharmacokinetic data in early and late times after dose prevented the estimation of the absorption constant and of the second volume of distribution of varenicline, respectively. A second major limitation of the work resides in the assumption that the participants had good adherence to the treatment and that all doses were taken by the participants. It must be mentioned that, although adherence cannot be ascertained, all participants were asked about the actual intake of varenicline at each visit. Third, in this analyzed population most of the participants were abstinent after a 3-month treatment period.

Despite these limitations, our study is the first to analyze the genetically-based variability of varenicline pharmacokinetic. This work showed that *UGT2B7* polymorphism and BW can influence varenicline concentrations. During smoking cessation program, in case of lack of abstinence in overweight individuals or apparition of side effects in underweight individuals, dose adjustment based on BW and, if available, on *UGT2B7* genotype, might be useful to improve clinical efficacy and tolerability of varenicline.

IV.1.6. SUPPLEMENTARY MATERIAL

Table S4.2.1: Parameter estimates and their bootstrap evaluations of the final CO turnover model.

Parameter	Final population parameter		Bootstrap evaluation (n=2000 samples)		Difference (%)[e]
	Estimate	RSE (%)[a]	Median	$CI_{95\%}$	
A (ppm/d)	5.9	13	6.0	(5.1; 7.2)	1.7
EFFCPD (ppm/d)	2.8	15	2.2	(1.0; 5.2)	-21
Effect on EFFCPD of[d]					
Score of irritation	-20	12	-27	(-60; 88)	35
Score of insomnia	51	24	43	(-56; 148)	-16
Score of depression	24	39	39	(-32; 419)	63
Sex (in female individuals)	-63	8	-53	(-11; 88)	-16
Kout (day^{-1})	2.77	-	-	-	-
IIV Kin (CV%)[b]	45	15	47	(31; 77)	4.4
σ (ppm)[c]	2.1	1	1.9	(1.1; 2.7)	-9.5

Final model: Kin (ppm/d) = 5.9 + 2.8 x (1-Q1 x 0.63) x (1+0.51 x (Score insomnia - 5)/5) x (1-0.20 x (Score irritation-5)/5) x (1+0.24 x (Score of depression -4)/4) x CPD with Q1=0 if male subjects and Q1=1 if female subjects and CPD, the number of cigarettes smoked per day

a. Relative standard errors of the estimates (SE) defined as SE/estimate directly retrieved from NONMEM®.

b. Intersubject variability defined as CVs (%).

c. Additive residual intrasubject variability.

d. Relative deviation of EFFCPD due to score of irritation, insomnia or depression and sex.

e. Difference (%) = (bootstrap median value – typical value from final model)/ typical value from final model x 100

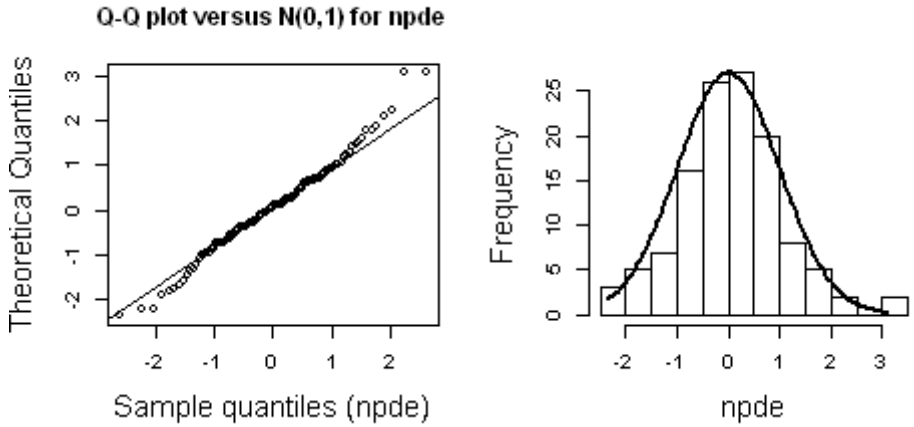

Figure S4.2.1: QQ-plot (left panel) and histogram (right panel) of the NPDEs computed for our final model versus, respectively, the theoretical quantiles and the density of a standard normal distribution. The graphs were obtained with the adds-on R package NPDE.[45]

