Étude clinique et pharmacogénétique sur la réponse et les effets secondaires des médicaments psychotropes

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Abréviations

ABC	Adenosine triphosphate–binding cassette
ADME	Absorption, Distribution, Métabolisme et Elimination
ADN	Acide désoxyribonucléique
ANOVA	Analyse de variance
AP	Antipsychotiques atypiques
BDNF	Brain-derived neurotrophic factor
CART	Cocaine- and amphetamine-regulated transcript
CHUV	Centre hospitalier universitaire vaudois
CI	Intervalle de confiance
CREB	cyclic AMP response element-binding protein
CRTC1	CREB-regulated transcription coactivators 1
CYP	Cytochrome P450
DRD	Récepteur dopaminergique
EDTA	Acide éthylène diamine tétracétique
FTO	Fat mass and obesity associated
GABA	Acide gamma-amminobutyrique
GWA	Genome wide association study
HDL	Cholestérol HDL (High Density Lipoprotein)
HPLC	Chromatographie liquide à haute performance
IMC	Indice de masse corporelle
KISS1	Kisspeptin-1
KO	Knock-out
LC	Chromatographie liquide
LEP	Leptine
LEPR	Récepteur de la leptine
MC4R	Récepteur Melanocortin-4
mRNA	Acide ribonucléique messager
MS	Spectrométrie de masse
NCBI	National Center for Biotechnology Information
NMDA	Récepteurs N-methyl-D-aspartate
NR	Récepteur nucléaire
OHRIS	9-hydroxy-risperidone
PCR	Polymerase chain reaction
PEPCK	Phosphoenolpyruvate carboxykinase
P-gp	Glycoprotéine de perméabilité ou glycoprotéine P
PPAR	Récepteurs du peroxisome proliferator-activated
PXR	Récepteurs du pregnane X
RIS	Risperidone
RNA	Acide ribonucléique
SD	Déviation standard
SNP	Single-nucleotide polymorphism
SH2B1	SH2B adaptor protein 1, également Src homolgy 2
TDM	Suivi thérapeutique des médicaments
UCP2	Uncoupling protein 2
UGT	UDP–glucuronosyltransferase
UPPC	Unité de pharmacogénétique et psychopharmacologie clinique
5HTR	Gène du récepteur sérotoninergique

Préface

Le présent travail de thèse a été réalisé dans l'Unité de Pharmacogénétique et Psychopharmacologie Clinique (UPPC; anciennement unité de biochimie et psychopharmacologie clinique) du Département de Psychiatrie de Centre Hospitalier Universitaire Vaudois (CHUV) en collaboration avec le laboratoire de chimie analytique et pharmaceutique de la section des sciences pharmaceutiques de l'Université de Genève et de l'Université de Lausanne.

Le laboratoire UPPC comprend différents secteurs d'activités, il fournit notamment des prestations médico-techniques dans le domaine du suivi thérapeutique du médicament (TDM pour Therapeutic Drug Monitoring) qui comprend le dosage plasmatique des médicaments, l'interprétation des résultats avec éventuellement des conseils pharmacologiques et également dans le domaine de la biologie moléculaire liée à la pharmacogénétique et du dépistage urinaire semi-quantitatif de drogues. Un programme de pharmacovigilance (Sécurité du médicament en psychiatrie) ainsi que des conseils et/ou intervisions de psychopharmacologie clinique sont également proposés aux différents services du département de psychiatrie du CHUV, aux médecins d'autres institutions, et aux médecins privés.

En recherche clinique et pharmacogénétique, l'UPPC s'intéresse tout particulièrement au métabolisme des psychotropes (principalement les antidépresseurs, les antipsychotiques, les pro-cognitifs, la méthadone et les substitutions nicotiniques), et à l'influence des facteurs génétiques sur la variabilité des taux sanguins des médicaments, sur la réponse thérapeutique et sur la survenue d'effets indésirables.

Dans le but d'améliorer la prise en charge médicamenteuse, les approches suivantes sont utilisées:

- Développement de méthodes de dosage de médicaments psychotropes et de substrats des cytochromes P450 pour des tests de phénotypage (tests pharmacogénétiques) dans différents matrices biologiques. Des méthodes de génotypage pour les cytochromes P450 et d'autres protéines sont également développées.
- Etudes du métabolisme (en particulier par les cytochromes P450) et du transport (par exemple par la glycoprotéine P) des médicaments.
- Etudes cliniques pharmacocinétiques - pharmacodynamiques pour étudier, chez les patients sous traitement, les relations entre les taux plasmatiques de médicaments psychotropes et les effets cliniques (effets thérapeutiques et effets indésirables), en tenant compte des facteurs génétiques.

Les traitements médicamenteux des patients psychiatriques demandent à être optimisés et adaptés individuellement, car leur succès dépend notamment de facteurs génétiques, environnementaux, psychologiques, et/ou sociaux Une prise de poids et des complications métaboliques sont associées à l'utilisation des antipsychotiques atypiques (AP) et de certains stabilisateurs de l'humeur (lithium, valproate) [1,2]. Bien que connues, l'intensité et la survenue de ces effets secondaires restent très variables d'un patient à l'autre. Une augmentation des risques cardiovasculaires et de la mortalité est ainsi décrite dans les populations psychiatriques par rapport à la population générale [3-5]. Dans ce contexte, le présent travail de thèse s'inscrit dans la même ligne de recherches cliniques et pharmacogénétiques effectuées à l'UPPC, et a pour but d'évaluer les facteurs prédictifs de l'apparition des complications métaboliques et de prévoir les patients les plus susceptibles d'en souffrir. A cette fin, trois études cliniques et pharmacogénétiques ont été conduites par l'UPPC, avec une inclusion à l'heure actuelle de plus de 500 patients.

Une première étape de ce travail de thèse a consisté à développer les méthodes analytiques permettant le dosage de psychotropes.
Les manuscrits I et II réunissent les méthodes analytiques développées. La quantification de ces différentes médications psychotropes a permis d'élargir le panel de médicaments proposés par notre laboratoire dans le cadre du dosage de médicaments psychotropes (TDM). Ces dosages ont notamment permis d'identifier les patients faisant partie des études cliniques avec un taux plasmatiques suffisamment bas pour suspecter un problème de compliance ou d'interaction pharmacologique ou génétique.

Une seconde étape de ce travail de thèse a été de définir la prévalence de risques cardiovasculaires et les facteurs cliniques prédictifs chez les patients psychiatriques. Le manuscrit III présente une revue sur la mortalité et les comorbidités de patients psychiatriques prenant des AP ainsi que le suivi somatique de certains facteurs métaboliques au cours du traitement. Le manuscrit IV décrit une population ambulatoire psychiatrique spécifique. Une prévalence élevée de différents risques cardiovasculaires est observée et certains facteurs cliniques associés à une prise de poids ont été déterminés.

La dernière étape de ce travail de thèse présente l'influence de différents polymorphismes de gènes associés à la réponse thérapeutique et aux effets secondaires de psychotropes. Une étude basée sur le risperidone dépôt, un antipsychotique atypique administré en injection toutes les 2 semaines, fait l'objet du manuscrit V alors que le manuscrit VI se focalise sur l'influence du *CRTC1*, un gène associé

chez la souris à la régulation de l'appétit, sur l'indice de masse corporel chez des patients psychiatriques

Finalement, le manuscrit VIII donné en annexe, est une revue sur l'énantiosélectivité potentielle de la protéine de transport glycoprotéine P, sur les polymorphismes génétiques associés et les conséquences cliniques observées chez l'être humain.

Chapitre I

Introduction

1. Observations cliniques

La prise de poids et les complications métaboliques consécutives à la prescription d'antipsychotiques atypiques et d'autres psychotropes sont un problème de santé important en psychiatrie [1,6].

Les risques et bénéfices sur la mortalité associée à une utilisation de l'antipsychotique atypique clozapine ont par exemple été estimés sur une période de 10 ans [7]. Il a été calculé que la réduction du taux de mortalité par suicide, grâce au traitement de clozapine, serait quasi entièrement contrebalancée par l'augmentation de la mortalité due aux effets délétères d'une prise de poids de 10 kg, ce qui est fréquent avec ce médicament [7-9].

Cette problématique de prise de poids est aussi illustrée par les 2 cas cliniques ci-après. Une patiente psychiatrique âgée de 37 ans, est hospitalisée pour la 9ème fois en raison d'idéation suicidaire et d'une symptomatologie dépressive. La patiente est traitée depuis de nombreuses années par un stabilisateur de l'humeur, le valproate, et par des antipsychotiques : olanzapine, rispéridone, clotiapine, amisulpride et par des antidépresseurs : citalopram, fluoxétine. A l'anamnèse, la patiente indique qu'il y a 10 ans, elle pesait 51 kg. Son poids a fluctué jusqu'à atteindre la valeur actuelle de 117 kg, montrant une augmentation durant cette période de 129 %.

Chez cette patiente, compte tenu des données de la littérature et d'une chronologie suggestive bien que peu précise, l'imputabilité des différents traitements psychotropes dans la survenue de la prise de poids et la persistance de l'obésité sur plusieurs années, a été estimée de l'ordre du possible.

D'autres prises de poids importantes sous psychotropes ont été signalées. Notamment, dans le cadre de conseils pharmacologiques proposés par l'UPPC, il a été mentionné le cas d'une patiente psychiatrique traitée par rispéridone et une benzodiazépine depuis 2 ans. Bien que les comédications ne soient pas connues précisément durant ces 2 années, la patiente indique qu'elle pesait 50 kg à l'initiation du traitement à la risperidone. Son poids actuel est de 100 kg, montrant une augmentation de 100 %. Son médecin actuel demande un conseil thérapeutique pour améliorer la situation clinique c'est-à-dire, une gestion de l'obésité de sa patiente.

Les questions sous-jacentes sont les suivantes: aurait-il été possible de prédire que ces patientes étaient à risque d'une prise de poids cliniquement importante et aurait-il été possible de l'éviter ? Ces questions sont à la base de ce travail de thèse et les manuscrits cliniques III et IV ainsi que le manuscrit pharmacogénétique VI apportent un début d'élément de réponse.

2. Pharmacogénétique

2.1. Introduction

Au début du 21ème siècle, l'entier du génome humain a été séquencé dans le cadre du « Human Genome Project ». Le génome humain est composé de 3 milliards de paires de bases, 30 à 40 mille gènes codant pour des protéines et 99.9% du génome est commun à tous les êtres humains [10,11]. Les variations dans la séquence des acides nucléiques restantes sont composées essentiellement de mutations génétiques comprenant la substitution d'un seul nucléotide, l'insertion, ou la délétion d'un ou plusieurs nucléotides. Il peut également survenir la répétition de séquences de nucléotides (tandem repeat), la délétion ou la duplication (copy number variant) du gène en entier [10,12].

Les *Single Nucleotide Polymorphisms* (SNPs) sont les variations génétiques les plus courantes (90%) et représentent une différence d'une base (allèle) à un endroit particulier de la séquence d'ADN. Il est estimé que les SNPs avec une fréquence allélique de ≥ 1% sont présents en moyenne tous les 300 bases dans le génome. De plus, une combinaison de différents allèles situés sur un même chromosome peut être transmis ensemble et ainsi former un haplotype [12].

Ces variations peuvent affecter la façon dont l'être humain peut développer une maladie ainsi que dans sa réponse à un traitement donné [11].En effet, pour la plupart des médicaments, à une même dose standard, une certaine proportion d'individus va s'éloigner de la réponse attendue, en présentant soit une diminution ou une absence d'efficacité soit au contraire des effets indésirables et/ou une toxicité. Cette variabilité est liée en partie à des facteurs physiologiques (par exemple : âge, sexe, stade de la maladie) ou environnementaux (par exemple : alimentation, interaction médicamenteuse, tabagisme), et est également influencée par les variations génétiques.

La pharmacogénétique est l'étude de la variabilité génétique de la réponse thérapeutique à un médicament donné [13,14]. La connaissance de facteurs génétiques ayant une influence sur la réponse thérapeutique permet potentiellement d'individualiser et d'optimiser un traitement. L'intérêt principal de cette médecine personnalisée réside dans l'amélioration de la prise en charge des patients et une optimisation des décisions thérapeutiques (dose et choix du médicament prescrit) en fonction du génome de l'individu afin d'obtenir la meilleure réponse thérapeutique avec le moins d'effets secondaires possibles. La pharmacogénétique couvre aussi bien l'étude des gènes liés à la pharmacocinétique, notamment le métabolisme ou le transport des médicaments, que les gènes liés à la pharmacodynamie des médicaments [13].

Les prémices de cette science sont apparues au début du 20ème siècle avec un concept de chimie individuelle proposé par A.Garrod suite à l'observation d'individus très vulnérables ou exceptionnellement résistants à une même substance [15]. Par la suite, plusieurs observations de métabolisme lent et rapide pour différents médicaments dans les années 1950 ont été rapportées [16-19] et finalement, F.Vogel a défini le terme « pharmacogénétique » en 1959 [13,20].

2.2. Stratégies de détection d'un gène d'intérêt

Plusieurs stratégies permettent d'étudier l'influence des variations génétiques sur un phénotype. Dans ce travail de thèse, les approches suivantes ont été utilisées :

- L'étude de gènes candidats dont l'implication a déjà été montrée, notamment dans la pharmacocinétique ou dynamique du médicament
- L'analyse de gènes codant pour des protéines participant à la régulation de l'appétit et au contrôle de l'homéostasie énergétique (comme la leptine) chez l'être humain ou dans des modèles animaux (souris *knock-out, KO*)
- L'étude de gènes identifiés dans des études d'épidémiologie génétique et dans des *genome wide association studies* (GWAs). Cette dernière approche implique le balayage rapide de marqueurs génétiques de l'ensemble du génome d'un grand nombre d'individus dans le but de trouver des variations génétiques associées à un phénotype (comme l'obésité) [21]. Généralement, les GWAs n'ont pas d'à priori sur les gènes impliqués dans un phénotype. Les variations génétiques identifiées par cette approche ne sont pas nécessairement la cause du phénotype mais peuvent être liées au variant causal et sont principalement des marqueurs du phénotype [21].

3. Maladies psychiatriques: la schizophrénie et les troubles bipolaires

La schizophrénie est un trouble psychiatrique dont la prévalence est d'environ 1% [22]. Cette maladie peut être caractérisée par des symptômes positifs (hallucinations auditives et visuelles, idées délirantes, troubles du comportement), et négatifs (apathie, repli sur soi, anhédonie). Elle peut également altérer les fonctions cognitives et affectives. Les débuts des symptômes apparaissent généralement à la fin de l'adolescence ou au début de l'âge adulte et l'évolution est généralement chronique, conduisant à une utilisation à long terme des traitements pharmacologiques [23].

Un rôle prépondérant de la dopamine est postulé. Par l'intermédiaire d'une dérégulation du circuit de la dopamine dans le système mésocortical ou mésolimbique, celle-ci pourrait induire les

symptômes de la maladie. Un fonctionnement abaissé des récepteurs N-methyl-D-aspartate (NMDA) dans le système glutaminergique contribuerait également à l'apparition des symptômes positifs [22].

Les troubles bipolaires quant à eux font partie des troubles de l'humeur et sont caractérisés par la perturbation de l'humeur qui peut osciller entre des phases d'excitation intense (phase maniaque) pouvant présenter les symptômes positifs de la psychose et des phases de dépression sévère, entrecoupées parfois de périodes de stabilité. Face à un large spectre de symptômes, cette maladie peut être traitée aussi bien par des antidépresseurs, des stabilisateurs de l'humeur que par des antipsychotiques.

4. Antipsychotiques atypiques

L'introduction des antipsychotiques atypiques (AP) a représenté une étape importante dans le traitement de la schizophrénie. En effet, ces médicaments se montrent remarquablement efficaces à la fois sur les symptômes positifs et négatifs de la schizophrénie avec une incidence moindre d'effets secondaires extrapyramidaux [24] par rapport aux antipsychotiques classiques. Bien qu'initialement destinée au traitement de la schizophrénie, l'utilisation de certains AP s'est élargie au traitement des troubles bipolaires et de l'irritabilité dans les troubles autistiques [22]. Cependant, ils ne sont pas dénués d'effets secondaires [22] et ceux-ci seront décrits plus en détail ultérieurement.

4.1. Pharmacocinétique

Les deux principaux types de protéines impliqués dans le processus d'Absorption, Distribution, Métabolisme et Elimination des médicaments (principe de l'ADME) sont les enzymes métaboliques et les transporteurs [25].

Les facteurs pharmacocinétiques peuvent influencer la prise de poids induite par une médication psychotrope en modulant sa concentration dans les tissus et la circulation sanguine [26]. Ainsi, une même dose de médicament induira des taux sanguins extrêmement variables selon les individus, cette variabilité interindividuelle dans la pharmacocinétique est influencée par des facteurs environnementaux (par exemple : âge, alimentation, comédications) et par des facteurs génétiques, notamment au niveau du métabolisme [27].

4.1.1. Métabolisme

Les réactions enzymatiques du métabolisme sont destinées à rendre plus polaire un composé hydrophobe afin de permettre son élimination. Les réactions de phase I sont l'oxydation, la réduction et l'hydrolyse du composé tandis que les réactions de phase II comprennent l'ajout d'un groupe polaire par différents types de conjugaison sur le composé. Ces réactions se déroulent principalement dans le foie mais également dans d'autres organes tels que les reins, le tractus gastro-intestinal et les poumons [27]. Les principales enzymes du métabolisme sont les cytochromes P450 (CYPs) impliqués dans la phase I, et les enzymes uridine dinucleotide phosphate glucuronosyltransferases (UGT) impliquées dans la phase II [28].

La famille des CYPs comprend 57 membres groupés en 27 familles, dont les familles 1 à 3 métabolisent 90 % des médicaments [28,29]. Les CYPs sont sujets à d'importants polymorphismes, un résumé des différents polymorphismes peut être trouvé sur le site de « *Human Cytochrome P450 Allele Nomenclature Committee* » (www.cypalleles.ki.se). Les CYPs impliqués dans le métabolisme des sept AP disponibles en Suisse sont listés dans le Tableau 1, la voie métabolique majeure est soulignée.

Tableau 1: Propriétés pharmacocinétiques des antipsychotiques atypiques [2,30].

Antipsychotique atypique (AP)	Intervalle thérapeutique	Temps de demi-vie ($t_{1/2}$)	Métabolisme	Inhibition
Olanzapine	20-80 ng/mL	29-39h	CYP1A2, CYP2D6	-
Clozapine	350-600 ng/mL	12h (6-26h)	CYP1A2, CYP2C19, CYP3A4, CYP2D6	-
Rispéridone 9-hydroxyrispéridone	20-60 ng/mL	3h 24h	CYP2D6, CYP3A4	CYP2D6
Quétiapine	70-170 ng/mL	7h	CYP3A4, CYP2D6	CYP1A2, CYP2C9, CYP2C19
Aripiprazole	150-300 mg/mL	3-6j	CYP2D6, CYP3A4	-
Amisulpride	100-400 ng/mL	12h	pas d'interaction avec les CYPs	-
Sertindole	50-100 ng/mL	3j	CYP2D6	-

Les polymorphismes génétiques du *CYP1A2*, enzyme principale du métabolisme de la clozapine, ont notamment montré une influence sur les taux de clozapine [31-33] et sur la réponse au traitement. En effet, les patients porteurs du polymorphisme *CYP1A2*1C*, associé à une diminution de l'activité enzymatique, ont présenté une concentration plus élevée de clozapine et de son métabolite dans le sang ainsi qu'un risque augmenté de résistance à l'insuline, un effet secondaire de la clozapine potentiellement dose-dépendant [33]. L'allèle **1F*, associé à une activité métabolique supérieure chez les fumeurs et les non fumeurs homozygotes, a été associé à une non-réponse au traitement de clozapine [32,34].

4.1.2. Transport

Les protéines de transport sont impliquées dans l'absorption et la distribution de certains médicaments. Elles peuvent influencer leurs taux dans le sang et dans les organes cibles. La plus étudiée est la glycoprotéine de perméabilité (P-gp), une protéine d'efflux codée par le gène *ABCB1*.

La P-gp peut influencer les taux sanguins, mais surtout les taux cérébraux des médicaments substrats, du fait de son activité au niveau de la barrière hémato-encéphalique [35-40]. La majorité des AP sont substrats et le gène codant pour la P-gp est polymorphique [38,41,42].

Une influence sur la réponse au traitement a été décrite pour le polymorphisme *C1236T* et la rispéridone ainsi que les polymorphismes *C1236T, G2677T, C3435T* et l'olanzapine [43,44].

4.1.3. Récepteurs nucléaires

Les récepteurs nucléaires peuvent moduler l'expression de gènes. Le *pregnane X receptor* (PXR) est codé par le gène *NR1I2* et fait partie de la famille des récepteurs nucléaires (Figure 1) [45]. Le PXR est notamment important dans la régulation de l'expression de certaines protéines du métabolisme et de transport [45].

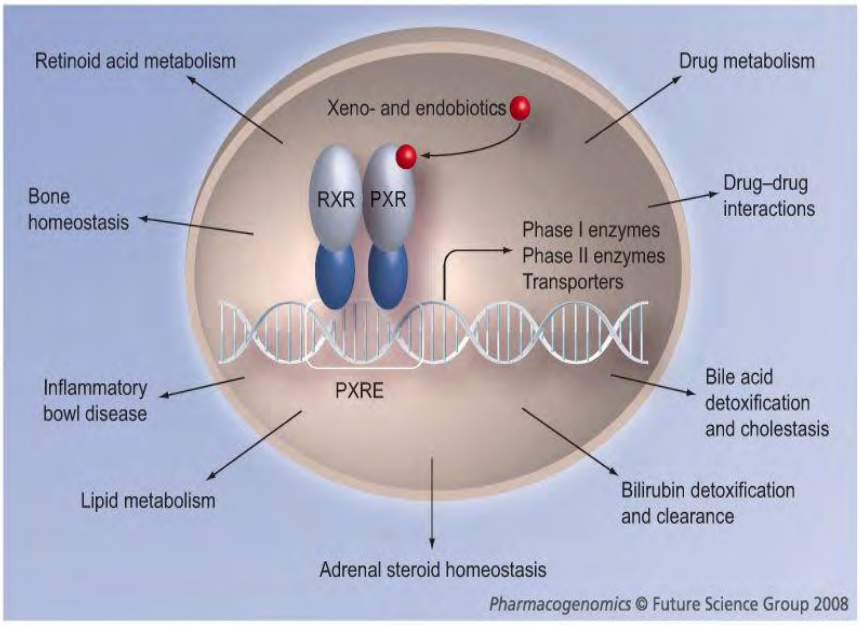

Figure 1 : Fonction biologique du PXR [45].

Il régule également certaines voies physiologiques dont le métabolisme des lipides [45].

Par une liaison sur le promoteur de certains gènes, le PXR peut induire leur expression, notamment

l'expression des enzymes CYP3A5, CYP3A7, CYP2C9, UGT et du transporteur P-gp [29,45,46]. Le PXR est activé par des ligands aussi bien endogènes qu'exogènes comme certains médicaments [47]. Il est majoritairement exprimé au niveau de l'intestin, du foie et des reins [47,48]. Certains polymorphismes du *NR1I2* ont été associés à une différence de transcription du gène ou à une différence d'activité du PXR [45,49,50] et seront décrits dans le manuscrit V. A l'heure actuelle, aucune influence des polymorphismes du *NR1I2* n'a été mise en évidence sur la pharmacocinétique et/ou la pharmacodynamie des médicaments psychotropes.

4.2. Pharmacodynamie

Les propriétés pharmacodynamiques des différents AP varient énormément d'une molécule à l'autre, montrant des affinités diverses dans leur profil de liaison aux récepteurs. Ces affinités influencent la réponse au traitement ainsi que la prévalence des effets secondaires (Tableaux 2, 3).

Le mécanisme d'action des AP est lié essentiellement à un effet sur le système dopaminergique par un effet antagoniste D_2. Les AP se distinguent des antipsychotiques classiques par une affinité différente pour certains récepteurs, tels que : un effet antagoniste conjoint sur les récepteurs sérotoninergiques $5HT_{2A}$ et dopaminergiques D_2, un effet agoniste partiel sur les récepteurs D_2 ou $5HT_{1A}$, une rapide dissociation des récepteurs D_2 ou/et un effet sur le récepteur $5HT_{2C}$ [22].

Tableau 2 : Affinités des antipsychotiques atypiques pour divers récepteurs [22].

Récepteur	Antipsychotique atypique						
	Olanzapine	Clozapine	Rispéridone	Quétiapine	Aripiprazole	Amisulpride	Sertindole
Dopaminérgique							
D_1	+	+					
D_2	+	+	+	+	+	+	+
D_3	+	+			+	+	+
D_4	+	+					
Sérotoninergique							
5-HT_{1A}		+		+	+		
5-HT_{2A}	+	+	+	+	+		+
5-HT_{2C}	+	+		+			+
Adrénergique							
α_1	+	+	+	+			+
α_2		+	+	+			
Histaminergique							
H_1	+	+		+			
Muscarinique							
M_1	+	+		+			
M_3	+	+					

Tableau 3 : Implication potentielle de divers récepteurs sur les effets secondaires [51,52].

Activité du récepteur	Effet(s) secondaire(s)
Dopaminérgique	
antagoniste D_2	Prise de poids, effet endocrine
Sérotoninergique	
antagoniste 5-HT_{2C}	Prise de poids, diabètes
Adrénergique	
antagoniste α_1	Prise de poids, sédation, hypotension orthostatique
Histaminergique	
antagoniste H_1	Prise de poids, sédation
Muscarinique	
antagoniste M_1	Effet anticholinergique (xérostomie, vision trouble, constipation)
antagoniste M_3	Diabètes

4.2.1. Effet thérapeutique

Les SNPs des gènes des récepteurs dopaminergiques D_2, D_3 et D_4 ont largement été testés quant à leurs effets possibles sur la réponse au traitement. Les résultats provenant d'études de différents designs, composées de populations d'ethnies, de médicaments et de durées de traitement différents rendent les comparaisons difficiles [53-56]. Les SNPs des gènes des récepteurs *Taq1A* et *-141C Ins/Del* du D_2, *Ser9Gly* du D_3, *-1438A>G* du $5HT_{2A}$ font partie des SNPs les plus fréquemment étudiés, mais de nombreux résultats contradictoires ont été publiés à leur sujet [56].

4.2.2. Effets secondaires

Un certain nombre d'effets secondaires sont reportés sous AP, tels qu'une agranulocytose avec la clozapine, un allongement de l'intervalle QT avec le sertindole, ou un syndrome neuroleptique malin, qui survient rarement mais peut être potentiellement mortel, avec les AP en général [2].

Une hyperprolactinémie peut être associée à des perturbations du cycle menstruel, à des dysfonctions sexuelles, à l'apparition de gynécomastie et/ou de galactorrhée [57-59]. L'augmentation de la prolactine par un effet antagoniste D_2 est un effet secondaire associé notamment à l'amisulpride et la rispéridone [2,60]. Ces derniers peuvent également induire des symptômes parkinsoniens qui sont dose-dépendants [2,61].

4.2.3. Effets secondaires liés à la prise de poids

La prise de poids, l'élévation de la lipidémie et la résistance à l'insuline varient selon les AP. La clozapine et l'olanzapine sont associées à une prise de poids importante, alors que la quetiapine et la rispéridone sont associées à une prise de poids intermédiaire. Finalement, l'amisulpride et l'aripiprazole semblent ne pas entraîner de prise de poids ou alors seulement de façon modeste (Tableau 4) [3,6,9,62,63].

Tableau 4 : Prise de poids potentielle par année en fonction du traitement (en livre, 10 lb correspondant à 4.5 kg environ) [9].

Potential Weight Change/year (lb)	0-5	0	1-5	6-10	11-15	>15
DRUG CLASS						
Antidepressants SSRI TCA MAOI SNRI Other	Bupropion Fluoxetine	Citalopram Duloxetine Escitalopram Fluvoxamine Nefazodone Selegiline Sertraline Trazodone Venlafaxine	Desipramine Nortriptyline Paroxetine Protriptyline	Amitriptyline Doxepin Imipramine Mirtazapine Phenelzine Tranylcypromine		
Antipsychotics Older Newer	Molindone	Aripiprazole Ziprasidone	Fluphenazine Haloperidol Paliperidone Perphenazine	Quetiapine Risperidone Thioridazine		Clozapine Olanzapine
Mood stabilizers Antiseizure Other	Topiramate	Lamotrigine Oxcarbazepine	Carbamazepine	Gabapentin	Lithium Valproate	

La prise de poids, les complications métaboliques (notamment hyperglycémie) et les anomalies lipidiques pouvant être associées à une augmentation des comorbidités (diabètes, hypertension, hypercholestérolémie, hyperlipidémies, maladies cardiovasculaires) sont particulièrement préoccupantes (Figure 2). Elles peuvent influencer la tolérance, l'acceptabilité et donc la compliance des patients envers leur traitement [64-66.]

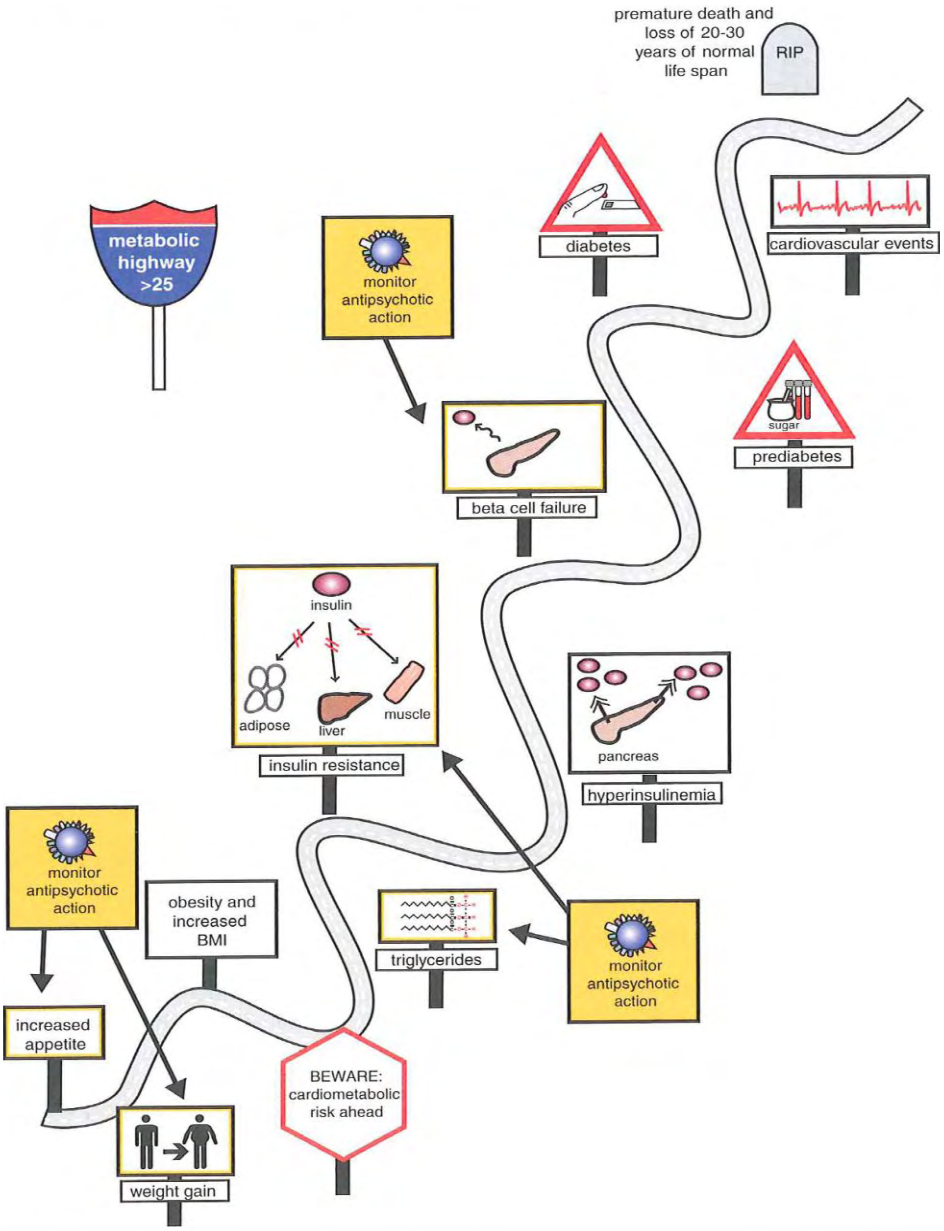

Figure 2 : Evolution des changements métaboliques sous AP avec une augmentation progressive des risques cardiovasculaires [22].

Les changements métaboliques peuvent débuter par une augmentation de l'appétit et une prise de poids, ce qui entrainent une augmentation de l'IMC au-dessus de 25 kg/m^2 (correspondant à un surpoids), pouvant évoluer jusqu'à une obésité, une résistance à l'insuline, une dyslipidémie comprenant une augmentation des taux de triglycérides. Finalement, une hyper-insulinémie peut progresser vers une dysfonction des cellules β pancréatiques, un pré-diabète et l'apparition d'un diabète. Ce dernier augmente les risques cardiovasculaires et peut conduire à une augmentation des événements cardiovasculaires, parfois mortels.

Malgré un nombre croissant de publications sur le sujet, le mécanisme sous-jacent entrainant une prise de poids sous AP est encore peu connu et serait certainement multifactoriel [67-70]. Les facteurs couramment mentionnés dans la littérature sont décrits ci-dessous.

4.2.3.1. Facteurs cliniques et récepteurs

La prise de poids peut être influencée par des facteurs cliniques tels qu'un jeune âge et un indice de masse corporel (IMC) bas en début de traitement, un sexe féminin, une activité sportive insuffisante, une diète calorique (*fast-food*), une longue durée de maladie psychiatrique et de traitement psychotrope. La consommation de tabac, la réponse au traitement ainsi que le profil pharmacologique de l'AP prescrit peuvent également prédire une modification du poids [71-73]. La pharmacologie apporte quelques pistes quant à l'implication de certains récepteurs et neurotransmetteurs dans le mécanisme de la prise de poids (Tableau 3).

Ainsi, le système sérotoninergique est connu comme étant associé à l'appétit, principalement au niveau de l'hypothalamus. La clozapine et l'olanzapine, les deux AP associés à la prise de poids la plus importante, ont une affinité importante pour le récepteur $5HT_{2C}$. De plus, des études menées sur des souris *KO* pour le gène $5HT_{2C}$ ont montré une hyperphagie ainsi qu'une obésité [74]. Le SNP *-759C>T* du gène $5HT_{2C}$, localisé dans la région du promoteur et entraînant une diminution de l'expression du gène, a été associé à une prise de poids dans de nombreuses études chez les patients prenant des AP [67]. L'allèle *-759C* montre dans la majorité des cas une association avec une plus grande prise de poids allant jusqu'à 1.1 unité d'IMC en plus [75-78]. L'allèle *-759T* a également été montré comme étant plus fréquent chez les sujets non-obèses que chez les sujets obèses [79,80].

Les récepteurs histaminergiques et adrénergiques peuvent être impliqués dans la prise de poids induite par les psychotropes [67,71,81,82]. L'activité des récepteurs adrénergiques module le poids corporel par une activation ou une inhibition de la lipolyse [83] alors que les *peroxisome proliferator-activated receptors* (PPAR) jouent un rôle essentiel dans la différentiation des adipocytes [84]. La plupart des analyses sur les SNPs des gènes codant pour les récepteurs autres que celui du $5HT_{2C}$ n'ont pas montré d'association avec la prise de poids. De plus, peu d'études ont été conduites pour chacun de ces gènes [67]. Plusieurs études sur les gènes des récepteurs histaminergiques H_1 [85], sérotoninergiques $5HT_{1A}$ et $5HT_{2A}$ [85,86], adrénergiques $\alpha_{1A,}$ α_{2A} et β_3 [85,87] et dopaminergiques D_2 et D_4 [88,89] n'ont pas permis d'identifier de SNPs impliqués de façon significative dans la prise de poids [67].

4.2.3.2. Régulation de l'appétit et contrôle de l'homéostasie énergétique

L'obésité est le résultat de l'équilibre entre apport énergétique (prise alimentaire) et dépense énergétique conduisant au stockage de l'énergie sous forme de graisse, principalement dans le tissu adipeux [90]. Un grand nombre de facteurs régulent la prise alimentaire et le poids [91]. Les signaux indiquant un excès d'énergie semblent moins puissants que ceux indiquant un déficit énergétique,

suggérant un biais dans l'homéostasie énergétique vers une protection contre un déficit énergétique [91].

La leptine (LEP), qui est produite par les tissus adipeux, est un des facteurs clé dans la régulation de la prise alimentaire et la consommation d'énergie (Figure 3) [67,92,93]. Elle joue un rôle majeur dans la régulation du poids corporel par son effet anorexigène. Ses taux sont altérés lors de l'initiation d'un traitement avec un AP [94]. Les souris déficientes pour la leptine (souris ob/ob) ont par ailleurs un phénotype obèse [95]. Cette hormone agit sur l'hypothalamus par l'intermédiaire de son récepteur (LEPR) et régule la dépense énergétique. Des mutations sur le gène de la leptine et sur le gène du récepteur à la leptine peuvent provoquer une obésité chez l'être humain [90,93].

L'allèle muté (Q223R) et l'allèle 656Lys (Lys656Asn) du gène du récepteur de la leptine [96,97] ont été associés à une prise de poids chez les patients prenant des AP, ainsi que l'allèle *G* du polymorphisme *-2548A>G* du gène de la leptine pour des traitements de courtes [77] et de longues durées [98].

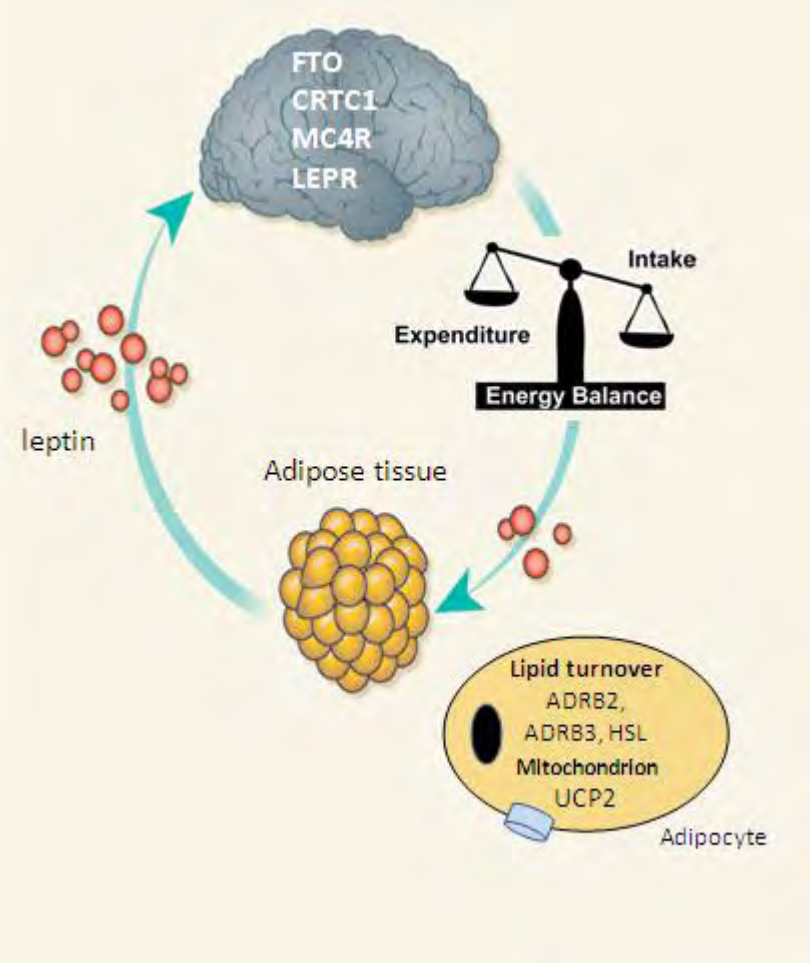

Figure 3 : Homéostasie énergétique et régulation de l'appétit [90,99,100].

La leptine (LEP) est principalement secrétée par le tissu adipeux et sa sécrétion est notamment stimulée par l'accumulation de graisse. Elle agit par son récepteur (LEPR) directement sur certaines zones de l'hypothalamus dont le noyau arqué. Celui-ci a des projections neuronales dans différentes zones de l'hypothalamus conduisant à l'activation de certains récepteurs et

à la sécrétion de neuropeptides associés la régulation de l'appétit et de l'homéostasie énergétique. Le *melanocortin-4 receptor* (MC4R) et le *cyclic AMP response element-binding protein-regulated transcription coactivators* (CRTC1) sont notamment régulés par la leptine [91,101,102]. Abréviations : UCP2, uncoupling-*protein* 2*;* FTO, *fat mass and obesity associated;* ADRB2 et ADRB3, récepteurs adrénergiques β2 et β3*;* HSL, lipase hormone-sensible.

Le *cyclic AMP response element-binding protein* (CREB)-*regulated transcription coactivator* 1 (CRTC1) est localisé sous sa forme inactive dans le cytosol. Après activation, il entre dans le noyau de la cellule, s'associe à CREB et augmente la transcription des gènes dépendants de CREB [103-105]. Le CRTC1 est majoritairement exprimé dans le cerveau et a été associé récemment à la régulation de l'appétit et de l'énergie dans des modèles animaux [102]. Les souris *KO* pour le gène *CRTC1* montrent une hyperphagie et développent sur le long terme une obésité et une hypercholestérolémie [102]. La leptine activerait le CRTC1 ce qui conduirait à une altération de l'expression de peptides anorexigènes et serait à la base de son effet. Aucun SNP lié à une prise de poids ou à l'IMC n'a été décrit pour l'instant dans le *CRTC1*.

Le gène *Uncoupling protein 2 (UCP2)* code pour un transporteur anionique au niveau des mitochondries des adipocytes [90]. L'UCP2 joue un rôle dans la transformation de l'énergie en chaleur et son expression serait associé à la variation de production de leptine [93,106]. Ce gène est associé à l'obésité chez l'être humain [93,107,108].

4.2.3.3. Gènes associés à l'obésité dans des GWAs et des études épidémiologiques

Environ 40-70% du phénotype de l'obésité est transmis par le bagage génétique [92]. La détermination des gènes associés à l'obésité est effectuée sur des populations générales non psychiatriques et elle permet d'améliorer les connaissances sur les mécanismes de la régulation du poids [93]. Il sera intéressant de déterminer si cette association n'est pas aggravée par une médication induisant potentiellement une prise de poids comme les AP. Quelques gènes sont souvent cités pour leur association à l'obésité comme le *Fat mass and obesity associated gene (FTO)* [109-112], le *Melanocortin-4 Receptor (MC4R)* [112-114] et le *Src homolgy 2* (*SH2B1)*. Les gènes associés au phénotype de l'obésité peuvent contribuer seuls (obésité monogénique avec un impact majeur sur le phénotype par un seul gène, par exemple, lors de déficience complète de *LEP, LEPR* et *MC4R*) ou ils peuvent contribuer de façon synergique (obésité polygénique avec une faible contribution de plusieurs gènes, notamment pour l'*UCP2*) [93]. Le *FTO* est un des gènes fréquemment cité dans la littérature [109-112]. Une étude GWAs a montré l'association de certains SNPs du *FTO* avec l'obésité et a confirmé les résultats chez les

caucasiens, les afro-américains et les hispano-américains [110]. Ces gènes sont impliqués dans l'homéostasie de l'énergie de la façon suivante:

- Le FTO est exprimé au niveau de l'hypothalamus, son rôle reste à élucider mais il pourrait activer la déacétylation des acides nucléiques et faire partie de la famille des oxygénases. Son expression est régulée par les périodes d'alimentation et de jeun [115].
- Le *MC4R* est un gène dont la déficience induit chez l'humain une des obésités autosomales les plus courantes [92,116]. Ce gène joue un rôle dans la régulation de la température corporelle et de l'appétence [92]. Les souris *KO* pour ce gène sont hyperphagiques, obèses et présentent une hyper-insulinémie [117].
- Le *SH2B1* code pour une tyrosine kinase pouvant augmenter la sécrétion de leptine [118]. Une méta-analyse comprenant 15 GWAs l'a identifié comme un des six gènes qui prédispose à une obésité chez l'être humain [119].

5. Les stabilisateurs de l'humeur

Le lithium et le valproate ont été incorporés dans ce travail de recherche en raison de leurs effets sur la prise de poids (Tableau 4) [2,9]. Ils ont en commun le fait d'être utilisés dans le traitement des troubles de l'humeur et d'avoir un mécanisme d'action peu connu, tant du point de vue thérapeutique que des effets secondaires [22,120].

Le lithium est un ion utilisé depuis plus de 50 ans dans le traitement des troubles bipolaires, particulièrement lors de phases maniaques, qui a montré son efficacité sur la diminution de la résurgence des symptômes. Son mécanisme d'action passerait par une inhibition des neurotransmetteurs impliquant des messagers secondaires associés à la voie du phosphatidyl-inositol. Le lithium a un index thérapeutique faible avec des concentrations plasmatiques de 0.5 à 1.2 mmol/L [22,120,121]. Il a un temps de demi-vie d'élimination d'environ 24 heures, n'est pas métabolisé et est éliminé à 95% par les reins [2].