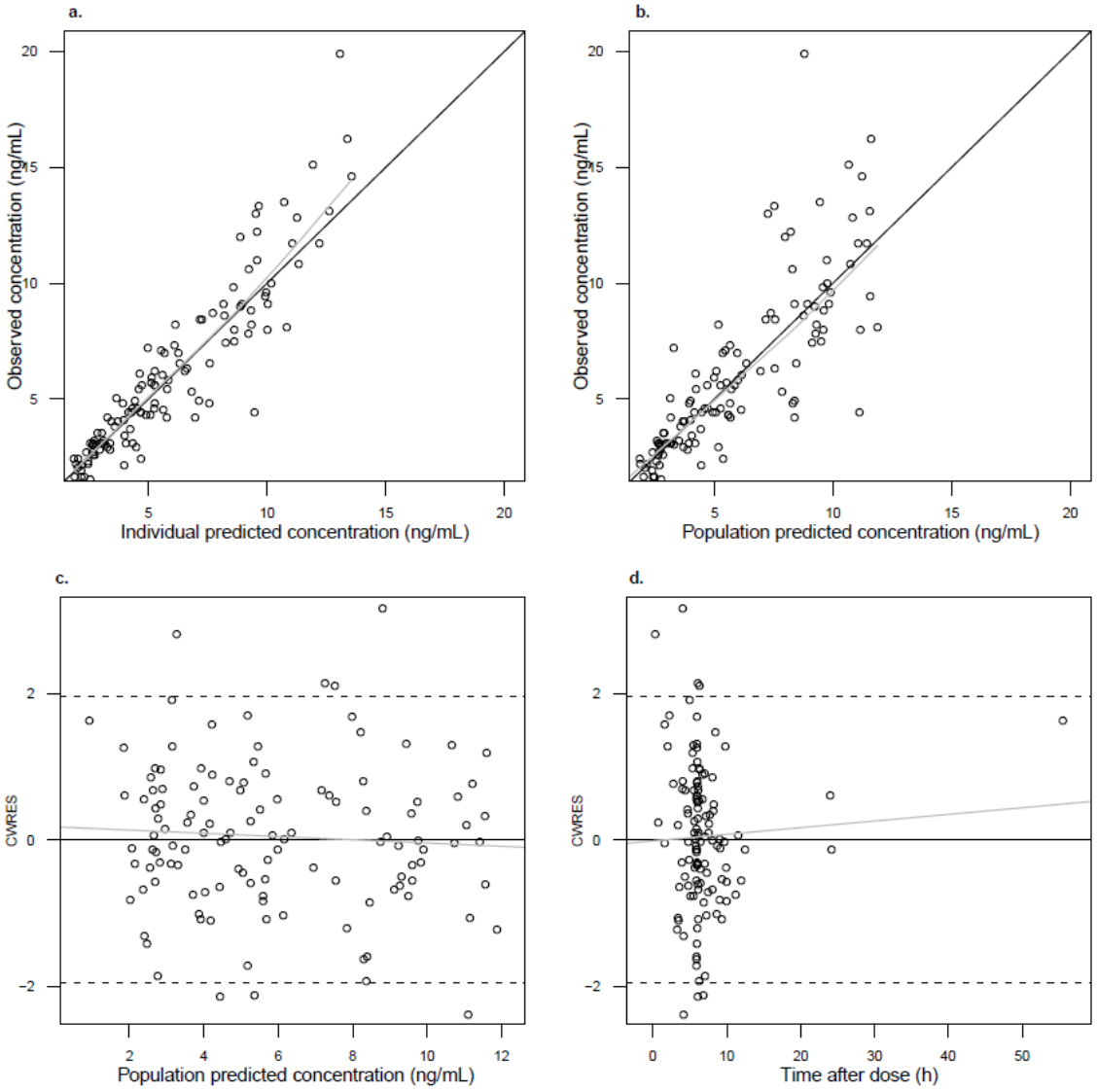

Figure S4.2.2: Goodness-of-fit plots of final population pharmacokinetic model. Loess smooth curves of the ordinate values are printed in grey. a) Observations vs. individual predictions; b) Observed concentrations vs. population predictions c) Conditional weighted residuals (CWRES) vs. population predictions. d) CWRES vs. time after dose.

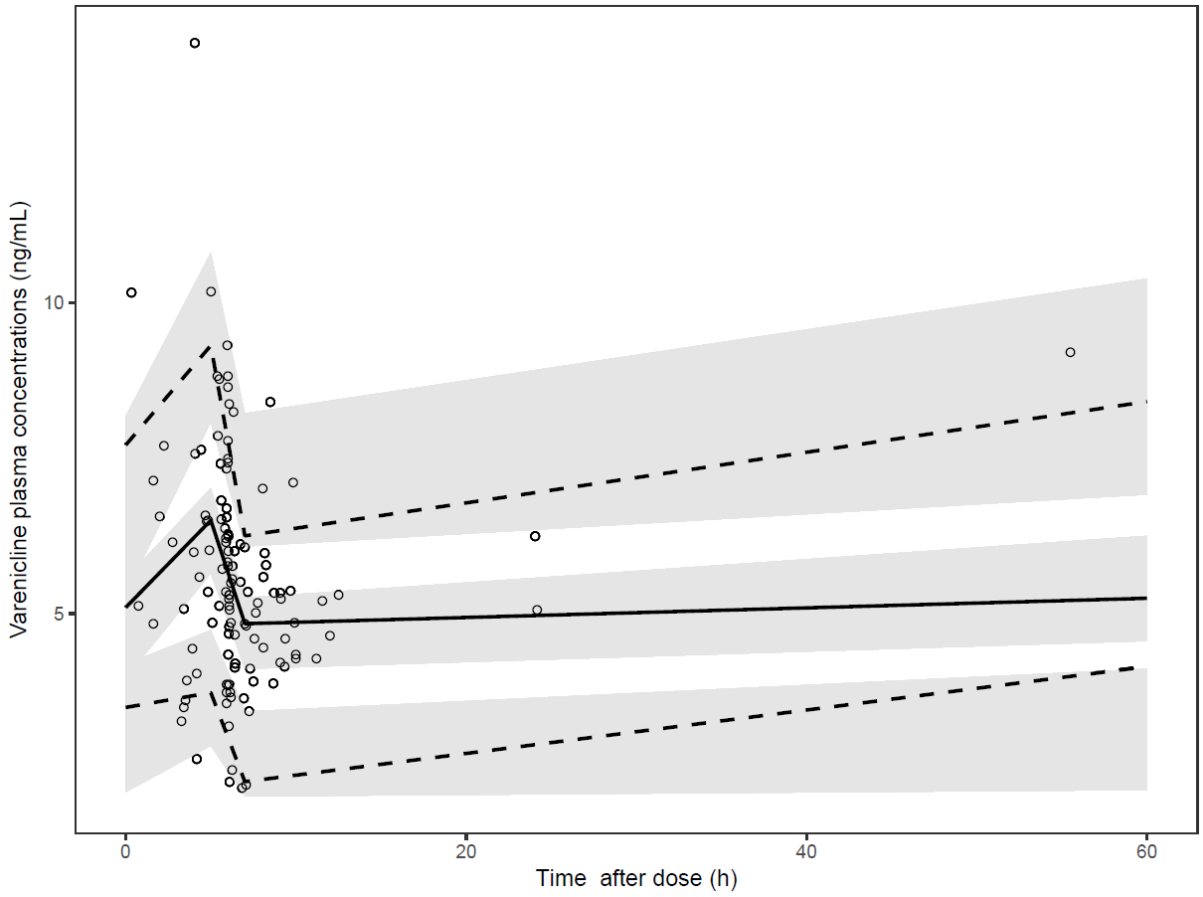

Figure S4.2.3: pvcVPC of the pharmacokinetic final model (n=82). Open circles represent varenicline plasma concentrations. The continuous line represents the median observed plasma concentration and the dashed lines represent the observed 5% and 95% percentiles. Shaded areas represent a simulation-based 95% confidence interval for the median, the 5% and 95% percentiles.