Le valproate (aussi nommé acide valproique) fait partie de la classe des antiépileptiques. Son mécanisme d'action reste à élucider mais pourrait agir, entre autre, par une inhibition des canaux sodiques voltages dépendants ou par une stimulation de l'action des neurotransmetteurs du type acide gamma-amminobutyrique (GABA). Ce serait par ce biais que le valproate pourrait agir sur les symptômes maniaques des troubles bipolaires [22]. La fenêtre thérapeutique est de 50 à 100 µg/mL, les principales voies métaboliques sont la β-oxydation et la glucuroconjugaison, son temps de demi-vie est d'environ 11 heures et il est éliminé majoritairement par les reins sous forme de métabolites [2,121].

Chapitre II

Buts

La prise de poids est un des effets secondaires importants des antipsychotiques atypiques. Celle-ci est particulièrement préoccupante en termes de morbidité et mortalité à long terme puisqu'elle peut être associée à une obésité abdominale, une altération du profil lipidique, une hyperglycémie et un syndrome métabolique. Des études montrent une prévalence deux à quatre fois plus élevée des problèmes métaboliques et une prévalence environ double de décès dus à un problème cardiovasculaire dans une population de patients schizophrènes par rapport à la population générale. Cet excès de mortalité, lié en partie à la maladie et aux comportements qui peuvent y être associés, est aussi en grande partie lié à l'utilisation des antipsychotiques atypiques. D'autres médicaments faisant partie de l'arsenal thérapeutique psychiatrique sont également associés à une prise de poids. Certains stabilisateurs de l'humeur (notamment le valproate et le lithium) ainsi que certains antidépresseurs (notamment la mirtazapine et l'amitriptyline) sont connus pour induire une prise de poids. Cette prise de poids, bien que très variable d'un individu à l'autre, peut être importante au point d'avoir fait l'objet d'une annonce de pharmacovigilance par l'UPPC au centre national de pharmacovigilance Swissmedic pour un cas extrême (voir chapitre I, point 1).

Le but de ce travail de thèse consiste à rechercher des facteurs prédictifs de la prise de poids, des différentes complications métaboliques ainsi que d'autres effets secondaires (par exemple, élévation du taux de prolactine) pouvant survenir dans une population psychiatrique sous antipsychotique atypique et/ou stabilisateur de l'humeur. Ces facteurs peuvent expliquer, en partie, la variabilité observée dans la fréquence et l'intensité des effets secondaires sous psychotropes.

Ce travail se base sur les trois études cliniques et pharmacogénétiques initiées par notre unité. La variabilité interindividuelle face à ces effets secondaires a été évaluée par l'analyse des facteurs de risques cliniques ainsi que par l'analyse de l'influence de polymorphismes de certains gènes liés à la pharmacocinétique et à la pharmacodynamique des médicaments psychotropes. Des gènes candidats ont également été identifiés dans des études d'association entre la prise de poids et la prescription d'AP, dans des études GWAs sur l'obésité, et/ou des modèles animaux sur l'obésité.

Le but à long terme initié dans ce travail est d'améliorer la sécurité d'utilisation de ces médicaments grâce à une meilleure compréhension des facteurs pouvant induire une prise de poids et d'autres effets secondaires métaboliques, de pouvoir détecter les patients à risque de ce genre de complications, et de prévenir l'apparition de ces effets secondaires.

Les buts spécifiques des différentes études étaient les suivants:

Manuscrits I et II : Développer des outils analytiques pour déterminer les concentrations plasmatiques de nombreux médicaments psychotropes prescrits aux patients dans les études pharmacogénétiques. Ces méthodes permettent notamment de contrôler la compliance au traitement ainsi que d'offrir un suivi thérapeutique médicamenteux.

Manuscrit III : Revue de la littérature pour mettre en évidence la mortalité et les comorbidités augmentées des patients sous psychotropes en comparaison à la population générale, et souligner la nécessité d'une prise en charge globale du patient comprenant en sus de la partie psychiatrique, un suivi somatique des paramètres métaboliques. Démontrer par quelques exemples, le rôle des SNPs dans la variabilité de la prise de poids.

Manuscrit IV : Identifier la prévalence de certains risques cardiovasculaires dans une population psychiatrique et mettre en perspective des facteurs cliniques pouvant influencer l'IMC et la prise de poids.

Manuscrit V : Analyser l'influence de polymorphismes de différents gènes liés au métabolisme et au transport de la risperidone et de son métabolite principal sur les taux plasmatiques ainsi que leurs impacts sur la réponse au traitement. L'influence des SNPs dans les gènes du *CYP2D6, CYP3A4, CYP3A5, CYP3A7, NR1I2, ABCB1* a été ainsi investiguée.

Manuscrit VI : Etudier l'influence sur l'IMC de certains polymorphismes du gène *CRTC1* dans deux populations psychiatriques.

Annexe :

Manuscrit VII : Présenter une revue de la littérature sur l'énantiosélectivité potentielle de la protéine de transport P-gp, sur les polymorphismes génétiques associés et sur les conséquences cliniques observées chez l'être humain.

Chapitre III

Méthodes

1. Etudes cliniques

Trois études cliniques ont été élaborées et menées par l'UPPC. Elles ont toutes été acceptées par les comités d'éthiques des centres dans lesquelles ces études ont été réalisées. Un consentement écrit a été obtenu de la part de chaque participant. Ces études sont présentées ci-dessous.

A. Etude prise de poids Genève

Il s'agit d'une étude transversale rétrospective observationnelle (sans dose ni médication recommandées) conduite dans deux secteurs ambulatoires de l'Hôpital Universitaire de Genève. Les patients âgés de 18 à 65 ans traités avec la clozapine, l'olanzapine, la quetiapine, la risperidone, le lithium et/ou le valproate depuis au moins 3 mois ont été recrutés. Au total, 196 patients ont été inclus dans cette étude entre 2006 et 2008. Les manuscrits IV et VI se réfèrent à cette population.

B. Etude Risperdal Consta

Il s'agit d'une étude transversalle observationnelle. Cette étude s'est déroulée dans les centres psychiatriques des Hôpitaux de Lausanne, Genève, Marsens et Königsfelden. Les patients psychotiques âgés de 18 ans et plus, traités actuellement par la risperidone sous forme d'un dépôt injectable (Risperdal Consta®), pour au minimum 2 mois, avec au moins 4 injections à la même dose ont été recrutés. Au total, 42 patients ont été inclus entre 2007-2009. Cette population est décrite dans le manuscrit V.

C. Suivi métabolique Lausanne

Il s'agit d'une étude longitudinale observationnelle. Les patients provenant de toutes les unités hospitalières et ambulatoires du Département de Psychiatrie - CHUV de Lausanne débutant un traitement de lithium, valproate, mirtazapine et/ou d'un antipsychotique atypique (amisulpride, aripiprazole, clozapine, olanzapine, quetiapine, risperidone, sertindole) ont été recrutés. Cette étude a débuté en 2007 et est toujours en cours. Lors de la rédaction de ce manuscrit, plus de 500 patients ont été inclus dans l'étude. Le manuscrit VI se réfère à cette population.

L'étude sur le suivi métabolique Lausanne se base sur une directive de bonnes pratiques cliniques visant à améliorer la prise en charge somatique de personnes traitées par les médicaments pouvant induire une prise de poids. Bien que les conséquences cliniques d'un syndrome métabolique soient reconnues par le corps médical, la mise en place d'un tel programme de suivi, a nécessité de grands efforts, particulièrement durant les 2 premières années (formation des différents intervenants, aide et soutien aux équipes, suivi du projet, etc.).

A notre connaissance, le Département de Psychiatrie - CHUV est le premier hôpital en Suisse à introduire dans sa routine clinique un programme de soins de cette envergure.

2. Méthodes analytiques

2.1. Le suivi thérapeutique des médicaments

Le suivi thérapeutique des médicaments (TDM de l'anglais *therapeutic drug monitoring*) consiste d'abord à doser le médicament d'intérêt dans le sang, et consiste ensuite à interpréter les concentrations obtenues en vue d'individualiser la posologie [121]. Bien que l'adaptation de la dose d'un médicament permette généralement d'atteindre l'effet thérapeutique souhaité, le TDM est recommandé notamment en l'absence de réponse thérapeutique ou en présence d'effets secondaires. Il a également montré son utilité pour le contrôle de la compliance, pour déterminer le taux associé à une rémission de la maladie chez un patient donné, en cas de suspicion d'une interaction médicamenteuse ou pour mettre en évidence un métabolisme particulier (métabolisme rapide ou déficient) [121,122].

Une fenêtre thérapeutique a été proposée pour la majorité des psychotropes dont ceux compris dans ce travail de thèse [121,122]. L'importance du TDM varie selon la médication. Ainsi, le dosage du lithium est obligatoire [2] et s'explique par sa fenêtre thérapeutique étroite, celui de la clozapine et de l'olanzapine est fortement recommandé tandis que les dosages des autres antipsychotiques atypiques et du valproate sont reconnus comme apportant une aide significative dans le traitement [121].

Les études montrent entre 30 et 60 % de mauvaise ou non-compliance pour des antidépresseurs ou antipsychotiques après 6 à 12 semaines de traitement [123-126]. Il était donc crucial dans les trois études présentées au chapitre III, point 1 de pouvoir exclure les patients ne présentant pas d'effets secondaires ou ne répondant pas au traitement en raison d'un manque de compliance. Le TDM nécessite des méthodes analytiques fiables et robustes, indispensables dans l'optique d'une prise en charge optimale des patients.

2.2. Méthodes analytiques

Une méthode d'analyse de molécules dans des échantillons biologiques à des fins de TDM nécessite une validation afin de s'assurer que chaque mesure réalisée ultérieurement sera proche de la valeur réelle et comprise dans des limites d'acceptabilité définies pour une application donnée. Les critères de validation utilisés dans ce travail pour des échantillons plasmatiques sont adaptés des documents

officiels de la Food and Drug Administration [127] et de la commission de la Société Française des Sciences Techniques Pharmaceutiques [128,129].

2.3. Critères de validation

Le processus de validation permet de déterminer l'erreur totale d'une méthode analytique. Celle-ci se base sur la combinaison d'une erreur systématique (justesse) et d'une erreur aléatoire (fidélité ou précision). Tandis que la première permet de déterminer le biais, soit l'étroitesse entre le résultat trouvé et la valeur considérée comme vraie, la deuxième permet d'évaluer la dispersion des données. Trois paramètres sont inclus dans la précision. La répétabilité correspond à la variabilité déterminée par comparaison de résultats obtenus au cours d'une même expérience. La fidélité intermédiaire correspond à la comparaison de résultats obtenus par la même méthode au cours de plusieurs expériences successives par des opérateurs différents. Ces deux premiers paramètres permettent de déterminer l'erreur aléatoire d'une procédure analytique au sein d'un même laboratoire. La reproductibilité correspond à la variabilité mesurée entre deux laboratoires et permet le transfert de méthodes.

La validation comprend également l'évaluation de la spécificité de la méthode, soit la capacité à déterminer de façon univoque la présence de la substance dosée. Les limites de quantification basse et haute permettent quant à elles, de déterminer un domaine de concentrations répondant aux critères de la validation.

Bien que faisant partie des critères additionnels pouvant être évalués durant la validation, la stabilité des échantillons a été ajoutée pour répondre aux besoins spécifiques du laboratoire. La stabilité est évaluée par comparaison d'une solution de référence avec des échantillons ayant subi des conditions de conservation pouvant survenir dans la pratique quotidienne du laboratoire. Ce procédé permet de déterminer les conditions spécifiques de transport et de stockage pour les méthodes utilisées.

2.4. La validation

La validation est basée sur l'utilisation du profil d'exactitude comprenant des limites d'acceptabilité de 15% conformément aux limites généralement utilisées dans les analyses biomédicales. Un seuil de 20% est toléré pour la limite basse de quantification. Les courbes d'étalonnage sont construites à partir du rapport de l'aire du signal de l'analyte sur celle du standard interne. La justesse, la répétabilité et la fidélité intermédiaire sont déterminées pour chaque niveau de concentration.

Dans le cadre de ce travail, le but est de valider la méthode de quantification sur un domaine de concentrations comprenant la fenêtre thérapeutique ainsi qu'une limite de quantification basse

proche de 1 ng/mL. Ce seuil minimal, parfois très inférieur à la fenêtre thérapeutique, permet de mieux différencier un problème de compliance d'une potentielle interaction médicamenteuse et/ou d'un métabolisme rapide. Face à une population psychiatrique souvent poly-médiquée, le critère de sélectivité a été investigué de façon détaillée, les potentielles interférences analytiques ont été déterminées.

Le laboratoire UPPC, accrédité selon les normes ISO 17025 et ISO 15189, a augmenté le nombre de médicaments proposés pour le TDM. Les plasmas des patients inclus dans les études effectuées durant ce travail de thèse ont été dosés (plus de 500 échantillons à la rédaction de ce manuscrit).

3. Manuscrit I : Therapeutic drug monitoring of 7 psychotropic drugs and 4 metabolites in human plasma by HPLC-MS

Le manuscrit I décrit une méthode de quantification dans le plasma de quatre antipsychotiques atypiques : l'aripiprazole, la clozapine, le sertindole et l'olanzapine ; deux antidépresseurs : la duloxetine et la venlafaxine et un médicament utilisé dans le traitement de l'hyperactivité : l'atomoxetine ainsi que leurs principaux métabolites. La méthode permet de les doser simultanément et dans un domaine couvrant leur fenêtre thérapeutique et descendant jusqu'à une limite basse proche de 1 ng/mL. Répondant aux critères de la validation, cette méthode a été introduite dans les analyses accréditées proposées par le laboratoire. Les plasmas des patients inclus dans les différentes études et traités par ces psychotropes ont été dosés.

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Therapeutic drug monitoring of seven psychotropic drugs and four metabolites in human plasma by HPLC–MS

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ABSTRACT

A simple and sensitive LC–MS method was developed and validated for the simultaneous quantification of aripiprazole (ARI), atomoxetine (ATO), duloxetine (DUL), clozapine (CLO), olanzapine (OLA), sertindole (STN), venlafaxine (VEN) and their active metabolites dehydroaripiprazole (DARI), norclozapine (NCLO), dehydrosertindole (DSTN) and O-desmethylvenlafaxine (OVEN) in human plasma. The above mentioned compounds and the internal standard (remoxipride) were extracted from 0.5 mL plasma by solid-phase extraction (mix mode support). The analytical separation was carried out on a reverse phase liquid chromatography at basic pH (pH 8.1) in gradient mode. All analytes were monitored by MS detection in the single ion monitoring mode and the method was validated covering the corresponding therapeutic range: 2–200 ng/mL for DUL, OLA, and STN, 4–200 ng/mL for DSTN, 5–1000 ng/mL for ARI, DARI and finally 2–1000 ng/mL for ATO, CLO, NCLO, VEN, OVEN. For all investigated compounds, good performance in terms of recoveries, selectivity, stability, repeatability, intermediate precision, trueness and accuracy, was obtained. Real patient plasma samples were then successfully analysed.

1. Introduction

Therapeutic drug monitoring is a useful tool for the clinical management of patients receiving a pharmacotherapy, particularly in psychiatry. Recommended target plasma concentrations for psychoactive drugs have already been published [1]. Aripiprazole, clozapine, olanzapine, and sertindole are so-called second generation or atypical antipsychotics. Compared to first generation antipsychotics, they have greatly improved the response to treatment of schizophrenia spectrum disorders by their efficacy on negative symptoms [2]. Venlafaxine and duloxetine are second generation antidepressants, with a selective inhibitory activity on serotonine and noradrenaline reuptake, which present a safer profile than the tricyclic antidepressants. Atomoxetine is a new noradrenaline reuptake inhibitor used for the treatment of attention deficit and hyperactivity disorder. The chemical structures and calculated physico-chemical properties of these seven studied psychotropic drugs and their active metabolite are presented in Fig. 1 and Table 1, respectively.

Most of the published methods allow quantification of a single compound, sometimes with their related metabolite [3–6]. Simultaneous quantification of various psychotropic drugs have also been published [7], mostly clozapine in combination with olanzapine [8–12], or the antidepressant venlafaxine with other drugs in the same therapeutic class [13–16]. For a monitoring service aiming to cover a large panel of psychotropic drugs, the opportunity of simultaneous quantification is very attractive also in terms of practical aspect and labour time. Although some methods have been published using gas chromatography [17–19], separation was mainly performed by HPLC coupled with UV [20–24], MS [5,11,25] and MS–MS detection [22,26–30]. For mass spectrometry, electrospray ionization in the positive mode (ESI+) was mainly used, rather than atmospheric pressure chemical ionization [31,32]. Therefore, a development on a LC–MS in ESI+ is considered suitable.

ESI mode has been reported to be particularly sensitive to the matrix effects when biological matrix such as plasma are analysed [33–35]. This matrix effects may influence the quality of a quantitative bioanalysis. They are reported as being the origin of possible co-elution of endogenous matrix components which can lead to unpredictable alteration of the MS signal [33–35]. Therefore, a clean sample extraction process such as LLE and SPE is mandatory to reduce this undesirable effect.

Liquid–liquid extraction (LLE) remains an attractive approach for extracting the molecules of interest from complex matrix such as plasma [3]. Recently, methods with on-line [36] or off-line solid-phase extraction (SPE) procedures [19] have been proposed. In this paper, the development and validation of a method is reported for

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Table 1
Relevant LC–MS characteristics.

Compound	pK_a basic[a]	log P[a]	log D pH 8[a]	$[M+H]^+$	Voltage [V]	t_R [min][b]
VEN	9.26	2.91	1.63	278	160	6.8
OVEN	9.33	2.26	0.91	264	160	4.7
OLA	7.77	3.29	3.05	313	140	8.1
DUL	10.02	3.73	1.74	298	80	8.4
CLO	7.14	3.47	3.42	327	40	9.7
NCLO	7.94	3.08	2.81	313	140	7.5
STN	9.06	5.26	4.18	441	150	10.7
DSTN	8.74	6.62	5.82	439	150	11.3
ARI	6.71	5.59	5.57	446	150	12.7
DARI	6.71	5.63	5.61	448	150	11.7
ATO	10.12	3.28	1.2	256	140	7.9

[a] pK_a basic, log P and log D at pH 8 were calculated using Advanced Chemistry Development Software V8.14 for Solaris (ACD/Labs, Toronto, Canada).
[b] t_R: Retention time.

the simultaneous determination of new psychotropic drugs ($n=7$) and their respective active metabolites ($n=4$), using SPE prior to LC–MS analysis.

2. Experimental

2.1. Chemicals and reagents

The drugs were kindly provided by their manufacturers: aripiprazole (ARI) and dehydroaripiprazole (DARI) by BMS (New Brunswick, USA); atomoxetine (ATO), duloxetine (DUL), and olanzapine (OLA) by Eli Lilly (Indianapolis, USA); clozapine (CLO) and norclozapine (NCLO) by Novartis (Basel, Switzerland); sertindole (STN) and dehydrosertindole (DSTN) by Lundbeck (Copenhagen, Denmark); venlafaxine (VEN) and O-desmethylvenlafaxine (OVEN) by Wyeth Ayerst (Princeton, USA); and the internal standard (IS) remoxipride (RMO) by Astra-Zeneca (Södertälje, Sweden). Hydrochloric acid (HCl 37%) was purchased from Merck (Darmstadt, Deutschland), and acetonitrile (ACN) and methanol (MeOH) both in gradient HPLC grade from J.T. Baker (Deventer, Holland). Ammonium hydroxide (25%), ammonium acetate for MS, and citric acid monohydrate 99–102% were bought from Sigma–Aldrich (Steinheim, Deutschland). Ultrapure water was obtained from a Milli-Q® RG with a QPAQ2 column system (Millipore, MA, USA). Other chemicals were of analytical grade. For the preparation of calibration and validation standards, more than 10 different batches of human plasma were obtained from the Hospital's blood transfusion center (CHUV, Lausanne, Switzerland).

2.2. Standard solution, working solution

Standard stock solutions of each analyte at 1 mg/mL were prepared by dissolving the adequate amount of pure analyte in MeOH and stored for a maximum of 1 year at −20 °C. Two working solutions were obtained by diluting the stock solutions with 0.01N HCl to 10 ng/μL for DUL, OLA, STN, DSTN and to 50 ng/μL and subsequently to 10 ng/μL for ATO, CLO, NCLO, ARI, DARI, VEN and OVEN according to their plasma concentration ranges. These solutions were divided into aliquots (0.5 mL) and stored at −20 °C. The two working solutions were then both diluted to 1.0 and 0.2 ng/μL to prepare calibration standards or validation standards at the

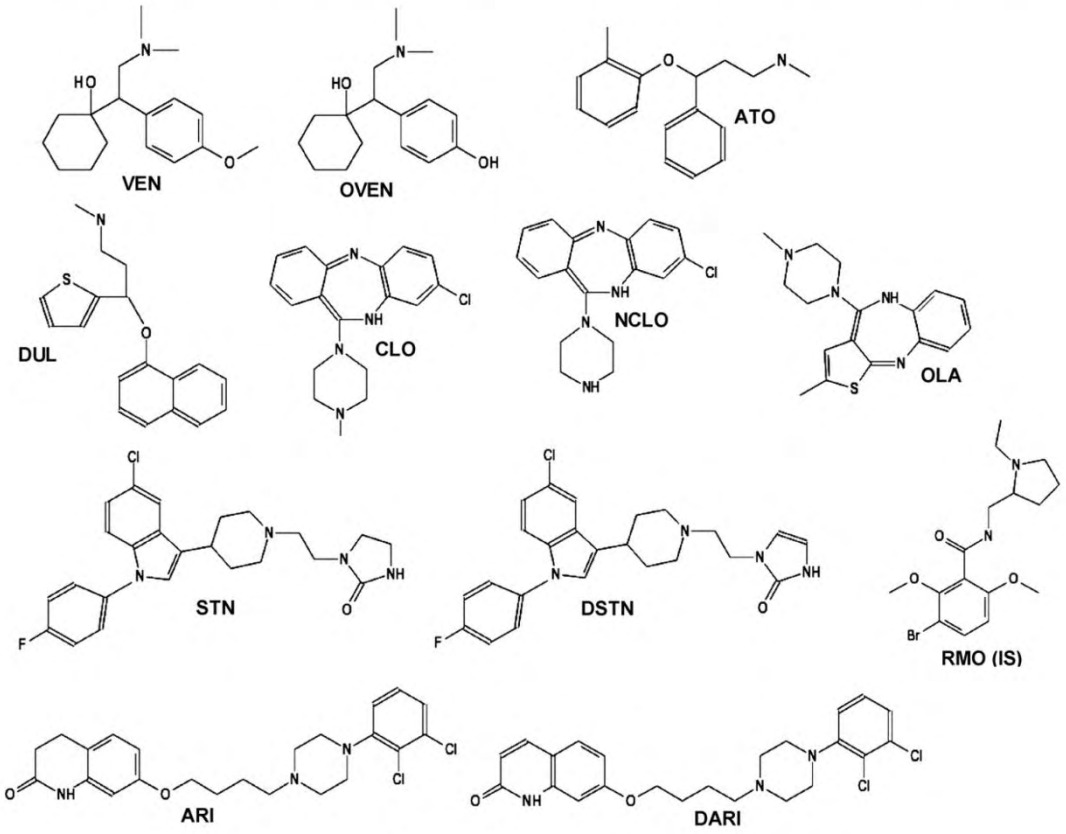

Fig. 1. Structure of molecules of interest.

appropriate concentration in plasma. Two different batches were prepared, one for preparation of the calibration standards and the other one for the validation standards. Finally, IS solution was prepared at 2 ng/mL in MeOH and stored at −20 °C.

2.3. Equipment

The liquid chromatography system consisted of an Agilent HP1100 binary pump equipped with a 100-vial autosampler (Agilent Technologies, Walldbronn, Deutschland), with a measured dwell volume of 1.15 mL. The system was coupled to a single quadrupole mass spectrometer (Agilent MSD), with electrospray ionization in the positive mode. Data acquisition, data handling and instrument control were performed by Chemstation 8.01.01 (Agilent Technologies). The whole system was maintained at 22 °C in an air conditioned room. The best chromatographic parameters were determined using HPLC modeling software (Osiris 4.1.1.2, Datalys, Grenoble, France), on the basis of two generic gradients that only differed in slope. The buffer capacity was carefully checked using PHoEBuS software version 1.3 (Analis, Namur, Belgium).

Separation was carried out on a Xbridge C18 column (2.1 × 100 mm; 3.5 μm) (Waters, Milford, MA, USA) equipped with a guard cartridge (2.1 × 10 mm; 3.5 μm) containing the same packing material. A 5 μL sample was injected into the system at a flow rate of 300 μL/min. Ammonium acetate 20 mM adjusted to pH 8.1 with ammonium hydroxide 25% (A) and ACN (B) was used as the mobile phase with the following gradient program: 16% of B at 0 min, 33.5% of B at 1.31 min, 60% of B maintained from 7.51 to 10.9 min, followed by a washing step at 85% of B from 11 to 13 min and finally, a 5 min reconditioning step at the initial conditions. The stability of the buffer solution was checked and found to remain stable for at least 2 weeks.

Analytes were quantified in the single ion monitoring (SIM) mode. All results were based on the peak area ratio between the

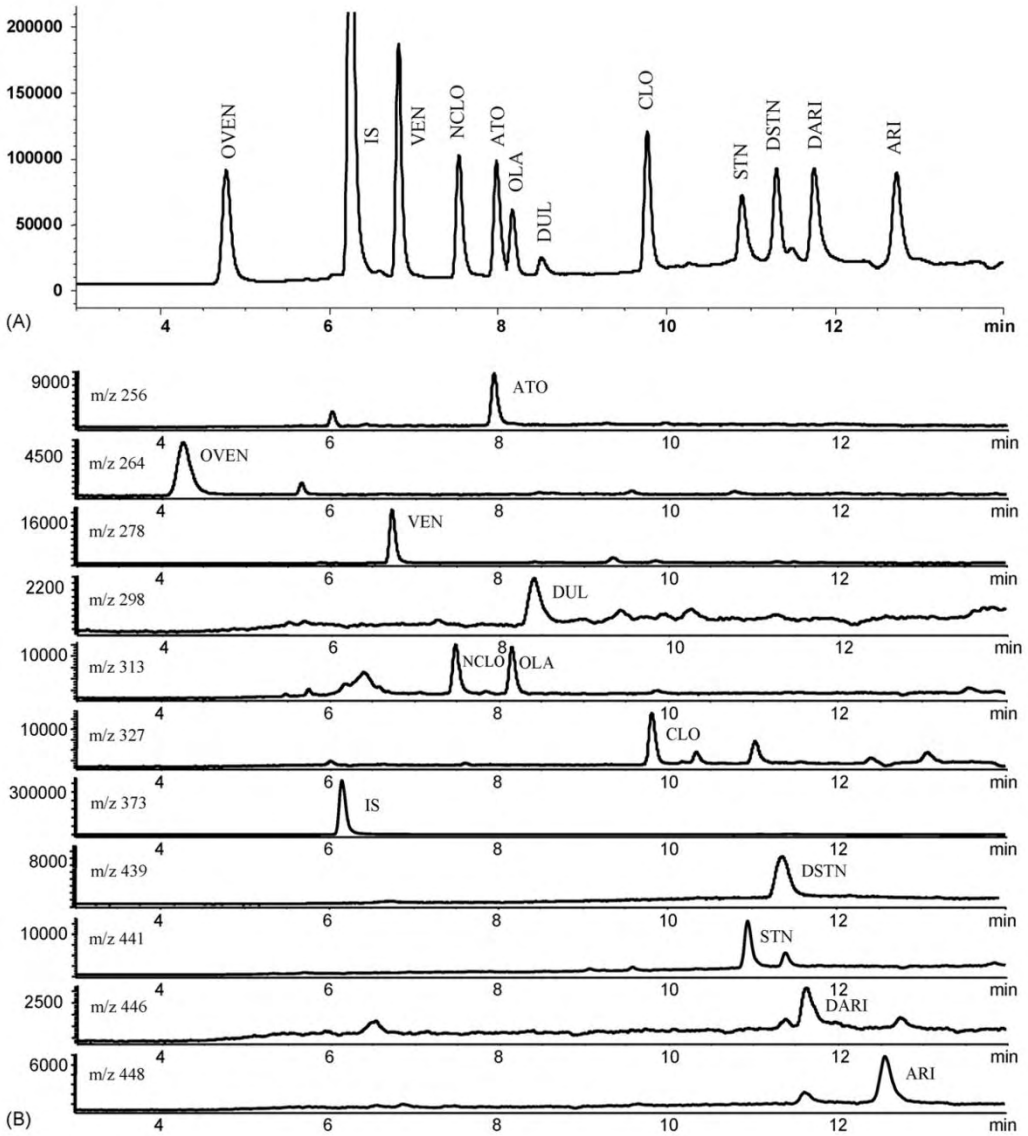

Fig. 2. Total ion current chromatogram of a plasma extract containing drugs at 50 ng/mL (OVEN, VEN, NCLO, ATO, CLO, DARI, ARI) or 20 ng/mL (OLA, DUL, STN, DSTN) and 100 ng/mL IS. Extracted ion chromatograms at 5 ng/mL. Note the isotopic contribution peak of DSTN and DARI on STN and ARI, respectively.

Table 2
Recoveries, matrix effect and process efficiency at 10 ng/mL except for the IS at 100 ng/mL.

Compound	Process efficiency (%)	Matrix effect (%)	Extraction recovery (%)
VEN	105	99	106
OVEN	106	99	107
OLA	131	118	111
DUL	107	116	92
CLO	103	101	102
NCLO	91	94	97
STN	126	133	95
DSTN	97	102	95
ARI	131	122	108
DARI	147	120	123
ATO	97	95	102
RMO (IS)	91	91	100

drug and the IS. The MS conditions were set as followed: drying gas flow 8 L/min, nebulizer pressure 40 psi, drying gas temperature 350 °C, capillary voltage 2000 V, dwell time 24 ms. Table 1 lists the m/z ratios measured, as well as the optimal fragmentor voltages for each $[M+H]^+$.

2.4. Sample preparation

Plasma calibration standards and plasma validation standards were extracted by SPE. The loading, washing, elution, evaporation and reconstitution steps of the sample preparation used during the SPE process were systematically investigated and the final process is reported below. First, 100 ng IS was added to 500 μL of plasma sample. The mixture was then diluted with 500 μL 1 M citric acid in water, vortexed and 1000 μL was loaded onto a SPE 96-well plate Oasis MCX support 10 mg (Waters, Milford, MA, USA), previously conditioned with 500 μL MeOH followed by 500 μL 1 M citric acid in water. A washing step consisting of 1 mL of 1 M citric acid in water followed by 1 mL MeOH was performed prior to elution. The compounds were then eluted with 500 μL MeOH–ammonium hydroxide 25% (94:6, v/v). After each step, a slow vacuum was applied until the wells were dry. The extracted samples were evaporated to dryness (40 °C, N_2 flow), and the residue was reconstituted in 250 μL mobile phase, i.e. ammonium acetate (pH 8.1; 20 mM)–ACN (84:16, v/v) and 5 μL was injected into the LC–MS system.

2.5. Method validation

The validation of this method was based on the guidelines of the Food and Drug Administration (FDA) and the recommendations of the "Société Française des Sciences et Techniques Pharmaceutiques" (SFSTP) [37]. The conference report of Viswanathan et al. from the workshop held on the same topic was also considered [38]. Three validation series were assessed to determine selectivity, repeatability and intermediate precision, trueness and accuracy on the basis of peak area ratio of drug and IS.

Method selectivity was ascertained for each validation series by analysing two different blank plasmas extracted and injected at the beginning of the HPLC analysis. In addition, after the highest validation standard, blank plasma was injected to determine any possible carryover effect. Drugs usually prescribed and/or taken in combination with the molecules of interest as well as some metabolites were tested. Plasma spiked with these potential interfering drugs were extracted and analysed. In case of similar retention factors, the potential signal suppression was carefully assessed by comparing peak area of the analyte alone and with two increasing concentrations of the potential interference.

Matrix effects were qualitatively estimated by simultaneously post-infusing a standard solution of the analytes and the IS [39]. Different batches of extracted blank plasma ($n = 6$) and blank plasma containing IS ($n = 6$) were analysed. An alteration of the m/z ratio baseline of the studied analytes at its retention time was considered as a matrix effect [33]. Matrix effects were quantitatively investigated at low (10 ng/mL) and high concentrations (150 ng/mL for DUL, OLA, STN, DSTN and 800 ng/mL for ATO, CLO, NCLO, VEN, OVEN, ARI, DARI) on the basis of the procedure proposed by Matuszewski et al. [40].

The variability of the peak areas was evaluated by calculation of the relative standard deviation (RSD) value on an assay done in triplicate for all analytes with a pool of five different sources of blank plasma. The process efficiency was obtained as the ratio between peak area of the plasma spiked before extraction and a standard solution at the corresponding level directly injected. Matrix effect was established using the peak area ratio between a plasma spiked after extraction and a standard solution directly injected. Extraction recoveries were defined as the ratio between plasmas spiked before and after the extraction.

Table 3
Drugs tested for selectivity assays.

Therapeutic class	Compound	t_R (min)[a]
Analytes and IS	ODVenlafaxine	4.71
	Remoxetine	6.17
	Venlafaxine	6.75
	Norclozapine	7.43
	Atomoxetine	7.98
	Olanzapine	8.05
	Duloxetine	8.45
	Clozapine	9.62
	Sertindole	10.71
	Dehydro-Sertindole	11.14
	Dehydro-Aripiprazole	11.58
	Aripiprazole	12.58
Antidepressants	Clomipramine	nd
	Desmethy-clomipramine	nd
	Imipramine	nd
	NODDVenlafaxine	3.35
	NDVenlafaxine	6.02
	Desmethyl-mirtazapine	6.34
	Nortriptyline	8.44
	Desmethyl-citalopram	7.12
	Citalopram	7.67
	Reboxetine	8.02
	Mirtazapine	8.39
	Desipramine	8.65
	Desmethyl-trimipramine	8.65
	Norfluoxetine	8.66
	Trazodone	8.86
	Fluoxetine	8.91
	Amitriptyline	9.83
	Sertraline	10.38
	Trimipramine	10.89
	Mianserine	11.29
Anxiolitics-hypnotics	Midazolam	8.88
Antipsychotics	Chlorpromazine	nd
	Sulpiride	nd
	Amisulpride	4.65
	9-OH Risperidone	6.90
	Risperidone	7.40
	Loxapine	7.54
	Haloperidol	8.69
	Quetiapine	8.97
	Ziprasidone	10.08
	Norsertindole	10.30
	Clopenthixol	10.55
Pro cognitifs	Galanthamine	4.21
	Memantine	6.38
	Rivastigamine	6.39
	Donepezil	8.77
Other drug	Methadone	9.05

[a] t_R: Retention time.

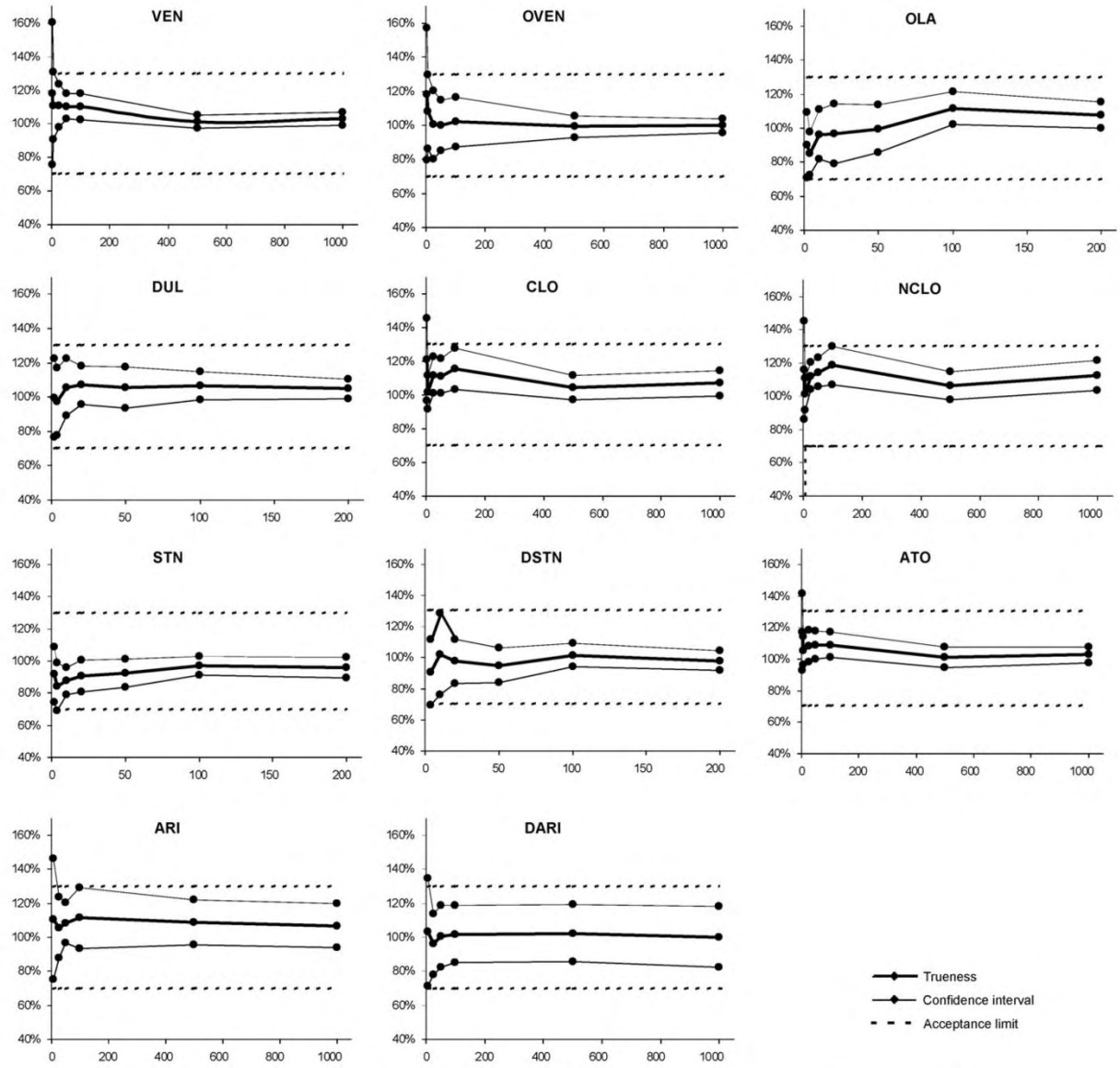

Fig. 3. Accuracy profiles within the acceptance limit ($\lambda = \pm 30\%$), and with confidence interval ($\alpha = 0.05$) calculated for each target in the dosing range.

Calibration standards (CS) were set in duplicate at the following concentrations to cover the plasma therapeutic range and expected patient's plasma values [1,21,41–43]: 2, 4, 20, 200 ng/mL for OLA, DUL, STN, DSTN and 2, 5, 500, 1000 ng/mL for ARI, DARI, ATO, CLO, NCLO, VEN, OVEN and validation standards (VS) were performed in quadruplicate at seven concentrations: 2, 4, 10, 20, 50, 100, 200 ng/mL for OLA, DUL, STN, DSTN and 2, 5, 25, 50, 100, 500, 1000 ng/mL for ARI, DARI, ATO, CLO, NCLO, VEN, OVEN. These solutions were independently prepared for each series using a pool of 6 different plasmas. The lowest and highest levels included in the VS, with respect to an acceptable repeatability, intermediate precision and trueness were considered as the lower limit of quantification (LLOQ) and the upper limit of quantification, respectively.

A 2-fold dilution with water of VS containing the analytes at twice the concentration of the highest CS was also included in the process to evaluate the plasma dilution effect with water in case of concentrations above the determined range of quantification. For stability tests, five blank plasmas were spiked at a low and high concentration with respect to their calibration ranges: 6 and 850 ng/mL for ATO, CLO, NCLO, VEN, OVEN, 10 and 850 ng/mL for ARI, DARI, 6 and 170 ng/mL for DUL, OLA, STN, and 8 and 170 ng/mL for DSTN. Freshly spiked plasmas were divided into 1 mL aliquots. One set of aliquots ($n = 5$) from each concentration was analysed immediately and the determined concentration was defined as the nominal level. Sets of aliquots were quantified after storage at room temperature for 24, 72 h and at +4 °C for 72 h, and after 1 and 3 freeze-thaw cycles.

Post-preparative stability in the autosampler was also assessed by leaving the extracted sample at room temperature and at +4 °C for 36 h. Finally, the long term stability was also assessed by keeping one set of aliquots at −20 °C for 2 months. For

Table 4
Assay validation parameters.

	Concentration (ng/mL)	Trueness (%)	Precision	
			Repeatability (%)	Intermediate precision (%)
VEN	2	118.2	16.2	19.5
	5	110.9	9.1	9.9
	25	110.9	2.5	6.4
	50	110.5	1.7	3.8
	100	110.4	1.7	3.9
	500	101.4	2.1	4.2
	1000	102.8	2.1	4.0
	2000/2	100.7	2.4	5.5
OVEN	2	118.4	12.3	17.8
	5	108.2	7.3	10.9
	25	100.4	3.2	10.9
	50	99.9	2.3	8.1
	100	102.0	1.3	7.7
	500	99.4	3.4	5.3
	1000	99.8	2.2	4.1
	2000/2	99.1	3.4	6.0
OLA	2	90.1	8.4	11.6
	4	85.1	5.4	8.2
	10	96.4	6.1	8.2
	20	96.8	7.9	9.9
	50	99.7	6.5	7.8
	100	111.7	4.7	4.9
	200	107.7	4.0	4.2
	400/2	100.8	5.5	5.2
DUL	2	99.6	11.0	12.5
	4	97.3	6.9	10.9
	10	105.6	7.2	8.6
	20	107.1	4.9	5.7
	50	105.4	4.6	6.2
	100	106.6	4.1	4.2
	200	104.8	3.0	3.9
	400/2	103.0	3.5	3.5
CLO	2	121.4	11.0	11.0
	5	101.6	5.3	5.5
	25	111.9	3.5	5.2
	50	111.1	2.8	5.0
	100	115.4	3.0	5.7
	500	104.3	3.8	5.4
	1000	107.1	3.8	8.2
	2000/2	105.5	3.4	8.7
NCLO	2	115.8	13.8	14.0
	5	101.1	5.4	5.1
	25	112.2	2.8	4.0
	50	114.5	2.8	4.1
	100	118.5	3.6	5.4
	500	106.2	4.4	6.9
	1000	112.7	4.4	4.9
	2000/2	105.5	8.7	8.6
STN	2	91.8	7.5	10.2
	4	84.0	10.0	3.8
	10	87.6	4.4	5.3
	20	90.6	5.7	5.8
	50	92.2	5.4	5.2
	100	97.1	3.4	3.7
	200	96.0	3.6	4.6
	400/2	98.8	4.5	4.5
DSTN	2	–	–	–
	4	90.5	7.8	12.8
	10	102.2	9.2	14.0
	20	97.6	4.8	7.8
	50	94.9	4.8	6.4
	100	101.5	4.0	4.4
	200	98.0	3.5	4.6
	400/2	98.5	3.8	4.4
DARI	2	–	–	–
	5	103.1	10.4	16.8
	25	96.1	9.1	10.1
	50	100.7	7.8	9.8
	100	101.8	7.5	9.0
	500	102.3	9.0	7.8
	1000	100.3	9.8	9.7
	2000/2	103.5	11.0	10.9
ARI	2	–	–	–
	5	110.8	10.3	17.5
	25	105.6	7.4	9.2
	50	108.5	4.9	5.9
	100	111.4	6.7	8.7
	500	108.9	6.6	7.0
	1000	106.7	6.6	6.0
	2000/2	108.0	7.6	6.8
ATO	2	117.1	12.9	11.4
	5	105.4	4.1	4.6
	25	108.3	3.5	5.0
	50	108.9	3.1	4.5
	100	109.0	2.9	4.0
	500	101.1	3.4	3.7
	1000	102.7	2.6	4.0
	2000/2	101.9	2.9	3.9

all experiments, difference in analyte concentration was determined as the ratio between the obtained level after storage and the nominal level. The RSDs of the set of 5 samples were also calculated.

3. Results and discussion

3.1. Choice of the experimental conditions in HPLC and MS

Due to the limited mass resolution (around *m/z* 1 FWHM) of the single quadrupole analyser, special attention was paid to the chromatographic separation of compounds with close *m/z* ratios. For instance, a baseline separation of OLA and NCLO by RPLC is mandatory, as the difference of *m/z* ratios (around 0.4 units) is not sufficient for an unambiguous differentiation. In addition, an isotopic peak contribution of DSTN (*m/z* 439) and DARI (*m/z* 446) could interfere with STN (*m/z* 441) and ARI (*m/z* 448), respectively. In agreement with other studies [44,45], improvement of the peak shape, better selectivity, higher retention of basic polar metabolites and superior signal responses, leading to a better chromatographic profile, could be observed at alkaline pH. A signal enhancement in basic conditions for basic drugs was attributed to a better desolvatation and spray stability in ESI when the drug is eluted with a higher ACN content [44,45]. Therefore, the chromatographic separation was investigated at different alkaline pH. For this purpose, various pH buffers between 8.1 and 10, with ammonia concentration ranging from 10 to 50 mM, were evaluated. According to PHoEBuS software calculation, each prepared buffer had a capacity at least equal to 6 mM/pH units. Thanks to the HPLC modeling software, the optimal separation was obtained at pH 8.1, in agreement with a study at pH 8 reported elsewhere for STN [21].