Annex 4.2.1 NONMEM code for the final varenicline PK model

```
$PROBLEM PopPK study VARENICLINE

$INPUT ID II SS Dose=DROP AMT DAT1=DROP TIME CMT DV NDV EVID FLAG SMOKE BW AGE RACE SEX HG CockG MDRD CONTR APXR SIOCT SNP1 SNP2 SNP3 SNP4 SNP5 SNP6 SNP7 SNP8 SNP9 SNP10 SNP11 SNP12 SNP13 SNP14 SNP15

$DATA VARE.csv IGNORE=#

$SUBROUTINE ADVAN2 TRANS2;

$PK
 IF (AMT.GT.0) THEN
 TDOS=TIME
 TAD=0.0
 ENDIF
 IF (AMT.EQ.0) TAD=TIME-TDOS

 Q1      = 0
 Q2      = 0
 IF (SNP4.EQ.3) Q1=1
 IF (SNP5.EQ.3)    Q2=2
 TVCL    = THETA(1)*((BW/70)**0.75)*(1+Q1*THETA(4))
 CL      = TVCL*EXP(ETA(1))
 V       = THETA(2)
 KA      = THETA(3)
 KE      = CL/V
 TMAX    = (LOG(KA)-LOG(KE))/(KA-KE)
 S2=V/1000

$ERROR
 DEL     = 0
 IF (F.EQ.0) DEL = 1
```

```
IPRED   = F
W       = F+DEL
Y       = F + W*ERR(1)
IRES    = DV-IPRED
IWRES   = IRES/W

$THETA
(0,10)   ; CL
(0,400)  ; V
0.98 FIX ; Ka
0.1      ; SNP4 on CL

$OMEGA
0.1   ; ETA(1)

$SIGMA
0.1

$EST METHOD=1 INTERACTION MAXEVAL=8000 PRINT=5 MSFO=run34

$COVARIANCE

$TABLE ID TIME TAD AMT EVID FLAG IPRED IRES IWRES CWRES NPDE EPRED EWRES ECWRES     ONEHEADER
NOPRINT FILE=sdtab34 ESAMPLE=3000
$TABLE ID CL V KA TMAX ETA(1) NOAPPEND ONEHEADER NOPRINT FILE=patab34
$TABLE ID SNP4 SNP5 NOAPPEND ONEHEADER NOPRINT FILE=catab34
$TABLE ID BW NOAPPEND ONEHEADER NOPRINT FILE=cotab34
```

Annex 4.2.2 NONMEM code for the final varenicline-CO model

```
$PROBLEM    PKPD study VARENICLINE

$INPUT      ID DROP TTTDUR TIME UNDERTTT DOSE FREQ DOSEPERDAY CONCV TAD CL KE ETACL AUC CAV
CO=DV COW1 LCO COTININE OHCOTI NICOTINE LCOTI CPD CPDLW SCORE_DEPRES SCORE_CRAV SCORE_IRRIT
SCORE_CONC SCORE_APP SCORE_INSOM SCORE_TOT SNP4_1 SNP4_2 CHRNA4 NMR NMSM NMSM2 THERALL
AGE SEX BW TTTPER1 TTTPER2 MAXOBSTIME CAVN ABSTCOTI ABSTCO MDV

$DATA      PKPD_9_EDA_dataset_NM.csv IGNORE=# IGNORE(CPD.EQ.-99) IGNORE(MDV.EQ.1)

$SUBROUTINES ADVAN13 TOL=9   ;TOL=6 in PK model

$MODEL NCOMP=1

     COMP (EFFECT)

$PK

Q1 = 0

IF (SEX.EQ.1) Q1=1

EFFSEX=THETA(5)

EFFSCOIN=THETA(6)

EFFSCOI=THETA(7)

EFFSCOD=THETA(8)

BASE=THETA(1)

EFFCPD=THETA(4)*(1+Q1*EFFSEX)*(1+EFFSCOIN*LOG(((SCORE_INSOM-5)/5)))*(1+EFFSCOI*((SCORE_IRRIT-
5)/5))*(1+EFFSCOD*((SCORE_DEPRES-4)/4))

TVKIN=BASE+EFFCPD*CPD

KIN = TVKIN*EXP(ETA(1))

KOUT = THETA(2)*EXP(ETA(2))     ; CO elimination rate

IF(A_0FLG.EQ.1) THEN

A_0(1)=KIN/KOUT

ENDIF
```

```
$DES

DADT(1) =  KIN-KOUT*A(1) ; Stimulation of Kin by the craving (score)


$ERROR

COP   = A(1)

IPRED =COP

IRES  =DV-IPRED

W     =THETA(3)

IWRES =IRES/W

Y     =IPRED+W*ERR(1) ; additive RE (if log scale > proportional in natural scale)


$THETA

3.4           ;BASE

2.77 FIX     ;KOUT  t1/2CO=6h -> KOUT=log(2)/(6/24)

(0,2.5)      ;RE ADD on natural scale

2.2           ;EFFCPD

1             ;EFFSEX

1             ;EFFSCOIN

1             ;EFFSCOI

1             ;EFFSCOD


$OMEGA

0.1    ;ETA1

0 FIX  ;ETA2


$SIGMA  1  FIX


$ESTIMATION METHOD=1 INTERACTION MAXEVAL=9900 PRINT=5 MSFO=run625 NSIG=3 POSTHOC ATOL=9


$COVARIANCE PRINT=E MATRIX=S


$TABLE ID TIME TTTDUR IPRED IRES IWRES CWRES NPDE ONEHEADER NOPRINT FILE=sdtab812

$TABLE ID TIME TTTDUR IPRED KE CAV CAVN CPD CO KIN BASE EFFCPD KOUT ETA(1) ETA(2) NOAPPEND
ONEHEADER NOPRINT FILE=patab812
```