No influence on the separation was observed for a pH range between 7.6 and 8.2 and ammonium buffer concentration between 15 and 25 mM. In addition, the separation was not affected by a change of the temperature in a range of ±5 °C. With the selected LC conditions, the run time was 13 min with a final 2 min flush step at 85% ACN, followed by a re-equilibration time of 5 min at the initial buffer composition. The average retention times of all analytes are listed in Table 1.

A typical total ion current (TIC) chromatogram and the respective extracted ion current (XICs) of the 11 investigated compounds

Table 5
Stability testing of drugs analysed in this study ($n = 5$).

Drug	Venlafaxine				O-desmethylvenlafaxine				Olanzapine			
Nominal conc. (ng/mL)	6		850		6		850		6		170	
	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %
Room temperature, 24 h	2	0	1	1	1	−3	1	2	1	−2	2	−1
Room temperature, 72 h	3	1	2	2	5	6	2	5	6	−14	2	1
4 °C, 72 h	1	2	1	2	1	6	2	3	3	−15	5	5
Freeze/thaw												
1 cycle	2	−1	2	3	4	2	2	2	2	−2	2	−6
3 cycles	2	−4	2	4	2	−3	2	3	2	−17	1	−4
Stability in storage (−20 °C, 2 months)	2	−3	2	9	2	−1	1	8	8	−8	5	7
Autosampler												
Room temperature, 36 h	4	1	3	−4	3	−17	4	−5	3	44	14	1
4 °C, 3 6h	3	0	2	−1	2	0	2	1	2	37	5	1

Drug	Duloxetine				Clozapine				Norclozapine			
Nominal conc. (ng/mL)	6		170		6		850		6		850	
	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %
Room temperature, 24 h	5	−2	1	−2	1	−1	1	4	3	−5	2	−1
Room temperature, 72 h	5	−17	1	−15	2	−3	1	−1	5	−13	2	−7
4 °C, 72 h	2	−15	3	−13	2	−4	2	−2	2	−12	3	−6
Freeze/thaw												
1 cycle	2	−14	2	−15	2	−9	3	0	3	−10	3	−2
3 cycles	2	−12	2	−13	1	−10	2	2	2	−14	2	0
Stability in storage (−20 °C, 2 months)	11	13	4	4	5	−10	1	−6	4	−10	4	8
Autosampler												
Room temperature, 36 h	11	10	4	−5	3	8	7	−8	5	−6	7	−3

Drug	Sertindole				Dehydrosertindole				Atomoxetine			
Nominal conc. (ng/mL)	6		170		8		170		6		850	
	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %
Room temperature, 24 h	7	5	4	7	4	2	4	3	3	−1	1	0
Room temperature, 72 h	5	2	8	8	8	11	8	11	2	−7	1	−3
4 °C, 72 h	7	6	7	8	4	13	7	12	1	−7	2	−4
Freeze/thaw												
1 cycle	3	−3	4	0	8	0	3	−3	0	1	3	2
3 cycles	4	−2	2	2	3	6	2	−2	2	0	2	3
Stability in storage (−20 °C, 2 months)	12	5	4	−9	3	−18	3	−11	4	−15	1	11
Autosampler												
Room temperature, 36 h	6	13	6	−6	2	17	8	−4	4	−10	3	−4

Drug	Aripiprazole				Dehydroaripiprazole			
Nominal conc. (ng/mL)	10		850		10		850	
	RSD %	var. %	RSD %	var. %	RSD %	var. %	RSD %	var. %
Room temperature, 24 h	4	6	3	5	1	−2	5	−3
Room temperature, 72 h	3	7	4	−6	4	−28	3	5
4 °C, 72 h	8	−1	4	−6	2	−12	5	17
Freeze/thaw								
1 cycle	3	2	4	−4	4	−2	4	11
3 cycles	3	3	3	−1	3	−21	4	14
Stability in storage (−20 °C, 2 months)	13	−4	2	−16	5	−16	2	−20
Autosampler								
Room temperature, 36 h	5	6	4	1	8	−9	8	−8

are presented in Fig. 2. Although stable isotope labelled IS are highly recommended, in this method, all of the compounds of interest are simultaneously analysed during the same run and the choice of a stable isotope labelled IS for each drug was thus not considered. Therefore, special attention was paid to the matrix effects. Different analogs, which may also compensate for matrix effects [35,46], were investigated during the development. Remoxipride, an antipsychotic agent, which presents the advantage that it cannot be present in the blood of patients as it was withdrawn from the market several years ago, was chosen as IS.

3.2. Development of a solid-phase extraction procedure

An easily automatable SPE process was selected as the sample preparation procedures. Since the compounds of interest possess a basic function, the SPE sorbent chosen combines cation exchange and hydrophobic interactions and leads to an enhancement of selectivity. However an endogenous plasma compound interfered with NCLO and OLA (m/z 313) during the development. To overcome this problem, different washing steps were tested with ACN, MeOH, different ratios of MeOH/isopropanol, 2% formic acid and an increasing

Table 6
Levels of psychotropic drugs determined in human plasma in ng/mL.

	n	Calibration range validated	Therapeutic range	Real samples	
				Active drugs	Metabolites
Venlafaxine	31	2–1000	200–400[a]	5–413	37–783
Olanzapine	34	2–200	20–80	2–76	–
Duloxetine	8	2–200	20–80	13–93	–
Clozapine	58	2–1000	350–600	14–895	6–566
Sertindole[b]	3	2–200	50–100	2–8	4–15
Aripiprazole	30	5–1000	150–300	9–441	6–492

[a] The therapeutic range is the sum of VEN and its metabolite OVEN.
[b] The validated range is 4–200 ng/mL for metabolite DSTN.

percentage of alkaline solution in MeOH. An attempt was made to add a protein precipitation prior to the SPE process as already reported elsewhere [47]. A satisfactory step was obtained with citric acid as already reported for OLA [48]. A better recovery was obtained using MeOH rather than ACN in the elution step. Good extraction performance in terms of plasma purity, recoveries and repeatability were hence obtained. Extraction recovery are shown in Table 2.

3.3. Validation

No interference was observed in the different blank plasmas as well as no carryover between injections during all analyses. The 2 min flush at a high percentage of ACN at the end of the run was hence found satisfactory. In order to assess the selectivity, the method was applied to plasmas spiked with antidepressants, anxiolitics, hypnotics, antipsychotics, pro-cognitive, some metabolites and other drugs susceptible to be used as co-medication. Four substances co-eluted with the compounds of interest, namely risperidone with NCLO, reboxetine with OLA, amisulpride with OVEN and nortryptiline with DUL but were distinguished by the MS detection. In addition, no clinically significant signal suppression effect was found, as the effect was below 4.5% for OLA, OVEN and DUL and below 11% for NCLO (data no shown). Therefore, no interferences of drugs usually associated with the studied analytes were observed (Table 3).

The post-infusion tests were applied to all analytes and the IS. No qualitative interferences were observed on the different sources of blank plasma since no alteration of the MS signal at the retention time of the analyte was observed (data no shown). The results of quantitative assessment of process efficiency, matrix effect and extraction recovery are reported in Table 2. Consistent results were obtained at high concentration but for sake of clarity, only low concentration is shown. They were repeatable (CV < 15% for $n = 3$) in all the quantitative matrix effects observations.

The process efficiency, which represents the combined effects of the extraction recoveries and the matrix effect [35], generally ranged from 91% to 107%. Four values were observed above 120%, namely STN (126%), OLA (131%), ARI (131%) and DARI (147%). A greater response of OLA in biological matrix was previously reported [7]. The signal response enhancement leads to an overestimation of a maximum of 14.7 ng/mL instead of 10 ng/mL for DARI, the drug with the highest effect, which is not of clinical significance. The matrix effect ranged between 91% and 120% except for STN (133%) and ARI (122%). Finally extraction recoveries ranged from 92% to 111% with a 123% for DARI.

The validation process was initially performed with 7 calibration standard levels ($n = 2$): 2, 5, 25, 50, 100, 500, 1000 ng/mL for ATO, CLO, NCLO, VEN, OVEN, ARI, DARI and 2, 4, 10, 20, 50, 100, 200 ng/mL for DUL, OLA, STN, DSTN. Several regression models were tested, and the most suitable model was obtained with 4 calibration levels. Thus, the calibration curve from 2 to 200 ng/mL was suitably fitted by a linear regression forced through 0 for DUL, OLA, STN, DSTN. A quadratic regression response model was mandatory as calibration for ATO, CLO, NCLO, VEN, OVEN, ARI, DARI. The accuracy profiles within the acceptance limit ($\lambda = \pm 30\%$), and with confidence interval ($\alpha = 0.05$) calculated for each target in the dosing range, are shown in Fig. 3. The LLOQ was established at 2 ng/mL for all drugs except 4 ng/mL for DSTN, and 5 ng/mL for ARI and its metabolite DARI.

As reported in Table 4, trueness, repeatability and intermediate precision were in the acceptance criteria over the evaluated assay range. A 2-fold dilution of twice the highest concentration was found to be in the accepted range of the accuracy profile. Therefore, a plasma dilution with water could be performed if required. The room temperature stability test demonstrated that all compounds were stable up to 72 h storage with the exception of DUL and DARI, which were stable only up to 24 h storage (Table 5).

At low concentrations of DUL and DARI, a difference of 17% and 28% respectively, were calculated at 72 h. The stability after 1 freeze-thaw cycle was confirmed for all compounds. For 3 cycles, DARI and OLA presented some degradation (−21%, −17%, respectively). The instability of OLA is consistent with another stability report [49]. Extracted plasmas were all stable except for OLA, which presented a significant signal enhancement at low concentration (6 ng/mL), at room temperature and at 4 °C. In the case of an HPLC–MS analysis performed 36 h after extraction (following a technical failure for example), OLA requires re-extraction prior to LC–MS analysis. Some discrepant results have been published concerning the stability of OLA [7,26,49], and the option of adding some ascorbic acid was proposed to decrease OLA degradation and tested without success [11,23]. The metabolites DARI and DSTN presented a decreased amount after 2 months at −20 °C. All the details of stability tests are presented in Table 5.

4. Application to samples from psychiatric patients

The developed method was used to quantify the plasma drug level of 177 patients (Table 6). CLO, VEN, ARI, OLA were the most prescribed drugs most prescribed drugs. ATO has only just been introduced on to the Swiss market and therefore no patient plasma could be collected at the time of these analysis. The plasma levels were within the calibration ranges. In two cases, they were prediluted 2-fold for concentration of CLO 1380 ng/mL and 1620 (not accounted for in Table 6). No concentration above 2000 ng/mL was observed. Therefore, the method was suitable for therapeutic drug monitoring. Integrated external quality controls samples from 2 providers (Health Control Therapeutic Drug Scheme, Cardiff Bioanalytical Services Ltd., United Kingdom and UTAK, Radolfzell-Bohringen, Germany) as well as patient plasma samples from another hospital lab were successfully quantified.

5. Conclusions

A simple HPLC–MS method was developed and validated according to FDA guidelines and SFSTP protocols for the quantification of ATO, ARI, DARI, DUL, CLO, NCLO, OLA, STN, DSTN, VEN and OVEN in human plasma. The SPE process allows to efficiently remove endogenous interfering substances from the matrix while the HPLC–MS method permits the quantification of seven substances and their metabolites over the wide concentration range usually measured in psychiatric patients. Finally, this method has been successfully used for quantification of real plasma samples.

Conflict of interest

The authors declare no conflicts of interest.

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References

[1] P. Baumann, C. Hiemke, S. Ulrich, G. Eckermann, I. Gaertner, M. Gerlach, H.J. Kuss, G. Laux, B. Müller-Oerlinghausen, M.L. Rao, P. Riederer, Pharmacopsychiatry 37 (2004) 243–265.
[2] A.F. Lehman, J.A. Lieberman, L.B. Dixon, T.H. McGlashan, A.L. Miller, D.O. Perkins, et al., Am. J. Psychiatry 161 (2004) 1–56.
[3] F. Saint-Marcoux, F.L. Sauvage, P. Marquet, Anal. Bioanal. Chem. 388 (2007) 1327–1349.
[4] J. Bhatt, A. Jangid, G. Venkatesh, G. Subbaiah, S. Singh, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 829 (2005) 75–81.
[5] H.A. Niederländer, E.H. Koster, M.J. Hilhorst, H.J. Metting, M. Eilders, B. Ooms, G.J. de Jong, J. Chromatogr. B 834 (2006) 98–107.
[6] M. Kubo, T. Koue, A. Inaba, H. Takeda, H. Maune, T. Fukuda, J. Azuma, Drug Metab. Pharmacokinet. 20 (2005) 55–64.
[7] H. Kirchherr, W.N. Kuhn-Velten, J. Chromatogr. B 843 (2006) 100–113.
[8] G. Zhang, A.V. Terry Jr., M.G. Bartlett, Rapid Commun. Mass Spectrom. 21 (2007) 920–928.
[9] K. Titier, S. Bouchet, F. Pehourcq, N. Moore, M. Molimard, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 788 (2003) 179–185.
[10] C. Sanchez de la Torre, M.A. Martinez, E. Almarza, Forensic Sci. Int. 155 (2005) 193–204.
[11] Z. Zhou, X. Li, K. Li, Z. Xie, Z. Cheng, W. Peng, F. Wang, R. Zhu, H. Li, J. Chromatogr. B 802 (2004) 257–262.
[12] C. Kratzsch, F.T. Peters, T. Kraemer, A.A. Weber, H.H. Maurer, J. Mass Spectrom. 38 (2003) 283–295.
[13] H. Juan, Z. Zhiling, L. Huande, J. Chromatogr. B 820 (2005) 33–39.
[14] S.M. Wille, K.E. Maudens, C.H. Van Peterghen, W.E. Lambert, J. Chromatogr. A 1098 (2005) 19–25.
[15] C. Frahnert, M.L. Rao, K. Grasmader, J. Chromatogr. B 794 (2003) 35–47.
[16] A. de Castro, M.d.M. Ramirez Fernandez, M. Laloup, N. Samyn, G. De Boeck, M. Wood, V. Maes, M. Lopez-Rivadulla, J. Chromatogr. A 1160 (2007) 3–12.
[17] S. Ulrich, Ther. Drug Monit. 27 (2005) 463–468.
[18] T.A. Jennison, P. Brown, J. Crossett, M. Kushnir, F.M. Urry, J. Anal. Toxicol. 19 (1995) 537–541.
[19] H.C. Huang, C.H. Liu, T.H. Lan, T.M. Hu, H.J. Chiu, Y.C. Wu, Y.L. Tseng, J. Chromatogr. B 856 (2007) 57–61.
[20] L. Mercolini, R. Mandrioli, R. Cazzolla, M. Amore, M.A. Raggi, J. Chromatogr. B 856 (2007) 81–87.
[21] M. Canal-Raffin, E. Deridet, K. Titier, E. Frakra, M. Molimard, N. Moore, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 814 (2005) 61–67.
[22] C. Patel, M. Patel, S. Rani, M. Nivsarkar, H. Padh, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 850 (2007) 356–360.
[23] O.V. Olesen, K. Linnet, J. Chromatogr. B 714 (1998) 309–315.
[24] Y. Shimokawa, H. Akiyama, E. Kashiyama, T. Koga, G. Miyamoto, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 821 (2005) 8–14.
[25] W. Liu, F. Wang, H.D. Li, J. Chromatogr. B 850 (2007) 183–189.
[26] R.V. Nirogi, V.N. Kandikere, M. Shukla, K. Mudigonda, S. Maurya, R. Boosi, A. Yerramilli, J. Pharm. Biomed. Anal. 41 (2006) 935–942.
[27] J.M. Sauer, A.J. Long, B. Ring, J.S. Gillespie, N.P. Sanburn, K.A. DeSante, D. Petullo, M.R. VandenBranden, C.B. Jensen, S.A. Wrighton, B.P. Smith, H.A. Read, J.W. Witcher, J. Pharmacol. Exp. Ther. 308 (2004) 410–418.
[28] S.P. Senthamil, K.V. Gowda, U. Mandal, W.D. Sam Solomon, T.K. Pal, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 858 (2007) 269–275.
[29] S.D. Menacherry, G.E. Stamm, S.-Y. Chu, J. Liquid Chromatogr. Related Technol. 20 (1997) 2241–2257.
[30] M. Kubo, Y. Mizooku, Y. Hirao, T. Osumi, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 822 (2005) 294–299.
[31] J.H. Mullen, R.L. Shugert, G.D. Ponsler, Q. Li, B. Sundaram, H.L. Coales, J.E. Yakupkovic, R.M. Lelacheur, W.J. Wheeler, F.J. Belas, J.M. Sauer, J. Pharm. Biomed. Anal. 38 (2005) 720–733.
[32] M.J. Bogusz, K.D. Krüger, R.D. Maier, R. Erkwoh, F. Tuchtenhagen, J. Chromatogr. B 732 (1999) 257–269.
[33] S. Souverain, S. Rudaz, J.L. Veuthey, J. Chromatogr. A 1058 (2004) 61–66.
[34] W. Xie, J. Pawliszyn, W.M. Mullett, B.K. Matuszewski, J. Pharm. Biomed. Anal. 45 (2007) 599–608.
[35] P.J. Taylor, Clin. Biochem. 38 (2005) 328–334.
[36] K.M. Kirschbaum, M.J. Müller, G. Zernig, A. Saria, A. Mobascher, J. Malevani, C. Hiemke, Clin. Chem. 51 (2005) 1718–1721.
[37] P. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuizet, P. Chiap, N. Cohen, P.A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, STP Pharma Pract. 13 (2003) 101–138.
[38] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Am. Assoc. Pharm. Sci. 9 (2007) E30–E42.
[39] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, Rapid Commun. Mass Spectrom. 13 (1999) 1175–1185.
[40] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019–3030.
[41] M. Fric, B. Pfuhlmann, G. Laux, P. Riederer, G. Distler, S. Artmann, M. Wohlschlager, M. Liebmann, J. Deckert, Pharmacopsychiatry 41 (2008) 151–155.
[42] K.M. Kirschbaum, M.J. Muller, J. Malevani, A. Mobascher, C. Burchardt, M. Piel, C. Hiemke, World J. Biol. Psychiatry 9 (2008) 212–218.
[43] J.M. Sauer, B.J. Ring, J.W. Witcher, Clin. Pharmacokinet. 44 (2005) 571–590.
[44] L. Peng, T. Farkas, J. Chromatogr. A 1179 (2008) 131–144.
[45] J. Schappler, R. Nicoli, D.T.T. Nguyen, S. Rudaz, J.L. Veuthey, D. Guillarme, Talanta 78 (2009) 377–387.
[46] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Pharm. Res. 24 (2007) 1962–1973.
[47] S. Souverain, S. Rudaz, J.L. Veuthey, J. Pharm. Biomed. Anal. 35 (2004) 913–920.
[48] C. Chin, Z.P. Zhang, H.T. Karnes, J. Pharm. Biomed. Anal. 35 (2004) 1149–1167.
[49] S. Heller, C. Hiemke, G. Stroba, A. Rieger-Gies, E. Daum-Kreysch, J. Sachse, S. Härtter, Ther. Drug Monit. 26 (2004) 459–461.

4. Manuscrit II : Quantification of 4 antidepressants and a metabolite by LC-MS for therapeutic drug monitoring

La méthode décrite dans l'article II permet le dosage dans le plasma de quatre antidépresseurs : le bupropion, le moclobemide, la reboxetine et le trazodone et un métabolite dans un domaine couvrant leur fenêtre thérapeutique. Le développement est similaire au précédent avec la différence de l'ajout d'un ion de confirmation permettant de garantir d'autant plus la sélectivité. Répondant aux critères de la validation, cette méthode a été introduite dans les analyses accréditées. Une année d'analyses dans les conditions d'une routine de laboratoire sur des échantillons de patients ont montré la fiabilité de la méthode.

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Quantification of 4 antidepressants and a metabolite by LC–MS for therapeutic drug monitoring

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ABSTRACT

A liquid chromatography method coupled to mass spectrometry was developed for the quantification of bupropion, its metabolite hydroxy-bupropion, moclobemide, reboxetine and trazodone in human plasma. The validation of the analytical procedure was assessed according to Société Française des Sciences et Techniques Pharmaceutiques and the latest Food and Drug Administration guidelines. The sample preparation was performed with 0.5 mL of plasma extracted on a cation-exchange solid phase 96-well plate. The separation was achieved in 14 min on a C18 XBridge column (2.1 mm × 100 mm, 3.5 μm) using a 50 mM ammonium acetate pH 9/acetonitrile mobile phase in gradient mode. The compounds of interest were analysed in the single ion monitoring mode on a single quadrupole mass spectrometer working in positive electrospray ionisation mode. Two ions were selected *per* molecule to increase the number of identification points and to avoid as much as possible any false positives. Since selectivity is always a critical point for routine therapeutic drug monitoring, more than sixty common comedications for the psychiatric population were tested. For each analyte, the analytical procedure was validated to cover the common range of concentrations measured in plasma samples: 1–400 ng/mL for reboxetine and bupropion, 2–2000 ng/mL for hydroxy-bupropion, moclobemide, and trazodone. For all investigated compounds, reliable performance in terms of accuracy, precision, trueness, recovery, selectivity and stability was obtained. One year after its implementation in a routine process, this method demonstrated a high robustness with accurate values over the wide concentration range commonly observed among a psychiatric population.

1. Introduction

Antidepressant treatments are widely prescribed for depressive and other psychiatric disorders. They are classified into five classes. Among them, moclobemide (MOC) is a reversible and selective monoamine oxydase A inhibitor, bupropion (BUP) and its main active metabolite 2-hydroxybupropion (OHBUP) are selective inhibitors of catecholamine recapture (noradrenalin and dopamine), reboxetine (REB) is a selective inhibitor of noradrenalin recapture and finally trazodone (TRAZ) inhibits the serotonin recapture and antagonises serotonin receptors [1,2].

Because of the risk of non-compliance, drug interaction and inter individual variability in dose–response, therapeutic drug monitoring (TDM) is of interest to optimise the pharmacological treatment. Although a therapeutic window has not been clearly defined for all antidepressants, some range indications were reported for the drugs of interest [3,4]. Additional studies are still required to better define the therapeutic window, in particular for new molecules with low plasma concentrations such as BUP. A low limit of quantification (LLOQ) close to the ng/mL, is therefore necessary to discriminate between low plasma concentrations due for example to a rapid metabolism status and non-compliance suggested by the total absence of the considered therapeutic agent. Furthermore, wide calibration ranges are necessary to cover the range of plasma concentrations measured in clinical practise including cases of potential overdose.

BUP, MOC, REB, and TRAZ are currently quantified separately in plasma by different approaches such as gas chromatography (GC) coupled to mass spectrometry (MS) or nitrogen phosphorus detection (NPD) and liquid chromatography (LC) coupled to

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ultraviolet detection, MS or MS/MS [2,5,6], generally in positive electrospray ionization (ESI) mode. Some stereoselective methods were reported for BUP and REB, using either LC–MS/MS [7,8] or capillary zone electrophoresis [9]. New approaches were described for the simultaneous dosage of several antidepressants [10–14], in which one quantifies three of the five targeted molecules (REB, MOC, and TRAZ) with high reported LLOQ (10 ng/mL for REB and 100 ng/mL for MOC and TRAZ) [14]. High throughput and time saving methods are increasingly necessary for TDM laboratory. However, to our knowledge, no method was reported for BUP with simultaneous quantification of other antidepressants in plasma.

The aim of this work was, to provide for the routine use in a TDM laboratory, a simultaneous quantification of selected antidepressants by LC–MS with respect to the current validation procedures.

2. Experimental

2.1. Chemicals and reagents

Bupropion hydrochloride and its metabolite 2-hydroxybupropion were kindly provided by GlaxoSmithKline (Franklin Plaza, PA, USA), moclobemide by Roche (Basel, Switzerland), and trazodone hydrochloride by Vifor (Villars-sur-Glâne, Switzerland). Reboxetine mesylate hydrate was purchased at Sigma–Aldrich (St. Louis, MO, USA). Litracen (LIT) from Lundbeck (Glattburg, Switzerland) and remoxipride (REMO) from Astra Zeneca (London, England) were used as internal standards (IS).

A hydrochloric acid (HCl) solution at 0.01 N was prepared with 37% HCl provided by Merck (Darmstadt, Germany) diluted with ultrapure water obtained from a Milli-Q® RG with a QPAQ2 column system from Millipore (Billerica, MA, USA). The mobile phase was acetonitrile (ACN) from J.T. Baker (Deventer, Netherland) and ammonium acetate buffer (pH 9, 50 mM) from ammonium acetate puriss p.a. for MS provided by Sigma–Aldrich (Steinheim, Germany). Methanol (MeOH), formic acid, 25% ammonium hydroxide and isopropanol from Sigma–Aldrich and 85% ortho-phosphoric acid from Merck were used for the solid phase extraction. For the preparation of calibration and control samples, human plasma of different origins ($n > 10$) were tested and pooled. All plasma samples were obtained from the Hospital's blood transfusion centre (CHUV, Lausanne, Switzerland).

2.2. Working solution

Working solutions of 1 mg/mL of BUP, OHBUP, MOC, REBOX and TRAZ were prepared in MeOH. Drugs were divided into two groups ranging from 1 to 400 ng/mL for REB and BUP and 2–2000 ng/mL for OHBUP, MOC, and TRAZ, according to their therapeutic and observed plasma concentration ranges [3,4]. Plasma samples were spiked at the appropriate concentration by freshly prepared subsequent dilutions of the working solution. Calibration standards (CS) and validation standards (independent seeded controls, VS), were obtained by using different batches. CS and VS were independently prepared. Finally, internal standard (IS) solutions of REMO and LIT were prepared at 1 μg/mL in HCl 0.01 N. Working solutions, IS solution and spiked plasma were all stored at −20 °C prior to analyses. No degradation was observed for the target drugs at −20 °C after one year of storage for the working solutions and IS solution and after two months storage for the spiked plasma samples.

2.3. Equipment

2.3.1. Sample pre-treatment and extraction

A solid phase extraction was performed on plasma samples after thawing and storage at room temperature. The sample extraction was carried out onto a 10 mg SPE 96-well plate Oasis MCX support from Waters (Milford, MA, USA). 50 ng of REMO and LIT were added to 500 μL of plasma sample before a dilution with 4% H_3PO_4 (1:1). After vortexing, the sample was loaded onto a SPE 96-well plate previously conditioned with 500 μL of MeOH and 500 μL of H_2O. Three successive washing steps consisting of 500 μL of 2% formic acid in H_2O (v/v), and two times 250 μL of MeOH were applied on the sample. The elution step was achieved with two times 125 μL of 5% ammonium hydroxide in MeOH/isopropanol (1:1, v/v) followed by 250 μL of H_2O prior to injection into the HPLC–MS system. Between each extraction step, the wells were slowly dried.

2.3.2. HPLC–MS analysis

The liquid chromatography system consisted of an Agilent HP1100 binary pump equipped with a 100-vial autosampler from Agilent Technologies (Santa Clara, CA, USA) coupled to a MSD Agilent simple quadrupole mass spectrometer equipped with an ESI source working in the positive ionization mode. The system was controlled by the Chemstation 8.01.01 from Agilent Technologies. The system was maintained at 20 °C in an air conditioned room. Optimal chromatographic conditions were determined using Osiris software version 4.1 from Datalys (Saint Martin d'Hères, France) as previously described [15], and MS signal response was optimised thanks to an experimental design strategy obtained with StatGraphics Plus 5.1 from Statistical Graphics Corp. (Herndon, VA, USA). Drying gas flow, nebulizer pressure, drying gas temperature, capillary voltage, were set at 13 L/min, 40 psig, 350 °C, 1250 V, respectively. The m/z ratios used were: 240, 256, 269, 314, 372 for BUP, OHBUP, MOC, REB and TRAZ, respectively. Fragmentor voltage was set at 80 V except for TRAZ and LIT which was at 60 V.

Separation was carried out (5 μL of sample) on a XBridge C18 column (2.1 mm × 100 mm, i.d. 3.5 μm) equipped with a XBridge guard cartridge (2.1 mm × 10 mm, i.d. 3.5 μm) provided by Waters (Milford, MA, USA). Ammonium acetate 50 mM adjusted to pH 9 with 25% ammonium hydroxide (A) and ACN (B) were used as the mobile phase with a flow rate of 300 μL/min with the following gradient program: 26% to 60% of B from 0 to 4.3 min, then 60% B was maintained from 4.4 to 7.4 min. Finally, a washing step at 90% of B was applied until 8.4 min followed by 5 min of reconditioning with the initial mobile phase condition. All analytes were quantified by the peak area ratio between the drug and the IS (LIT) in the single ion monitoring mode (SIM).

2.4. Method validation

The method validation was performed according to the recommendations of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) regarding total error concept [16,17] and the last Food and Drug Administration (FDA) proposal [18]. The selectivity, trueness, repeatability and intermediate precision were evaluated on three different validation series. Accuracy profile based on tolerance intervals was used to select the best calibration function and to determine the validated concentration ranges. The tolerance probability β was set at 80% and the acceptance limit at ±30% [17]. Matrix effects were qualitatively and quantitatively analysed. The qualitative part of the matrix effect evaluation was performed by the post-infusion of a standard solution of the analytes at 2 μL/min in the HPLC–MS system while different blank plasma ($n = 6$) were injected according to the HPLC–MS method described herein [19]. A concentration of 0.1 ng/mL was used corresponding to the lower end concentration signal response (approximately 5 ng/mL). Any alteration of the signal in the detection window of the studied antidepressants was considered as a harmful matrix effect as it leads to a modification of the peak area of the compound of interest [20].

The quantitative assay was inspired from the procedure reported by Matuszewski et al. [21,22]. Low (10 ng/mL) and high concentrations (340 ng/mL for REB, BUP, OHBUP and 1700 ng/mL for MOC, TRAZ) were prepared in triplicate with a pool of six different batches of blank plasma. Extraction recoveries were established as the ratio between plasma samples at the same corresponding levels spiked before and after the extraction. Matrix effect was determined by using the peak area ratio between plasma spiked after extraction and a standard solution directly analysed. The process efficiency was defined in the Matuszewski procedure as the ratio between peak areas from the plasma spiked before extraction and from a standard solution at the corresponding concentration directly analysed [21,22]. It incorporates the effects of both the extraction recoveries and the matrix effect [22,23]. A maximum relative standard deviation (RSD) value of 15% was accepted for each concentration.

For each validation series, the selectivity was assessed by injecting two different extracted blank plasma samples at the beginning of the series and after the highest concentration standards. Thus, any eventual carryover effect was detected. Potential drugs ($n = 60$) which could be prescribed together with the studied antidepressants as well as some of their metabolites were investigated (see Supplementary Materials). These molecules, spiked at 100 ng/mL in human plasma, were extracted and analysed with regards to their retention factor. In case of co-elution, the MS signal alteration was carefully studied by comparing peak area of the antidepressant alone and together with the potential interference spiked at two different concentrations.

CS at three concentrations were performed in duplicate at the following concentrations: 1, 200, 400 ng/mL for BUP, REB and 2, 1000, and 2000 ng/mL for MOC, TRAZ, and OHBUP. VS were prepared in quadruplicate at the five following concentrations: 1, 4, 10, 100, and 400 ng/mL for BUP, REB and 2, 25, 100, 1000, and 2000 ng/mL for MOC, TRAZ, and OHBUP. It can be mentioned that OHBUP is the main metabolite of BUP and could present a concentration 3 to 14 fold higher than BUP [24]. These standards were independently prepared for each series using a pool of six different human plasma samples. The concentrations were chosen to cover the plasma therapeutic range and expected patients' plasma levels [3,25].

The concentrations of the VS were back-calculated using the best calibration function and analysed during the same run. The lowest and the highest levels included in the VS with respect to the latest criteria [16–18,26] were considered as the limit of quantification. A dilution step with ultrapure water (1:1) was performed during each validation series to demonstrate the possibility to dilute a sample presenting a concentration above the validated range.

For the following stability tests, five different batches of blank plasma were selected and spiked at different concentrations depending on the drug calibration range. Low stability control was set at 6 ng/mL for all target drugs and high stability control at 320 ng/mL for BUP, REB and 1600 ng/mL for MOC, TRAZ, and OHBUP. They were immediately separated into eight aliquots of 0.5 mL for each concentration. One set of aliquots was directly analysed and was considered as the nominal concentration. The other sets of aliquots were then quantified after storage at room temperature for 24 h, 72 h, at 4 °C for 72 h and after one and three freeze–thaw cycles. The long term stability was also investigated by keeping the two remaining sets of aliquots at −20 °C for two months. The extracted samples were also analysed after 36 h at room temperature in order to evaluate the post-preparative stability. For all stability tests, the variation in antidepressant concentration was determined by the ratio between the level after storage and the nominal level.

2.5. Confirmation ions

In order to detect an influence on the quantification process from an unknown interference, a confirmation ion is used for each drug when required. The presence of an interference (i.e. comedication) with similar retention factor and m/z ratio will potentially lead to an overestimation of the drug concentration. The confirmation run allowing a peak identification/discrimination in HPLC–MS was performed using the same HPLC–MS as previously described, with the addition of the confirmation ions (m/z ratios: 184 for BUP, 238 for OHBUP, 182 for MOC, 176 for REB, 373 for TRAZ). The mean relative ion intensity was calculated as a ratio between the peak areas of the confirmation and the original ions, with an accepted range of ±30%.

3. Results and discussion

3.1. Sample preparation

Co-elution of endogenous compounds was reported to be responsible for erroneous results especially with an ESI source [20,22,23,27–30]. A fast and easily automatable SPE process was chosen among other purification procedures. All analytes have a pKa above 6 and so were extracted in acidic conditions (pH < 2) in their ionised form using a mixed mode cation exchange 96-well plate (MCX). An optimal recovery of the analytes was obtained by using a mixture of ammonium hydroxide 5% in MeOH/isopropanol (1:1, v/v) followed by an addition of the same volume of water prior to injection into the LC–MS.

3.2. HPLC–MS

Improvement of selectivity, signal response and peak feature were reported in alkaline pH for basic compounds due to the relatively large amount of organic solvent in the mobile phase [31,32]. Therefore, the separation was developed with various alkaline pH buffers and different ammonium solution concentrations. The optimal separation was found at pH 9. It was particularly important to obtain a baseline separation of the analytes because the loss of the hydroxyl group (m/z 16) of OHBUP (m/z 256) gives a peak contribution on the BUP channel (m/z 240). Moreover, TRAZ (m/z 372) and REMO (m/z 373) gave an isotopic peak contribution to each other. Since the single quadrupole has a low mass resolution, these two pairs of compounds should be baseline separated by the LC for an unambiguous discrimination.

The run time was 14 min including a washing step at 90% ACN which allowed to maintain assay robustness and HPLC performance. A reconditioning step of 5 min with the initial mobile phase composition achieved the run. A typical single ion monitoring (SIM) of the selected antidepressants is presented in Fig. 1. No interferences were observed in the total ion current chromatograms (TIC) of blank plasma during the selectivity and matrix effect assays.

Analogue molecules with similar physico-chemical properties and ionisation fractionation, which may also compensate for matrix effects, were tested as IS [23,33]. Litracen (m/z 278) and remoxipride (m/z 373), both antipsychotic agents, which present the advantage that they cannot be present in the patients' blood as they were withdrawn from the market several years ago, were found the best fit for the purpose.

3.3. Method validation

A clean baseline was recorded for each compound m/z ratio for all the blank plasma tested, without carryover between injections. The selectivity was further investigated by analyzing plasma samples spiked with sixty drugs susceptible to be taken together

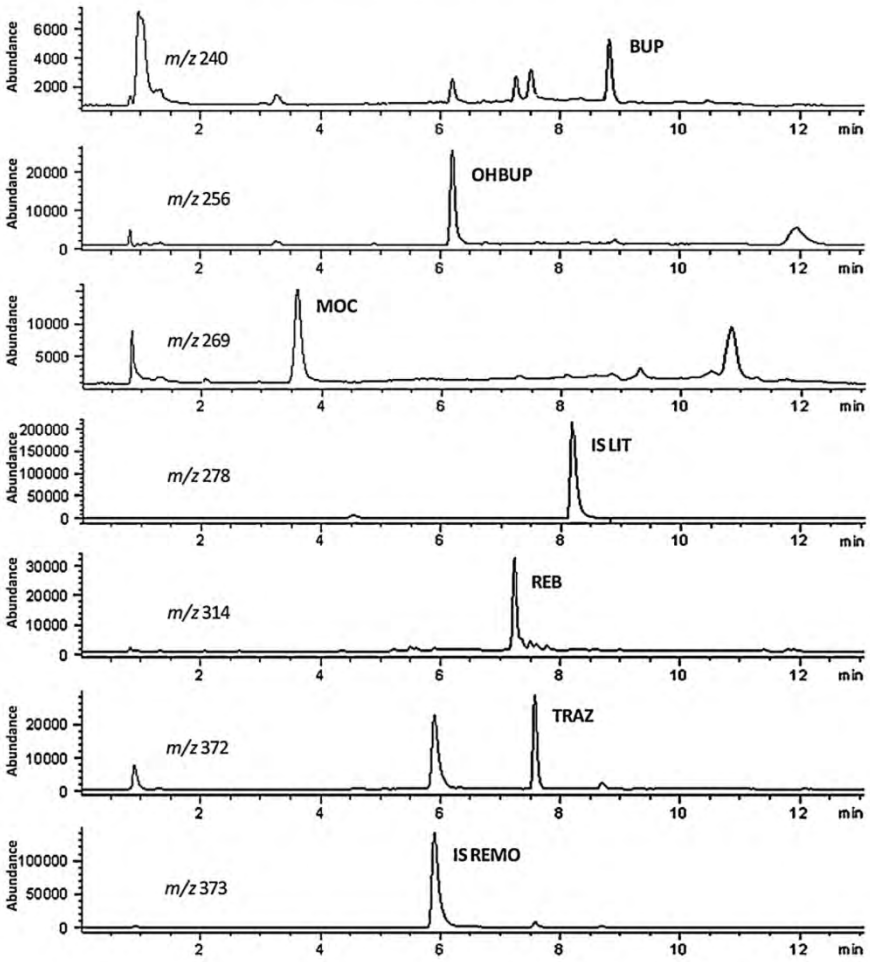

Fig. 1. Single ion monitoring chromatogram of a human plasma sample containing 10 ng/mL of bupropion (BUP, Rt = 8.9), reboxetine (REB, Rt = 7.3), moclobemide (MOC, Rt = 3.7), hydroxybupropion (OHBUP, Rt = 6.3), trazodone (TRAZ, Rt = 7.7), and 50 ng/mL of IS remoxipride (IS REMO, Rt = 5.6), IS litracen (IS LIT, Rt = 8.3). Note the contribution peak of IS REMO and OHBUP on TRAZ and BUP, respectively. Rt: retention time.

with the drugs of interest as well as some of their metabolites. No significant interference was observed for all the tested molecules (range: −4% to 2%). The highest variation was observed for BUP with methadone (−15%) which remains largely inferior to the clinical significance. Regarding matrix effect, no qualitative interferences were observed at the retention time of interest during the post-infusion test. Furthermore, on the different batches of blank plasma incorporated in every validation series either at the beginning of the series or after the highest CS, no interfering peaks were noticed for the selected antidepressants (data not shown).

The outcome of quantitative assessment for extraction recovery, matrix effect, and process efficiency is presented in Table 1. The process efficiency was highly repeatable (RSD ≤ 5%, $n = 3$) and ranged from 89 to 106% at high concentration and from 108 to 121% at low concentration. This increase at low concentration is predominantly due to the matrix effect measured between 116 and 130%. However, no clinically significant influence should be expected since the enhancement leads to an average maximum overestimation in absolute concentration of about 2 ng/mL. The matrix effects were observed with a maximum RSD of 8%. Finally, extraction recoveries remain very consistent at low and high concentration with a range between 88 to 96% and RSD ≤ 10%.

The best model was selected through the most favourable accuracy profile. The calibration curves were transformed with a quadratic regression by three calibration levels at 1, 200, 400 ng/mL for BUP, REB and 2, 1000, 2000 ng/mL for MOC, TRAZ, and OHBUP. LLOQ were set at 1 for BUP, REB and 2 ng/mL for MOC, TRAZ, and OHBUP. Good linearity was observed between calculated concentration and concentration of the analyte in the sample for the three validation series with a mean slope and determination coefficient higher than 0.95 and 0.99, respectively. All the validation standards remain in the acceptance criteria in terms of trueness, repeatability, and intermediate precision within tolerance interval ($\beta = 80\%$) and acceptance limit (±30%) as shown in the accuracy profiles presented in Fig. 2. The results for accuracy profiles are presented in Table 2.

No significant difference was found neither between a twofold dilution of 400 ng/mL and 200 ng/mL for BUP and REB nor between a twofold dilution of 4000 ng/mL and 2000 ng/mL for MOC, TRAZ and OHBUP ($n = 12$, with all t-test p values above 0.3). A twofold dilution with water was therefore found to be in the accepted range of the accuracy profile. Thus, when needed, a dilution of patient plasma with ultrapure water could be performed. Narrow accuracy profile with better LLOQ was found with LIT than REMO. Therefore, LIT was

Table 1
Recoveries, matrix effects and process efficiencies. Low concentration is defined as 10 ng/mL and high concentrations are defined as 340 ng/mL for BUP, OHBUP, and REB, and 1700 ng/mL for MOC and TRAZ. The IS are tested at the concentration used in the method namely 50 ng/mL for LIT and 100 ng/mL for REMO.

	Extraction recovery (%)		Matrix effect (%)		Process efficiency (%)	
	Low	High	Low	High	Low	High
BUP	93 ± 9	91 ± 1	116 ± 9	98 ± 3	108 ± 2	89 ± 4
OHBUP	94 ± 9	95 ± 0.3	125 ± 10	110 ± 1	117 ± 3	105 ± 1
MOC	95 ± 9	96 ± 1	128 ± 8	110 ± 3	121 ± 4	106 ± 2
REB	92 ± 9	93 ± 1	130 ± 11	109 ± 2	120 ± 3	102 ± 1
TRAZ	88 ± 8	95 ± 1	128 ± 9	109 ± 5	113 ± 3	104 ± 4
LIT (IS)	108 ± 1		96 ± 1		104 ± 3	
REMO IS)	109 ± 2		94 ± 4		103 ± 3	

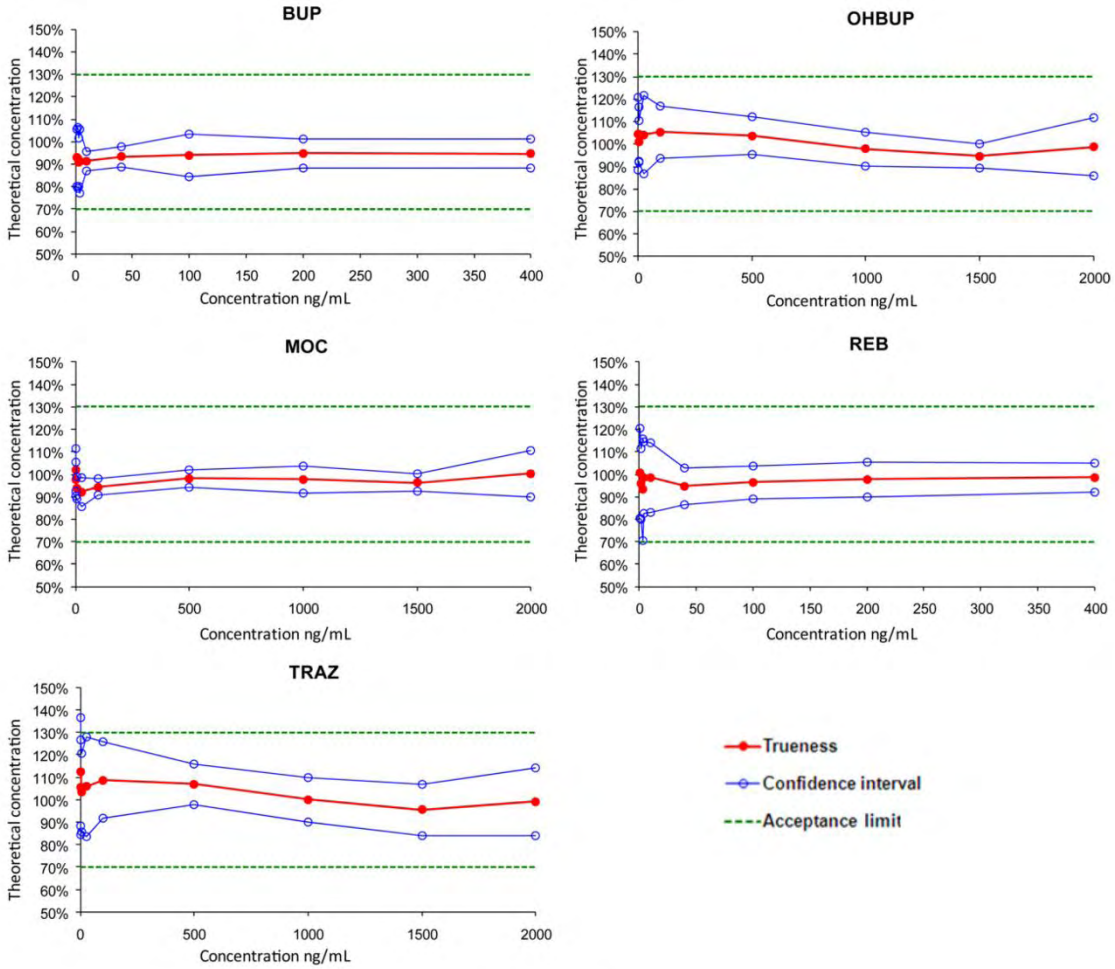

Fig. 2. Accuracy profiles.

chosen as IS for all the selected drugs and REMO remained in case of an unexpected event with LIT. REMO can also constitute a good alternative in case of supply problem of the former IS.

Low and high concentrations of each antidepressant underwent the stability tests as reported in Table 3. All tests were performed in quintuplicate for each antidepressant and at two concentrations. The target drugs remained stable (bias ≤ 15%) at room temperature for 24 h and 72 h as well as at 4 °C for 72 h with the exception of BUP. After 72 h at room temperature, more than 30% of BUP is lost. Special caution should thus be taken for the pre-analytic part for this drug and consequently BUP should be analysed within 48 h following blood sampling if the sample cannot be stored at 4 °C. Another study found that REB was stable at room temperature for seven days [34]. No significant difference was observed after one freeze–thaw cycle for all drugs. At low concentration, BUP presented a bias of 14% after three cycles. TRAZ remained stable (within 15% of the nominal concentration) for a maximum of two freeze–thaw cycles (data not shown) while presented a bias of −20% after three cycles. MOC, REB, OHBUP remained in the stability criteria. The long stability test of two months at −20 °C was successfully completed for all the drugs. Finally, extracted plasma remained stable for 36 h at room temperature for each target drug.

Table 2
Validation assay parameters.

Validation criterion	BUP	REB	Validation criterion	OHBUP	MOC	TRAZ
Calibration range (ng/mL)	[1; 400]	[1; 400]	Calibration range (ng/mL)	[2; 2000]	[2; 2000]	[2; 2000]
LLOQ (ng/mL)	1	1	LLOQ (ng/mL)	2	2	2
Trueness			Trueness			
Relative bias (%)			Relative bias (%)			
1 ng/mL	−7.2	0.6	2 ng/mL	4.6	2.0	12.5
4 ng/mL	−8.8	−1.6	25 ng/mL	4.2	−7.9	5.8
10 ng/mL	−8.7	−1.5	100 ng/mL	5.4	−5.7	8.7
100 ng/mL	−6.1	−3.6	1000 ng/mL	−2.2	−2.2	0.0
400 ng/mL	−5.3	−1.4	2000 ng/mL	−1.2	0.4	1.0
Precision			Precision			
Repeatability (%)/intermediate precision (%)			Repeatability (%)/intermediate precision (%)			
1 ng/mL	5.8/6.4	7.0/9.3	2 ng/mL	7.0/8.1	5.3/6.3	10.6/12.0
4 ng/mL	4.3/6.2	6.9/8.0	25 ng/mL	2.2/5.9	2.2/3.6	2.6/7.2
10 ng/mL	2.2/2.3	5.6/7.3	100 ng/mL	2.0/4.2	2.0/2.3	2.9/6.2
100 ng/mL	4.8/4.9	3.9/3.9	1000 ng/mL	4.1/4.1	4.3/4.3	5.2/5.2
400 ng/mL	2.0/2.9	3.3/3.3	2000 ng/mL	6.9/6.9	7.2/7.2	7.6/7.9
Accuracy			Accuracy			
Lower and upper confidence limits of total error (%)			Lower and upper confidence limits of total error (%)			
1 ng/mL	[−16.6; 2.2]	[−14.2; 15.3]	2 ng/mL	[−7.5; 16.7]	[−7.5; 11.5]	[−5.7; 30.6]
4 ng/mL	[−19.0; 1.5]	[−13.5; 10.3]	25 ng/mL	[−7.5; 16.0]	[−14.2; −1.6]	[−8.9; 20.4]
10 ng/mL	[−11.9; −5.4]	[−13.0; 10.1]	100 ng/mL	[−2.7; 13.4]	[−9.2; −2.1]	[−3.0; 20.4]
100 ng/mL	[−13.1; 1.0]	[−9.2; 2.0]	1000 ng/mL	[−8.0; 3.6]	[−8.3; 3.9]	[−7.4; 7.4]
400 ng/mL	[−10.0; −0.6]	[−6.2; 3.4]	2000 ng/mL	[−11.0; 8.6]	[−9.9; 10.7]	[−12.4; 10.5]

Table 3
Stability assay. The bias/RSD are presented in percent. The low levels are defined as 6 ng/mL for each molecule and high levels are defined as 320 ng/mL for BUP and REB and 1600 ng/mL for OHBUP, MOC, and TRAZ according to the validated range.

Stability assay	Level	BUP	OHBUP	MOC	REB	TRAZ
Short term stability, bias %(RSD)						
20 °C, 24 h	Low	−7 (5)	6 (3)	−5 (1)	4 (3)	−10 (4)
	High	−3 (2)	−2 (4)	−3 (3)	−3 (1)	−6 (4)
20 °C, 72 h	Low	−45 (9)	8 (0)	−5 (3)	7 (1)	−8 (3)
	High	−37 (14)	−13 (2)	1 (1)	0 (1)	11 (1)
4 °C, 72 h	Low	−15 (5)	8 (6)	−1 (4)	8 (6)	−10 (6)
	High	−5 (1)	−9 (2)	0 (1)	0 (1)	7 (2)
Long term stability, bias %(RSD)						
−20 °C, 2 months	Low	3 (4)	12 (3)	4 (5)	14 (5)	13 (4)
	High	−11 (4)	−15 (2)	−12 (2)	5 (2)	−11 (2)
Autosampler, bias %(RSD)						
20 °C, 24 h	Low	−15 (9)	0 (3)	−5 (3)	4 (3)	−14 (1)
	High	−14 (4)	1 (5)	−2 (3)	−3 (2)	−1 (2)
Freeze/thaw stability, bias %(RSD)						
1 cycle	Low	−11 (6)	9 (2)	0 (3)	13 (1)	−6 (2)
	High	−4 (2)	−10 (2)	5 (2)	3 (1)	15 (1)
3 cycles	Low	−14 (6)	9 (3)	−1 (2)	12 (2)	−4 (2)
	High	−10 (4)	−8 (1)	9 (4)	3 (3)	20 (6)

4. Clinical application

After the validation process, the method was applied on a routine basis for TDM. A quality control chart (QC) was also established. In each batch, three QC at low, medium and high concentrations were randomly inserted between patient samples. According to the therapeutic and validated range, the concentrations corresponded to 5, 80, 300 ng/mL for BUP, REB and 10, 700, 1500 ng/mL for MOC, TRAZ, and OHBUP. The mean bias and the RSD during the first year (50 series) for the three QC levels of all target drugs were at a maximum of 7% and 10%, respectively. All QC at any levels showed stable results throughout the year (Table 4).

The presence of an interference with a similar retention factor and m/z ratio would potentially lead to an increase of the target drug peak area which could consequently result in an overestimation of drug concentration. The mean relative ion intensities were based on the ratios between the peak areas acquired with the quantification method and with the confirmation method. The ratios were calculated on the data obtained during the validation procedure and the first year of IC and were of 9% for BUP, 18% for OHBUP, 2% for MOC, 10% for REB, 29% for TRAZ. The ratio was considered in acceptance range because it was within ±30% of the mean relative ion intensities.

The quantifications of BUP and TRAZ were the two most requested analyses. In total, the method was used to analyse these drugs in the plasma of 199 patients (Table 4). In eight cases (5%), plasma samples were diluted twofold before the extraction to be within the calibration range. No concentration above 800 ng/mL for BUP, REB and 4000 ng/mL for MOC, TRAZ, and OHBUP was observed, the patient plasma levels were then all within the validated range. As expected, the concentrations range of the drug of interest measured in the samples of patients receiving these drugs was found to be large (Table 4). External quality control samples were provided by two quality service centres (Arvecon, Gesellschaft für Toxicologische und Forensische Chemie, Walldorf, Germany and UTAK Laboratories, SL Marketing GbR, Utak Generalvertretung Radolfzell-Bohringen, Germany). These external controls were successfully quantified (data not shown). In addition, patients' plasma samples provided by another hospital laboratory working on a GC with nitrogen phosphorus detection were also analysed. The

Table 4
One year analysis with uncertainty assessment of internal control (IC) and drug plasma concentrations from TDM requests. Bias and RSD are presented in percent. Number of patients (n) is reported with the median (min–max) drug concentrations.

	BUP	OHBUP	MOC	REB	TRAZ
Relative bias, %(RSD)					
Low IC	4.3 (10.0)	7.5 (7.7)	0.8 (8.6)	5.0 (9.0)	8.0 (8.0)
Medium IC	−3.1 (4.4)	3.6 (5.2)	1.9 (5.9)	−0.6 (6.2)	3.6 (4.6)
High IC	−4.4 (4.0)	−1.6 (5.7)	−0.4 (5.0)	−1.3 (4.5)	−2.4 (5.6)
Real samples					
n	127	127	5	18	49
Drug levels	16 (1–119)	718 (2–1970)	1083 (51–1434)	144 (4–297)	621 (70–1851)

plasma concentrations obtained by the two methods were found similar (bias $\leq$ 15%, data not shown).

5. Conclusion

A quantification method based on an SPE cation exchange extraction followed by an analysis by HPLC–MS of several antidepressants (BUP and it main active metabolite OHBUP, MOC, REB, and TRAZ) was developed. The analytical procedure was validated using the latest recommendations. Moreover, the method was successfully implemented in a therapeutic drug monitoring laboratory for routine quantification of the drug of interest in patients' plasma. One year of analysis demonstrated a high robustness of the method and shows a wide range of plasma concentrations as measured in a psychiatric population.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2011.03.049.

References

[1] S.M. Stahl, Stahl's Essential Psychopharmacology—Neuroscientific Basis and Practical Applications, Cambridge University Press, New York, 2008.
[2] F. Saint-Marcoux, F.L. Sauvage, P. Marquet, Anal. Bioanal. Chem. 388 (2007) 1327.
[3] P. Baumann, C. Hiemke, S. Ulrich, G. Eckermann, I. Gaertner, M. Gerlach, H.J. Kuss, G. Laux, B. Müller-Oerlinghausen, M.L. Rao, P. Riederer, Pharmacopsychiatry 37 (2004) 243.
[4] S.H. Preskorn, Psychopharmacol. Bull. 27 (1991) 637.
[5] J.M. Hoskins, A.S. Gross, G.M. Shenfield, L.P. Rivory, J. Chromatogr. B 754 (2001) 319.
[6] M.A. Martinez, C. Sanchez de la Torre, E. Almarza, J. Anal. Toxicol. 28 (2004) 174.
[7] R. Coles, E.D. Kharasch, J. Chromatogr. B 857 (2007) 67.
[8] J.C. Fleishaker, M. Mucci, C. Pellizzoni, I. Poggesi, Biopharm. Drug Dispos. 20 (1999) 53.
[9] R. Mandrioli, M.A. Raggi, Electrophoresis 27 (2006) 213.
[10] K. Titier, S. Bouchet, F. Pehourcq, N. Moore, M. Molimard, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 788 (2003) 179.
[11] U. Gutteck, K.M. Rentsch, Clin. Chem. Lab. Med. 41 (2003) 1571.
[12] S.M.R. Wille, K.E. Maudens, C.H. Van Peterghen, W.E. Lambert, J. Chromatogr. A 1098 (2005) 19.
[13] A. de Castro, M.d.M. Ramirez Fernandez, M. Laloup, N. Samyn, G. De Boeck, M. Wood, V. Maes, M. Lopez-Rivadulla, J. Chromatogr. A 1160 (2007) 3.
[14] H. Kirchherr, W.N. Kuhn-Velten, J. Chromatogr. B 843 (2006) 100.
[15] E. Choong, S. Rudaz, A. Kottelat, D. Guillarme, J.L. Veuthey, C.B. Eap, J. Pharm. Biomed. Anal. 50 (2009) 1000.
[16] P. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuizet, P. Chiap, N. Cohen, P.A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, STP Pharma Pract. 13 (2003) 101.
[17] E. Rozet, A. Ceccato, C. Hubert, E. Ziemons, R. Oprean, S. Rudaz, B. Boulanger, P. Hubert, J. Chromatogr. A 1158 (2007) 111.
[18] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Am. Assoc. Pharm. Sci. 9 (2007) E30.
[19] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, Rapid Commun. Mass Spectrom. 13 (1999) 1175.
[20] S. Souverain, S. Rudaz, J.L. Veuthey, J. Chromatogr. A 1058 (2004) 61.
[21] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[22] I. Marchi, V. Viette, F. Badoud, M. Fathi, M. Saugy, S. Rudaz, J.L. Veuthey, J. Chromatogr. A 1217 (2010) 4071.
[23] P.J. Taylor, Clin. Biochem. 38 (2005) 328.
[24] Compendium Suisse de médicaments, Documed S.A., Basel, 2008.
[25] P.B. Mitchell, Clin. Chem. Lab. Med. 42 (2004) 1212.
[26] P. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, N. Cohen, P.A. Compagnon, W. Dewe, M. Feinberg, M. Laurentie, N. Mercier, G. Muzard, L. Valat, E. Rozet, J. Pharm. Biomed. Anal. 48 (2008) 760.
[27] C.R. Mallet, Z. Lu, J.R. Mazzeo, Rapid Commun. Mass Spectrom. 18 (2004) 49.
[28] E. Chambers, D.M. Wagrowski-Diehl, Z. Lu, J.R. Mazzeo, J. Chromatogr. B 852 (2007) 22.
[29] I. Marchi, S. Rudaz, M. Selman, J.L. Veuthey, J. Chromatogr. B 845 (2007) 244.
[30] W. Xie, J. Pawliszyn, W.M. Mullett, B.K. Matuszewski, J. Pharm. Biomed. Anal. 45 (2007) 599.
[31] L. Peng, T. Farkas, J. Chromatogr. A 1179 (2008) 131.
[32] J. Schappler, R. Nicoli, D.T.T. Nguyen, S. Rudaz, J.L. Veuthey, D. Guillarme, Talanta 78 (2009) 377.
[33] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Pharm. Res. 24 (2007) 1962.
[34] S. Heller, C. Hiemke, G. Stroba, A. Rieger-Gies, E. Daum-Kreysch, J. Sachse, S. Härtter, Ther. Drug Monit. 26 (2004) 459.

Chapitre IV

Résultats

1. Partie clinique

1.1. Manuscrit III (revue) : Suivi du syndrome métabolique induit par les antipsychotiques atypiques : recommandations et perspectives pharmacogénétiques.

L'article III décrit une revue de la littérature sur les effets des AP, particulièrement sur leurs effets métaboliques. En passant des antipsychotiques classiques aux AP, un transfert de la fréquence des effets secondaires graves, soit des effets extrapyramidaux vers des complications métaboliques, a été observé. Une prévalence de risques cardiovasculaires supérieure à la normale et une augmentation de la mortalité somatique (autre que suicides) sont observées dans les populations psychiatriques [130,130-132] Les recommandations adoptées par le Département de Psychiatrie – CHUV [1,6,133,134] prévoient le contrôle des paramètres métaboliques (taux de cholestérol, triglycérides, glucose, etc.) à certains intervalles de temps. Certains gènes, dont celui de la leptine et le récepteur serotoninergique $5HT_{2C}$, illustrent l'influence du bagage génétique sur la prise de poids pour les individus traités par AP [5,67,135].

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Suivi du syndrome métabolique induit par les antipsychotiques atypiques : recommandations et perspectives pharmacogénétiques

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Malgré une tolérance globale améliorée par rapport aux antipsychotiques classiques, les atypiques ne sont pas dénués d'effets secondaires, notamment métaboliques (prise de poids, altération du profil lipidique et glycémique). Ceux-ci sont particulièrement préoccupants en termes de morbidité et mortalité à long terme. En se basant sur des consensus récemment publiés, le département de psychiatrie du Centre hospitalier universitaire vaudois (CHUV) a établi une directive sur le suivi clinique des patients recevant des atypiques, directive que nous présentons ici. En outre, des études en pharmacogénétique montrent que la prise de poids et les altérations métaboliques induites par ce type de médication peuvent être influencées par le patrimoine génétique des patients. Ceci permet d'espérer que, dans un avenir proche, le traitement pourra être adapté individuellement à chaque patient.

introduction

L'arrivée sur le marché des antipsychotiques de seconde génération (dits «atypiques») a représenté un progrès significatif dans le traitement des psychoses. En comparaison des antipsychotiques de première génération, la réponse au traitement des troubles du spectre de la schizophrénie a été améliorée. Ces antipsychotiques ont une efficacité comparable à celle des neuroleptiques typiques sur les symptômes positifs et une moindre tendance à aggraver les symptômes négatifs ou à induire des effets secondaires, en particulier extrapyramidaux.[1] En conséquence, les recommandations actuelles, tant européennes que nord-américaines, proposent les antipsychotiques atypiques comme médicaments de premier choix dans le traitement de la schizophrénie et des autres troubles psychotiques. Cependant, des publications récentes ont montré que leur prescription peut entraîner un syndrome métabolique avec une prise de poids parfois importante, une modification de l'indice de masse corporelle (IMC), une obésité abdominale, une altération du profil lipidique et glycémique et/ou une hypertension. Des consensus publiés par différentes sociétés médicales soulignent l'importance d'un suivi efficace et attentif des effets secondaires induits par cette médication, en particulier le syndrome métabolique.[2-4] Suite à ces recommandations, une directive a été établie pour le suivi clinique des patients du département de psychiatrie du CHUV recevant un antipsychotique atypique.

antipsychotiques atypiques et syndrome métabolique

Des études montrent une prévalence deux à quatre fois plus élevée de problèmes métaboliques et une prévalence environ double de décès dus à un problème cardiovasculaire dans une population de patients schizophrènes par rapport à la population générale.[5] Les maladies cardiovasculaires représentent ainsi une des causes principales de l'excès de mortalité chez les patients souffrant de schizophrénie.[5,6] Cet excès de mortalité, associé à une plus grande prévalence de facteurs de risques cardiovasculaires (obésité, dyslipidémie, diabète, hypertension, inactivité physique, tabagisme), est en partie liée à la maladie et aux comportements qui peuvent y être associés, mais aussi en grande partie, à l'utilisation des antipsychotiques.[7,8] Ainsi, de nombreuses publications récentes montrent qu'un traitement avec

l'olanzapine et la clozapine et, dans une moindre mesure, avec la quétiapine et la rispéridone, entraîne une prise de poids associée à des désordres métaboliques tels qu'une hyperglycémie, une hypercholestérolémie et une hypertriglycéridémie. Ces effets n'ont, par contre, pas ou peu été observés avec l'aripiprazole.[9] Plusieurs revues de la littérature sur la prise de poids et le syndrome métabolique induits par les antipsychotiques atypiques ont été publiées.[9-12]

recommandations sur le suivi du syndrome métabolique

La procédure pratique visant à dépister la survenue de troubles métaboliques s'inscrit dans le contexte d'une prise en charge plus large de la santé physique des patients souffrant de troubles psychiatriques en général et de psychose en particulier. Les directives relatives au suivi des effets secondaires métaboliques sont décrites dans le tableau 1. Ce schéma est utilisé lors de l'instauration d'un traitement antipsychotique atypique ou lors d'un changement de traitement antipsychotique. Dans ce dernier cas, on considère comme début du traitement le moment effectif du changement, avant de commencer le croisement entre les deux médicaments. Après la première année, si les valeurs sont stables et dans les normes, les contrôles vont s'effectuer environ une fois par trimestre ou une fois par an.[2,3] Des valeurs limites ont été définies pour le syndrome métabolique (tableau 2).[13]

Tableau 1. Recommandations sur le suivi du syndrome métabolique chez les patients recevant des antipsychotiques atypiques (AA)

		Historique familial et personnel[a]	Poids, IMC[b]	Tour de taille	Tension artérielle	Glycémie[c]	Profil lipidique[c,d]
Première année	Avant AA	X	X	X	X	X	X
	1 mois		X				
	2 mois		X				
	3 mois		X		X	X	X
1 x trimestre			X				
1 x an		X		X	X	X	(X)

[a] Historique familial et personnel sur : obésité, diabète, dyslipidémie, hypertension ou problèmes cardiovasculaires.
[b] IMC : indice de masse corporelle : poids (en kg) / taille^2 (en m^2). Un IMC de 25 à 30 est synonyme de surpoids, supérieur à 30 d'obésité et supérieur à 40 d'obésité morbide.
[c] A jeun.
[d] Profil lipidique : cholestérol total, HDL, LDL et triglycérides.
(X) Pour les patients avec un profil lipidique normal, une mesure tous les cinq ans est conseillée.

Tableau 2. Caractérisation du syndrome métabolique (présence de trois des éléments suivants)[13]

Tour de taille • Femmes > 88 cm • Hommes > 102 cm	Tension artérielle > 130/85 mmHg
Triglycérides • Femmes > 1,54 mmol/l • Hommes > 1,82 mmol/l	Glycémie (à jeun) > 5,8 mmol/l
Cholestérol HDL • Femmes < 1,3 mmol/l • Hommes < 1,0 mmol/l	

Des contrôles plus fréquents que ceux mentionnés dans le tableau 1 peuvent être envisagés en fonction de l'état clinique du patient, des résultats des contrôles des paramètres cliniques et biologiques (c'est-à-dire

en cas d'obésité, d'hyperglycémie, d'hyperlipidémie, etc.) lors de certaines associations médicamenteuses (par exemple, prescriptions de comédications susceptibles d'induire une prise de poids ou un syndrome métabolique tels que le valproate, le lithium ou la mirtazapine). Il faut cependant relever que certains antipsychotiques classiques peuvent également induire une prise de poids significative.[11] De manière générale, une implication du médecin généraliste traitant est encouragée, de manière à favoriser un cadre de suivi optimal de cet aspect du traitement.

Il est conseillé, à titre préventif, mais en tout cas lors d'une augmentation égale ou supérieure à 5% du poids initial, ou en cas d'altérations significatives et durables de la glycémie et du profil lipidique, d'instaurer des mesures d'hygiène de vie (régime, activité sportive, consultation diététique).[2] Lors de telles altérations, le bilan risques/bénéfices du traitement doit aussi être analysé (par exemple, risque de rechute de la maladie psychiatrique versus risque cardiovasculaire à long terme) afin d'évaluer l'indication d'un changement de médication. Dans l'évaluation du risque cardiovasculaire, le tour de taille est le paramètre corrélant le mieux avec ce type de risque. Cependant, l'utilisation de l'IMC demeure intéressante, en particulier pour les patients réticents au contact physique lié à la mesure de leur tour de taille.[13,14]

autres contrôles effectués lors du suivi

En présence d'effets secondaires ou d'absence de réponse au traitement, la mesure du taux plasmatique de l'antipsychotique (Therapeutic drug monitoring) est recommandée. Ce taux permet de vérifier la présence d'un métabolisme particulier (par exemple, métabolisme ultrarapide ou métabolisme déficient) ou de contrôler la compliance.[15] Il est utile de rappeler que la non-compliance à un traitement médicamenteux peut atteindre un taux de 50% à 60% par exemple chez des patients schizophrènes.[16] A cet égard, la survenue d'effets secondaires tels qu'une prise de poids importante est un facteur de risque important pour une mauvaise ou une non-compliance.

Des contrôles plus fréquents qu'indiqués dans le tableau 1 sont nécessaires pour certains médicaments (par exemple, contrôle de la formule sanguine en raison du risque d'agranulocytose pour la clozapine). D'autres effets secondaires particuliers nécessitent un suivi spécifique. Ainsi, certains antipsychotiques, tels que la rispéridone ou l'amisulpride, peuvent entraîner une hyperprolactinémie dont les signes évocateurs sont les troubles sexuels, l'altération du cycle menstruel ou l'apparition d'une galactorrhée. Il est important, dans de tels cas, d'exclure une cause somatique (adénome). Un contrôle du taux sanguin de prolactine est indiqué chaque fois que de tels symptômes cliniques sont présents. Si l'antipsychotique est à l'origine de tels effets secondaires, il est conseillé d'évaluer l'indication à changer d'antipsychotique. D'autre part, certains antipsychotiques sont connus pour leur tendance à induire des modifications de l'ECG. Un contrôle ECG est recommandé pour certains antipsychotiques (par exemple, pimozide) ou obligatoire pour d'autres (par exemple, dropéridol, sertindole) en raison du risque de prolongation de l'intervalle QTc. En présence des facteurs prédisposant à une prolongation de l'intervalle QTc (congénital, pathologie cardiaque, coprescriptions médicamenteuses à risque, hypokaliémie, etc.), il est conseillé d'éviter ces médicaments. En cas d'intervalle QTc prolongé (> 450 msec chez les hommes, > 470 msec chez les femmes), une consultation avec un cardiologue devrait être planifiée. Le tableau 3 recense d'autres effets secondaires associés à la prise d'antipsychotiques atypiques.

Tableau 3. Autres effets secondaires induits par les antipsychotiques atypiques (liste non exhaustive)

Effets secondaires	Antipsychotiques concernés
Prolongation de l'intervalle QT	• Sertindole
Agranulocytose	• Clozapine
Hyperprolactinémie	• Rispéridone • Amisulpride
Baisse du seuil épileptogène	• Clozapine

rôle de la génétique dans la survenue d'un syndrome métabolique sous traitement antipsychotique

La variabilité interindividuelle dans la prise de poids induite par une médication est une réalité bien connue

des prescripteurs. Elle peut être expliquée par des facteurs environnementaux (âge, alimentation, comédication, etc.) mais également par des facteurs génétiques. Au cours des dernières années, des études en pharmacogénétique ont permis de mettre en évidence l'implication de différents gènes dans la prise de poids et les altérations métaboliques induites par les antipsychotiques atypiques. Plusieurs mécanismes peuvent jouer un rôle dans l'émergence d'un syndrome métabolique sous traitement antipsychotique.

Influence des taux plasmatiques de médicament

Les enzymes cytochromes P450 (CYP) sont majoritairement responsables du métabolisme et donc du taux plasmatique des antipsychotiques. Ils peuvent jouer un rôle lorsque l'effet secondaire est dépendant de la dose et du taux. Ainsi une étude récente montre qu'un taux beaucoup plus élevé en clozapine, en insuline et en triglycérides est détecté chez les patients sous clozapine ayant la mutation CYP1A2*1D et/ou CYP1A2*1C. De ce fait, des personnes porteuses de ces deux polymorphismes présentent un plus grand risque de développer des résistances à l'insuline.[17]

Influence des récepteurs, neurotransmetteurs et hormones

Les mécanismes pharmacodynamiques pouvant expliquer la prise de poids sous antipsychotiques n'ont pas été clairement établis.[18-20] Cependant, le profil pharmacologique des médicaments amène quelques pistes quant à l'implication de certains récepteurs et neurotransmetteurs.[21] Le système sérotoninergique (5HT) est ainsi connu comme étant associé à l'appétence, principalement au niveau de l'hypothalamus où se situent les centres de régulation de l'appétit. La clozapine et l'olanzapine, les deux antipsychotiques provoquant la plus grande prise de poids, ont une forte affinité pour ces récepteurs. Ce récepteur semble montrer, pour un de ses variants, un effet protecteur contre la prise de poids. Des études effectuées dans différents pays sur de jeunes psychotiques chinois,[22] coréens[23] ou caucasiens[24] ont ainsi montré que les porteurs de l'allèle T du polymorphisme 759 C/T prennent significativement moins de poids sous antipsychotiques atypiques que les autres. Trois autres polymorphismes du récepteur $5HT_{2C}$, associés à l'augmentation du tour de taille, sont déterminants pour la survenue d'un syndrome métabolique.[25]

Les médicaments qui sont antagonistes des récepteurs histaminergiques H_1, récepteurs influençant l'appétit, ont été également associés à une prise de poids.[26] D'autres récepteurs, tels que les récepteurs histaminergiques H_2, adrénergiques (ADR), les récepteurs du peroxisome proliferator activated receptor (PPAR) ou symptosomal associated protein peuvent également être impliqués dans la prise de poids induite par les psychotropes.[21,28] Ainsi, l'activité des récepteurs ADR module le poids corporel par une activation ou une inhibition de la lipolyse[27] alors que les PPAR jouent un rôle essentiel dans la différentiation des adipocytes.[30] Certaines mutations des récepteurs $ADR_{\beta 1}$ sont corrélées à un taux plus élevé d'insuline et à une résistance à l'insuline, pendant que d'autres mutations montrent une association avec une augmentation de la pression systolique.[27] D'autres polymorphismes ADR sont encore corrélés avec le phénotype de l'obésité.[29] Certaines mutations du gène PPAR semblent avoir un effet protecteur contre le diabète de type 2 et contre l'apparition de maladie coronarienne.[30] La leptine est une hormone produite par les tissus adipeux qui régule la prise d'aliments et la consommation d'énergie. Des polymorphismes génétiques soit sur le gène codant pour la leptine, soit sur le gène du récepteur à la leptine ont été montrés comme pouvant influencer la prise de poids lors d'un traitement antipsychotique.[31,32] Un résumé (tableau 4) donne quelques exemples de facteurs pharmacodynamiques pouvant moduler la prise de poids ou le syndrome métabolique (pour une revue complète, voir la revue de Chagnon et coll.).[20]

Tableau 4. Facteurs pharmacodynamiques pouvant moduler la prise de poids ou le syndrome métabolique
IMC : indice de masse corporelle.

Gène	Protéine	Mécanisme	Effet	Réf.
LEP	Leptine	Contrôle l'appétence et la consommation d'énergie	↑↓ Poids selon les variants génétiques	24
LEP R	Récepteur de la leptine	Contrôle l'appétence et la consommation d'énergie	↑↓ Poids selon les variants génétiques	31
ADR	Récepteur adrénergique	Module la lipolyse	↑ Risque de résistance à l'insuline ↑ Pression systolique ↑ Taux de graisse et ↑ IMC	27 29
BDNFα	Brain-derived neurotrophic factor	Module l'appétence et le métabolisme	↑ Poids	33
PPAR	Peroxysome proliferator activated receptor	Taille et différentiation des adipocytes	Effet protecteur contre le diabète type 2 et contre les maladies cardiovasculaires	30
H1R	Récepteur histaminergique H_1	Contrôle l'appétence	↑ Poids	20
HTR_{2C}	Récepteur sérotoninergique $5HT_{2C}$	Contrôle l'appétence et la satiété	↑↓ Poids et tour de taille selon les variants génétiques ↑ IMC	22-25
$CYP_{450}1A2$	CYP1A2	Métabolisme de médicaments dont celui de la clozapine et de l'olanzapine	↑ Résistance à l'insuline	17

conclusion

L'impact potentiel des effets indésirables métaboliques produits par les antipsychotiques atypiques sur la morbidité et mortalité à long terme dans la population de patients traités avec ces médicaments, exige un suivi clinique de ces effets. L'identification des facteurs génétiques influençant la survenue de ce type d'effets secondaires pourrait permettre une meilleure adaptation pharmacologique du traitement.

Bibliographie : 1 Lehman AF, Lieberman JA, Dixon LB, et al. Practice guidelines for the treatment of patients with schizophrenia, second edition. Am J Psychiatry 2004;161:1-56. 2 ** American Diabetes Association. Consensus development conference on antipsychotic drugs and obesity and diabetes. Diabetes Care 2004;27:596-601. 3 ** Goff DC, Cather C, Evins AE, et al. Medical morbidity and mortality in schizophrenia : Guidelines for psychiatrists. J Clin Psychiatry 2005;66:183-94. 4 Meyer JM, Davis VG, Goff DC, et al. Change in metabolic syndrome parameters with antipsychotic treatment in the CATIE schizophrenia trial : Prospective data from phase 1. Schizophr Res 2008;101:273-86. 5 Osby U, Correia N, Brandt L. Mortality and causes of death in schizophrenia on Stockholm country, Sweden. Schizophr Res 2000;45:21-8. 6 Casey DE, Hansen. Excessive mortality and morbidity in schizophrenia. In : Meyer JM, Nasrallah HA, editors. Medical illness and schizophrenia. Washington DC : American Psychiatric Publishing, 2003;13-4. 7 Ryan MC, Collins P, Thakore JH. Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. Am J Psychiatry 2003;160:284-9. 8 ** Heiskanen T, Niskanen L, Lyytikainen R, et al. Metabolic syndrome in patients with schizophrenia. J. Clin Psychiatry 2003;63:575-9. 9 Casey DE. Metabolic issues and cardiovascular disease in patients with psychiatric disorders. Am J Med 2005;118(Suppl. 2):15S-22S. 10 Haupt DW. Differential metabolic effects of antipsychotic treatments. Eur Neuropsychopharmacol 2006; 16 (Suppl. 3):S149-S55. 11 Wirshing DA. Schizophrenia and obesity : Impact of antipsychotic medications. J Clin Psychiatry 2004;65 (Suppl. 18):13-26. 12 Newcomer JW. Metabolic considerations in the use of antipsychotic medications : A review of recent evidence. J Clin Psychiatry 2007;68(Suppl. 1):20-7. 13 Third report of the National cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult treatment panel III). Final report. Circulation 2002;106:3143-421. 14 Wajchenberg BL. Subcutaneous and visceral adipose tissue : Their relation to the metabolic syndrome. Endocr Rev 2000;21:697-738. 15 * Baumann P, Hiemke C, Ulrich S, et al. Therapeutic monitoring of psychotropic drugs – An outline of the AGNP-TDM expert group consensus guideline. Ther Drug Monit 2004;26:167-70. 16 Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005;353:487-97. 17 Melkersson KI, Scordo MG, Gunes A, Dahl ML. Impact of CYP1A2 and CYP2D6 polymorphisms on drug metabolism and on insulin and lipid elevations and insulin resistance in clozapine-treated patients. J Clin Psychiatry 2007;68:697-704. 18 Baptista T. Body weight gain induced by antipsychotic drugs : Mechanisms and management (review). Acta Psychiatr Scand 1999;100:3-16. 19 Taylor DM, McAskill R. Atypical antipsychotics and weight gain – a systematic review. Acta Psychiatr Scand 2000;101:416-32. 20 ** Chagnon YC. Susceptibility genes for the side effect of antipsychotics on body weight and obesity. Current Drug Targets 2006;7:1681-95. 21 Zimmermann U, Kraus T, Himmerich H, Schuld A, Pollmächer T. Epidemiology, implications and mechanisms underlying drug-induced weight gain in psychiatric patients. J Psychiatr Res 2003;37:193-220. 22 Reynolds GP, Zhang ZJ, Zhang XB. Polymorphism of the promoter region of the serotonin 5-HT2C receptor gene and clozapine-induced weight gain. Am J Psychiatry 2003;160:677-9. 23 Ryu S, Cho EY, Park T, et al. -759 C/T polymorphism of 5-HT2C receptor gene and early phase weight gain associated with antipsychotic drug treatment. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:673-7. 24 Templeman LA, Reynolds GP, Arranz B, San L. Polymorphism of the 5-HT2C receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis. Pharmacogenetics and Genomics 2005;15:195-200. 25 Mulder H, Franke B, van der-Beek van der AA, et al. The association between HTR2C gene polymorphisms and the metabolic syndrome in patients with schizophrenia. J Clin Psychopharmacol 2007;27:338-43. 26 Kroeze WK, Hufeisen SJ, Popadak BA, et al. HI-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. Neuropsychopharmacology 2003;28:519-26. 27 Lima JJ, Feng H, Duckworth L, et al. Association analyses of adrenergic receptor polymorphisms with obesity and metabolic alterations. Metabolism 2007;56: 757-65. 28 Muller DJ, Klempan TA, De Luca V, et al. The SNAP-25 gene may be associated with clinical response and weight gain in antipsychotic treatment of schizophrenia. Neurosci Lett 2005;379:81-9. 29 Yasuda K, Matsunaga T, Adachi T, et al. Adrenergic receptor polymorphisms and autonomic nervous system function in human obesity. Trends Endocrinol Metab 2006;17:269-75. 30 Liu Y, Yuan Z, Liu Y, et al. PPARgamma gene C161T substitution is associated with reduced risk of coronary artery disease and decreased proinflammatory cytokine expression. Am Heart J

2007;154:718-24. 31 Ellingrod VL, Bishop JR, Moline J, et al. Leptin and leptin receptor gene polymorphisms and increases in body mass index (BMI) from olanzapine treatment in persons with schizophrenia. Psychopharmacol Bull 2007; 40:57-62. 32 Yevtushenko OO, Cooper SJ, O'Neill R, et al. Influence of 5-HT2C receptor and leptin gene polymorphisms, smoking and drug treatment on metabolic disturbances in patients with schizophrenia. Br J Psychiatry 2008;192:424-8. 33 Zhang XY, Zhou DF, Wu GY, et al. BDNF Levels and genotype are associated with antipsychotic-induced weight gain in patients with chronic schizophrenia. Neuropsychopharmacology 2008;33:2200-5. * à lire ** à lire absolument

1.2 Manuscrit IV: Psychotropic drug induced weight gain and other metabolic complications in a Swiss psychiatric population. Manuscrit soumis pour publication.

L'article IV décrit les comorbidités somatiques observées dans une population psychiatrique. La prise de poids et les risques cardiovasculaires rencontrés chez des individus traités au long cours par des psychotropes induisant potentiellement une prise de poids sont discutés. Au total, les échantillons provenant de 196 patients ont été analysés (patients Etude prise de poids Genève). A l'instar d'autres études menées dans d'autres pays, cette étude montre que la prédisposition à des risques cardiovasculaires est élevée pour les individus prenant un traitement psychotrope, avec notamment une prévalence de 38% de patients obèses et 21% de patients avec une hypercholestérolémie. Une prise de poids supérieure à 10% du poids initial est rencontrée chez 47% des patients. Une augmentation d'appétit provoquée par l'instauration du traitement, le type de médication utilisé et le genre du patient sont associés à l'évolution de l'IMC. Dans une population psychiatrique où une mortalité plus élevée que dans la population générale a été reportée, nous montrons ainsi une prévalence élevée de différents risques cardiovasculaires et une prise de poids cliniquement importante suite à l'instauration du traitement psychotrope. Cette étude confirme la nécessité de contrôler les paramètres métaboliques chez les individus traités par certains psychotropes, comme les antipsychotiques atypiques, le lithium et/ou le valproate.

Psychotropic drug induced weight gain and other metabolic complications in a Swiss psychiatric population

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Manuscrit soumis pour publication.

Abstract:

The aim of the study was to describe the weight gain-related side-effects of psychotropic drugs and their consequences on metabolic complications (hypercholesterolemia, obesity) in a Swiss cohort of psychiatric patients. A cross-sectional observational study was performed in two out-patient psychiatric centers with patients receiving for more than 3 months the following drugs: clozapine, olanzapine, quetiapine, risperidone, lithium, and/or valproate. Clinical measures and lifestyle information (smoking behaviour, physical activity) were recorded. In total, 196 were included in the study. Weight gain (≥10% of initial weight) following drug treatment was reported in 47% of these patients. Prevalence of obesity (BMI≥ 30), hypercholesterolemia (≥ 6.2 mmol/L) and low HDL-cholesterol (< 1.0 mmol/L in men, < 1.3 mmol/L in women) were present in 38%, 21%, and 27% of patients, respectively. A higher standardised dose, an increase of appetite following medication introduction, the type of medication (clozapine or olanzapine > quetiapine or risperidone > lithium or valproate), and the gender were shown to be significantly associated with evolution of BMI. In conclusion, high prevalence of obesity and hypercholesterolemia was found in an outpatient psychiatric population and confirms drug-induced weight gain complications during long-term treatment. The results support the recently published recommendations of monitoring of metabolic side effects during treatment with atypical antipsychotics. Moreover, the weight gain predictors found in the present study could help to highlight patients with special health care management requirement.

1. Introduction

Weight gain related side effect was shown for several therapeutic classes including anti-HIV drugs, antidepressants (in particular the tricyclics), second generation antipsychotics (SGA) or so-called atypical antipsychotics, and some mood stabilizers (MS). The atypical antipsychotics commonly used as first line treatment are not themselves devoid of side-effects and many recent publications have drawn attention to the emerging risk of developing metabolic complications including weight gain, diabetes or dyslipidemia (De Hert et al., 2009;Stahl, 2008;American Diabetes Association et al., 2004). Thus, clozapine and olanzapine are frequently associated with the highest weight gain values, followed by quetiapine and risperidone and finally, aripiprazole, amisulpride and sertindole are also associated with weight gain risks, but to a lesser extent and for a smaller percentage of patients (Hasnain et al., 2009;Komossa et al., 2010;Meyer, 2007;McIntyre et al., 2001;Wirshing et al., 1999;Basson et al., 2001;Newcomer & Haupt, 2006;Leucht et al., 2009;Tschoner et al., 2007). Expectedly, longer SGA treatment durations are associated with increased risks of weight gain (Zheng et al., 2009). In addition, other drugs such as valproate and lithium, two mood stabilizers (MS), can equally contribute to metabolic complications in psychiatric patients, especially through an increase in body weight (Vieweg et al., 2008;Compendium Suisse de Médicaments®, 2010).

Drug induced weight gain is a challenging issue for physicians. Not only because it can jeopardise the patients self-confidence and increase the risk of relapse by compromising treatment compliance (Weiden et al., 2004), but it could also be considered as the first step towards the development of obesity and other metabolic complications, such as hyperlipidemia, diabetes mellitus or cardiovascular heart diseases, which can ultimately reduce life expectancy by several years (Lakka et al., 2002;Isomaa et al., 2001;Stahl, 2008). Thus, an increase in the natural mortality rate of 1.5 to 2-fold as compared to the general population was reported for depressed, bipolar and schizophrenic patients (Laursen et al., 2007;Angst et al., 2002), which appears to be mainly due to a two-fold excess of cardiovascular risk factors (McEvoy et al., 2005;Osby et al., 200;Musselman et al., 1998;Van der Kooy et al., 2007;Birkenaes et al., 2007;Cohn & Remington, 2003). Thus, several studies reported a higher prevalence of MetS in bipolar and schizophrenic patients as compared to the general population, suggesting that the difference in the vulnerability to MetS between various psychiatric disorders should be investigated in more details (Fiedorowicz et al., 2008;Correll et al., 2008;Sicras et al., 2008;Birkenaes et al., 2007;van Winkel et al., 2008b). As a consequence, consensus reports have recommended to monitor closely for iatrogenic weight gain side effects and other metabolic complication in psychiatric patients receiving SGAs (De Hert et al., 2009;American Diabetes Association et al., 2004).

The major aim of the present study was to estimate the prevalence of obesity and hypercholesterolemia, two metabolic complications, in a chronic psychiatric population from Switzerland and to evaluate the influence of SGAs and MS on weight gain in this population.

2. Materials and methods

2.1. Subjects

Patients between 18-65 years old with a prescription for at least 3 months of one or several of the following psychotropic drugs were recruited: clozapine, olanzapine, quetiapine, risperidone, lithium, and/or valproate. No exclusion criterion was set for this study. All concomitant medications were allowed. The study was conducted in two out-patient psychiatric centres of the Geneva University hospital (Jonction and Servette). Diagnosis was made by their current psychiatric physician according to the ICD-10 diagnostic scale.

Enrolment occurred between June 2006 and May 2008 during one of the patients usual follow-up examination. The visit could take place at any time during the day regardless of the drug and food intake. This cross-sectional study performed in a naturalistic setting was approved by the Ethics Committee of the University of Geneva. All patients gave written consent to participate in the study after oral and written information. In the case of a patient with a legal tutor, the approval of the tutor was obtained as well.

2.2. Measurements

Body weight, sex, age, height, previous and current psychotropic treatment and comedications were extracted from the medical record. Different lifestyle factors, tobacco consumption, physical activity defined as any daily sport and/or walking activity (to go to work, to the bus, climbing stairs, etc.), for < 1H or > 1H were based on patient declaration. Self-reported appetite modification (decreased appetite, no change, increased appetite), baseline weight and weight change after 1, 3, and 6 months under SGA and MS treatment were directly obtained from the patient.

Blood samples were collected and samples were sent to the laboratory of the unit of pharmacogenetics and clinical psychopharmacology and stored at –20 °C until analysis. Measurements of SGAs concentrations were performed by liquid chromatography – mass spectrometry as described previously (Choong et al., 2009;Albrecht et al., 2004). The validated method for risperidone determination allows the simultaneous dosage of quetiapine (data available on request). Due to the instability of olanzapine (Choong et al., 2009;Heller et al., 2004) and the long duration between blood samplings and plasma level determinations, this drug could not be

measured for the patients included in the present study. Except for patients with evidence of side-effects (i.e. weight gain) following the introduction of olanzapine (n= 19), the patients receiving this drug were not included in the statistical analyses of drug induced weight gain (i.e. we could not be certain that no-weight gain could not be due to low or undetected plasma levels, see below). Measurement of lithium plasma levels was performed with an ion specific electrode apparatus (EasyLyte, Medica, Châtel-St-Denis, Switzerland). Total cholesterol (CHOLtot), HDL cholesterol (HDL) and valproate plasma levels were quantified by a Cobas Integra 400 (Roche Diagnostic, Basel, Switzerland).