IV.2.7 REFERENCES

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CHAPTER V. DISCUSSION AND PERSPECTIVES

V.I Discussion

The psychiatric population is more likely to suffer from somatic diseases leading to an increase mortality.[1, 2] In particular, schizophrenic individuals have a risk of being obese increased by 3 to 4 times, of cardiovascular diseases by 1.5 to 3-times, and of sudden cardiac death by 2 to 4 times compared to the general population. In addition, the prevalence of type 2 diabetes mellitus is increased by two to three times. Relevant risk factors are unhealthy lifestyle including smoking as well as psychotropic drugs.[2] To stop smoking is often a hard challenge with repeated cycles of abstinence and relapse and smokers may wait to have problematic complications of smoking like cardiovascular diseases to attempt to quit.[3] For treating smoking, several pharmacotherapies have been approved by the FDA (nicotine replacement therapy (NRT), varenicline and bupropion). However, these treatments have a modest efficacy. NRT (combination of nicotine patch and nicotine gum or lozenge) and varenicline are reported to have a 6-month abstinence of 30-40% after treatment.[4] Enhancement of the efficacy of these treatments is possible by increasing the treatment duration or the dose in the limit of safety or by finding factors (demographic, clinical or genetic) influencing the treatment efficacy. Treatments will be adapted to the patient depending on these factors.

As compared to first-generation antipsychotics, second generation antipsychotics induce less extra-pyramidal side effects but have propensity for inducing cardiometabolic side effects which are important for some antipsychotics.[5, 6] In a psychiatric population having chronic cardiometabolic complications caused by the disease itself[2], administration of drugs that worsen the metabolic abnormalities can cause substantial complications, even more in the elderly.[7] Efforts are undertaken by researchers to understand the heterogeneity of weight gain in patients by studying the factors influencing this adverse effect. Young age, female gender and first psychotic episode are already known risk factors for an important weight gain. A low and a high baseline BMI is a risk factor for rapid and longer term weight gain, respectively during antipsychotic treatment.[5] Finally, gaining weight appears to be more important in case of combination of SGAs.[8, 9]

Optimized prescription is essential in order to maximize efficacy and tolerability of treatments. This can be reached by understanding the influence of environmental factors (e.g comedications and smoking habit) and clinical susceptibilities of the patient such as age, gender and morbidities (e.g cardiovascular diseases, hepatic or renal impairments) on the pharmacokinetics and pharmacodynamics of psychotropic drugs.

The overall aim of the present thesis was to identify opportunities of optimization of smoking cessation treatments (nicotine replacement therapy and varenicline) and two common antipsychotic drugs (amisulpride and quetiapine) by the study of their pharmacokinetics, pharmacodynamics and pharmacogenetics. We identified factors influencing effectiveness of smoking cessation treatments, pharmacokinetics of varenicline, amisulpride and quetiapine as well as adverse events, notably weight gain and hyperprolactinemia.

The influence of the nicotine metabolizer status determined either by phenotype or by some genetic polymorphism of *CYP2A6,* the main cytochrome metabolizing nicotine (70-80%), was explored on the effectiveness of NRT and varenicline.[10] Very recently, a weighted genetic risk score (wGRS) including seven genetic variants of *CYP2A6* has been developed in Europeans to predict nicotine metabolism quit rate after treatment by nicotine patches or varenicline.[11] The wGRS alone and the wGRS plus age, sex and BMI explained 34% and 41% respectively of the nicotine-metabolite ratio variance. This wGRS may thus be a useful tool for smoking personalized pharmacotherapy and improve efficacy of smoking cessation treatments.