Body mass index (BMI) was calculated as weight/height^2 (kg/m^2) and was considered as normal (BMI 18.5 to <25 kg/m^2), overweight (BMI 25 to <30 kg/m^2), obesity (BMI ≥ 30 kg/m^2) (from here-after called normal, intermediate and high BMI) (National Institute of Health, 2002). Target levels for CHOLtot and HDL were defined as CHOLtot ≤ 6.2 mmol/L, and HDL as ≥ 1.3 mmol/L for women and as ≥ 1.0 mmol/L for men according to the National Cholesterol Education Program (National Institute of Health, 2002). Dyslipidemia criteria were met when CHOLtot levels and/or HDL were outside the target limits and/or when lipid lowering agent was prescribed. As blood samples were taken at anytime regardless of fasting period, triglycerides were not measured in the present study.

An increase of 10% of baseline weight was considered as an important weight gain. An arbitrary threshold at 10% of the minimal therapeutic drug plasma concentration (Baumann et al., 2004) (i.e. 35, 2, 7, 2 ng/mL, 0.05 mmol/L and 5 mg/L for clozapine, olanzapine, quetiapine, risperidone + hydroxy-risperidone, lithium, and valproate) was chosen to indicate a suspicion of compliance issue and/or a rapid metabolism and/or pharmacokinetics drug interaction and/or low standardised dose prescription (e.g. prescription of 50 mg/day of quetiapine for sleep disorders). Such patients (n= 21) were included in the epidemiology analyses but were not considered for weight gain analyses.

2.3. Statistical analysis

Dose standardization (i.e. z-score transformation to obtain a mean of 0 and a standard deviation of 1, obtained by the mean dose subtracted from the raw dose and divided by the standard deviation of the dose) was done for each medication and each inclusion site, which allows using this standardised dose in the analyses regardless of the medication type. Thus a mean of 0 indicated that the administered dose was equal to the mean dose of the centre.

Kruskal-Wallis, Mann-Whitney tests as well as Pearson's chi-squared and Fisher exact tests (whenever the Pearson's chi-square is not anymore reliable) were used to assess any association between metabolic complication in continuous traits (weight gain, CHOLtot levels, HDL levels) and in

categorical traits (BMI categories (BMI < 25, 25-30, ≥ 30 kg/m^2), obesity (BMI≥ 30), important weight gain (> 10% of baseline weight), dyslipidemia, and studied medication) with clinical variables (gender, age, diagnosis, smoking status, standardised doses, treatment duration, etc.). Moreover, categorised variables such as important weight gain were analysed using logistic regressions by controlling the model with specification link test for single-equation models (linktest command in Stata) (Pregibon, 1979;Pregibon, 1980;Tukey, 1949). Finally, correlations between continuous traits were performed using Spearman correlation test (r_s). Measurements are given as median with the interquartile range unless otherwise stated.

Because of the complicated evolution trend of weight gain, a generalized additive mixed model was used which allows a semi-parametric flexible trend for weight gain versus time (Wood, 2006). The test was performed to fit the response variable (i.e. BMI which was log transform to obtain parametric data) for patients with drug plasma levels above the value corresponding to 10% of the minimal therapeutic level (see above) from both centres, taking into account several covariates (age, age at the beginning of the treatment, gender, standardised dose, current studied medication, comedication with a potential impact on weight (concomitant drugs potentially causing an increased of weight: other SGA, mirtazapine, clomipramine, mianserine, cetirizine, tibolone; concomitant drugs potentially causing a decreased of weight: fluoxetine, venlafaxine, metformin, topiramate (Compendium Suisse de Médicaments®, 2010), treatment durations, physical activity, smoking behaviour, alteration of appetite following MS or SGA introduction, and initial BMI). The validity of the model was assessed by graphical residual diagnostics. The psychotropic drugs were considered separately or in 3 categories grouped according to their expected effects on weight: clozapine with olanzapine, risperidone with quetiapine, lithium with valproate (Leucht et al., 2009;Newcomer, 2005;Tschoner et al., 2007;McIntyre & Konarski, 2005) or according to their therapeutic classes (SGA vs. MS). The medication with the longest treatment duration was considered as the influential medication in the analyses while the other drug(s) of interest (clozapine, olanzapine, quetiapine, risperidone, lithium, and/or valproate) were considered as concomitant drugs potentially causing an increased of weight.

The significance threshold is fixed at p-value of ≤ 0.05. Statistical analyses were performed using Stata 10 (StataCorp, College Station TX, USA) and using R environment for statistical analysis version 2.11.1 for model-fitting purposes (2010).

3. Results

3.1. Patient characteristics

Among the 200 included patients, 4 were withdrawn (not completed report form (n= 1), treatment duration shorter than 3 months (n= 1), treatment not taken into account in the protocol (i.e. amisulpride, n= 2) leading to a total of 196 patients. Extremely low drug plasma concentrations (see Material and Methods) were measured in 21 patients and their data were not included in the assessment of the weight gain-related side effect but were used for all other analyses (lipid levels analysis, standardised dose, obesity prevalence, etc.). The whole cohort is composed of 86% Caucasian, 55% male, with a median age of 41 years (range: 34-48) (women 43 y, range: 35-49; men 40 y, range: 33-47). Patients met the following ICD-10 diagnosis: mood affective disorder (ICD-10: F30-F39; n= 89), schizophrenia, schizotypal and delusional disorders (F20-F29; n= 87), neurotic, stress-related and somatoform disorders (F40-F48; n= 7), adult personality and behavioural disorders (F60-F69; n= 6) including one patient with an alcohol dependence syndrome, organic including symptomatic disorders, mental disorders (F00-F09; n= 3), mental and behavioural disorders due to psychoactive substance use (F10-F19; n= 2), and pervasive developmental disorders (F80-F89; n= 1). Finally, the diagnosis for 1 patient was not defined. The population characteristics are summarised in Table 1. The median of current SGA and/or MS treatment duration was 2.3 years (range: 0.9-5.6). SGA were prescribed to 136 patients, with the following distribution: risperidone (21%), quetiapine (18%), clozapine (14%), and olanzapine (16%). The remaining 60 patients were receiving a mood stabilizer with the following distribution: lithium (18%), valproate (13%). No significant difference of prescription was found between genders ($p>0.1$).

The mean daily doses are given in Table 2. Among the 196 patients, 18% of patients had a second study medication and 3% had in total 3 studied drugs. The most frequent association were quetiapine-lithium (n= 8), risperidone-lithium (n= 5), and valproate-clozapine (n= 8). Prescription of more than 1 studied drug is higher for MS than SGA ($p< 0.001$) with 48% of patients on MS compared to 11% on SGA taking a second drug of interest. The present cohort has in majority experienced other antipsychotic drugs since the psychiatric onset. A percentage of 26% having previously been prescribed a MS and 57% a SGA with 4% and 16% of patients declared having previously experienced 2 different MS or SGA, respectively before the current medication (called from hereafter previous treatment). No previous treatment was recorded for 67 patients. Regarding the current prescription, patients received one concomitant drug potentially causing an increased of weight (amisulpride n= 10, aripiprazole n= 3, mirtazapine n= 8, clomipramine n= 4, mianserine n= 1, cetirizine n= 1, tibolone n= 1). On the other hand, some patient received comedication inducing weight loss (fluoxetine n= 3, venlafaxine n= 24, metformin n= 3, topiramate n= 4). A few of them had two concomitant drugs that

could alter the weight, by inducing either an increase (mirtazapine with amisulpride, n= 1), a decrease (fluoxetine with topiramate, n= 1), or in an opposing way (venlafaxine with aripiprazole n= 1, venlafaxine with mirtazapine n= 3, topiramate with mirtazapine n= 1, and metformin with amisulpride n= 1) (Compendium Suisse de Médicaments®, 2010). Finally, some patients were also receiving pharmacological treatments in relation to the metabolic syndrome, with a lipid lowering agent, antihypertensive, or antidiabetic drugs prescribed to 4% (4 women; 4 men), 7% (5 women; 9 men) and 6% (7 women; 4 men) of patients, respectively.

3.2. Metabolic complications

The median BMI in the whole group is 27.9 kg/m^2 (range: 24.2 - 31.4), which is in line with other European studies with schizophrenic patients (Bobes et al., 2007) or with patients with mood disorders (Salvi et al., 2008;McElroy et al., 2002;Sicras et al., 2008). Approximately 30% of patients in the cohort were overweight (n= 59, 22% of women and 37% of men) and 38% were obese (n= 74, 32% of women and 43% of men). As expected, median BMI was higher in males compared to females (25.8 kg/m^2, range: 22.5 - 31.0 for women compared to 28.4 kg/ m^2, range: 25.5 - 31.6 for men, p= 0.015) (Schokker et al., 2007). Accordingly, frequencies of overweight and obese patients varied between gender, with 37% and 22% of overweight, and 43% and 32% of obese male and female patients, respectively (p< 0.001). No significant differences between patients taking MS and those taking SGA were found regarding obesity on a whole (p= 0.6) or for patients receiving a single drug (p= 0.8). Although no control group was included in the present study, we found an approximately twice higher prevalence of obesity when compared to several Swiss general population cohorts, with prevalence between 5 to 14% for women and 11 to 18% for men of similar or slightly older age (Figure 1) (Chiolero et al., 2008;Firmann et al., 2008;Office féderal de la statistique, 2009;Fondation Suisse de Cardiologie, 2002). On the other hand and interestingly, the prevalence of overweight patients in the present cohort is similar to or slightly lower than the general population (Figure 1).

High CHOLtot levels were observed in 21 % of the patients (20% in women, 22% in men), and low HDL levels were observed in 27 % of patients (30% in women, 25% in men) (Table 1) (National Institute of Health, 2002). Taking into account that lipid lowering agents were prescribed to 4% of patients, dyslipidemia (see materiel and methods) was observed in up to 47% of patients (43% in women, 57% in men). Finally, 60% of patients were smokers (median number of cigarettes per day, range: 20, 20-30 cigarettes), which adds another cardiovascular risk factor.

3.3. Weight gain during treatment

Self-reported body weights were evaluated for reliability in the subset of patients for whom body weights were recorded in the medical files. Estimated baseline body weight and at different time points after treatment initiation were thus compared to body weight found in medical records for 29 patients (mean treatment duration: 2 years; range: 0.25-14 years). An excellent concordance was found (r_s = 0.97, p < 0.0001) between weight modification estimated by the patient (median: 6 kg; range: 3 kg - 11 kg) and the one recorded in the medical file (median: 6 kg; range: 1 kg - 10 kg). Moreover, baseline self-reported and recorded body weight were not found to be significantly different (p= 0.3).

Regarding the patients estimation on the drug being responsible for their greatest weight gain (during the past and current treatment prescription), 58%, 54%, 43%, 32%, 26% and 21% of patients receiving olanzapine, clozapine, risperidone, valproate, quetiapine and lithium, respectively, stated that this drug was the drug which induced the most severe weight gain. Weight modification from drug initiation to inclusion in the study was available for 157 patients. Among them, 142 had blood levels above the minimal threshold (see material and methods) and were subsequently tested for the weight gain analyses. Wide interindividual variability was found for weight gain with a change from -29 kg to + 51 kg (min/max) during a median treatment length of 2.3 (range: 0.9 - 5.6) years. No difference in weight change was found between genders (p= 0.9), between Caucasians (n= 125) and any other ethnic population (n= 17, p= 0.9), smokers and non-smokers (p= 0.8), and groups with and without physical activity (p= 0.8). In marginal analyses, weight gain shows a tendency for a positive correlation with treatment duration (r_s= 0.15, p= 0.08) while it shows no significant correlation with the age at the beginning of treatment (r_s= -0.10 , p= 0.2), or with the current age (r_s= -0.04 , p= 0.6).

Since 23% of the whole cohort had more than one studied drug (19% had two studied drugs and 4% had 3 studied drugs), the additional impact of concomitant drugs potentially causing an increase of weight was also examined. No evidence was found to confirm the influence of additional medication on the drug-induced weight gain with 6 kg (n= 105, range: 1-13) and 9 kg (n= 37, range: 5-13) in patient receiving one and more than one drug, respectively (p= 0.15) while the duration of treatment was not significantly different between patients with one or more than one studied drugs (p= 0.3). When taking all comedications known to potentially alter body weight into account (see 3.1 Patient characteristics), a non-significant difference (p= 0.2) was found between the groups of patients receiving medication inducing weight loss, medication with no influence on weight and medication inducing weight gain (5.9 kg (n= 14, range: 2-9); 6.5 kg (n= 77, range: 2-13) and 8.4 kg (n= 51, range: 4-13), respectively).

Comparing patients receiving MS to patients receiving SGA, no significant difference in weight gain was found between the two groups (6 kg (n= 53, range: 2.5-13) vs. 7 kg (n= 89, range: 2-15), respectively, p= 0.5), while both groups had an almost similar treatment duration (2.7 years, range: 0.9-7.2 vs. 2.1 years, range: 1.0-5.3, p= 0.5, respectively). In the whole collection of data, the weight change was not significantly associated with the type of studied drugs when analysed separately (p= 0.21), however, considering the subgroups of patients free of other drugs potentially linked to weight gain (n= 91), a significant weight gain difference was found between all studied drugs (p= 0.035), despite similar treatment durations (p= 0.07). In addition, grouping the type of medication by their expected impact on weight gain (see Material and Methods), the body change remained significant (p= 0.043), with a median weight change for clozapine or olanzapine, risperidone or quetiapine, lithium or valproate of 8.2 kg (n= 28, range: -4.2 kg - +16.0 kg), 5.3 kg (n= 45, range: 2.0 - 15.5 kg), and 2.4 kg (n= 18, range: 0.0-7.0 kg), respectively.

The weight gain induced by previous treatments shows a significant negative association with the weight gain induced by the present treatment (n= 134, r_s= -0.21, p= 0.016), i.e. patients who had already experienced a strong weight increase during a previous treatment tended to gain less weight on their current medication, when compared to patients who experienced a weaker weight gain during a previous treatment. Thus, dividing patients into three groups of equal size, based on the severity of their weight gain induced by previous treatment (low, intermediate, and high previous weight gain), their median weight gain during the current treatment was found to be 8 kg (n= 57, range: 3-15), 8 kg, (n= 35, range: 5-13), 3.7 kg, (n= 42, range: -2 - +12), respectively (p= 0.033). Similarly, the BMI at the beginning of the treatment was significantly correlated with BMI change (r_s= -0.18, p= 0.030). Patients with lower baseline BMI experienced a higher increase of BMI (p= 0.035), with BMI gain of 2.7 kg/m^2 (n= 72, range: 1.4 - 4.7), 2.4 kg/m^2 (n= 51, range: 0.7-5.0), and 0.3 kg/m^2 (n= 19, range: -2.3 kg/m^2 - +2.7 kg/m^2) for normal, overweight, and obese features at baseline; this finding is in line with a report showing that lower baseline BMI is a predictor of more important weight gain (Kinon et al., 2001;Basson et al., 2001).

An important weight gain at the final follow-up (≥ 10% of the baseline body weight) was found in 47% patients (n= 67, see Table 1). A logistic regression was used to investigate the combined effect of age, sex, past weight gain, medication (according to their worsening effect on weight), and treatment duration on an increased weight of at least 10%. The contributions of medication and of previous weight gain were found significant in the model, adjusted for age, sex and treatment duration, with a pseudo-R^2 of 15%. Compared to clozapine or olanzapine, both the prescription of risperidone or quetiapine (p= 0.034), and of lithium or valproate (p= 0.008) were found to be less likely to induce an

important weight gain (odds ratio = 0.29 (CI: 0.09-0.91) and 0.17 (CI: 0.04-0.63), respectively). Moreover, the past weight gain showed to be a protective factor (odds ratio 0.93 (CI: 0.87-0.98), p= 0.011).

Finally, using either weights recorded in the medical files or those self-reported, weight increase at 3 (n= 44, 8 recorded, rs= 0.61, p< 0.0001) and 6 months of treatment (n= 48, 9 recorded, rs= 0.70, p< 0.0001) were found to be good predictor for long term weight gain (> 6 months), but not at 1 month (n= 42, 1 recorded) (r_s= 0.17, p= 0.27). Figure 2 presents the median weight gains after 3 months and 6 months, and the weight gain at the time of inclusion, when grouping the weight gains after 3 months of treatment into 3 tertiles (low, intermediate and high increases of weight).

3.4. Appetite modification

More than half of 193 patients reported a change in their feelings of satiety following the introduction of their current treatment, with 48% having an increase of appetite, and 5% describing a diminution of appetite; regardless of the medication. The weight gain was significantly associated with the appetite change, with weight change of 10 kg (n= 68, range: 6-18 kg), 4 kg (n= 67, range: 1-9 kg) and -2 kg (n= 5, range: -20 kg - +5 kg) for patients experiencing an increase of appetite, no change in appetite and a decrease of appetite (p= 0.0001). In the whole group, no difference of self-reported appetite was found when examining each drug (p= 0.6) or each class (i.e. SGA vs. MS, p= 0.26) separately. On the other hand, considering the subgroup of patient with one single drug linked to weight, an increase of self-reported appetite was reported by 50% of patients treated with a SGA (n= 111) compared to only 21 % patients treated with a MS (n= 19, p= 0.025).

3.5. Mixed-model approach

A mixed-model was used to investigate which clinical variables can influence BMI evolution under treatment. The effect of the studied medication (grouped according to their expected impact on weight), standardised doses, concomitant drugs potentially causing an increase in weight, age, gender, appetite change, physical activity, duration of treatment and the drug plasma levels threshold corresponding to 10% of the minimal therapeutic level were considered. The model allowed to take into account the whole cohort (n= 196). A significant association was found between BMI during treatment and the type of studied medication, with a significantly lower BMI with lithium or valproate (p= 0.005), and risperidone or quetiapine (p= 0.032) as compared to clozapine or olanzapine. Moreover, gender (p<0.001), appetite change (p= 0.027), standardised dose (p<0.001), and drug plasma levels threshold (p= 0.034) were found to influence BMI evolution but not physical activity (p= 0.22) and concomitant drugs potentially causing an increase in weight (p= 0.84). Based on

the model, predicted BMIs after 24 months with the current treatment are shown in Table 3. Evolution of BMI controlling for age over treatment duration for each gender regardless of the studied drugs is shown in Figure 3 (p= 0.008). Since appetite was self-reported and several factors might influence it, we performed the mixed model analysis without this factor. Similar results were obtained (data not shown). Physical activity was significantly associated to BMI when the standardised dose is not taken into account (p= 0.020). All positive associations found in the present report for drug-induced weight gain were independent of the psychiatric diagnostic of the patients (p> 0.2) or the number of studied drugs (p= 0.6).

4. Discussion

Increased mortality observed in psychiatric patients can be, in part, explained by the high prevalence of cardiovascular risk factors. Besides psychiatric illnesses and other environmental and life-style factors, an important modulator of cardiovascular risks is the prescription of psychotropic drugs. To our knowledge, this is the first study to examine the prevalence of weight gain and subsequent cardiovascular risk factors in a Swiss psychiatric population under long-term SGA and/or MS treatment. The present results showed a mean BMI of 28 kg/m^2, corresponding to an overweight status, a high prevalence of patients having high CHOLtot levels (21%), dyslipidemia (47%), and smoking habits (60%), thus confirming the high prevalence of cardiovascular risk factors found in other European psychiatric populations (De Hert et al., 2010;van Winkel et al., 2008a;Garcia-Portilla et al., 2009;Birkenaes et al., 2007;Salvi et al., 2008;De Hert et al., 2006). It should be noted that the prevalence of cardiovascular risk factors in this Swiss psychiatric population is higher than in the general Swiss population of the same average age, with obesity and smoking prevalence being about 1.5-2 times more frequent.

Of note, according to the cardiovascular risk management proposed by the European psychiatric, cardiology and diabetes societies (De Hert et al., 2009), statins may be required to achieve CHOLtot ≤ 5 mmol/L. In the present cohort, only 4% of the patients received lipid lowering agents while 21% patients had high CHOLtot levels (mean CHOLtot: 7.1 ± 0.7 mmol/L for patients above the threshold). This underlies the underestimated risk of somatic disease management in psychiatric population.

In the present study, the percentage of obese patients was 38% with a median treatment duration of 2.7 years (min=0.3, max=14 years). Although 66% of patients were previously treated with other SGA (56%) and/or MS (26%) in the past, the participants remain vulnerable to weight gain during their current treatment. Interestingly, past weight gain somehow masked the impact of current treatment as previous weight gain was negatively correlated with weight gain induced by the current drug. On the same line, a low baseline BMI was found to be a risk factor for a stronger weight gain during drug

treatment, which is in agreement with some (Umbricht et al., 1994;Wetterling & Mussigbrodt, 1999) but not all reports (Gebhardt et al., 2009). In the present study, both the baseline BMI before treatment and the weight gain at 3 and 6 months of treatment were predictive for the long term weight gain. The latter result is in agreement with reports showing that the weight change is mostly noticeable during the first year of treatment, the weight often reaching thereafter a plateau (Kinon et al., 2001;Zimmermann et al., 2003;Wirshing et al., 1999).

A mixed model showed that BMI was associated with gender, type of medication, standardised dose, appetite and higher blood levels. In another study, a positive association between higher blood levels and weight gain was reported for clozapine and olanzapine although no clear association was found with their daily dose and with the other atypical antipsychotics (Simon et al., 2009). We used the mathematical tool "standardisation" to allow comparison between the different drug doses. Our results suggested a positive association between BMI and increased standardised dose but this can however not be extrapolated to the daily dose for each specific treatment. The model showed that the strongest increase of appetite was constantly and significantly associated with the greatest weight gain. The effects of SGA on appetite change, has already been described (McIntyre et al., 2001;Theisen et al., 2003;Gebhardt et al., 2007). The drug action on satiety feeling and the weight gain mechanisms are only partially understood (Stahl, 2008). Several studies suggest the involvement of hypothalamic receptors involved in the energy expenditure and satiety pathway, such as the histamine H_1 and serotoninergic $5HT_2$ receptors (Baptista, 1999;Taylor & McAskill, 2000;Chagnon, 2006;Zimmermann et al., 2003;Stahl, 2008). Clozapine and olanzapine, as strong antagonists of these receptors, are associated with the highest weight gain. Interestingly, physical activity is significantly and negatively correlated with drug induced weight gain, although this association became no more significant when drug standardised doses were taken into account. For clinical purposes, we calculated predicted BMI at 24 months for less or more than 1 hour of daily physical activity (including sport but also softer activity such as walking). Although our result did not reach significance, a 0.7 kg/m^2 lower predicted BMI value was found for patient practising more than one hour of daily physical activity. This is in line with reports recommending regular exercises as prevention and treatment of established obesity (Alvarez-Jimenez et al., 2008;De Hert et al., 2009).

The limitations of the study should be discussed. Firstly, the design of the study, with a cross-sectional analysis, heterogeneous treatment durations, and the absence of a control group implies that the different causes of weight gain could only be hypothesised but not be demonstrated with certainty. The present results are however in agreement with previously published studies. Secondly, the patients were recruited in out-patient psychiatric centres from a single city and the results should

be generalized with caution. Thirdly, the effect of weight gain could be masked by previous treatment impact as well of years of illness as suggested by our results. Thus, the majority of patients in our cohort are not drug naive; therefore the impact of medication on weight might be more important than what we have found. The impact of previous treatments is also possibly contributing to the strong weight losses observed in some patients, probably when switching from a drug inducing a strong increase of weight to another with a more favourable profile concerning this side-effect. Finally, this cohort is only constituted of patients wishing to be enrolled in a study examining the prevalence of weight gain induced by drugs, which might introduce a bias towards increased reports and increased frequencies of such side-effects. On the other hand, patients who had previously presented severe side-effects in relation to the metabolic syndrome may have been switched to other drugs less likely to induce metabolic risks and might be therefore under-represented in this cohort.

Some important clinical studies have already investigated the prevalence of metabolic risk factors in psychiatric populations, from the US (McEvoy et al., 2005;Stroup et al., 2009;American Diabetes Association et al., 2004) and Europe (De Hert et al., 2010;van Winkel et al., 2008a;Garcia-Portilla et al., 2009;Birkenaes et al., 2007;Salvi et al., 2008) which emphasises the worrisome prevalence of metabolic syndrome. Indeed, metabolic syndrome prevalence in other European countries was reported to be between 18 to 25% (De Hert et al., 2010;van Winkel et al., 2008a;Garcia-Portilla et al., 2009;Birkenaes et al., 2007;Salvi et al., 2008). This study proposes a snap shot of a cohort with psychiatric out-patients in Switzerland, with multiple treatment histories, taking into account the country health care system, lifestyle, diet habit and aetiology. Given the high prevalence of cardiovascular risk factors in this sensitive population, the importance of an adequate somatic care and general health monitoring should be addressed. Finally, it must be mentioned that consequences of weight gain are not limited to cardiovascular problem: adherence to treatment is also jeopardised with all the subsequent possible human, social, and financial consequences of relapse (Weiden et al., 2004)

5. Conclusion

The present study found a high prevalence of cardiovascular risks factors in a Swiss psychiatric population treated with SGA and/or MS, with increased frequencies of obesity, dyslipidemia and smoking behaviour. It highlights the importance of an adequate somatic care and general health monitoring in this type of population.

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Reference List

Albrecht A, Morena PG, Baumann P, Eap CB. High dose of depot risperidone in a nonresponder schizophrenic patient. Journal of Clinical Psychopharmacology 2004; 24:673-674.

Alvarez-Jimenez M, Hetrick SE, Gonzalez-Blanch C, Gleeson JF, McGorry PD. Non-pharmacological management of antipsychotic-induced weight gain: systematic review and meta-analysis of randomised controlled trials. British Journal of Psychiatry 2008; 193:101-107.

American Diabetes Association, American Psyhiatric Association, American Association of Clinical Endocrinologists, North American Association for the Study of Obesity. Consensus

development conference on antipsychotic drugs and obesity and diabetes. Diabetes Care 2004; 27:596-601.

Angst F, Stassen HH, Clayton PJ, Angst J. Mortality of patients with mood disorders: follow-up over 34-38 years. J Affect Disord 2002; 68:167-181.

Baptista T. Body weight gain induced by antipsychotic drugs: mechanisms and management. Acta Psychiatr Scand 1999; 100:3-16.

Basson BR, Kinon BJ, Taylor CC, Szymanski KA, Gilmore JA, Tollefson GD. Factors influencing acute weight change in patients with schizophrenia treated with olanzapine, haloperidol, or risperidone. J Clin Psychiatry 2001; 62:231-238.

Baumann P, Hiemke C, Ulrich S, Gaertner I, Rao ML, Eckermann G, Gerlach M, Kuss HJ, Laux G, Müller-Oerlinghausen B, Riederer P. Therapeutic monitoring of psychotropic drugs - An outline of the AGNP-TDM expert group consensus guideline. Therapeutic Drug Monitoring 2004; 26:167-170.

Birkenaes AB, Opjordsmoen S, Brunborg C, Engh JA, Jonsdottir H, Ringen PA, Simonsen C, Vaskinn A, Birkeland KI, Friis S, Sundet K, Andreassen OA. The level of cardiovascular risk factors in bipolar disorder equals that of schizophrenia: a comparative study. J Clin Psychiatry 2007; 68:917-923.

Bobes J, Arango C, Aranda P, Carmena R, Garcia-Garcia M, Rejas J. Cardiovascular and metabolic risk in outpatients with schizophrenia treated with antipsychotics: results of the CLAMORS Study. Schizophr Res 2007; 90:162-173.

Chagnon YC. Susceptibility genes for the side effect of antipsychotics on body weight and obesity. Current Drug Targets 2006; 7:1681-1695.

Chiolero A, Prior J, Bovet P, Masson JC, Darioli R. Expectation to improve cardiovascular risk factors control in participants to a health promotion program. J Gen Intern Med 2008; 23:615-618.

Choong E, Rudaz S, Kottelat A, Guillarme D, Veuthey JL, Eap CB. Therapeutic drug monitoring of seven psychotropic drugs and four metabolites in human plasma by HPLC-MS. Journal of Pharmaceutical and Biomedical Analysis 2009; 50:1000-1008.

Cohn TA, Remington G. Risk factors for coronary heart disease in longer term patients on antipsychotic medication: high prevalence of syndrome X. Schizophr.Res. 2003; 60:353-354.

Compendium Suisse de Médicaments®. Bâle (Suisse): Documed S.A., 2010.

Correll CU, Frederickson AM, Kane JM, Manu P. Equally increased risk for metabolic syndrome in patients with bipolar disorder and schizophrenia treated with second-generation antipsychotics. Bipolar Disord 2008; 10:788-797.

De Hert M, Dekker JM, Wood D, Kahl KG, Holt RI, Moller HJ. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric

Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). Eur Psychiatry 2009; 24:412-424.

De Hert M, Mittoux A, He Y, Peuskens J. Metabolic parameters in the short- and long-term treatment of schizophrenia with sertindole or risperidone. In: 2010.

De Hert M, van Winkel R, Van Eyck D, Hanssens L, Wampers M, Scheen A, Peuskens J. Prevalence of diabetes, metabolic syndrome and metabolic abnormalities in schizophrenia over the course of the illness: a cross-sectional study. Clin Pract Epidemol Ment Health 2006; 2:14.

Fiedorowicz JG, Palagummi NM, Forman-Hoffman VL, Miller dD, Haynes WG. Elevated prevalence of obesity, metabolic syndrome, and cardiovascular risk factors in bipolar disorder. Ann Clin Psychiatry 2008; 20:131-137.

Firmann M, Mayor V, Vidal PM, Bochud M, Pecoud A, Hayoz D, Paccaud F, Preisig M, Song KS, Yuan X, Danoff TM, Stirnadel HA, Waterworth D, Mooser V, Waeber G, Vollenweider P. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord 2008; 8:6.

Fondation Suisse de Cardiologie. Chiffres et données sur les maladies cardio-vasculaires en Suisse. 2002.

Garcia-Portilla MP, Saiz PA, Bascaran MT, Martinez AS, Benabarre A, Sierra P, Torres P, Montes JM, Bousono M, Bobes J. Cardiovascular risk in patients with bipolar disorder. J Affect Disord 2009; 115:302-308.

Gebhardt S, Haberhausen M, Heinzel-Gutenbrunner M, Gebhardt N, Remschmidt H, Krieg JC, Hebebrand J, Theisen FM. Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. J Psychiatr Res 2009; 43:620-626.

Gebhardt S, Haberhausen M, Krieg JC, Remschmidt H, Heinzel-Gutenbrunner M, Hebebrand J, Theisen FM. Clozapine/olanzapine-induced recurrence or deterioration of binge eating-related eating disorders. J Neural Transm 2007; 114:1091-1095.

Hasnain M, Vieweg WV, Fredrickson SK, Beatty-Brooks M, Fernandez A, Pandurangi AK. Clinical monitoring and management of the metabolic syndrome in patients receiving atypical antipsychotic medications. Prim Care Diabetes 2009; 3:5-15.

Heller S, Hiemke C, Stroba G, Rieger-Gies A, Daum-Kreysch E, Sachse J, Härtter S. Assessment of storage and transport stability of new antidepressant and antipsychotic drugs for a nationwide TDM service. Therapeutic Drug Monitoring 2004; 26:459-461.

Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001; 24:683-689.

Kinon BJ, Basson BR, Gilmore JA, Tollefson GD. Long-term olanzapine treatment: weight change and weight-related health factors in schizophrenia. J Clin Psychiatry 2001; 62:92-100.

Komossa K, Rummel-Kluge C, Schmid F, Hunger H, Schwarz S, Srisurapanont M, Kissling W, Leucht S. Quetiapine versus other atypical antipsychotics for schizophrenia. The Cochrane Collaboration 2010.

Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. Jama: Journal of the American Medical Association 2002; 288:2709-2716.

Laursen TM, Munk-Olsen T, Nordentoft M, Mortensen PB. Increased mortality among patients admitted with major psychiatric disorders: a register-based study comparing mortality in unipolar depressive disorder, bipolar affective disorder, schizoaffective disorder, and schizophrenia. J Clin Psychiatry 2007; 68:899-907.

Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet 2009; 373:31-41.

McElroy SL, Frye MA, Suppes T, Dhavale D, Keck PE, Jr., Leverich GS, Altshuler L, Denicoff KD, Nolen WA, Kupka R, Grunze H, Walden J, Post RM. Correlates of overweight and obesity in 644 patients with bipolar disorder. J Clin Psychiatry 2002; 63:207-213.

McEvoy JP, Meyer JM, Goff DC, Nasrallah HA, Davis SM, Sullivan L, Meltzer HY, Hsiao J, Scott ST, Lieberman JA. Prevalence of the metabolic syndrome in patients with schizophrenia: baseline results from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) schizophrenia trial and comparison with national estimates from NHANES III. Schizophr Res 2005; 80:19-32.

McIntyre RS, Konarski JZ. Tolerability profiles of atypical antipsychotics in the treatment of bipolar disorder. J Clin Psychiatry 2005; 66 (Suppl 3):28-36.

McIntyre RS, Mancini DA, Basile VS. Mechanisms of antipsychotic-induced weight gain. J.Clin.Psychiatry 2001; 62 (Suppl. 23):23-29.

Meyer JM. Strategies for the long-term treatment of schizophrenia: real-world lessons from the CATIE trial. J Clin Psychiatry 2007; 68 Suppl 1:28-33.

Musselman DL, Evans DL, Nemeroff CB. The relationship of depression to cardiovascular disease: Epidemiology, biology, and treatment. Arch Gen Psychiatry 1998; 55:580-592.

National Institute of Health. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Final report. Circulation 2002; 106:3143-3421.

Newcomer JW. Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs 2005; 19 (Suppl 1):1-93.

Newcomer JW, Haupt DW. The metabolic effects of antipsychotic medications. Can.J.Psychiatry 2006; 51:480-491.

Office féderal de la statistique NS. Statistique de la santé. 2009.

Osby U, Correia N, Brandt L. Mortality and causes of death in schizophrenia on Stockholm County, Sweden. Schizophr.Res. 200; 45:21-28.

Pregibon D. Data analytic methods for generalized linear models. Toronto: University of Toronto, 1979.

Pregibon D. Goodness of link tests for generalized linear models. Applied Statistics 1980; 29:15-24.

R Development Core Team (2010), R: A language and environment for statistical computing, R Fondation for Statistical Computing,Vienna,Austria.ISBN 3-900051-07-0,URL. In: 2010.

Salvi V, Albert U, Chiarle A, Soreca I, Bogetto F, Maina G. Metabolic syndrome in Italian patients with bipolar disorder. General Hospital Psychiatry 2008; 30:318-323.

Schokker DF, Visscher TL, Nooyens AC, van Baak MA, Seidell JC. Prevalence of overweight and obesity in the Netherlands. Obes Rev 2007; 8:101-108.

Sicras A, Rejas J, Navarro R, Serrat J, Blanca M. Metabolic syndrome in bipolar disorder: a cross-sectional assessment of a Health Management Organization database. Bipolar Disord 2008; 10:607-616.

Simon V, van Winkel R, De Hert M. Are weight gain and metabolic side effects of atypical antipsychotics dose dependent? A literature review. J Clin Psychiatry 2009; 70:1041-1050.

Stahl SM. Stahl's Essential Psychopharmacology - Neuroscientific Basis and Practical Applications. New York: Cambridge University Press, 2008.

Stroup TS, Lieberman JA, McEvoy JP, Davis SM, Swartz MS, Keefe RS, Miller AL, Rosenheck RA, Hsiao JK. Results of phase 3 of the CATIE schizophrenia trial. Schizophr Res 2009; 107:1-12.

Taylor DM, McAskill R. Atypical antipsychotics and weight gain - a systematic review. Acta Psychiatr Scand 2000; 101:416-432.

Theisen FM, Linden A, Konig IR, Martin M, Remschmidt H, Hebebrand J. Spectrum of binge eating symptomatology in patients treated with clozapine and olanzapine. J Neural Transm 2003; 110:111-121.

Tschoner A, Engl J, Laimer M, Kaser S, Rettenbacher M, Fleischhacker WW, Patsch JR, Ebenbichler CF. Metabolic side effects of antipsychotic medication. Int J Clin Pract 2007; 61:1356-1370.

Tukey JW. One degree of freedom for non-additivity. Biometrics 1949; 5:232-242.

Umbricht DS, Pollack S, Kane JM. Clozapine and weight gain. J Clin Psychiatry 1994; 55 Suppl B:157-160.

Van der Kooy K, van Hout H, Marwijk H, Marten H, Stehouwer C, Beekman A. Depression and the risk for cardiovascular diseases: systematic review and meta analysis. Int J Geriatr Psychiatry 2007; 22:613-626.

van Winkel R, De Hert M, Van Eyck D, Hanssens L, Wampers M, Scheen A, Peuskens J. Prevalence of diabetes and the metabolic syndrome in a sample of patients with bipolar disorder. Bipolar Disord 2008a; 10:342-348.

van Winkel R, van Os J, Celic I, Van Eyck D, Wampers M, Scheen A, Peuskens J, De Hert M. Psychiatric diagnosis as an independent risk factor for metabolic disturbances: results from a comprehensive, naturalistic screening program. J Clin Psychiatry 2008b; 69:1319-1327.

Vieweg WVR, Levy JR, Fredrickson SK, Chipkin SR, Beatty-Brooks M, Fernandez A, Hasnain M, Pandurangi AK. Psychotropic drug considerations in depressed patients with metabolic disturbances. Am J Med 2008; 121:647-655.

Weiden PJ, Mackell JA, McDonnell DD. Obesity as a risk factor for antipsychotic noncompliance. Schizophr Res 2004; 66:51-57.

Wetterling T, Mussigbrodt HE. Weight gain: side effect of atypical neuroleptics? J Clin Psychopharmacol 1999; 19:316-321.

Wirshing DA, Wirshing WC, Kysar L, Berisford MA, Goldstein D, Pashdag J, Mintz J, Marder SR. Novel antipsychotics: comparison of weight gain liabilities. J.Clin.Psychiatry 1999; 60:358-363.

Wood SN. GAMMs with R. In: Carlin BP, Chatfield C, Tanner M, Zidek J, editors. Generalized Additive Models - An Introduction with R. Broken: Chapman & Hall/CRC, 2006. p. 319-324.

Zheng L, Mack WJ, Dagerman KS, Hsiao JK, Lebowitz BD, Lyketsos CG, Stroup TS, Sultzer DL, Tariot PN, Vigen C, Schneider LS. Metabolic changes associated with second-generation antipsychotic use in Alzheimer's disease patients: the CATIE-AD study. Am J Psychiatry 2009; 166:583-590.

Zimmermann U, Kraus T, Himmerich H, Schuld A, Pollmächer T. Epidemiology, implications and mechanisms underlying drug-induced weight gain in psychiatric patients. J.Psychiatr.Res. 2003; 37:193-220.

Table 1: Patient characteristics

Data are expressed as number (percentage) of patient or as median (interquartile range).

Characteristics	Drugs						
	Clozapine	**Olanzapine**	**Quetiapine**	**Risperidone**	**Lithium**	**Valproate**	**All**
	n=28	n=31	n=35	n=42	n=35	n=25	n=196
Sex,male	13 *(46)*	20 *(65)*	19 *(54)*	24 *(57)*	21 *(60)*	11 *(44)*	108 *(55)*
Caucasian ethnicity	24 *(86)*	28 *(90)*	31 *(89)*	29 *(69)*	34 *(97)*	23 *(92)*	169 *(86)*
Smoker	15 *(54)*	16 *(52)*	20 *(57)*	25 *(60)*	23 *(66)*	18 *(72)*	117 *(60)*
Age,median (range),y	35 *(29-44)*	41 *(33-46)*	45 *(38-51)*	40 *(35-47)*	44 *(34-49)*	44 *(40-49)*	41 *(34-48)*
Concomitant studied drug[⍰]	6 *(21)*	1 *(3)*	2 *(6)*	6 *(14)*	20 *(57)*	9 *(36)*	44 *(22)*
Cholesterol mmol/L							
median (range)	5.3 *(4.4-6.1)*	5.5 *(4.8-6.2)*	4.9 *(4.4-5.8)*	5.3 *(4.6-6.1)*	5.5 *(4.6-6.3)*	4.9 *(4.4-6.1)*	5.3 *(4.5-6.1)*
level at risk*	5 *(18)*	*7* (23)	3 *(9)*	10 *(24)*	11 *(31)*	6 (24)	42 (21)
Cholesterol-hdl mmol/L							
median (range)	1.2 *(1.0-1.6)*	1.2 *(1.1-1.4)*	1.3 *(1.0-1.5)*	1.2 *(1.1-1.5)*	1.4 *(1.2-1.7)*	1.2 *(0.9-1.6)*	1.3 *(1.0-1.6)*
level at risk*	9 *(32)*	9 *(29)*	9 *(26)*	12 *(29)*	4 *(11)*	10 *(40)*	53 *(27)*
BMI kg/m^{2‡}							
median (range)	28 *(24-31)*	29 *(25-31)*	29 *(25-32)*	27 *(24-31)*	26 *(23-32)*	29 *(25-32)*	28 *(24-31)*
<25	10 *(36)*	9 *(29)*	8 *(23)*	14 *(33)*	15 *(43)*	7 *(28)*	63 *(32)*
25-30	9 *(32)*	10 *(32)*	12 *(34)*	14 *(33)*	7 *(20)*	7 *(28)*	59 *(30)*
>30	9 *(32)*	12 *(39)*	15 *(43)*	14 *(33)*	13 *(37)*	11 *(44)*	74 *(38)*
Weight gain>10% during the present drug treatment	10 *(53)*	12 *(75)*	6 *(30)*	16 *(47)*	16 *(50)*	7 *(33)*	67 *(47)*

*Total cholesterol ≥6.2 mmol/L and cholesterol-hdl <1mmol/l for men and <1.3mmol/l for women are considered as cardiovascular risk factors

‡BMI is considered as normal when <25, overweight when 25-30 and obese when BMI>30 kg/m^2

⍰Number of patients with prescription of more than 1 studied drug (i.e. clozapine, olanzapine, quetiapine, risperidone, lithium, valproate)

Table 2: Treatment duration, daily dose and drug plasma concentration

	Drugs																			
							Risperidone													
	Clozapine		Olanzapine		Quetiapine		oral		injection		Lithium		Valproate		All					
	n=28		n=31		n=35		n=27		n=15		n=35		n=25		n=196					
Treatment duration,y																				
median (range)	4.8	*(1.7-7.8)*	1.8	*(1.1-7.0)*	1.1	*(0.7-3.7)*	3.0	*(1.0-5.8)*	1.0	*(0.4-2.8)*	3.1	*(1.0-10.9)*	1.7	*(0.9-3.7)*	2.3	*(0.9-5.6)*				
Dose, median (range), mg/day *	300	*(200-350)*	10	*(5-15)*	200	*(100-400)*	2	*(1-4)*	38	*(37.5-50)*	24	*(22-30)*	1500	*(1000-1500)*						
Plasma level,ng/mL †																				
median (range)	249	*(165-408)*			42	*(10-194)*	3	*(0.7-8)*	7	*(4-10)*	0.8	*(0.6-0.8)*	65	*(36-80)*						
metabolite, median (range)	135	*(87-180)*					10	*(5-21)*	19	*(10-33)*										

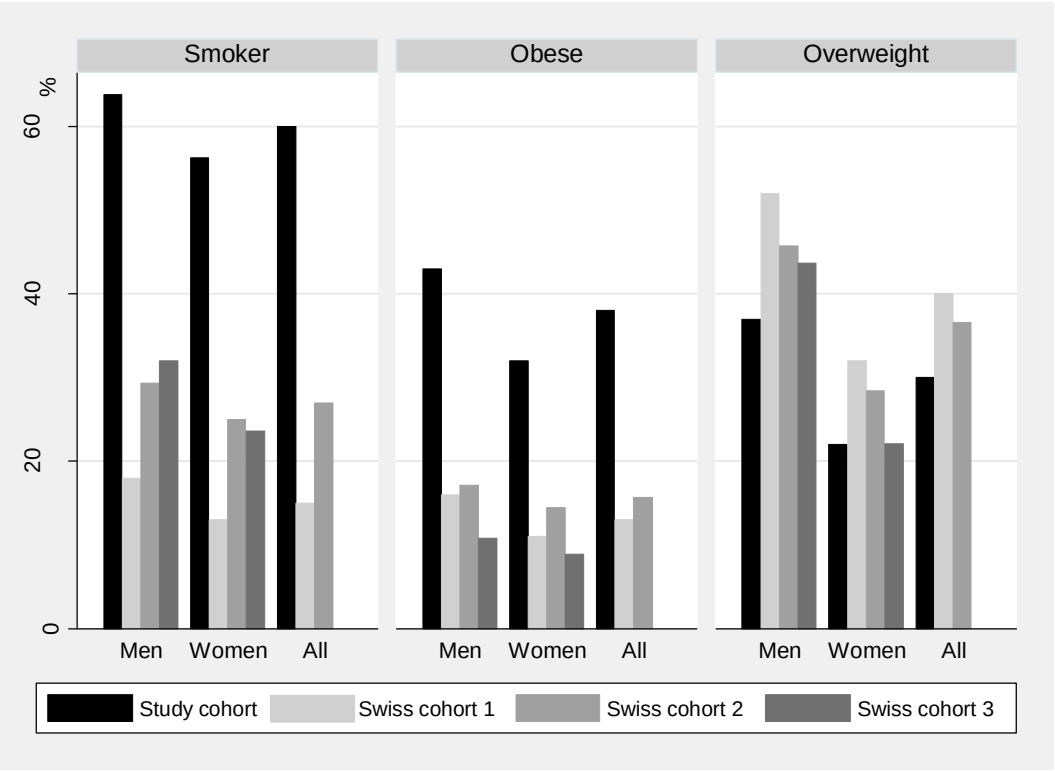
*Lithium is expressed as mmol/day and risperidone depot as

†Lithium is expressed as mmol/L and valproate as mg/L

Table 3: Predicted BMI after 24 months with the current treatment for different covariables.

CI: 95% confidence intervals

		24 months of treatment		
Covariables assessed		predicted BMI	Lower CI	Upper CI
a)	Clozapine or olanzapine	26.30	25.07	27.53
	Quetiapine or risperidone	25.63	24.42	26.83
	Lithium or valproate	24.45	23.01	25.89
b)	Men	28.09	26.84	29.33
	Women	26.30	25.07	27.53
c)	Increased appetite	27.27	25.95	28.59
	No change or decreased	26.30	25.07	27.53
d)	plasma threshold >10%	26.30	25.07	27.53
	plasma threshold <10%	24.49	22.70	26.29
e)	Daily physical activity <60min	26.30	25.07	27.53
	Daily physical activity >60min	25.59	24.17	27.01



Swiss cohort 1 (Chiolero et al., 2008), Swiss cohort 2 (Firmann et al., 2008), Swiss cohort 3 (Office féderal de la statistique, 2009).

Figure 1: Prevalence (%) of smoking, obesity and overweight by gender in the study cohort of psychiatric patients and in other Swiss general population cohorts.
Note that data for men and women combined in Swiss cohort 3 is not available.

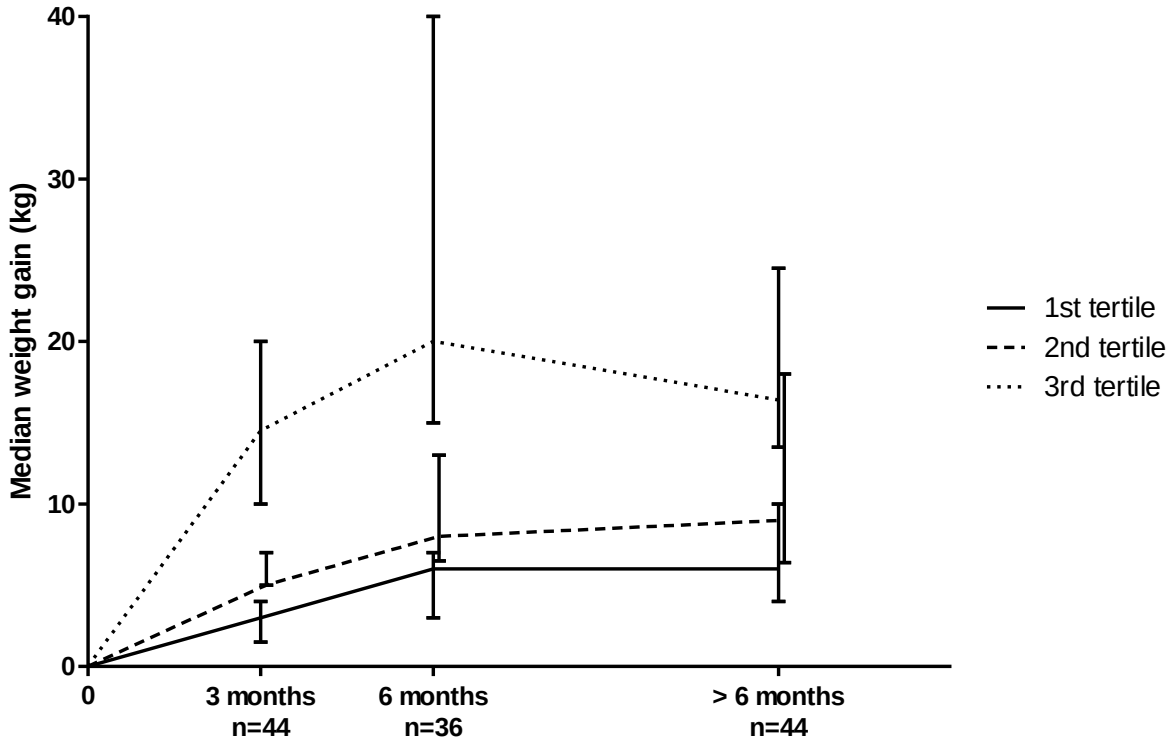


Figure 2: Weight gains after 3 months, 6 months, and weight gain at the time of inclusion, when grouping the weight gains after 3 months of treatment into 3 tertiles (high, intermediate and low increases of weight for 3rd, 2nd and 1st tertile, respectively). Median weight gain and interquartile ranges are shown.

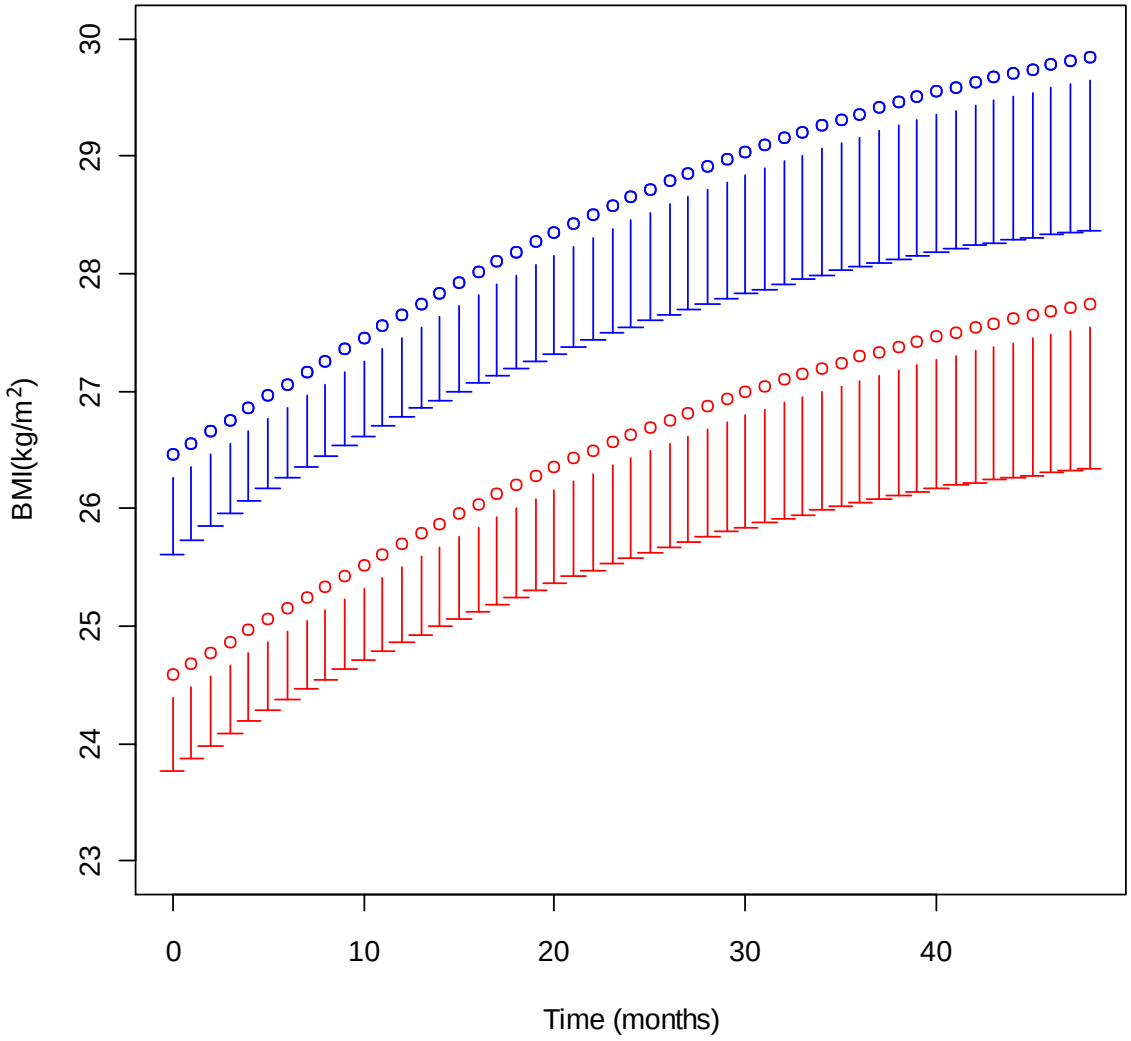


Figure 3: Predicted evolution of BMI controlling for age over treatment duration for each gender regardless of the studied drugs (Top curve: men, bottom curve: women).

2. Partie pharmacogénétique

2.1. Manuscrit V: Pharmacogenetic study on risperidone long-acting injection: influence of cytochrome P450 2D6 and Pregnane X receptor on risperidone exposure and drug-induced side-effects. Manuscrit en préparation.

Le Risperdal Consta® a la particularité d'être un antipsychotique atypique administré sous forme injectable toutes les deux semaines. Il ne subit pas l'effet de premier passage hépatique et peu de données pharmacogénétiques existent pour cette formulation. L'article V traite de l'influence des polymorphismes des gènes liés à la pharmacocinétique de la risperidone (*CYP2D6, CYP3A, ABCB1 et NR1I2*) et de l'activité du CYP3A sur les taux plasmatiques de risperidone et de son métabolite ainsi que sur les effets secondaires. La population étudiée est celle de l'Etude Risperdal Consta (N=42) et l'activité enzymatique du CYP3A est déterminée par phénotypage en utilisant le midazolam comme substrat. Le rôle principal du gène *CYP2D6* dans le métabolisme de la risperidone, montré pour la risperidone orale, a été confirmé pour la forme injectable. Les taux plasmatiques de risperidone sont influencés significativement par le génotype du *CYP2D6*. Le polymorphisme du gène *NR1I2 (rs7643645A>G)* influence également les taux plasmatiques du métabolite 9-hydroxy-rispéridone et de la fraction active (la somme de rispéridone et de 9-hydroxy-rispéridone). Cette influence a été confirmée dans une seconde cohorte de patients traités par le Risperdal Consta et sélectionnés dans les cohortes Prise de poids Genève et Suivi métabolique Lausanne (N=20). Les résultats obtenus sont en accord avec une diminution d'activité démontrée pour l'allèle G du SNP *rs7643645* dans des tests *in vitro* et des taux de 9-hydroxy-rispéridone et de fraction active plus basse chez les individus GG que pour les individus porteur de l'allèle A. Le phénotype du CYP3A, les polymorphismes des *CYP3A* et de *l'ABCB1* n'ont pas montré d'association avec les taux de risperidone. Concernant les effets secondaires, le génotype *rs2472677CC (C>T)* du *NR1I2* a été associé à des effets secondaires extrapyramidaux (échelle standardisée Simpson-Angus) et à un taux de prolactine plus élevé. L'activité du CYP3A est corrélée au taux de cholestérol-HDL. A notre connaissance, ceci est la première étude à montrer l'influence de polymorphismes du *NR1I2* sur les taux et les effets secondaires (extrapyramidaux, élévation de la prolactine) de la risperidone en forme injectable.
Le manuscrit V est actuellement revu par les co-auteurs et sera soumis prochainement.

Pharmacogenetic study on risperidone long-acting injection: influence of cytochrome P450 2D6 and Pregnane X receptor on risperidone exposure and drug-induced side-effects

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Manuscrit en préparation

Abstract

Risperidone is metabolised by polymorphic enzymes and a large variability in plasma concentration and therapeutic response is observed. Risperidone long-acting injection (RLAI) avoids the first-pass effect and little is known about the influence of genes polymorphisms involved in its pharmacokinetics. The influence on plasma concentrations of risperidone (RIS), its metabolite 9-hydroxy-risperidone (OHRIS) and on side-effects were investigated for polymorphisms of *cytochrome P450 2D6* (*CYP2D6) (*3, *4, *5, *6), CYP3A (CYP3A4*1B, CYP3A4 rs4646437, CYP3A5*3, CYP3A7*1C), ABCB1 (1236C>T, 2677G>T, 3435C>T*), *NR1I2* coding for pregnane X receptor *(rs1523130, rs2472677, rs7643645),* and for CYP3A activity measured by a phenotyping test. Forty-two patients with at least 4 consecutive unchanged doses of RLAI were included in a multi-centre cross-sectional study. A 55% lower dose-adjusted plasma levels of RIS were observed for CYP2D6 ultra rapid metabolizers as compared to CYP2D6 intermediate metabolizers (P<0.007). *NR1I2* polymorphism (*rs7643645A>G*) influenced risperidone exposure with a 2.6-fold lower active moiety (P=0.031) in GG compared to the AA genotype. This was confirmed in a second independent cohort (n=20). Furthermore, cholesterol-HDL was positively correlated with CYP3A activity (P=0.01) and the *NR1I2* (*rs2472677*) polymorphism was associated with different side-effects including extrapyramidal symptoms and prolactin plasma levels, when adjusted for age and gender. In conclusion, our results confirmed the influence of *CYP2D6* genotype on plasma levels of risperidone. This is the first report on the influence of *NR1I2* polymorphisms on RLAI exposure and on drug-induced side-effects. Further studies will have to confirm these results.

1. Introduction

Risperidone (RIS), an atypical antipsychotic, is metabolized in the organism into its principal active metabolite 9-hydroxyrisperidone (OHRIS), both substances contributing to the pharmacological activity, their sum being called “active moiety”. Risperidone improves both the positive and negative symptoms of schizophrenia and produces a relatively low incidence of extrapyramidal symptoms at low doses. However, a relationship between occurrence of parkinsonian side effects and plasma levels of RIS and OHRIS has been reported[1] and risperidone produces dose-related increases in plasma prolactin levels both in men and women.[2]

Several in vitro and in vivo studies showed that the cytochrome P-450 enzyme 2D6 (CYP2D6), and to a lesser extent CYP3A, play a major role in the metabolism of RIS to OHRIS.[3-6] Thus, concomitant administration of CYP2D6 inhibitors such as paroxetine or fluoxetine significantly increases RIS and OHRIS plasma concentrations.[5,7] Such an increase of the RIS/OHRIS ratio in plasma following the administration of CYP2D6 inhibitor does not necessarily result in an increase of side effects[5] but could be a risk factor, as suggested by the appearance of parkinsonian symptoms in one patient two weeks after the introduction of 20mg/day of paroxetine and an important increase of the active moiety plasma levels.[7] In vivo, the CYP3A-inhibitor itraconazole increases, while the CYP3A-inducers carbamazepine and rifampicine reduce the plasma concentrations of RIS, OHRIS and of the active moiety.[8-12]

Accordingly, several studies have shown that the genetic polymorphism of *CYP2D6* gene, leading either to a CYP2D6 poor, intermediate, extensive or ultrarapid metabolizer status, significantly contributes to the wide interindividual variability of RIS and OHRIS plasma concentrations on same oral dose.[13,14] With regard to the metabolism by CYP3A, a term that in humans reflects the collective activity of CYP3A4, CYP3A5, and CYP3A7, the large interindividual variability of these isoforms is poorly determined by genotyping procedures and is presently better assessed by a phenotyping test using for example midazolam as probe.[15] To our knowledge, no study has ever attempted to determine if, and to what extent, the interindividual variability of CYP3A activity as measured by a phenotyping test could contribute to the interindividual variability of RIS and OHRIS plasma levels.

Besides CYP2D6 and CYP3A, risperidone is a substrate of the drug efflux transporter P-glycoprotein (P-gp) encoded by the *ABCB1* gene. It has been shown that the genetic polymorphism of *ABCB1* (*C3435T* and *G2677T/A*) does not influence the steady-state plasma concentrations of RIS and OHRIS after the oral intake of 3 mg/day of risperidone.[16] However, as P-gp is expressed at the blood brain

barrier, it has been suggested that this protein plays a much more important role in modulating drug concentrations in the brain than in the plasma.[17-19]

In addition, attention has been recently drawn to modulators of gene expression in phase I and II metabolism and in drug transport. Pregnane X receptor (PxR) encoded by *NR1I2* gene mediates induction of several drug detoxification genes including *CYP3A4/5* and *ABCB1.*[20,21] *NR1I2* mRNA is predominantly expressed in intestine, liver and kidney[22,23] and *NR1I2* is activated by many endogenous and exogenous ligands. Single nucleotide polymorphisms (SNP) of the *NR1I2* gene in promoter and intron 1 have been associated with alteration of CYP3A activity in vitro, the most consistent results being found for SNPs *rs1523130, rs2472677*, and *rs7643645.*[24]

Risperidone is also available as a long-acting depot formulation (Risperdal Consta®, Janssen-Cilag, Baar, Switzerland), with a recommended injection every two weeks. The long-acting depot preparations bypass the deactivating process of the intestines and of the liver, which has been reported to lead to more predictable and stable plasma concentration of the drug.[25] In contrast, to the multiple studies aimed to examine the genetic factors influencing the plasma levels of RIS and OHRIS following the oral intake of risperidone, very few studies have been carried out in patients receiving risperidone long acting injection (RLAI). One study showed a lower active moiety level in a patient taking RLAI and carbamazepine, a CYP3A inducer, compared to others patients receiving the same RLAI dose.[26] In addition, we reported a case of rapid risperidone elimination in one patient necessitating a very high dosage of RLAI (125 mg/2 weeks) before obtaining a substantial improvement of his clinical state.[27] This highlights the need for a better understanding of the in vivo influence of genetic and environmental factors on RIS and OHRIS plasma levels after injection of risperidone depot.

The major aim of this study is to analyse the influence of gene polymorphisms linked to risperidone metabolism and transport (genotyping of *CYP2D6, CYP3A, ABCB1, NR1I2,* and phenotyping of CYP3A) in a group of patients receiving RLA in steady-state conditions. For this purpose, RIS and OHRIS plasma levels were measured at two time points, the day of injection and seven days later, and the potential side effects induced by this drug (extrapyramidal symptoms, weight gain, lipid profile and prolactin levels) were also recorded.

2. Materials and Methods

Subject and study design

Clinical diagnosis was based on ICD-10 criteria.[28] All the patients between 18-65 years old presenting with a psychotic disorder and receiving RLAI were screened from February 2007 to December 2009. Because pregnancy was an exclusion criteria, a pregnancy test was performed at day 0 before inclusion of women of childbearing age. No change in the dosage of the co-medications was allowed for at least 1 week before inclusion in the study (2 months for fluoxetine) and RLAI have been administered for at least 2 months (4 injections) at constant dose. At inclusion, patients had no history of substance dependence, organic psychiatric illness or uncontrolled medical illness and were not taking oral risperidone. Complete inclusion comprised a visit on the day of risperidone depot injection (day 0), and seven days later (day 7). No fasting period was required.

This naturalistic cross-sectional study was conducted in four psychiatric centres in Switzerland (Lausanne, Geneva, Königsfelden, Marsens) and was approved by the respective ethic committees as well as by the Swiss Agency for Therapeutic Products (Swissmedic). The study was performed according to the Good Clinical Practices and written informed consent was obtained from all patients.

A second group of patients receiving RLAI was used to replicate the positive findings found for the *NR1I2* gene. These patients were selected from two independent clinical studies which were approved by their respective ethics committee (Ethics Committee of Geneva and Lausanne University hospitals, respectively) and written informed consent was given by all subjects.

Measurements

Somatic disorders, co-medications and clinical variables were recorded from medical files. Subjects were asked to report any change in their medication or life-style during the study. The year of onset of psychiatric disorder was found in the medical files, otherwise an estimation was given by the patients. An evaluation of the global illness severity was performed using the clinical global impression scale (CGI scale from 1 to 7, with 7 being the most severe presentation).[29] Extrapyramidal side effects were assessed by the Simpson-Angus scale (SAS).[30,31] Ten items ranging from 0 to 4 and linked to movement disorders (parkinsonism, akathisia, dystonia and tardive dyskinesia) were evaluated and the item scores were summed to provide a raw score ranging from 0 to 40.[30] SAS scores below 3 were reported to be considered as normal.[30] Furthermore, spontaneous self-reported adverse events and side effects were also collected. All investigators involved in the rating scales assessments were trained prior to recruitment to minimize inter-rater variability.

Blood samples were collected at day 0 and day 7 and were stored at –20 °C until analysis. RIS and OHRIS plasma concentrations, CYP3A activity and prolactin levels were measured at day 0 and day 7 while total cholesterol and HDL cholesterol were measured at day 0. Quantification of RIS and OHRIS plasma levels were measured by HPLC-MS (Eap et al, unpublished method, detailed method available on request). Briefly, a liquid-liquid extraction step was performed prior to inject the extract into a C18 Nucleosil column (EC 125/2 Nucleosil 100-5, Macherey-Nagel, Oensingen, Switzerland) using a mobile phase containing 35% tetrahydrofuran, 65% ammonium nitrate 4 mM, and 1.5% methanol. The compounds of interest were analysed in the single ion monitoring mode on a single quadrupole MS analyser working in the positive ESI mode (Agilent Series 1100 MSD single quadrupole, Agilent Technologies, Geneva, Switzerland). Intraday and interday coefficients of variation for the determination of RIS and OHRIS were between 1% and 3% for both substances. The low limit of quantification was 0.1 and 0.2 ng/mL for RIS and OHRIS, respectively. External quality controls (LGC Standards Proficiency Testing, United Kingdom) were successfully quantified since the analytical procedure validation

The active moiety and the RIS/OHRIS ratio were calculated for day 0 and day 7. Plasma concentrations were corrected by the dose and are hereafter called dose-adjusted. The evolution of plasma concentration between day 0 and day 7 was estimated using the ratio of day 7 to day 0 for RIS, OHR and the active moiety. A ratio above 1 was considered as an increase of the plasma levels at day 7 compared to day 0.

Total cholesterol (CHOLtot) and HDL cholesterol (HDL) levels were quantified by a Cobas Integra 400 (Roche Diagnostic, Basel, Switzerland). Prolactin levels were quantified by immunoassay on an Abbott Axsym system (Abbott, Wiesbaden, Germany). High total cholesterol levels were defined as ≥ 6.2 mmol/L, and normal HDL levels as ≥ 1.3 mmol/L for women and as ≥ 1.0 mmol/L for men.[32] Dyslipidemia was defined as a concentration of total and/or HDL cholesterol outside the limits, and/or when cholesterol-lowering drug is prescribed. High level of prolactin was defined as a prolactin ≥ 50 ng/mL for women and ≥ 40 ng/mL for men, which represents twice the threshold for normal values.[33,34] Hyperprolactinemia was defined as high prolactin level and/or by the presence of clinical symptoms such as gynecomastia, and/or galactorrhea and/or amenorrhea. BMI is defined as body weight/height^2 (kg/m^2) and BMI is considered as normal <25, overweight 25-30 and obese ≥30 kg/m^2. An increase of at least 10% of the baseline weight was considered as a relevant weight gain.

CYP3A phenotyping was performed on day 0 and day 7. For this purpose, patients received an oral micro dose (75 µg) of midazolam and a blood sample was collected 30 minutes later. The midazolam,

and its metabolite, 1-hydroxymidazolam plasma levels were measured by GC-MS, using a method described previously.[35,36] The 1-hydroxymidazolam / midazolam ratio (MR), a marker of CYP3A activity[37] was calculated for all subjects. A weak CYP3A activity was defined as MR<1.9, an extensive activity as MR ≥1.9.[15]

Genomic DNA was extracted from the EDTA blood samples using the FlexiGene DNA Kit (Qiagen, Hombrechtikon, Switzerland). SNPs selected from literature were analysed by real-time polymerase chain reaction with the 5'-nuclease allelic discrimination assays according to previous studies or according to the manufacturer instructions (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland).[15,37-39] The following SNPs were analyzed: *CYP2D6: allele *3, *4, *5, *6; CYP3A4*1B (rs776746), CYP3A4 rs4646437, CYP3A5*3 (rs776746), CYP3A7*1C (defined by the -262T>A, -270T>G), ABCB1 3435C>T (rs1045642), ABCB1 2677 G>T (rs2032582), ABCB11236C>T (rs1128503); NR1I2 rs1523130, NR1I2 rs2472677, NR1I2 rs7643645.* The *CYP2D6 *XN* gene duplication was analyzed by long PCR as previously described.[38] Patients were classified as CYP2D6 poor (PM), intermediate (IM), extensive (EM) or ultrarapid (UM) metabolizer according to the presence of none, one, two functional alleles or gene duplication (www.cypalleles.ki.se). All reagents were purchased from Applied Biosystems (Rotkreuz, Switzerland). Internal quality control samples of known genotype[40] were included in all analyses.

Statistical analysis

Spearman correlations were used to determine association whereas ordered logistic regression was performed when assessing risperidone depot dose requirement. Non-parametric analyses (Kruskal-Wallis and Wilcoxon/Mann-Whitney rank tests) were used to compare R, OHR, and the active moiety concentrations corrected by the dose between the different genotypes and clinical variables (age, gender, SAS scores etc) at each time point. Incidences of extrapyramidal side-effect, dyslipidemia, hyperprolactinemia, and of weight gain were compared between genotypes and clinical variables by Fischer $\chi 2$ test. To take into account the two time points, a multilevel mixed-effects linear regression was performed and when necessary, log, square or 1/square transformations were used to normalize the data. The model was always adjusted for age and gender, unless otherwise indicated. The validity of the model was assessed by graphical residual diagnostics. Measurements are given as median and interquartile range (IQR), except for the clinical characteristic of the patients (i.e. Table 1, which indicate the minimum, maximum range), or mean ± standard deviation for the result obtained according to the mixed-model.

Concomitant drugs that could modify the studied measurements were taken into account, including strong or moderate inhibitors of CYP3A and CYP2D6, and CYP3A inducers[41], lipid-lowering agents, drugs causing weight gain[42] as well as relevant clinical variables (i.e. age, sex). Patients receiving a strong CYP2D6 inhibitor were defined as CYP2D6 poor metabolizers. The statistical analyses were performed using Stata 10 (StataCorp, College Station TX, USA). The allele frequencies are reported with their 95% confidence interval (95%CI) calculated by the Adjusted Wald Method. The SNPs were tested for Hardy-Weinberg equilibrium using the *genhwi* function in Stata software. Linkage disequilibrium was calculated using r^2 parameter. All tests were two-sided and a P-value less than or equal to 0.05 was considered statistically significant.

3. Results

Patient characteristics

Sixty-seven patients receiving RLAI were screened for inclusion (15 refusals to participate, 10 not meeting all inclusion criteria). The present results are based on 42 patients (15 in Lausanne, 10 in Königsfelden, 10 in Marsens, 7 in Geneva), 12 women and 30 men, 32 Caucasians and 7 Africans, 2 Arabs and 1 Asian. Due to a possible intake of an oral dose of risperidone at day 0, data for 1 patient was considered only at day 7. Patients were diagnosed for schizoaffective disorder (F25, n=8) and for schizophrenia (F20, n=34). The median duration of RLAI treatment was 8.5 months (range: 4-29) and the time from the first psychiatric episode to the inclusion of the studied ranged from 1 to 42 years (median: 9 years). The main clinical characteristics of the patients are shown in Table 1.

The second group of patients receiving RLAI and being incorporated to test results replication was composed of 75% men, with a median age of 37 years (range: 21-63), a median BMI of 27.8 kg/m^2 (range: 24-34). Twenty-six percent were obese, the median cholesterol total was 5.3 mmol/L (range: 3.8-7.7) and 28% were smoker. Among them, 50% were Caucasian, 20% African and 30% of various origins. The patient were taking a median dose of 37.5 mg/fortnightly (range: 25-100) for a median duration of treatment of 12 months (range: 3-45). No data was available on SAS score and prolactin levels.

Genotype frequencies

Allele and genotype frequencies are presented in Table 2. The observed allele frequencies in Caucasian are in line with reported results.[38,39,43-45] All studied SNPs were in Hardy-Weinberg equilibrium (P>0.05). Only one patient was genotyped as CYP2D6 poor metabolizer (PM), 15 as intermediate metabolizer (IM), 21 as extensive metabolizer (EM) and 5 patients as ultra rapid metabolizer (UM). Arbitrary, one patient with the **4/*XN* genotype was classified as an IM. In Caucasian individuals (n=32), the *CYP3A4*1B* was in linkage disequilibrium with *CYP3A5*3* (r^2= 0.35),

and the *CYP3A4 rs4646437* was linked to *CYP3A4*1B* (r^2= 0.39) and *CYP3A5*3* (r^2= 0.58), which is in accordance with previous reports.[39,46] In our study, CYP3A Caucasian allele frequencies were similar as those previously reported (i.e. 32 patients, 9.4%, 95%CI: 4-19% for CYP3A5*3).[45] A linkage disequilibrium was also noted between the analysed SNPs of *ABCB1* gene *(*r^2= 0.67 for *2677G>T* and *1236C>T*; r^2= 0.41 for *2677G>T* and *3435C>T*; r^2= 0.51 for *3435C>T* and *1236C>T*), which is in accordance with previous reports.[39,47] Finally, a moderate linkage disequilibrium was noted between the analyzed SNPs of the *NR1I2* gene (*rs2472677* and *rs1523130* r^2= 0.11). No associations were found for the haplotypes of *ABCB1, CYP3A* and *NR1I2* with risperidone exposure or clinical responses (data not shown).

Pharmacokinetic variability and associated factors

Plasma concentration

Five patients received one of the following CYP2D6 inhibitors: weak inhibitors (citalopram (n=1), escitalopram (n=2)), and strong inhibitors (paroxetine (n=1) and levomeprazine (n= 1)). One patient drank regularly grapefruit juice, a strong CYP3A inhibitor, while a second took grapefruit juice only at day 7. No CYP3A inducers were prescribed. The median RLAI dose ranged from 25-75 mg fortnightly (Table 1). The median baseline plasma concentration in the whole population for RIS, OHRIS and active moiety were 5 ng/mL (IQR: 3-9 ng/mL), 12 ng/mL (IQR: 8-18 ng/mL), and 21 ng/mL (IQR: 12-27 ng/mL), respectively. Plasma levels for each dose are shown in Table 3. Risperidone doses were significantly correlated with plasma levels of RIS, OHRIS and the active moiety, both at day 0 and day 7 (data not shown), which is in agreement with previous reports.[26,48] The dose-adjusted plasma concentrations displayed large interindividual variability (37-fold, 38-fold and 12-fold for RIS, OHRIS and active moiety, respectively at baseline; 18-fold, 10-fold, 6-fold, respectively at day 7).

Influence of *CYP2D6* and *CYP3A* genotypes

Because only one patient was genotyped as a PM, the statistical analyses were first performed with IM for reference. The *CYP2D6* genotypes significantly influenced the dose-adjusted RIS plasma levels (Test for trend, day 0 P=0.023; day 7 P=0.031; Figure 1), with dose-, age- and gender-adjusted RIS plasma concentration in UMs being 45% of IMs values (P=0.007). The RIS/OHRIS ratios were gradually decreased across IM, EM, and UM genotypes. The RIS/OHRIS ratios adjusted for age and gender was for UMs 36% of IMs values (P=0.006). No association was found with dose-adjusted OHRIS (P>0.7) or active moiety (P>0.1).

In a second step, data from the lone PM were combined with those of 2 patients receiving strong CYP2D6 inhibitors (paroxetine n=1, levomeprazine n=1). Using the mixed-model, these 3 patients,

with a presumably CYP2D6 PM status, had a 3.3-fold higher dose-corrected RIS level (P=0.001), 2.7-fold lower dose-corrected OHRIS level (P<0.001) and 9.1-fold higher RIS/OHRIS ratio (P<0.001) as compared to the other *CYP2D6* combined genotypes. All together, the present results with the RLAI formulation are in agreement with previously published results showing a major influence of CYP2D6 in the metabolism of RIS to OHRIS following the administration of the oral form. On the other hand, none of the analyzed *CYP3A* genetic polymorphism (*CYP3A4 rs4646437*, *CYP3A4*1B*, *CYP3A5*3*, and *CYP3A7*1C)* nor the CYP3A activity determined by the midazolam phenotyping test were found to be associated with RIS, OHRIS plasma levels, RIS/OHRIS ratios nor dose requirements (data not shown). These variables were also not significantly affected by grapefruit consumption (3 patients) when compared to the other patients (data not shown).

Influence of *ABCB1* and *NR1I2* genotypes

No association was found between *ABCB1* genetic polymorphism and dose-adjusted plasma levels of RIS, OHRIS, active moiety, and RIS/OHRIS ratios nor RLAI dose requirement (P>0.1). On the other hand, and interestingly, a significant association was found at baseline for *NR1I2 rs7643645A>G,* with a 2.5-fold lower dose-adjusted of OHRIS (P=0.033) and 2.6-fold lower active moiety (P=0.031) in *GG* compared to the *AA* genotype. Because of the novelty of this finding, we aimed to replicate this result in a second independent cohort. Twenty additional patients receiving RLAI (described in Subject and study design) from two other studies were analyzed (Choong et al., submitted manuscript)[49]. In line with the previous results, 2.5 lower dose-adjusted OHRIS (P=0.015) and active moiety (P=0.024) plasma levels were found with the *GG* genotype compared to *AA* carriers. In addition, similar result were obtained when the two cohorts were combined (n=61, OHR P=0.018, active moiety P=0.011).

Variation of RLAI over 1 week

The mean RIS/OHRIS ratio decreased significantly from day 0 to day 7 and RIS/OHRIS ratio at day 7 was 80% of day 0 (P>0.001 using the mixed-model), suggesting a decrease of RIS plasma level and/or an increase of its metabolite. Although dose-corrected RIS, OHRIS and active moiety plasma concentrations adjusted for age and gender were not significantly different between day 0 and day 7 (P>0.6, P>0.1 and P>0.4, respectively), 2 groups of patients can be distinguished. A first group of 22 patients had an approximately 2-fold increase, while a 30 to 50% decreased concentrations of RIS, OHRIS and active moiety were measured in the remaining 19 patients. No difference in term of age (P>0.6), concomitant CYP2D6 inhibitor (P=1.0) or CYP3A4/5 inhibitor prescription (P>0.5) could be found between both groups. Male patients were equally distributed while more women (82%) had increased concentrations on day 7 (P=0.038). Interestingly, the variation was associated with NR1I2

genetic polymorphism. Thus, a significantly higher increase of dose-adjusted RIS, OHRIS and active moiety from day 0 to day 7 was observed for *NR1I2 rs7643645 G* carriers compared to the *A* carriers (P<0.009 for all plasma concentrations, see Table 4). In addition, *C* carriers for *NR1I2 rs2472677* were more likely to increase their plasma concentrations the week after injection than the *T* carriers, with 1 out of 8 (13%) individuals with the *NR1I2 rs2472677TT* genotype having increased plasma levels, whereas 16 of 25 (64%) with the *TC* genotype, and 5 of 8 (63%) with the *CC* genotype having increased plasma levels of active moiety ($\chi 2$, P=0.034). Similar results were obtained for RIS and OHRIS separately (data not shown)

Pharmacodynamics variability and associated factors

Clinical response (CGI)

The median baseline CGI score was 3 points (IQR: 2-4), which corresponded to a mild psychiatric illness. Patients had very variable duration of treatment, and expectedly, no significant correlations were found between CGI scores and fortnightly dose (P>0.9), dose-adjusted RIS (P>0.1), OHRIS (P>0.7), active moiety (P>0.3) plasma levels at baseline and at day 7 (adjusted for age and gender in a mixed model, data not shown). No associations were found between CGI scores and other clinical demographic or genetic data (data not shown)

Extrapyramidal side effects (SAS)

The median baseline SAS score was 3 points (IQR: 1-5). No association was found with RLAI dose (P=0.3), treatment length (P>0.5), as well as RIS (P=0.4), OHRIS (P=0.3), active moiety (P=0.2) plasma levels at baseline, at day 7 and also using the mixed model (data not shown). Among patients, 55% had SAS scores >3 (n=20) or were receiving an anticholinergic agent (biperiden, n=6 among whom 3 had still a SAS score >3). *NR1I2 rs2472677CC* carriers had higher SAS score (n=9, median: 5, IQR: 4-7) than the *CT* and *TT* carriers (n=33, median 2, IQR: 0-5; P=0.03) at day 0 and at day 7 (P=0.03 on both cases), also in the mixed model (P=0.035). Finally, no associations were found between SAS scores and *CYP2D6* or *CYP3A* genotypes, and with CYP3A activity (P>0.3 for both days).

Weight gain and lipid profile

Several cardiovascular risk factors were observed in the present cohort, with a prevalence of obesity in 26% of the patients, 81 % being smokers, and 45% presenting a dyslipidemia. Treatment for metabolic complication were prescribed to 6 patients, the majority being lipid lowering drugs (n=4). A median weight gain of 6 kg (IQR: 0-16 kg) for a median duration of RLAI treatment of 8 months (IQR: 4-15) was found for the 39 patients with available data. It is noteworthy to mention that ten patients received other drugs potentially linked to weight gain (valproate n=4, clozapine n=1,

mirtazapine n=2, quetiapine n=2, mirtazapine and quetiapine in combination n=1). Weight gain was not correlated to fortnightly dose, RIS, OHRIS, and active moiety plasma levels in the whole population and in the subgroup without weight gain-related drug (P>0.1).

No evidence was found between weight gain and the selected polymorphisms for genes potentially involved in the pharmacokinetics of risperidone, nor with CYP3A activity. CHOLtot and HDL levels were not correlated with RIS, OHRIS, active moiety plasma levels (P>0.1) nor with fortnightly doses (P=0.9). The CYP3A activity and polymorphisms of the *CYP2D6* and *CYP3A* genes were not associated with CHOLtot and HDL levels (P=0.9). As expected, HDL levels differ significantly between gender (median HDL levels in women: 1.65 mmol/L, IQR: 1.23-2.05; in men: 1.11 mmol/L, IQR: 0.95-1.38, P=0.004). Interestingly, HDL levels were significantly correlated to CYP3A activity in the whole population (r_s= 0.49, P=0.001). Thus, in men, median HDL levels of 0.95 mmol/L (n=5, IQR: 0.9-1) and 1.19 mmol/ L (n=22, IQR: 0.98-1.46) were measured in the group with low CYP3A activity (<1.9 MR) as compared to the group with an extensive activity (≥1.9 MR; P=0.029), respectively. The lone woman with low CYP3A activity was also observed with a lower HDL level than those with extensive CYP3A activity: 1.1 mmol/L (n=1) and 1.68 mmol/L (n=10, IQR: 1.4-2.05), respectively. In the whole population, CYP3A activity adjusted for age and gender remained significantly associated with HDL levels (P=0.014). The result was similar when the subgroup without lipid lowering agent was analysed (P=0.029).

Prolactin plasma concentration

As expected, an important difference in prolactin level was found between men and women (baseline median prolactin level: n=30, 27 ng/mL (IQR: 19-45) in men; n=12, 75 ng/mL (IQR: 46-133) in women; P<0.001). High prolactin levels were measured in 17 patients (40%) at day 0 and 19 patients (45%) at day 7 and were more frequent in women (9/12 women, 75%) than in men (8/30 men, 27%; χ2, P=0.004), which is in agreement with previous studies.[33,34,50] Hyperprolactinemia symptoms were reported by 6 patients (3 women, 3 men; sexual dysfunction, n=3; galactorrhea, n=1; gynecomastia, n=1; and amenorrhea, n=1), which were not associated with particularly high prolactin levels (median: 37 ng/mL, IQR: 20-55).

Prolactin levels were significantly correlated with RIS, OHRIS and active moiety plasma levels (r_s=0.43, P=0.0047; r_s=0.33, P=0.037; r_s=0.38, P=0.013, respectively) at day 0. Significant association was found between risperidone exposure and prolactin levels using a mixed model adjusted for age and gender (RIS P=0.001, OHRIS P<0.001 and active moiety P<0.001). Of note, 47% of the variation of prolactin level can be explained by the model including age, sex and active moiety plasma level. On the other

hand, fortnightly dose does not improve the model and was not found to be associated with prolactin levels (P=0.47). Prolactin levels adjusted for age and gender were significantly associated with *NR1I2 rs2472677C>T* polymorphism (P=0.032). At day 0, 1.8-fold (in women) and 1.3-fold (in men) lower prolactin levels were found in carriers of the *CC* genotypes as compared to the *CT* and *TT* genotypes were found.

4. Discussion

This study aimed to analyse, in a group of patients receiving RLAI treatment, the influence of gene polymorphisms potentially linked to risperidone metabolism and transport, and of the CYP3A activity on the plasma levels of RIS and OHRIS, and on potential side effects induced by this drug.

Pharmacokinetic variability and associated factors

In agreement with a previous report,[26] a very large inter-individual variability was measured for dose-adjusted plasma concentrations of RIS, OHRIS and active moiety in patients receiving RLAI (37-, 38- and 12-fold variations, respectively). CYP2D6 activity was found to be a major determinant of risperidone pharmacokinetics, with the *CYP2D6* genotype showing a significant influence on dose-adjusted plasma levels of RIS, and RIS/OHRIS ratio but not on dose-adjusted OHRIS and active moiety. These results are similar to previously published results in patients receiving oral risperidone.[16,51-53] Our results are also in agreement with studies showing a minor influence of CYP2D6 on dose-adjusted active moiety concentration,[52-55] although some controversial results have been published.[13] Additionally, we confirmed the influence of strong CYP2D6 inhibitors on risperidone metabolism.[5,7]

Although not statistically significant, CYP2D6 PMs seemed to be underrepresented with only 1 (3%, 95%CI: 0-17%) patient with this genotype out of 32 Caucasians as compared to the 5-10% frequency expected in the Caucasian population.[56,57] In contrast, CYP2D6 UMs seemed to be overrepresented in the present study (5 patients, 12%, 95%CI: 6-32%), the frequency being more than 2 to 4-fold higher than the reported 3 to 5% in Caucasian populations.[56] Therefore, the possibility of an earlier treatment discontinuation in PM patients due to potential side effects is questioned. The PM phenotype was previously reported to increase the risk for side-effects and drug discontinuation in patients receiving oral risperidone.[58] On the other hand, the higher than expected prevalence of UM in the present study raise the question whether patients receiving antipsychotics metabolized mainly or partially by this enzyme (e.g. aripiprazole and to a lesser extent haloperidol, olanzapine) might respond inadequately and then be switched to an injection formulation because of suspicion of poor compliance.

In patients receiving oral risperidone, the administration of strong CYP3A inhibitors or inducers resulted in high or low plasma levels of RIS, OHRIS and of the active moiety, respectively.[8,11,12] In the present study with patients with depot formulation of risperidone, CYP3A activity did not predict dose-adjusted RIS, OHRIS, and active moiety plasma levels. As the midazolam ratio was suggested to reflect CYP3A activity both in the liver and in the intestines,[59] one may hypothesize that the depot formulation, by shunting the first pass effect, reduces the influence of CYP3A in the pharmacokinetics of injected risperidone.

In agreement with the direct determination of CYP3A activity, no associations were found between the analyzed *CYP3A* SNPs and risperidone pharmacokinetics. These results are in line with other studies,[53,60,61] while another study found higher OHRIS and active moiety plasma levels for *CYP3A5*3*3* compared to the other genotypes in patients receiving oral risperidone.[14] It has recently been shown that the genetic polymorphism of *ABCB1* does not influence or only moderately the steady-state plasma concentrations of RIS and OHRIS after the oral intake of risperidone.[13,16,53,61] In the present study with risperidone depot formulation, we showed that *ABCB1* genetic polymorphisms do not significantly contribute to variability of RIS and OHRIS in the blood.

Interestingly, we showed a significant association between *NR1I2* genetic polymorphism and risperidone pharmacokinetics. The *rs1523130*, *rs2472677*, and *rs7643645* SNPs were previously identified in transcription binding sites of *NR1I2* regulatory regions.[24] In patients with HIV treated with 400 mg/day of atazanavir, a P-gp and CYP3A substrate, the *rs2472677T* reduces the trough plasma concentration of this drug[44] which is in line with its reported increased activity.[24] In the present study, the *NR1I2 rs7643645G* allele, linked to a decreased transcription [24], was found to be associated with lower dose-adjusted OHRIS and active moiety plasma levels. Moreover, these results were replicated in a second independent cohort of patients receiving RLAI as well as when the two cohorts were combined. These results could be tentatively explained by the lower *CYP3A* and *ABCB1* transcription activity measured in subjects with the *G* allele.[24] However, because CYP3A activity measured by the phenotyping test was not found to be associated with risperidone pharmacokinetics, the influence of PxR observed in the present study could not be explained by the induction of CYP3A but rather by the induction of other drug metabolizing enzymes and/or P-gp and/or other transporters. The influence of *NR1I2* gene remains, however, to be demonstrated after an oral intake of this drug, due to the first pass effect and the involvement of other confounding factors.