The work on amisulpride and hyperprolactinemia in the present thesis and previous studies[12, 13] suggest that patients receiving amisulpride at therapeutic doses will have hyperprolactinemia. Thus, even if patients do not report early clinical symptoms, taking into account an amisulpride-induced hyperprolactinemia in the clinical evaluation will be beneficial for them in order to prevent longer-term complications. Some recommendations to reduce prolactin levels have been already proposed and should be considered given that amisulpride has been shown to be one of the most efficacious antipsychotics with a low potential to induce weight gain.[6] It has been suggested to add aripiprazole,

since it seems that a dose of 10 mg/day would be sufficient to decrease significantly the antipsychotic-induced hyperprolactinemia.[14] However, contrasting results are reported concerning efficacy and safety of aripiprazole in this context due to different study designs, but also because aripiprazole can cause decompensation of the psychotic disease due to its partial agonist activity.[14] In a case report, topiramate (an antiepileptic drug) was shown to decrease prolactin level and prolactin rebound was observed after discontinuation of topiramate treatment.[15] Besides, adding a dopaminergic agonist (such as bromocriptine and cabergoline) would have an inhibition action on prolactin secretion but may worsen psychotic symptoms and is thus not recommended by recent guidelines.[14, 16] Finally, it has been proposed to add oral contraceptive in premenopausal women to treat symptoms of oestrogen deficiency.[16] In the end, if possible with regard to therapeutic benefit, a switch to a prolactin sparing antipsychotic such as aripiprazole, olanzapine, quetiapine and clozapine, should be proposed keeping in mind the strong potential for inducing metabolic side-effects of the last three drugs.[16]

After 20 years of publications on antipsychotic-induced cardiometabolic adverse effects, psychiatrists should be aware of these adverse effects, educate patients and propose strategies for monitoring such side effects.[17-19] Worsening cardiometabolic abnormalities, reducing treatment adherence and decline of quality of life and self-esteem of patients are the consequences of antipsychotic-induced weight gain.[20] For these reasons, it is essential to control weight gain in order to improve clinical care of patients. Strategies to minimize weight gain during antipsychotic treatment include healthy life style counseling, switch to a low-risk antipsychotic for weight gain (like amisulpride, aripiprazole, brexpiprazole, cariprazine and lurasidone) and add a medication that reduce body weight.[21, 22] The British Association for Psychopharmacology proposed levels of recommendation based on evidence.[23] Lifestyle interventions, switch to an antipsychotic with a low propensity to induce weight gain, adjunction of two anti-diabetic drugs (metformin or liraglutide) had the highest level of recommendation for weight management.[23] In a more recent meta-review of 27 meta-analyses representing more than 47’000 patients, pharmacological and non-pharmacological interventions for weight gain management were compared. Individual lifestyle counselling was the most effective non

–pharmacological strategy.[24] Increasing the aripirazole dose was the most effective pharmacological intervention.[24] Adding topiramate, d-fenfluramine (an anorectic medication) or metformin in the treatment strategy was the most effective on weight gain.[24] In this thesis work, weight gain during quetiapine treatment seemed to depend on quetiapine exposure. Decreasing the dose of quetiapine may be another strategy for controlling weight gain. However, it remains to be determined whether the therapeutic (in particular antipsychotic and antimaniac) effects can be preserved while attaining clinically meaningful weight loss.

In the present thesis, genetic explained a modest part of heterogeneity in psychotropic drugs exposure. During decades, pharmacogenetic testing was a promise for a personalized prescription and an enhancement of the standard care of psychiatric patients.[25, 26] Today, there is still ambivalence on the utility of pharmacogenetic tests to the personalized prescription in psychiatry.[27, 28] This can be explained by a lack of scientific evidence showing their clinical utility and a lack of clinician training on how to adapt the medication based on the results of the pharmacogenetic tests.[29]

V.II Conclusion and perspectives

In conclusion, therapeutic drug monitoring by drug concentration interpretation and clinical evaluation of efficacy and tolerability of psychotropic drugs appears to be essential for treatment optimization in psychiatric patients. The pharmacokinetic models presented in this thesis could guide the individualisation of amisulpride and quetiapine prescription. As a perspective, the two pharmacokinetic models would be implemented in a Bayesian tool for dosage adjustment developed by the CHUV and the HEIG-VD. The software, Tucuxi©, is a user-friendly model-based TDM solution for practitioners. The pharmacokinetic-pharmacodynamic models of hyperprolactinemia during amisulpride treatment and weight gain during quetiapine treatment could improve the monitoring of these adverse events.

V.III References

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