Variation of RLAI over 1 week

This study also aimed to investigate the stability of RIS and OHRIS levels between the day of injection and 7 days later. Non significant differences were observed for RIS, OHRIS and active moiety between the two time points, which is in agreement with previously published results showing moderate (about 2-fold) plasma variations observed at steady state.[48,62] However, although the mean RIS, OHRIS and active moiety plasma levels did not vary overall between day 0 and day 7, 2 groups of patients can be distinguished, the first group of 22 patients having an approximately 2-fold increase while a second group of 19 patients had a 30 to 50% decrease concentration of RIS, OHRIS and active moiety.

Interestingly, the present study showed significant association of *NR1I2 rs2472677* and *rs7643645* with RIS, OHRIS and active moiety plasma evolution, with the *T* and *A* alleles, respectively, associated with decreased plasma levels from day 0 to day 7. These results are in agreement with results showing that these alleles are associated with increased CYP3A activity.[24] However, since CYP3A activity measured in the present study was not found to influence the evolution of RIS, OHRIS, and active moiety plasma levels between day 0 and day 7, the present results suggest that the induction of other drug metabolizing enzymes and/or transporters following the injection of RLAI leads to a decrease of dose adjusted RIS, OHRIS and active moiety plasma levels from the day of injection to seven days later.

Pharmacodynamic variability and associated factors

Clinical response (CGI)

In line with previous reports, no significant correlation was found between blood concentration and therapeutic response for RIS, OHRIS and active moiety.[7,63] This was expected as the naturalistic study design was not well fitted for this purpose (very variable treatment duration, simple global impression scale used which may not reflect the specific therapeutic action on psychotic symptoms, etc). The criteria of four injections might also have discarded non-responders with high CGI score, while the long treatment duration (median of 8.5 months) might contribute to the overall low CGI score in the group (median score of 3).

Extrapyramidal side effects (SAS)

Extrapyramidal side-effect is an important issue for risperidone treatment as 16% and 20% of the patients received anticholinergic drugs when treated with oral risperidone and RLAI, respectively, while 34% had extrapyramidal symptoms in the latter group.[64,65] In the present study, in accordance with previous reports on oral risperidone, no association was found between the different plasma

levels and other clinical variables with SAS scores.[1,10,66] Conflicting results were published on the relationship between the prevalence of EPS and risperidone dose or risperidone plasma concentrations.[1,5,7,58,67-70]

CYP2D6 PMs were suggested to be at higher risk for tardive dyskinesia, and risperidone discontinuation due to adverse drug reactions.[58,58,71] In the present study, as only one CYP2D6 PM was included, no conclusion can be drawn on this point. When taking into account the 3 patients with a presumably CYP2D6 PM status, no association was observed for the drug adverse reactions. Although increased extrapyramidal symptoms were noted in a patient with a concomitant administration of risperidone and strong CYP3A inhibitor,[11] we did not find any influence of *CYP3A* genotypes and SAS scores in the present study, which is in agreement with the lack of association between *CYP3A* genotypes and RIS plasma levels.

ABCB1, a gene that encodes an efflux transport at the blood brain barrier and which could therefore influence the brain levels of drugs, was hypothesised to influence EPS. A previous study with oral risperidone found that *ABCB1 2677T - 3435T* carriers presented an increased occurrence of extrapyramidal symptoms which were not linked to plasma concentrations.[13] No such associations were found in the present study, which differ by many variables (oral form, drug naïve patients, short term study with 8 weeks of follow up and a majority of women in the former study). Interestingly, we found a higher SAS score for *NR1I2 rs2472677*CC genotype, which was reported to be linked to a decreased activity,[24] as compared to the *T* carriers. Thus, one could hypothesize that decreased activity of P-gp and/or of other transporters at the blood brain barrier could increase RIS and OHRIS brain levels and therefore explain the observed increase of extrapyramidal symptoms.

Prolactin plasma concentration

In the present study, 43% of patients had elevated prolactin levels which is in accordance with the high occurrence found in previous reports with oral risperidone,[50,72,73] and with RLAI[74] although its intensity compared to oral risperidone is still not clearly demonstrated.[75] Interestingly, a significant positive correlation was found between prolactin levels and RIS, OHRIS and active moiety plasma levels. Prolactin level adjusted for age and gender was also significantly associated with *NR1I2 rs2472677C>T* polymorphism, but with lower levels in carriers of the *CC* genotypes as compared to the *CT* and *TT* genotypes. No explanation can be presently proposed to explain the apparent discrepancy that the same genotypes (i.e. *CC*) are associated with increased extrapyramidal symptoms and decreased prolactin levels. One can, however, mention that occurrence of extrapyramidal symptoms is thought to be mediated by D_2 receptor antagonism at the level of the

striatum while the release of prolactin is induced by D_2 blockade at the level of the pituitary.[76] Interestingly, while the striatum is located in the central nervous system part protected by the blood brain barrier, the pituitary is outside this barrier.[77,78] Thus, as polymorphisms of *NR1I2* affect the activities of both metabolic enzymes and transporters, the same genetic polymorphism could affect differently the activity of drugs depending on the location of the target organ. In addition, it remains to be determined whether the stronger increase of RIS, OHRIS and active moiety plasma levels observed in the carriers of the *C* allele of *NR1I2 rs2472677* between day zero and day 7, with therefore wider fluctuations of RIS and metabolite plasma levels during the 14 day interval between 2 injections, could contribute to the observed association with stronger plasma prolactin levels measured on day 0 as suggested elsewhere.[79]

Weight gain and metabolic profile

Median weight gain was 4.5 kg for a median duration of treatment of 8.5 months, which is in accordance with a previous report.[80] In total, 38% of patients gained more than 10% of their baseline weight. Only one patient was treated with a medication associated with weight loss (i.e. metformin), therefore no adjustment was carried out for this type of medication in the analyses. Increased cholesterol and decreased HDL levels were respectively found in 14% and 33% of the cohort, which are not a common side-effect related to oral risperidone treatment[81,82] nor to RLAI.[74] However, risperidone could indirectly influence the lipid pathway through the increased risk of weight gain in this group during long-term treatment. This is in agreement with results showing lipid parameter impairment during long-term risperidone monotherapy in an Asian population[83] and a rapid worsening of hypertriglyceridiemia in a geriatric patient for whom oral risperidone has been introduced.[84] Concerning HDL levels, the significant positive correlation found in the present study between CYP3A activity and higher HDL levels is in accordance with similar findings in a rat model. An increased HDL levels was observed in rats treated with inducers of CYP3A activity as compared to control rats, and this HDL level increase was proportional to the potency of each CYP3A inducer.[85] Moreover, the PXR inducer rifampin[86] was reported to increase HDL levels in mice while in PXR-knock-out mice no significant change was observed.[85] Similar effect is also suggested in humans: patients receiving anticonvulsant (i.e. phenobarbital, phenytoin, two CYP3A4/5 inducers[41]) showed increased HDL levels as compared to healthy matched controls.[87,88] A significant correlation was also found between HDL levels and the corresponding CYP content in 18 human livers obtained by percutaneous biopsy,[87,88] with CYP3A being the major CYP enzyme in the liver.[89]

Limitations

This study has several limitations: first, its naturalistic cross-sectional design which leads to a heterogeneous RLAI medication including different treatment durations, variable doses, and

psychiatric diagnostics. This, in addition to the fact that the CGI score is a very simple scale, limits the value of our data on therapeutic response as well as the statistical analysis performed on these data. On the other hand, this limitation should not apply to the blood levels of the RIS, OHRIS and active moiety, nor to the other clinical variables (prolactin and cholesterol levels, extrapyramidal symptoms). The second limitation is the modest number of patients included and the statistical analysis which were not corrected for the multiple tests performed. Thus, the new findings of the present study must be considered as preliminary and must be validated in other cohorts, although the significant association between *NR1I2* genetic polymorphism and RIS, OHRIS and active moiety have already been replicated in a second data set of patients receiving RLAI. On the other hand, the strength of this study on depot formulation is that by definition, the compliance of the patients is ascertained, which is important considering the poor compliance observed with oral formulation on long term treatment.[90,91]

Conclusion

In agreement with previous reports on oral risperidone, CYP2D6 was also found to significantly contribute to RIS pharmacokinetics in patients treated with RLAI. On the other hand, neither CYP3A activity measured by a phenotyping test nor genetic polymorphisms of *CYP3A*, and *ABCB1* genes were found to be associated with RIS, OHRIS or the active moiety plasma levels. Accordingly, side effects induced by risperidone may not be associated with these genetic polymorphisms. Prolactin levels were found to be related to risperidone exposure. Interestingly, genetic polymorphisms of the *NR1I2* gene were significantly associated with RIS, OHRIS and active moiety plasma levels, with variations of their levels during the week following the injection, and with the extrapyramidal side-effects, and with the prolactin levels. These results must be replicated in other studies with patients receiving RLAI, and it must also be determined whether these results remain valid in patients receiving the oral form of risperidone.

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Reference List

1. Spina E, Avenoso A, Facciolà G et al. Relationship between plasma risperidone and 9-hydroxyrisperidone concentrations and clinical response in patients with schizophrenia. Psychopharmacology 2001; 153:238-243.

2. Kleinberg DL, Davis JM, De Coster R et al. Prolactin levels and adverse events in patients treated with risperidone. J Clin Psychopharmacol 1999; 19(1):57-61.

3. Fang J. Metabolism of risperidone to 9-hydroxyrisperidone by human cytochromes P450 2D6 and 3A4. Naunyn-Schmiedeberg's Arch Pharmacol 1999; 359:147-151.

4. Yasui-Furukori N, Hidestrand M, Spina E et al. Different enantioselective 9-hydroxylation of risperidone by the two human CYP2D6 and CYP3A4 enzymes. Drug Metab Dispos 2001; 29(9):1263-1268.

5. Bondolfi G, Eap CB, Bertschy G et al. The effect of fluoxetine on the pharmacokinetics and safety of risperidone in psychotic patients. Pharmacopsychiatry 2002; 35(2):50-56.

6. DeVane CL, Nemeroff CB. An evaluation of risperidone drug interactions. J Clin Psychopharmacol 2001; 21(4):408-416.

7. Spina E, Avenoso A, Facciolà G et al. Plasma concentrations of risperidone and 9-hydroxyrisperidone during combined treatment with paroxetine. Ther Drug Monit 2001; 23:223-227.

8. Jung SM, Kim KA, Cho HK et al. Cytochrome P450 3A inhibitor itraconazole affects plasma concentrations of risperidone and 9-hydroxyrisperidone in schizophrenic patients. Clin Pharmacol Ther 2005; 78(5):520-528.

9. Spina E, Avenoso A, Facciolà G et al. Plasma concentrations of risperidone and 9-hydroxyrisperidone: Effect of comedication with carbamazepine or valproate. Ther Drug Monit 2000; 22:481-485.

10. Olesen OV, Licht RW, Thomsen E et al. Serum concentrations and side effects in psychiatric patients during risperidone therapy. Ther Drug Monit 1998; 20:380-384.

11. Bork JA, Rogers T, Wedlund PJ et al. A pilot study on risperidone metabolism: the role of cytochromes P450 2D6 and 3A. J Clin Psychiatry 1999; 60:469-476.

12. Kim KA, Park PW, Liu KY et al. Effect of rifampin, an inducer of CYP3A and P-glycoprotein, on the pharmacokinetics of risperidone. J Clin Pharmacol 2008; 48:66-72.

13. Jovanovic N, Bozina N, Lovric M et al. The role of CYP2D6 and ABCB1 pharmacogenetics in drug-naive patients with first-episode schizophrenia treated with risperidone. Eur J Clin Pharmacol 2010; 66(11):1109-1117.

14. Kang RH, Jung SM, Kim KA et al. Effects of CYP2D6 and CYP3A5 genotypes on the plasma concentrations of risperidone and 9-hydroxyrisperidone in Korean schizophrenic patients. J Clin Psychopharmacol 2009; 29(3):272-277.

15. Eap CB, Buclin T, Hustert E et al. Pharmacokinetics of midazolam in CYP3A4 and CYP3A5 genotyped subjects. Eur J Clin Pharmacol 2004; 60(4):231-236.

16. Yasui-Furukori N, Mihara K, Takahata T et al. Effects of various factors on steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone: lack of impact of MDR-1 genotypes. Br J Clin Pharmacol 2003; 57(5):569-575.

17. Lin JH, Yamazaki M. Role of p-glycoprotein in pharmacokinetics: clinical implications. Clin Pharmacokinet 2003; 42(1):59-98.

18. Marzolini C, Paus E, Buclin T et al. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther 2004; 75(1):13-33.

19. Wang JS, Ruan Y, Taylor RM et al. The brain entry of risperidone and 9-hydroxyrisperidone is greatly limited by P-glycoprotein. Int J Neuropsychopharmacol 2004; 7(4):415-419.

20. Crettol S, Petrovic N, Murray M. Pharmacogenetics of phase I and phase II drug metabolism. Curr Pharm Des 2010; 16(2):204-219.

21. Synold TW, Dussault I, Forman BM. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. Nat Med 2001; 7(5):584-590.

22. Kliewer SA, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr Rev 2002; 23(5):687-702.

23. Francis GA, Fayard E, Picard F et al. Nuclear receptors and the control of metabolism. Annu Rev Physiol 2003; 65:261-311.

24. Lamba J, Lamba V, Strom S et al. Novel single nucleotide polymorphisms in the promoter and intron 1 of human pregnane X receptor/NR1I2 and their association with CYP3A4 expression. Drug Metab Dispos 2008; 36(1):169-181.

25. McEvoy JP. Risks versus benefits of different types of long-acting injectable antipsychotics. J Clin Psychiatry 2006; 67 (Suppl 5):15-18.

26. Castberg I, Spigset O. Serum concentrations of risperidone and 9-hydroxyrisperidone after administration of the long-acting injectable form of risperidone - Evidence from a routine therapeutic drug monitoring service. Ther Drug Monit 2005; 27(1):103-106.

27. Albrecht A, Morena PG, Baumann P et al. High dose of depot risperidone in a nonresponder schizophrenic patient. J Clin Psychopharmacol 2004; 24(6):673-674.

28. ICD-10. 2011. Available at:

29. ECDEU assessment manual for psychopharmacology. In: Guy W, editor. Clinical Global Impressions. Rockville: National Institute of Mental Health, 1976: 218-222.

30. Hawley CJ, Fineberg N, Roberts AG et al. The use of the Simpson Angus Scale for the assessment of movement disorder: a training guide. Int J Psych Clin Pract 2003; 7:249-257.

31. Simpson GM, Angus JW. A rating scale for extrapyramidal side effects. Acta Psychiatr Scand Suppl 1970; 212:11-19.

32. National Institute of Health. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Final report. Circulation 2002; 106:3143-3421.

33. Halbreich U, Kahn LS. Hyperprolactinemia and schizophrenia: mechanisms and clinical aspects. J Psychiatr Pract 2003; 9(5):344-353.

34. Henderson DC, Doraiswamy PM. Prolactin-related and metabolic adverse effects of atypical antipsychotic agents. J Clin Psychiatry 2008; 69 Suppl 1:32-44.

35. Eap CB, Bouchoux G, Powell Golay K et al. Determination of picogram levels of midazolam, and 1- and 4-hydroxymidazolam in human plasma by gas chromatography-negative chemical ionization-mass spectrometry. Journal of Chromatography B 2004; 802:339-345.

36. Eap CB, Bender S, Jaquenoud Sirot E et al. Nonresponse to clozapine and ultrarapid CYP1A2 activity: Clinical data and analysis of the CYP1A2 gene. J Clin Psychopharmacol 2004; 24:214-219.

37. Eap CB, Fellay J, Buclin T et al. CYP3A activity measured by the midazolam test is not related to 3435 C >T polymorphism in the multiple drug resistance transporter gene. Pharmacogenetics 2004; 14(4):255-260.

38. Crettol S, Déglon JJ, Besson J et al. ABCB1 and cytochrome P450 genotypes and phenotypes: influence on methadone plasma levels and response to treatment. Clin Pharmacol Ther 2006; 80(6):668-681.

39. Crettol S, Venetz JP, Fontana M et al. CYP3A7, CYP3A5, CYP3A4, and ABCB1 genetic polymorphisms, cyclosporine concentration, and dose requirement in transplant recipients. Ther Drug Monit 2008; 30(6):689-699.

40. Oneda B, Crettol S, Bochud M, Besson J, Croquette-Krokar M, Hämmig R et al. b-arrestin2 influences the response to methadone in opioid dependent patients. Pharmacogenomics Journal. In press.

41. Service de pharmacologie et toxicologie clinique, Hôpitaux Universitaires Gèneve. Interactions medicamenteuses et cytochrome P450. 2010. Available at: http://www.pharmacoclin.ch/_library/pdf/cytp450.pdf. Accessed May 8, 2011.

42. Compendium Suisse de Médicaments®. 31e ed. Bâle (Suisse): Documed S.A., 2010.

43. Oneda B, Crettol S, Jaquenoud Sirot E et al. The P450 oxidoreductase is associated with CYP3A activity in vivo as measured by the midazolam phenotyping test. Pharmacogenetics and Genomics 2009; 19(11):877-883.

44. Siccardi M, D'Avolio A, Baietto L et al. Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C-->T) with reduced concentrations of unboosted atazanavir. Clin Infect Dis 2008; 47:1222-1225.

45. National Center for Biotechnology Information-dbSNP. 2011. Available at: http://www.ncbi.nlm.nih.gov/snp. Accessed May 8, 2011.

46. Dally H, Bartsch H, Jager B et al. Genotype relationships in the CYP3A locus in Caucasians. Cancer Lett 2004; 207(1):95-99.

47. Gex-Fabry M, Eap CB, Oneda B et al. CYP2D6 and ABCB1 genetic variability: influence on paroxetine plasma level and therapeutic response. Ther Drug Monit 2008; 30(4):474-482.

48. Eerdekens M, Van Hove I, Remmerie B et al. Pharmacokinetics and tolerability of long-acting risperidone in schizophrenia. Schizophr Res 2004; 70(1):91-100.

49. Choong E, Solida A, Lechaire C et al. Suivi du syndrome métabolique induit par les antipsychotiques atypiques: recomendations et perspectives pharmacogénétiques. Rev Med Suisse 2008; 4(171):1994-1999.

50. Melkersson KI. Prolactin elevation of the antipsychotic risperidone is predominantly related to its 9-hydroxy metabolite. Hum Psychopharmacol 2006; 21(8):529-532.

51. Scordo MG, Spina E, Facciolà G et al. Cytochrome P450 2D6 genotype and steady state plasma levels of risperidone and 9-hydroxyrisperidone. Psychopharmacology 1999; 147:300-305.

52. Roh HK, Kim CE, Chung WG et al. Risperidone metabolism in relation to CYP2D6*10 allele in Korean schizophrenic patients. Eur J Clin Pharmacol 2001; 57:671-675.

53. Gunes A, Spina E, Dahl ML et al. ABCB1 polymorphisms influence steady-state plasma levels of 9-hydroxyrisperidone and risperidone active moiety. Ther Drug Monit 2008; 30(5):628-633.

54. Heykants J, Huang ML, Mannens G et al. The pharmacokinetics of risperidone in humans: A summary. J Clin Psychiatry 1994; 55 (Suppl.):13-17.

55. Huang ML, Van Peer A, Woestenborghs R et al. Pharmacokinetics of the novel antipsychotic agent risperidone and the prolactin response in healthy subjects. Clin Pharmacol Ther 1993; 54:257-268.

56. Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. Clin Pharmacokinet 2009; 48(11):689-723.

57. de Leon J, Sandson NB, Cozza KL. A preliminary attempt to personalize risperidone dosing using drug-drug interactions and genetics: part II. Psychosomatics 2008; 49(4):347-361.

58. de Leon J, Susce MT, Pan RM et al. The CYP2D6 poor metabolizer phenotype may be associated with risperidone adverse drug reactions and discontinuation. J Clin Psychiatry 2005; 66(1):15-27.

59. Eap CB, Buclin T, Cucchia G et al. Oral administration of a low dose of midazolam (75 micrograms) as an in vivo probe for CYP3A activity. Eur J Clin Pharmacol 2004; 60:237-246.

60. Xiang Q, Zhao X, Zhou Y et al. Effect of CYP2D6, CYP3A5, and MDR1 genetic polymorphisms on the pharmacokinetics of risperidone and its active moiety. J Clin Pharmacol 2010; 50(6):659-666.

61. Leon J, Susce MT, Pan RM et al. A study of genetic (CYP2D6 and ABCB1) and environmental (drug inhibitors and inducers) variables that may influence plasma risperidone levels. Pharmacopsychiatry 2007; 40(3):93-102.

62. Bazire S. Psychotropic Drug Directory 2010. Aberdeen: HealthComm UK Ltd, 2010.

63. Kakihara S, Yoshimura R, Shinkai K et al. Prediction of response to risperidone treatment with respect to plasma concencentrations of risperidone, catecholamine metabolites, and polymorphism of cytochrome P450 2D6. Int Clin Psychopharmacol 2005; 20(2):71-78.

64. Taylor DM, Young CL, Mace S et al. Early clinical experience with risperidone long-acting injection: a prospective, 6-month follow-up of 100 patients. J Clin Psychiatry 2004; 65(8):1076-1083.

65. Canas F, Moller HJ. Long-acting atypical injectable antipsychotics in the treatment of schizophrenia: safety and tolerability review. Expert Opin Drug Saf 2010; 9(5):683-697.

66. Bondolfi G, Dufour H, Patris M et al. Risperidone versus clozapine in treatment-resistant chronic schizophrenia: a randomized double-blind study. Am J Psychiatry 1998; 155(4):499-504.

67. Chouinard G, Jones B, Remington G et al. A Canadian multicenter placebo-controlled study of fixed doses of risperidone and haloperidol in the treatment of chronic schizophrenic patients. J Clin Psychopharmacol 1993; 13:25-40.

68. Marder SR, Meibach RC. Risperidone in the treatment of schizophrenia. Am J Psychiatry 1994; 151:825-835.

69. Riedel M, Schwarz MJ, Strassnig M et al. Risperidone plasma levels, clinical response and side-effects. Eur Arch Psychiatry Clin Neurosci 2005; 255(4):261-268.

70. Yoshimura R, Ueda N, Nakamura J. Possible relationship between combined plasma concentrations of risperidone plus 9-hydroxyrisperidone and extrapyramidal symptoms. Preliminary study. Neuropsychobiology 2001; 44(3):129-133.

71. Köhnke MD, Griese EU, Stosser D et al. Cytochrome P450 2D6 deficiency and its clinical relevance in a patient treated with risperidone. Pharmacopsychiatry 2002; 35(3):116-118.

72. Kinon BJ, Gilmore JA, Liu H et al. Prevalence of hyperprolactinemia in schizophrenic patients treated with conventional antipsychotic medications or risperidone. Psychoneuroendocrinology 2003; 28 (Suppl. 2):55-68.

73. Young RM, Lawford BR, Barnes M et al. Prolactin levels in antipsychotic treatment of patients with schizophrenia carrying the DRD2*A1 allele. Br J Psychiatry 2004; 185:147-151.

74. Simpson GM, Mahmoud RA, Lasser RA et al. A 1-year double-blind study of 2 doses of long-acting risperidone in stable patients with schizophrenia or schizoaffective disorder. J Clin Psychiatry 2006; 67(8):1194-1203.

75. Chue P, Eerdekens M, Augustyns I et al. Comparative efficacy and safety of long-acting risperidone and risperidone oral tablets. Eur Neuropsychopharmacol 2005; 15(1):111-117.

76. Stahl SM. Stahl's Essential Psychopharmacology - Neuroscientific Basis and Practical Applications. Thrid edition ed. New York: Cambridge University Press, 2008.

77. Madhusoodanan S, Parida S, Jimenez C. Hyperprolactinemia associated with psychotropics--a review. Hum Psychopharmacol 2010; 25(4):281-297.

78. Barrett kE, Barman SM, Boitano S et al. Ganong's review of medical physiology. 23rd ed. McGraw-Hill companies,Inc., 2010.

79. Bai YM, Chen TT, Lin WK et al. Pharmacokinetics study for hyperprolactinemia among schizophrenics switched from risperidone to risperidone long-acting injection. J Clin Psychopharmacol 2007; 27(3):306-308.

80. Meyer JM. A retrospective comparison of weight, lipid, and glucose changes between risperidone- and olanzapine-treated inpatients: metabolic outcomes after 1 year. J Clin Psychiatry 2002; 63(5):425-433.

81. de Leon J, Susce MT, Johnson M et al. A clinical study of the association of antipsychotics with hyperlipidemia. Schizophr Res 2007; 92(1-3):95-102.

82. De Hert M, Mittoux A, He Y, Peuskens J. Metabolic parameters in the short- and long-term treatment of schizophrenia with sertindole or risperidone. Eur Arch Psychiatry Clin Neurosci. In press.

83. Murashita M, Inoue T, Kusumi I et al. Glucose and lipid metabolism of long-term risperidone monotherapy in patients with schizophrenia. Psychiatry Clin Neurosci 2007; 61(1):54-58.

84. Kohen I, Manu P. Rapidly worsening hypertriglyceridemia during treatment with risperidone. Am J Ther 2010; 17(2):216-218.

85. Bachmann K, Patel H, Batayneh Z et al. PXR and the regulation of apoA1 and HDL-cholesterol in rodents. Pharmacol Res 2004; 50(3):237-246.

86. Matheny CJ, Ali RY, Yang X et al. Effect of prototypical inducing agents on P-glycoprotein and CYP3A expression in mouse tissues. Drug Metab Dispos 2004; 32(9):1008-1014.

87. Luoma PV, Sotaniemi EA, Pelkonen RO et al. Plasma high-density lipoprotein cholesterol and hepatic cytochrome P-450 concentrations in epileptics undergoing anticonvulsant treatment. Scand J Clin Lab Invest 1980; 40(2):163-167.

88. Luoma PV. Cytochrome P450 and gene activation--from pharmacology to cholesterol elimination and regression of atherosclerosis. Eur J Clin Pharmacol 2008; 64(9):841-850.

89. Guengerich FP. Cytochrome P450s and other enzymes in drug metabolism and toxicity. AAPS J 2006; 8(1):E101-E111.

90. Lacro JP, Dunn LB, Dolder CR et al. Prevalence of and risk factors for medication nonadherence in patients with schizophrenia: a comprehensive review of recent literature. J Clin Psychiatry 2002; 63(10):892-909.

91. Dolder CR, Lacro JP, Dunn LB et al. Antipsychotic medication adherence: Is there a difference between typical and atypical agents? Am J Psychiatry 2002; 159(1):103-108.

Table 1: Clinical characteristics of the patients

Variables			
Prolactin [ng/mL]	(median; range)	34	(7-271)
High prolactin ‡	(n; %)	18	(43)
BMI [kg/m^2]			
Initial BMI *	(median; range)	24.8	(16.9-36.3)
Current BMI	(median; range)	27.1	(18.0-39.9)
Cardiovascular risks ¥			
Initial prevalence of obesity *	(n; %)	18	(23)
Current prevalence of obesity	(n; %)	22	(26)
Smoker	(n; %)	68	(81)
Total cholesterol [mmol/L]	(median; range)	4.8	(1.2-9.6)
High total cholesterol	(n; %)	12	(14)
HDL cholesterol [mmol/L]	(median; range)	1.2	(0.6-2.4)
Low HDL cholesterol	(n; %)	28	(33)
RLAI duration [months]	(median; range)	8.5	(2-78)
RLAI dose [mg/2weeks]	(median; range)	37.5	(25-75)

RLAI: risperidone long-acting injection

‡ High prolactin is considered as prolactin > 40 ng/mL for women and > 50 ng/mL for men

¥BMI is considered as normal <25, overweight 25-30 and obese >30, total cholesterol ≥6.2 mmol/L, and HDL cholesterol <1mmol/l for men and <1.3mmol/l for women are considered as cardiovascular risk factors.

* Variables before RLAI treatment initiation

Table 2: Genotype frequencies in the whole cohort (n=42).

	n	Frequency (%)	95% CI (%)		n	Frequency (%)	95% CI (%)		n	Frequency (%)	95% CI (%)
ABCB1 3435CT				***CYP3A4 *1B***				***NR1I2 rs1523130***			
CC	15	36	23-51	**1/*1*	35	83	69-92	*TT*	10	24	13-39
CT	19	45	31-60	**1/*1B*	5	12	5-25	*TC*	20	48	33-62
TT	8	19	10-34	**1B/*1B*	2	5	1-17	*CC*	12	29	17-44
ABCB1 1236CT				***CYP3A5 *3***				***NR1I2 rs7643645***			
CC	15	36	23-51	**1/*1*	5	12	5-25	*AA*	22	52	38-67
CT	22	52	38-67	**1/*3*	7	17	8-31	*AG*	16	38	25-53
TT	5	12	5-25	**3/*3*	30	71	56-83	*GG*	4	10	3-23
ABCB1 2677GT				***CYP3A7 *1C***				***CYP2D6****			
GG	19	45	31-60	**1/*1*	38	90	77-97	*PM*	1	2	0-13
GT	17	40	27-55	**1/*1C*	4	10	3-23	*IM*	15	36	23-51
TT	6	14	6-28	**1C/*1C*	0	0	-	*EM*	21	50	35-64
CYP3A4 rs4646437				***NR1I2 rs2472677***				*UM*	5	12	5-25
CC	26	62	47-75	*CC*	9	21	11-36				
CT	12	29	17-44	*CT*	25	60	44-73				
TT	4	10	3-23	*TT*	8	19	10-34				

*CYP2D6: PM**,** poor metabolizer: *4/*4, n=1; IM**,** intermediate metabolizer: *1/*3 n=1, *1/*4 n=11, *1/*5 n=2, *4/*XN n=1; EM, extensive metabolizer: *1/*1 n=21; UM, ultrarapid metabolizer: *1/*XN or *XN/*XN, n=5.

Table 3: Risperidone, OH-risperidone and active moiety (RIS + OHRIS) plasma levels at day of injection (day 0) and at day 7 according to the fortnightly dose of RLAI (median and interquartile range (IQR) are shown).

Dose	n	%	Plasma levels (ng/mL)					
			Risperidone		OH-risperidone		Active moiety	
Levels at day 0			median	IQR	median	IQR	median	IQR
25 mg	12	(29)	3	(0.8-13)	9	(1-38)	12	(4-47)
37.5 mg	9	(22)	4	(3-14)	16	(6-22)	21	(9-26)
50 mg	16	(39)	5	(2-59)	14	(7-53)	23	(9-81)
75 mg	4	(10)	6	(3-13)	18	(9-36)	27	(12-44)
Levels at day 7								
25 mg	13	(31)	3	(1-15)	9	(2-21)	12	(7-26)
37.5 mg	9	(21)	6	(2-9)	18	(8-27)	24	(10-30)
50 mg	16	(38)	5	(2-36)	16	(7-34)	23	(9-52)
75 mg	4	(10)	5	(3-20)	23	(20-26)	29	(26-40)

Table 4: Risperidone, OH-Risperidone and active moiety (RIS + OHRIS) plasma levels corrected by the dose at day of injection (day 0) and at day 7 according to the *NR1I2 rs7643645 genotypes* (median and interquartile range (IQR) are shown).

NR1I2 rs7643645		**Day 0 (ng/mL·mg)**		**Day 7 (ng/mL·mg) ***		**Ratio day 7/day 0**	
	n	plasma levels	IQR	plasma levels	IQR	ratio	IQR
Risperidone/dose							
AA	22	0.12	0.08-0.20	0.10	0.07-0.20	0.93	0.60-1.33
AG	15	0.12	0.10-0.36	0.13	0.08-0.54	0.80	0.40-2.25
GG	4	0.07	0.05-0.10	0.22	0.14-0.31	3.04	2.17-4.70
p		*0.140*		*0.342*		*0.009*	
OH-Risperidone/dose							
AA	22	0.34	0.21-0.45	0.33	0.27-0.48	1.09	0.72-1.50
AG	15	0.38	0.26-0.48	0.41	0.30-0.53	1.00	0.58-2.15
GG	4	0.13	0.08-0.23	0.59	0.30-0.75	3.48	2.31-4.52
p		*0.008*		*0.549*		*0.006*	
Active moiety/dose							
AA	22	0.51	0.32-0.64	0.47	0.37-0.64	1.09	0.69-1.43
AG	15	0.56	0.36-0.96	0.52	0.40-0.79	0.89	0.48-2.33
GG	4	0.18	0.16-0.30	0.82	0.50-1.00	3.25	2.37-4.50
p		*0.020*		*0.275*		*0.005*	

* one additional patient at day 7 (AG genotype) excluded from day 0 and day 7/day 0 ratio due to a suspicion of oral risperidone intake. Similar results for day 7 were obtained with or without this patient.

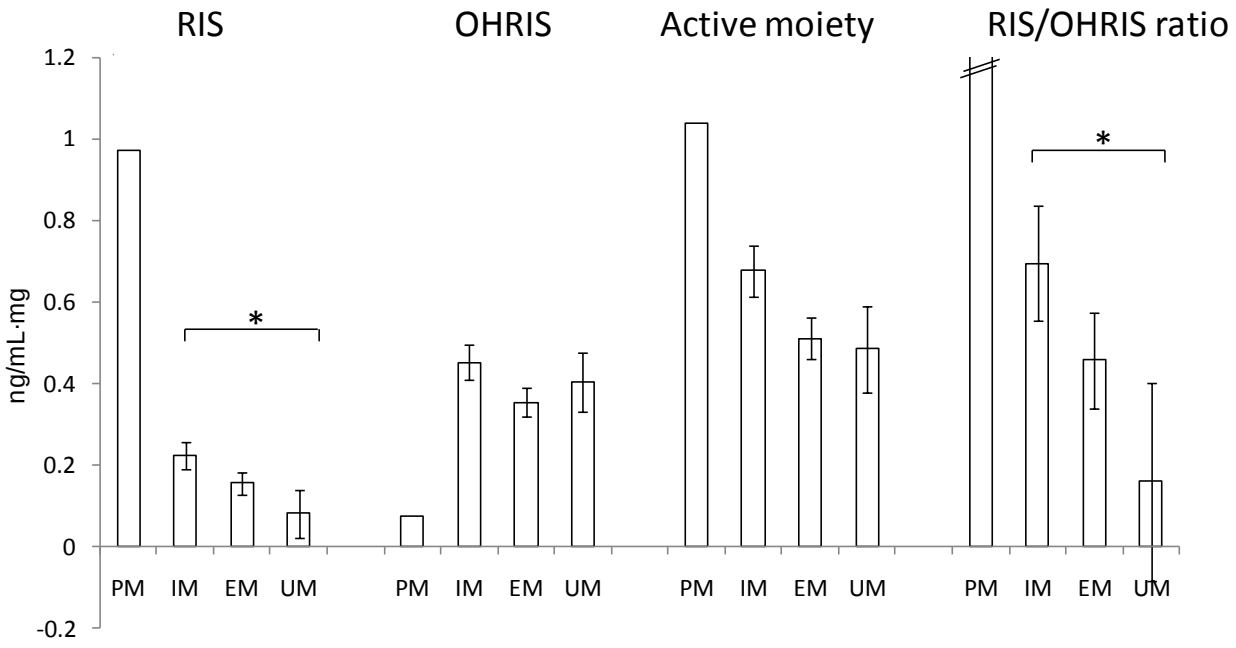

Fig 1: Plasma concentration corrected by the dose (ng/mL·mg) of risperidone (RIS), OH-risperidone (OHRIS), the active moiety (RIS + OHRIS) and RIS/OHRIS ratio adjusted by age and sex for CYP2D6 genotype. Data were obtained according to the mixed-model and showed as mean ± standard error.

*** p<0.01; PM: poor metabolizer; IM: intermediate metabolizer; EM: extensive metabolizer; UM: ultrarapid metabolizer**

2.2 Manuscrit VI: Influence of *CRTC1* polymorphisms on body mass index in patients with psychotropic treatments. Manuscrit en préparation.

Le CRTC1 a récemment été montré comme faisant partie du cycle de la leptine et jouant un rôle dans la régulation de la satiété et de l'homéostasie de l'énergie dans des modèles animaux. Cette étude décrit l'influence de trois SNPs du *CRTC1,* sélectionnés de façon exploratoire, sur l'IMC et la prise de poids dans deux populations différentes provenant de l'Etude prise de poids Genève (N=152) et de l'Etude Suivi métabolique Lausanne (N=174).

Le SNP *rs3746266A>G* du gène *CRTC1,* amenant à un changement d'acide aminé, a été associé à une différence de l'IMC. Les porteurs de l'allèle G ont un IMC significativement plus bas que les individus AA, le génotype GG étant donc associé à un effet protecteur.

Cet effet est plus marqué chez les femmes pré-ménopausées que chez les femmes post-ménopausées et n'est pas visible chez les hommes. Une différence allant jusqu'à 2.9 kg/m^2 entre les deux génotypes a ainsi été observée chez les femmes pré-ménopausées. Ce résultat positif trouvé dans la première cohorte a été répliqué dans la deuxième. Les deux autres SNPs sélectionnés (*rs10402536* et *rs8104411*) n'ont pas montré d'associations significatives avec l'IMC. Cette étude est, à notre connaissance, la première à démontrer l'influence d'un polymorphisme du *CRTC1* sur l'IMC des patients sous psychotropes.

Influence of *CRTC1* polymorphisms on body mass index in patients with psychotropic treatments.

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Manuscrit en préparation

Abstract

Weight gain is one of the most frequent side-effect associated with the use of atypical antipsychotics and/or mood stabilisers, with an important interindividual variability. The *CREB-regulated transcription coactivator* (*CRTC1*) gene was recently reported to be involved in energy balance and satiety in animal models. The present study investigates the role of *CRTC1* polymorphisms (*rs10402536*, *rs8104411* and the non-synonymous *rs3746266*) on body mass index (BMI) in patients treated with psychotropic drugs. Two independent Caucasian psychiatric populations were included (n_1=152 and n_2=174 patients) and mixed-model analysis controlling for age, gender, type of drug, dose, and comedication causing weight-gain was performed. In the first cohort, lower BMIs were measured in carriers of the *rs3746266G* allele than in *AA* genotypes (p_1=0.008), a finding confirmed in the second group (p_2=0.048) and in the combined cohort (p<0.001), with an increased BMI of 1.6, 1.0 and 1.3 kg/m^2, respectively. When combining both cohorts, lower BMI (1.5 kg/m^2, p=0.020) were measured in women carriers of the *rs3746266G* allele (n=40) than in *AA* genotypes (n=78), but not in men (p=0.17). The strongest effect was observed in the premenopausal women group with 2.9 kg/m^2 higher BMI in *AA* genotype (n=38) compared to *G* carriers (n=23, p=0.005), while no differences between genotypes were measured in the postmenopausal women group (p=0.30). In conclusion, *CRTC1 rs3746266* polymorphism was associated with differences in BMIs in patients receiving psychotropic drugs, an effect which seems to be restricted to premenopausal women.

1. Introduction

Several studies have demonstrated an increased mortality in psychiatric populations (1-3), due in part to the high cardiovascular risk factors linked to the illness and to several comorbidities including obesity, metabolic syndrome, lifestyle factors (smoking status) and also to the pharmacological treatment (Choong et al, submitted)(4-8). The strong effect on weight of atypical antipsychotics (AP) and of other drugs such as the mood stabilizers (MS) lithium and valproate (9-11) has been of growing concern for the health care management of patients. Thus, weight gain and obesity in the long term can lead to several metabolic complications such as dyslipidemia, insulin resistance, type 2 diabetes and cardiovascular diseases, which can ultimately reduce life expectancy by several years (12-15).This led to the establishments of guidelines to monitor the potential metabolic complications during AP treatment (16;17).

The cyclic AMP response element-binding protein (CREB)-regulated transcription coactivators (CRTCs, also called TORCs) comprise three members, namely *CRTC1, CRTC2*, and *CRTC3* that are expressed in different tissues (18). They are localised in the cytoplasm in a phosphorylated form (19). Following the activation by calcium or by cyclic AMP, CRTCs are dephosphorylated, shuttle to the nucleus, bind to CREB, and thereby enhance the transcription of CREB-dependent transcription genes (19-21). *CRTC1* gene is of particular interest since it was recently associated with energy balance in mice models (22). *CRTC1* knock-out mice were observed eating more and expending less energy, thus developing an obese feature under a normal diet (21;22). In the long term, the mutant mice presented a hypertriglyceridemia, a hyperglycemia and 2 to 3 times more white adipose tissue than their littermates (22). Because no difference was observed in other tissues, the authors concluded on a specific impact of CRTC1 on body weight, which might be specific to white adipose tissue.

Although the role of *CRTC1* in obesity has not yet been elucidated, it was suggested that it might alter the expression of anorexigen neuropeptides and be involved in satiety (22). The *CRTC1* is mainly expressed in the brain (22-24) and is a putative target for leptin in the hypothalamus, where it may transduce its anorexic effect by promoting the expression of neuropeptides mediating satiety (i.e. the cocaine- and amphetamine-regulated transcript CART and the brain derived neurotrophic factor BDNF (22;25). Interestingly, CRTC1 has been associated with the regulation of *CARTPT* (CART prepropeptide) gene expression *in vitro* and CART was reported to inhibit food intake in response to leptin in arcuate neurons (22). In addition, a role in reproduction was also reported for CRTC1 even if this point is more controversial (21;22). The *CRTC1* knockout mice were reported to be infertile and presented low luteinizing hormone (LH) levels (22) although this was not confirmed in another study

(21). The mechanism underlying infertility was supposed to be linked to the absence of CRTC1 stimulation on kisspeptin-1 (KISS1) expression (22).

The risk of obesity has a heritable component suggesting that it might be caused by genetic variations. In addition, significant inter-individual variability in weight gain was reported during psychotropic treatment, which might be due, among others, to genetic factors. Thus, a certain number of genes that showed consistent associations with antipsychotic-induced weight gain are already known (26-28). Given the strong effect of *CRTC1* in mice, this gene might also play a role in obesity in humans. The present work analyses the association of human *CRTC1* polymorphisms with BMI, weight gain during psychotropic treatment in two independent psychiatric populations treated with APs and/or with MS.

2 Materiel and Methods

2.1 Patients and study design

Subjects were selected from two independent studies (described below) which were approved by their respective ethics committee (Ethics Committee of Geneva and Lausanne University hospitals, respectively). Written informed consent was given by all subjects or their legal representative. All subjects were of Caucasian origin and were treated with a psychotropic drugs that could cause weight gain (AP and/or lithium and/or valproate) and weights for at least two different time points during their current psychotropic treatment were available. Patients were included in the present study only if their drug plasma levels were above an arbitrary threshold at 10% of the minimal therapeutic drug plasma concentration (29) (i.e. 15, 35, 2, 7, 2 5, 0.05 mmol/L and 5 mg/L for aripiprazole, clozapine, olanzapine, quetiapine, risperidone + 9-hydroxy-risperidone, sertindole, lithium, and valproate, respectively). This threshold was chosen to indicate a suspicion of compliance issue and/or a rapid metabolism and/or pharmacokinetics drug interaction and/or low dose prescription (e.g. prescription of 50 mg/day of quetiapine for sleep disorders). The demographic data and treatment histories were obtained from the medical files. Psychiatric diagnoses were established by physicians according to ICD-10. BMI was defined as weight/height^2, measured in kg/m^2 and values between 25-30 kg/m^2 and equal or higher than 30 kg/m^2 were used to define overweight and obese patients, respectively. Comedications described as inducing frequent (>1%) weight gain or weight loss were identified from relevant data bases (30;31). Patients characteristic are reported in Table 1.

Cohort 1

In a retrospective cross-sectional study, patients from two out-patient psychiatric centres were included from Geneva University hospital from 2006 to 2008. Patients treated for more than 3

months with clozapine, olanzapine, quetiapine, risperidone, lithium, and/or valproate were included (Choong et al., submitted). Patients' weight at the initiation of the current treatment or at different time during treatment were collected from the medical file or were self-reported (baseline weight was self-reported in 76% of the cases). The reliability of self-reported weights was ascertained among patients with weights also available from the medical files and an excellent concordance was found (n=29, r_s>0.9) (Choong et al., submitted). The median treatment duration was 30 months (range: 3-333) and the median current BMI (i.e. at time of inclusion) was 28.1 kg/m^2 (range: 17.1-42.3). The study comprised one single visit performed during the routine psychiatric follow-up. When two or more studied drugs were concomitantly prescribed, the medication with the longest treatment duration was considered in the model, while the others were classified as comedication possibly causing weight gain.

Cohort 2

In an ongoing follow-up study conducted since 2007 in all psychiatric wards of Lausanne University hospital (from paediatrics to geriatrics), patients with a new prescription of aripiprazole, amisulpride, clozapine, olanzapine, quetiapine, risperidone, sertindole, and/or lithium, valproate were recruited. Most of them had already received other psychotropic treatments and were included in the study after switching the medication (Choong et al., manuscript in preparation). No wash-out period was required. Weights and clinical variables were recorded at several time points during the first 12 months according to the currently recommended monitoring protocols (i.e. baseline before starting the current medication then, months 1, 2, 3, 6, 9, and 12) (17;32). The median treatment duration was 6 months (range: 1-12) with a median BMI of 24.2 kg/m^2 (range: 14.7-41.2). The studied drug, which was newly introduced at the time of inclusion, was considered in the model. In case of concomitant prescription of other studied drugs, the latter were classified as comedication potentially linked to weight gain.

2.2 Clinical measurements

Blood samples were stored at –20 °C until analysis. Quantification of psychotropic drugs in plasma was performed using validated analytical methods (Choong et al., in preparation) (33). Lithium plasma levels were determined using a specific electrode system (EasyLyte, Medica, Châtel-St-Denis, Switzerland) and valproate levels using a Cobas Integra 400 instrument (Roche Instrument Diagnostic, Basel, Switzerland).

2.3 Genotyping

Genomic DNA was extracted from the ethylenediaminetetra-acetic acid (EDTA) blood samples using the FlexiGene DNA Kit (Qiagen, Hombrechtikon, Switzerland). *CRTC1* polymorphisms were genotyped by real-time polymerase chain reaction (RT-PCR) with the 5'-nuclease allele discrimination assays (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland). A Custom Taqman SNP Assay including the primer sequences TGGCTCCTCTCCACAGCA (forward), CTGAGTGATGGGTGACAGC (reverse), and labelled probes FAM-ATCGCCCGCCCTGT and VIC-CATCGCCCACCCTGT was used to analyse *rs3746266A>G* polymorphism. Taqman SNP Genotyping Assay ID: C____26737143_10 and ID: C__32455030_10 were used for *rs8104411C>T* and *rs10402536G>A* SNPs, respectively. All reagents were obtained from Applied Biosystems (Rotkreuz, Switzerland), and genotyping was performed according to the manufacturer's protocol. Prior to the analyses of patients' samples, the genotyping assay was validated by sequencing control samples, using an ABI BigDye terminator version 1.1 kit on an ABI PRISM 3100 automated sequencer, as previously described (34). These control samples were added to each 96-reaction plates to ensure the quality of the results.

2.4 Statistical analysis

The association of *CRTC1* SNPs with the BMI was firstly assessed at the beginning of the treatment in Cohort 1, 2 and in the combined cohort using Mann-Whitney tests. Then, the influence of *CRTC1* SNPs on the BMI and weight gain during the current treatment was evaluated in Cohort 1. The influence of the three *CRTC1* SNPs on BMIs was assessed by fitting a Generalized Additive Mixed Model (GAMM) (43;44). Age, gender, studied drugs, psychiatric diagnosis, and standardised dose were controlled in the models for patient with no comedication that might cause weight gain. The same Generalized Additive Mixed Model was used for weight adding height to the previously considered covariables. Coefficients obtained from the mixed-model were used as it provides information on both the direction of the association between BMI and the genotype and on the magnitude of this change (the coefficient does not belong to a specific period of the treatment but represents an overall trend). The psychotropic drugs were classified into 2 categories, according to their therapeutic class (i.e. AP vs. MS) (30). A 45-year-old threshold was applied to categorise pre- and post menopausal women.

The positive result obtained with *rs3746266* was repeated in Cohort 2 for replication. Statistical analyses were also performed with data of the combined cohorts. For this purpose, because the 2 cohorts differ by the median length in treatment, only patients having been treated with the current psychotropic treatment for less than 24 months were taken into account to preserve homogeneity in

this group. The weight gain during treatment with psychotropic medication and in relation to *CRTC1* genotype was analysed in univariate analysis using Mann-Whitney tests and in linear regressions controlling for the covariables considered in the mixed-model for weight.
All SNPs were tested for Hardy-Weinberg equilibrium (HW) and linkage disequilibrium, as defined by the values of D' and r^2.

All tests were two-sided and a P-value less than or equal to 0.05 was considered statistically significant. All the analyses were performed using Stata 11 (StataCorp, College Station TX, USA), R version 2.11.1 software (45), and GraphPad Prism v5.04 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

3 Results

The present study is based on a total number of 326 Caucasian patients (152 in Cohort 1, 174 patients in Cohort 2), with 30% and 44% being overweighed and 40% and 18% being obese in Cohort 1 and 2, respectively. Thus, the prevalence of obesity in both cohorts was about 1.5 to 3-fold higher than in the general Swiss population of similar age (46-49), which is in agreement with other European studies on schizophrenic (50) or bipolar patients (8;51;52). The lower BMIs and lower frequency of obese patients measured in patients in Cohort 2 as compared to Cohort 1 may most probably be explained by the lower age and the lower treatment duration in the this group (see Table 1).

3.1 *CRTC1* polymorphisms

To our knowledge, no functional consequences of *CRTC1* genetic polymorphism have been reported in humans until now. For the present study, three *CRTC1* SNPs were selected. Thus, because CRTCs N and C terminal domains were reported to be important for the binding to CREB and for enhancing CREB activity via an association with another CREB coactivator ($TAF_{II}130$) (18), SNPs in the intron 1 (*rs10402536*) and in the 3'UTR region (*rs8104411*) with allelic frequencies in Caucasian population superior to 0.3 were selected. The third selected SNP was exonic (*rs3746266*) leads to an amino acid change from threonine to alanine.

The observed minor allele frequencies for *rs3746266* were similar between the two cohorts (20 % (95% confidence interval (CI): 16-25) and 18 % (95%CI: 13-21) in Cohort 1 and Cohort 2, respectively). To our knowledge, no frequency in a Caucasian population has been available so far. A frequency of 10 % (95%CI: 6-18) for this allele has been reported in NCBI (53), although the data were from a multi-ethnic population (n=46). The two other SNPs were analysed only in Cohort 1 and showed a minor allele frequency of 27 % (95%CI: 22-32) for *rs8104411* and 23 % (95%CI: 19-28) for

rs10402536. The frequencies were lower than those reported in NCBI (40 % and 34 %, respectively) but only one Caucasian population was available for each SNP (53). Moreover, the frequencies were similar to those found in a group of internal healthy controls (n=20), with a MAF of 32 % (95%CI: 21-46) for *rs8104411* and 27 % (95%CI: 16-41) for *rs10402536*. Allelic frequencies are reported in Table 2. The SNPs were in Hardy-Weinberg equilibrium (p>0.1). Comparison of the distribution of the different genotypes indicated high linkage disequilibrium between r*s3746266* and *rs8104411* and between *rs3746266* and *rs10402536*. The SNPs *rs10402536* and *rs8104411* were not in linkage disequilibrium (Table 2).

3.2 Association of *CRTC1* polymorphisms with BMI

3.2.1 Univariate analyses

The association of *CRTC1 rs3746266* polymorphism with the BMI was firstly assessed at the beginning of the treatment in Cohort 1, 2 and in the combined cohort. Because of the low number of mutated homozygous (n=6, n=10 for Cohort 1 and 2, respectively), this association was analyzed using a dominant model. The median BMI in Cohort 1 at the beginning of treatment was significantly lower for *G* carriers as compared to *AA* genotypes (p=0.012) with a BMI of 24.4 kg/m^2 (range: 17.1-39.8) and 26.2 kg/m^2 (range: 15.6-45.5), respectively. Lower median BMI was also observed in Cohort 2 at the beginning of treatment (22.3 kg/m^2 (range: 15.8-33.0) for *G* carriers as compared to 22.6 kg/m^2 (range: 14.7-41.9) for *AA* genotype), although the results were not significantly different (p=0.13). The BMI at the beginning of treatment for the combined cohort was 22.7 kg/m^2 (range: 15.8-33.1) for *G* carriers and 24.1 kg/m^2 (range: 14.7-45.5) for *AA* individuals (p=0.01). Figure 1 shows the BMIs at different time periods for *CRTC1 rs3746266* genotype in Cohorts 1 and 2 and in the combined group. No influence was observed for the *CRTC1 rs8104411* and *rs10402536* in the univariate analyses (data not shown).

3.2.2 Mixed model approach

Associations between *CRTC1 rs3746266* genotypes and BMI or body weight with a dominant model are reported in Table 3. The *rs3746266* polymorphism was significantly associated with BMI when controlling for cofactors (age, gender, type of studied drugs, psychiatric diagnosis, and standardised dose) for patients receiving no comedication that might cause weight gain. The *G* allele was found to be associated with a smaller BMI, with the *G* carriers having a 1.56 kg/m^2 lower BMI than the *AA* genotype (p=0.008). Similar results were found in Cohort 2 (p=0.048) and in the combined population (p<0.001), with carriers of the *G* allele having 1.0 and 1.3 kg/m^2 lower BMIs as compared to the *AA* genotype, respectively. In the combined cohort, the predicted BMI at the initiation of the current treatment was 24.4 kg/m^2 (95%CI: 23.4-25.4) and 23.1 (95%CI: 21.9-24.1) for the wild type genotype

(*AA*) as compared to the carriers of the *G* allele. The predicted BMI after 12 months of treatment with the current medication was 26.6 kg/m^2 (95%CI: 25.6-27.6) and 25.3 (95%CI: 24.0-26.3), respectively. In the combined cohort, the model integrating age, gender, type of studied drugs, psychiatric diagnosis, standardised dose and *CRTC1 rs3746266* explained 12% of the BMI variations. The variable significantly associated with a higher BMI in this model was a higher age (p<0.0001), while a tendency was observed for a prescription of atypical antipsychotics as compared to mood stabilisers (p=0.054) and for male gender (p=0.086). The predicted BMIs and weights, at baseline and after 12 months with the current treatment are shown in Table 4.

A stratified gender analysis was performed in the combined cohort. A different impact of *CRTC1 rs3746266* between men and women was observed (Table 3). The BMI remained significantly associated with the genotype when the comedications possibly inducing weight gain were taken into account in the mixed model analyses for the combined cohort (p=0.003, n=311) and for all women (p=0.017, n=152). Additional analyses taking into account the menopausal status of women were performed. As the menopausal status of the patients were not known, an arbitrary value of 45 years was used to classify pre-and post-menopausal women in order to obtain a similar number of patients in both groups. In premenopausal women (below 45 years old) the strongest protective effect of the *G* allele, with a higher coefficient BMI of 2.9 kg/m^2 in *AA* genotypes (n=38) compared to *G* carriers (n=23, p=0.004) was observed. On the other hand, only a trend was shown for the influence of *rs3746266* in women older than 45 years old (n=57, p=0.212) and no significant association was found in men (n=116, p=0.168). Similar results were obtained when using a value of 50 and 55 years to classify pre- and post- menopausal women. Indeed, the BMI coefficient was 2.04 (n=85, p=0.007) and 1.87 (n=95, p=0.011) for women with the *G* allele younger than 50 and 55 years old, respectively, as compared to those with the wild type genotype. Because neither *rs8104411* nor *rs10402536* showed a significant association with BMI in Cohort 1, these SNPs were not tested for replication in Cohort 2.

3.3 Association of *CRTC1* polymorphisms with weight gain during the current treatment

Among patients, 51 % and 30 % in Cohort 1 and 2 were found to have a weight gain during the current treatment corresponding to 10 % or more of their baseline weight. The weight gain (kg) over time was not found to be associated with the *CRTC1* genotypes in univariate and multivariate (adjusted for the covariables of the mixed-model) analyses in Cohort 1 and Cohort 2 and in the combined cohort for all studied SNPs (data not shown). These results are in agreement with the above mentioned results showing an effect of the CRTC1 genotype on the BMIs already at the initiation of the current treatment. Similar results were obtained considering the BMI change (data not shown).

4 Discussion

The present study is the first to suggest a role for *CRTC1* gene in the regulation of human body weight, which is consistent with data from animal models (21;22). In a cohort of 152 psychiatric patients the *CRTC1* non-synonymous polymorphism *rs3746266A>G* was associated with BMIs, with lower values measured in carriers of the *G* allele when compared to the *AA* genotypes. This result was replicated in a second independent cohort of 174 patients and in the combined population. In the stratified analysis with the combined population, a similar protective effect for the *G* allele was observed in women, while no effect was observed in men. Interestingly, the strongest and most clinically relevant effect was observed in supposedly premenopausal women (i.e. younger than 45 years) for whom a difference of BMI of 2.9 kg/m^2 was found between *G* carriers and *AA* genotype. The strong association found in women but not in men could have been caused by an enhancement of CRTC1 activity by leptin stimulation as this peptide might stimulate the satiety pathway differently in women as compared to men. Gender differences were reported for leptin levels (54;55), and female gender was shown as a clinical predictive factor for stronger weight gain during treatment with APs (56). Interestingly, other studies examining the influence of polymorphisms in the gene of leptin or of the leptin receptor on weight induced by APs also showed gender-specific differences (57;58).

The difference found between pre-and post-menopausal women, with the protective effect of the *G* allele being attenuated after menopause, suggests a complex mechanism closely linked to the endocrine system. A hypothetical mechanism is presented in Figure 2. Thus dopaminergic D_2 antagonists, such as the APs, can increase prolactin release. An elevated prolactin level is associated with decreased levels of the follicle-stimulating hormone (FSH) and of the luteinizing hormone (LH) (59) which stimulates the secretion of estrogens and progesterone (60). The estrogens were reported in animal model or in *in vitro* studies to have an intrinsic anorexigen effect (61;62), and to stimulate the secretion of leptin (54;55;63). In addition, it has been shown that estrogens levels correlated with the levels of the anorexigen BDNF (64). Interestingly, in humans, a recent meta-analysis of genome wide association studies reported an association of an intronic SNP of *CRTC1* (*rs10423674*) with the age of menarche (65). Thus, before menopause, a potential proper intrinsic anorexigen effect of estrogens (61;62) in conjunction with the increased CRTC1 activity associated with the G allele of *rs374266* might protect against the elevation of body weight. After menopause, the *CRTC1 rs3746266G* protection might be attenuated, as the endocrine hormones are not secreted anymore (60).

A high prevalence of overweight and obese patients was found in the present study, which is in agreement with other studies with psychiatric patients (4-8;67;68). Also, in agreement with the association reported between weight gain and treatment duration (69), the median BMI in Cohort 1

with the longest treatment duration was higher than the median value in Cohort 2 (Table 1). Because most of the patients had a long history of psychiatry illness, were not drug naïve and had previously received and experienced weight gain due to multiple treatment before the current one (Table 1), a high prevalence of overweight and obese subjects was also found at the initiation of the current treatment (Table 1). This might explain, at least in part, why no influence of the studied *CRTC1* polymorphisms was observed on weight gain during the current drug treatment. Thus the BMI at the beginning of treatment was already different between *CRTC1* genotypes, with the *G* carriers showing lower BMIs, which could be explained by the effects of previous drug treatments. However, in the present study including only psychiatric patients receiving weight gain inducing drugs, it cannot be determined whether the effect of *CRTC1* polymorphisms on BMIs is due to the protective effect of the *G* allele during weight gain inducing drug treatments, or if this effect could also be observed in healthy subjects without medications, or both.

Several limitations of this study need to be acknowledged. The hormonal status was not available in both cohorts, therefore, the 45-year-old threshold was established as an attempt to discriminate between pre- and post- menopausal women. This threshold is, however, in accordance with the common occurrence of menopausal symptoms between 46 and 55 years of age (60). In addition, similar results were obtained with a threshold of 50 and 55 years. Besides the variables examined in the present study which could influence the weights, other environmental and social factors were not accounted for due to the naturalistic design of the study. However, the fact that the results were replicated in two independent cohorts strengthens the validity of our data. Finally, as above-mentioned, the present study performed in psychiatric patients does not allow to determine whether the influence of *CRTC1* gene on weight was demonstrated because of the strong effect of psychotropic drug treatment on this variable or whether an influence can also be shown in healthy subjects without such treatment. This is presently investigated with data obtained from a genome wide scan study with 6000 subjects from the COLAUS study (47).

5 Conclusion

Obesity is influenced by a complex system of neurotransmitters including neuropeptides, hormones and immune related factors (28) which can regulate hunger, satiety and energy homeostasis (70). This is the first study to show an association between *CRTC1* polymorphisms and BMIs in humans. Our results thus suggest an influence of *CRTC1 rs3746266A>G* polymorphism in BMI regulation with a protective effect on body weight for the *G* allele. The strong influence of *CRTC1* in premenopausal women opens a new area of research on the interconnection between body weight and hormones. The implication of *CRTC1* as candidate gene in obesity risk and weight gain mechanisms should be

further studied in the general population, but also in psychiatric patients, since it may improve our approach towards better treatment strategies in the latter group.

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Reference List

(1) Osby U, Correia N, Brandt L. Mortality and causes of death in schizophrenia on Stockholm County, Sweden. Schizophr Res 200;45:21-8.

(2) Heiskanen T, Niskanen L, Lyytikainen R, et al. Metabolic syndrome in patients with schizophrenia. J Clin Psychiatry 2003;63:575-9.

(3) van Winkel R, De Hert M, Wampers M, Van Eyck D, Hanssens L, Scheen A, et al. Major changes in glucose metabolism, including new-onset diabetes, within 3 months after initiation of or switch to atypical antipsychotic medication in patients with schizophrenia and schizoaffective disorder. J Clin Psychiatry 2008 Mar;69(3):472-9.

(4) De Hert M, Mittoux A, He Y, Peuskens J. Metabolic parameters in the short- and long-term treatment of schizophrenia with sertindole or risperidone. Eur Arch Psychiatry Clin Neurosci. In press 2010.

(5) van Winkel R, De Hert M, Van Eyck D, Hanssens L, Wampers M, Scheen A, et al. Prevalence of diabetes and the metabolic syndrome in a sample of patients with bipolar disorder. Bipolar Disord 2008 Mar;10(2):342-8.

(6) Garcia-Portilla MP, Saiz PA, Bascaran MT, Martinez AS, Benabarre A, Sierra P, et al. Cardiovascular risk in patients with bipolar disorder. J Affect Disord 2009 Jun;115(3):302-8.

(7) Birkenaes AB, Opjordsmoen S, Brunborg C, Engh JA, Jonsdottir H, Ringen PA, et al. The level of cardiovascular risk factors in bipolar disorder equals that of schizophrenia: a comparative study. J Clin Psychiatry 2007 Jun;68(6):917-23.

(8) Salvi V, Albert U, Chiarle A, Soreca I, Bogetto F, Maina G. Metabolic syndrome in Italian patients with bipolar disorder. Gen Hosp Psychiatry 2008 Jul;30(4):318-23.

(9) Vieweg WVR, Levy JR, Fredrickson SK, Chipkin SR, Beatty-Brooks M, Fernandez A, et al. Psychotropic drug considerations in depressed patients with metabolic disturbances. Am J Med 2008 Aug;121(8):647-55.

(10) McIntyre RS, Konarski JZ. Tolerability profiles of atypical antipsychotics in the treatment of bipolar disorder. J Clin Psychiatry 2005;66 (Suppl 3):28-36.

(11) Yumru M, Savas HA, Kurt E, Kaya MC, Selek S, Savas E, et al. Atypical antipsychotics related metabolic syndrome in bipolar patients. J Affect Disord 2007 Mar;98(3):247-52.

(12) Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 2002 Dec 4;288(21):2709-16.

(13) Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001 Apr;24(4):683-9.

(14) Stahl SM. Stahl's Essential Psychopharmacology - Neuroscientific Basis and Practical Applications. Thrid edition ed. New York: Cambridge University Press; 2008.

(15) National Institute of Health. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Final report. Circulation 2002;106:3143-421.

(16) De Hert M, Dekker JM, Wood D, Kahl KG, Holt RI, Moller HJ. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). Eur Psychiatry 2009 Sep;24(6):412-24.

(17) American Diabetes Association, American Psyhiatric Association, American Association of Clinical Endocrinologists, North American Association for the Study of Obesity. Consensus development conference on antipsychotic drugs and obesity and diabetes. Diabetes Care 2004;27(2):596-601.

(18) Conkright MD, Canettieri G, Screaton R, Guzman E, Miraglia L, Hogenesch JB, et al. TORCs: transducers of regulated CREB activity. Mol Cell 2003 Aug;12(2):413-23.

(19) Screaton RA, Conkright MD, Katoh Y, Best JL, Canettieri G, Jeffries S, et al. The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. Cell 2004 Oct 1;119(1):61-74.

(20) Bittinger MA, McWhinnie E, Meltzer J, Iourgenko V, Latario B, Liu X, et al. Activation of cAMP response element-mediated gene expression by regulated nuclear transport of TORC proteins. Curr Biol 2004 Dec 14;14(23):2156-61.

(21) Breuillaud L, Halfon O, Magistretti PJ, Pralong FP, Cardinaux JR. Mouse fertility is not dependent on the CREB coactivator Crtc1. Nat Med 2009 Sep;15(9):989-90.

(22) Altarejos JY, Goebel N, Conkright MD, Inoue H, Xie J, Arias CM, et al. The Creb1 coactivator Crtc1 is required for energy balance and fertility. Nat Med 2008 Oct;14(10):1112-7.

(23) Wu Z, Huang X, Feng Y, Handschin C, Feng Y, Gullicksen PS, et al. Transducer of regulated CREB-binding proteins (TORCs) induce PGC-1alpha transcription and mitochondrial biogenesis in muscle cells. Proc Natl Acad Sci U S A 2006 Sep 26;103(39):14379-84.

(24) Kovacs KA, Steullet P, Steinmann M, Do KQ, Magistretti PJ, Halfon O, et al. TORC1 is a calcium- and cAMP-sensitive coincidence detector involved in hippocampal long-term synaptic plasticity. Proc Natl Acad Sci U S A 2007 Mar 13;104(11):4700-5.

(25) Finsterwald C, Fiumelli H, Cardinaux JR, Martin JL. Regulation of dendritic development by BDNF requires activation of CRTC1 by glutamate. J Biol Chem 2010 Sep 10;285(37):28587-95.

(26) Chagnon YC. Susceptibility genes for the side effect of antipsychotics on body weight and obesity. Current Drug Targets 2006;7:1681-95.

(27) Correll CU, Malhotra AK. Pharmacogenetics of antipsychotic-induced weight gain. Psychopharmacology 2004 Aug;174(4):477-89.

(28) Muller DJ, Muglia P, Fortune T, Kennedy JL. Pharmacogenetics of antipsychotic-induced weight gain. Pharmacol Res 2004 Apr;49(4):309-29.

(29) Baumann P, Hiemke C, Ulrich S, Gaertner I, Rao ML, Eckermann G, et al. Therapeutic monitoring of psychotropic drugs - An outline of the AGNP-TDM expert group consensus guideline. Ther Drug Monit 2004;26(2):167-70.

(30) Compendium Suisse de Médicaments®. 31e ed. Bâle (Suisse): Documed S.A.; 2010. http://www.kompendium.ch/Search.aspx?lang=fr. May 8, 2011.

(31) Thomson Reuters.Copyright © 1974-2011,. MICROMEDEX® 1.0 (Healthcare series). 2011. http://www.micromedex.com/. May 8, 2011.

(32) Choong E, Solida A, Lechaire C, Conus P, Eap CB. Suivi du syndrome métabolique induit par les antipsychotiques atypiques: recomendations et perspectives pharmacogénétiques. Rev Med Suisse 2008;4(171):1994-9.

(33) Choong E, Rudaz S, Kottelat A, Guillarme D, Veuthey JL, Eap CB. Therapeutic drug monitoring of seven psychotropic drugs and four metabolites in human plasma by HPLC-MS. J Pharm Biomed Anal 2009;50:1000-8.

(34) Oneda B, Crettol S, Bochud M, Besson J, Croquette-Krokar M, Hämmig R, et al. b-arrestin2 influences the response to methadone in opioid dependent patients. Pharmacogenomics Journal. In press 2010.

(35) Dobrinas M, Cornuz J, Oneda B, Kohler Serra M, Puhl M, Eap CB. Impact of smoking, smoking cessation, and genetic polymorphisms on CYP1A2 activity and inducibility. Clin Pharmacol Ther. In press 2011.

(36) Liu JS, Park EA, Gurney AL, Roesler WJ, Hanson RW. Cyclic AMP induction of phosphoenolpyruvate carboxykinase (GTP) gene transcription is mediated by multiple promoter elements. J Biol Chem 1991 Oct 5;266(28):19095-102.

(37) Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, et al. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 2001 Sep 13;413(6852):179-83.

(38) Iourgenko V, Zhang W, Mickanin C, Daly I, Jiang C, Hexham JM, et al. Identification of a family of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. Proc Natl Acad Sci U S A 2003 Oct 14;100(21):12147-52.

(39) Liu Y, Coello AG, Grinevich V, Aguilera G. Involvement of transducer of regulated cAMP response element-binding protein activity on corticotropin releasing hormone transcription. Endocrinology 2010 Mar;151(3):1109-18.

(40) Pasini E, Flati V, Paiardi S, Rizzoni D, Porteri E, Aquilani R, et al. Intracellular molecular effects of insulin resistance in patients with metabolic syndrome. Cardiovasc Diabetol 2010;9:46.

(41) Houseknecht KL, Portocarrero CP. Leptin and its receptors: regulators of whole-body energy homeostasis. Domest Anim Endocrinol 1998 Nov;15(6):457-75.

(42) Wan YJ, Han G, Cai Y, Dai T, Konishi T, Leng AS. Hepatocyte retinoid X receptor-alpha-deficient mice have reduced food intake, increased body weight, and improved glucose tolerance. Endocrinology 2003 Feb;144(2):605-11.

(43) Lin X, Zhang D. Inference in generalized additive mixed models by using smoothing splines. J R Statist Soc B 1999;61(2):381-400.

(44) Wood SN. GAMMs with R. In: Carlin BP, Chatfield C, Tanner M, Zidek J, editors. Generalized Additive Models - An Introduction with R.Broken: Chapman & Hall/CRC; 2006. p. 319-24.

(45) R Development Core Team,. R: A language and environment for statistical computing, R Fondation for Statistical Computing,Vienna,Austria. 2010. http://www.R-project.org. May 8, 2011.

(46) Chiolero A, Prior J, Bovet P, Masson JC, Darioli R. Expectation to improve cardiovascular risk factors control in participants to a health promotion program. J Gen Intern Med 2008 May;23(5):615-8.

(47) Firmann M, Mayor V, Vidal PM, Bochud M, Pecoud A, Hayoz D, et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord 2008;8:6.

(48) Fondation Suisse de Cardiologie,. Chiffres et données sur les maladies cardio-vasculaires en Suisse. 2002. www.swissheart.ch. May. 5, 2011.

(49) Office féderal de la statistique NS. Statistique de la santé. 2009. http://www.bfs.admin.ch/bfs/portal/fr/index.html. May. 5, 2011.

(50) Bobes J, Arango C, Aranda P, Carmena R, Garcia-Garcia M, Rejas J. Cardiovascular and metabolic risk in outpatients with schizophrenia treated with antipsychotics: results of the CLAMORS Study. Schizophr Res 2007 Feb;90(1-3):162-73.

(51) McElroy SL, Frye MA, Suppes T, Dhavale D, Keck PE, Jr., Leverich GS, et al. Correlates of overweight and obesity in 644 patients with bipolar disorder. J Clin Psychiatry 2002 Mar;63(3):207-13.

(52) Sicras A, Rejas J, Navarro R, Serrat J, Blanca M. Metabolic syndrome in bipolar disorder: a cross-sectional assessment of a Health Management Organization database. Bipolar Disord 2008 Jul;10(5):607-16.

(53) National Center for Biotechnology Information-dbSNP. 2011. http://www.ncbi.nlm.nih.gov/snp. May 8, 2011.

(54) Jockenhovel F, Blum WF, Vogel E, Englaro P, Muller-Wieland D, Reinwein D, et al. Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. J Clin Endocrinol Metab 1997 Aug;82(8):2510-3.

(55) Roemmich JN, Clark PA, Berr SS, Mai V, Mantzoros CS, Flier JS, et al. Gender differences in leptin levels during puberty are related to the subcutaneous fat depot and sex steroids. Am J Physiol 1998 Sep;275(3 Pt 1):E543-E551.

(56) Gebhardt S, Haberhausen M, Heinzel-Gutenbrunner M, Gebhardt N, Remschmidt H, Krieg JC, et al. Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. J Psychiatr Res 2009 Mar;43(6):620-6.

(57) Zhang XY, Tan YL, Zhou DF, Haile CN, Cao LY, Xu Q, et al. Association of clozapine-induced weight gain with a polymorphism in the leptin promoter region in patients with chronic schizophrenia in a Chinese population. J Clin Psychopharmacol 2007 Jun;27(3):246-51.

(58) Gregoor JG, Van Der Weide J, Mulder H, Cohen D, van Megen HJ, Egberts AC, et al. Polymorphisms of the LEP- and LEPR gene and obesity in patients using antipsychotic medication. J Clin Psychopharmacol 2009 Feb;29(1):21-5.

(59) Smith S, Wheeler MJ, Murray R, O'Keane V. The effects of antipsychotic-induced hyperprolactinaemia on the hypothalamic-pituitary-gonadal axis. J Clin Psychopharmacol 2002 Apr;22(2):109-14.

(60) Barrett kE, Barman SM, Boitano S, Brooks HL. Ganong's review of medical physiology. 23rd ed. McGraw-Hill companies,Inc.; 2010.

(61) Chi JH, Narita K, Ichimaru T, Murata T. Estrogen Increases c-Fos Expression in the Paraventricular Nucleus along with its Anorexic Effect in Developing Rats. J Reprod Dev 2011 Feb 25.

(62) Gotoh K, Masaki T, Chiba S, Higuchi K, Kakuma T, Shimizu H, et al. Hypothalamic neuronal histamine signaling in the estrogen deficiency-induced obesity. J Neurochem 2009 Sep;110(6):1796-805.

(63) Shi H, Seeley RJ, Clegg DJ. Sexual differences in the control of energy homeostasis. Front Neuroendocrinol 2009 Aug;30(3):396-404.

(64) Pluchino N, Cubeddu A, Begliuomini S, Merlini S, Giannini A, Bucci F, et al. Daily variation of brain-derived neurotrophic factor and cortisol in women with normal menstrual cycles, undergoing oral contraception and in postmenopause. Hum Reprod 2009 Sep;24(9):2303-9.

(65) Elks CE, Perry JR, Sulem P, Chasman DI, Franceschini N, He C, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet 2010 Dec;42(12):1077-85.

(66) Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001 Dec 13;414(6865):799-806.

(67) De Hert M, van Winkel R, Van Eyck D, Hanssens L, Wampers M, Scheen A, et al. Prevalence of diabetes, metabolic syndrome and metabolic abnormalities in schizophrenia over the course of the illness: a cross-sectional study. Clin Pract Epidemol Ment Health 2006;2:14.

(68) Casey DE, Hansen. Excessive mortality and morbidity in schizophrenia. In: Meyer JM, Nasrallah HA, editors. Medical illness and Schizophrenia.Washington DC: American Psychiatric Publishing; 2003. p. 13-4.

(69) Zheng L, Mack WJ, Dagerman KS, Hsiao JK, Lebowitz BD, Lyketsos CG, et al. Metabolic changes associated with second-generation antipsychotic use in Alzheimer's disease patients: the CATIE-AD study. Am J Psychiatry 2009 May;166(5):583-90.

(70) Muller EE. Neuroendocrine and autonomic control of metabolic functions: recent advances. Eat Weight Disord 2008 Sep;13(3):e61-e66.

Table 1. Patients' characteristics

Characteristics		**Cohort 1, n = 152**	**Cohort 2, n = 174**
Sex			
Male	(%)	52	49
Female	(%)	48	51
Age, years	(median; range)	42 (19-64)	35 (12-69)
Diagnosis			
Psychotic disorders	(%)	44	50
Mood disorders	(%)	51	41
Others diagnosis	(%)	5	9
BMI			
Initial BMI, kg/m^{2} ‡	(median; range)	25.2 (15.6-45.3)	22.4 (14.7-41.9)
Initial 25≥ BMI<30 kg/m^2	(%)	55	30
Initial BMI≥ 30 kg/m^2	(%)	20	12
Current BMI, kg/m^2	(median; range)	28.1 (17.1-42.3)	24.2 (14.7-41.2)
Current 25≥ BMI<30 kg/m^2	(%)	30	44
Current BMI≥ 30 kg/m^2	(%)	40	18
Weight increase*	(%; n)	51 (n=126)	30 (n=173)
Smoker	(%)	57	57
Prescribed psychotropic drug			
Amisulpride	(%)	-	9
Aripirazole	(%)	-	7
Clozapine	(%)	16	6
Olanzapine	(%)	12	16
Quetiapine	(%)	17	23
Risperidone	(%)	18	21
Lithium	(%)	22	12
Valproate	(%)	15	7
Treatment duration, months	(median; range)	30 (3-333)	6 (1-12)
Concomitant drugs linked to weight gain	(%)	31	33

* Weight increase required initial and present weight and is defined as an increase of ≥10 % of the initial weight during the current treatment.
‡ Before the current treatment.

Table 2. Top. Frequencies of *CRTC1* genotypes and Hardy Weinberg equilibrium (HWE) in the two separate cohorts and in the combined population.
Bottom. Linkage disequilibrium in Cohort 1 (D' and r^2).

Patients	n		Frequencies (%)		HWE
rs3746266		***AA***	***AG***	***GG***	
Cohort 1	152	64.5	31.6	3.9	1.0
Cohort 2	174	70.7	24.7	4.6	0.1
Combined cohort	326	67.9	27.8	4.3	0.2
rs8104411		***CC***	***CT***	***TT***	
Cohort 1	152	55.3	35.5	9.2	0.2
rs10402536		***GG***	***AG***	***AA***	
Cohort 1	152	59.2	34.9	5.9	0.8

D'	*rs3746266*	*rs8104411*	**r^2**	*rs3746266*	*rs8104411*
rs10402536	0.67	0.03	*rs10402536*	0.03	0.00
rs8104411	1.00		*rs8104411*	0.09	

Table 3. Associations between *CRTC1 rs3746266* and BMI and body weight in a dominant model.

	BMI (kg/m^2)				***Weight (kg)***			
rs3746266A>G	**AG, GG**	**AA**	***P***	**E. Var. (%)**	**AG, GG**	**AA**	***P***	**E. Var. (%)**
Cohort 1	Ref	1.56	*0.008*	20.5	Ref	4.03	*0.017*	44.8
Cohort 2	Ref	1.01	*0.048*	13.9	Ref	3.43	*0.024*	37.5
Combined cohort *	Ref	1.34	*<0.001*	11.7	Ref	3.80	*<0.001*	35.7
Combined cohort *, Men	Ref	0.57	*0.168*	7.6	Ref	2.07	*0.143*	30.5
Combined cohort *, Women	Ref	1.55	*0.020*	15.3	Ref	4.13	*0.021*	20.9
Combined cohort *, Women>45y	Ref	0.92	*0.212*	5.6	Ref	1.84	*0.304*	13.3
Combined cohort *, Women<45y	Ref	2.90	*0.004*	21.9	Ref	8.21	*0.005*	29.8

E. Var.: explained variance (%)

Results were obtained by fitting Generalized Additive Mixed Models for patient with no comedication causing weight-gain controlling for age, gender, studied drugs, and standardised doses.

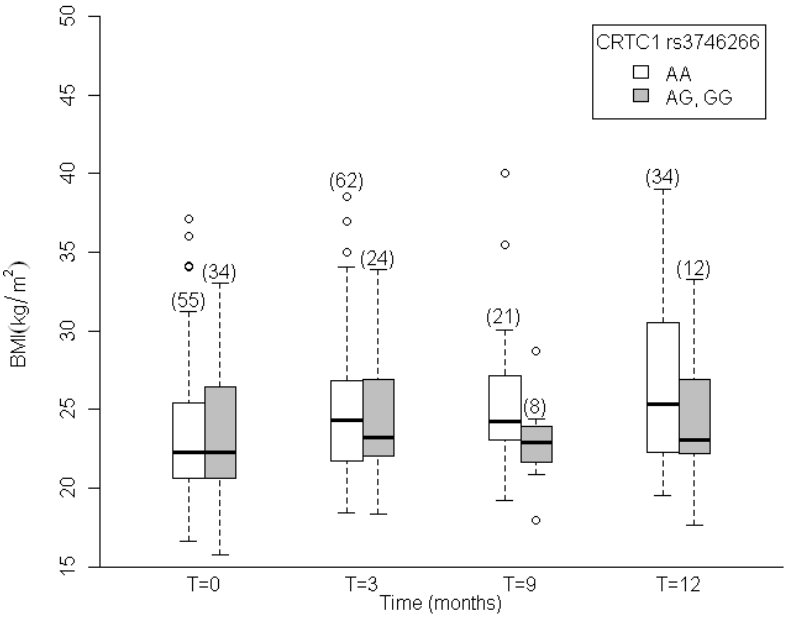
* To have similar treatment duration, only patients from cohort 1 treated for up to 24 months were included in the combined cohort

Table 4: Predicted BMI at baseline and after 12 months with the current treatment for the different *rs3746266* genotypes.

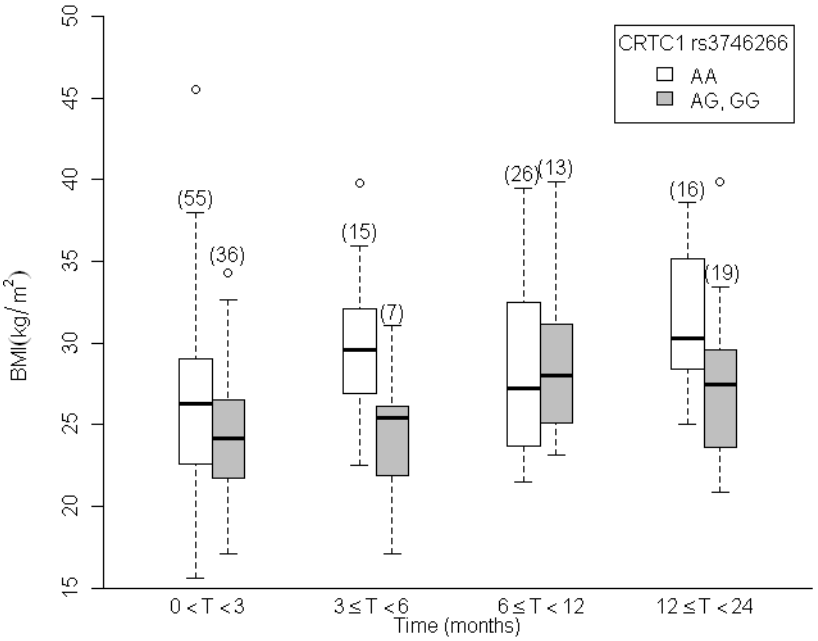
Cohort	*rs3746266*	Predicted BMI (CI), kg/m^2				Predicted weight (CI), kg			
		Baseline		12 months		Baseline		12 months	
Cohort 1, n= 152									
	AA	25.7	(24.0-27.3)	28.6	(26.9-30.2)	73.3	(68.0-77.9)	81.6	(76.3-86.2)
	AG, GG	24.4	(22.7-26.1)	27.3	(25.6-29.0)	69.7	(64.8-74.5)	78.0	(73.1-82.8)
Cohort 2, n= 174									
	AA	23.6	(22.5-24.6)	25.7	(24.6-26.8)	71.7	(67.5-75.8)	78.1	(73.9-82.3)
	AG, GG	22.5	(21.4-23.7)	24.7	(23.5-25.9)	68.3	(64.3-72.0)	74.7	(70.7-78.5)
Combined cohort *, n= 234									
	AA	24.4	(23.4-25.4)	26.6	(25.6-27.6)	72.8	(68.9-76.3)	79.1	(75.2-82.7)
	AG, GG	23.1	(21.9-24.1)	25.3	(24.0-26.3)	69.0	(65.2-72.6)	75.3	(71.5-78.9)

CI: 95% confidence intervals
Results were obtained by fitting Generalized Additive Mixed Models for patient with no comedication causing weight-gain controlling for age, gender, studied drugs, and standardised doses.
The results were adjusted for height in the weight analyses.
* To have similar treatment duration, only patients from cohort 1 treated for up to 24 months were included in the combined cohort

Cohort 1



Cohort 2



Combined cohort

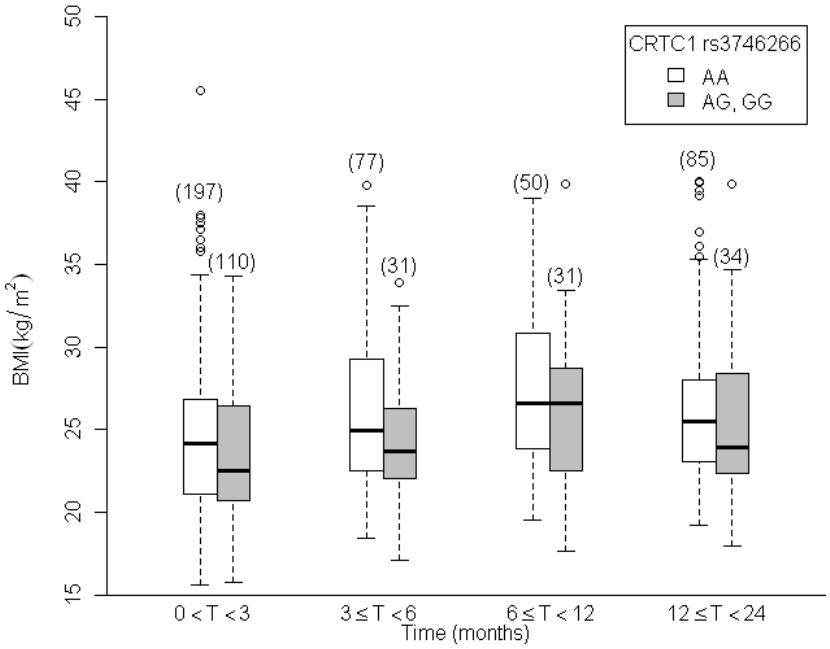
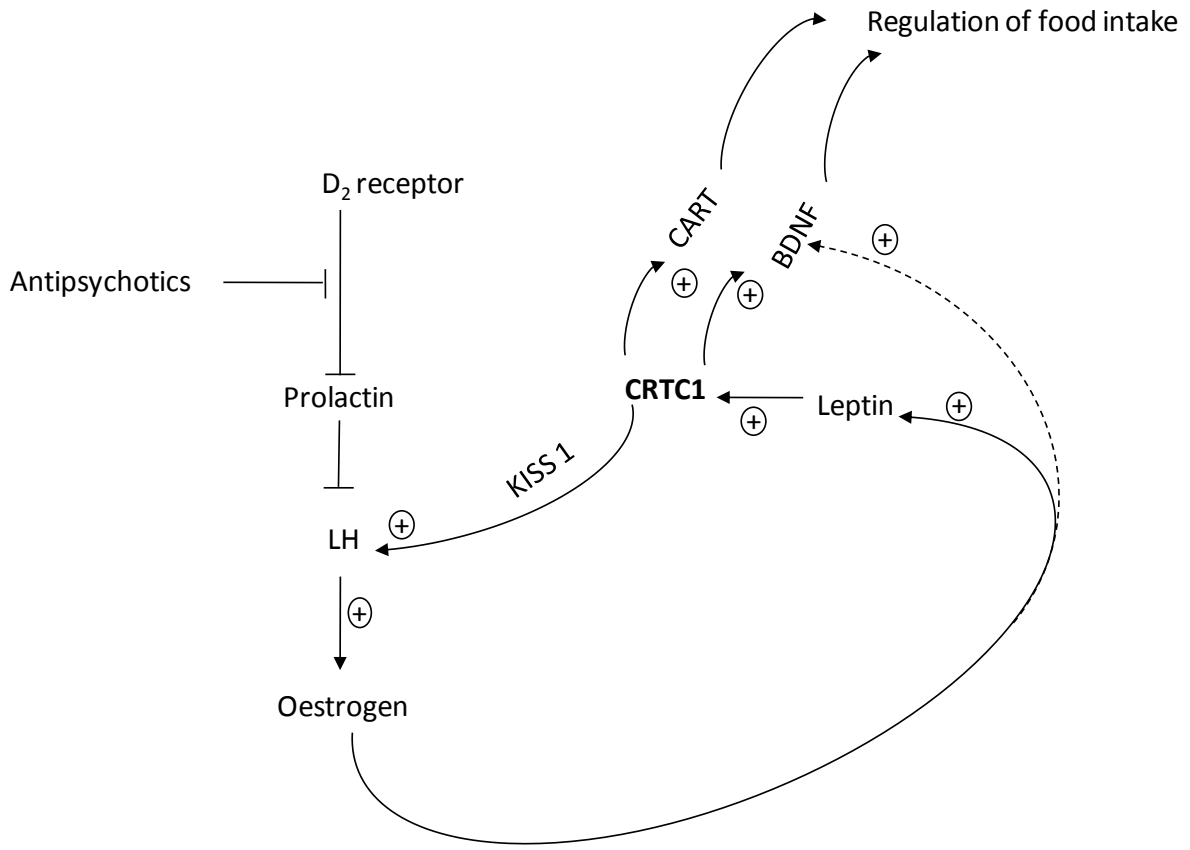


Figure 1. BMI in relation to *CRTC1 rs3746266* genotypes in Cohort 1, Cohort 2 and in combined cohort, presented at different time periods. Median and interquartile ranges are shown and number of BMI observations used for each box plot is indicated.



CRTC1: CREB-regulated transcription coactivator 1, CART: the cocaine- and amphetamine-regulated transcript, BDNF: brain-derived neurotrophic factor, KISS1: kisspeptin-1, LH: luteinizing hormone

Figure 2. Postulated role of CRTC1 for the regulation of food intake.

Chapitre V

Conclusions et perspectives

Les individus présentant une maladie psychiatrique, notamment la schizophrénie et les troubles bipolaires, ont été associés à une mortalité plus élevée que dans la population générale, notamment, en raison d'une plus grande prévalence de complications métaboliques conduisant à des problèmes cardiovasculaires. Les médicaments étudiés dans ce travail de thèse, les antipsychotiques atypiques, le lithium, et le valproate font tous partie de l'arsenal thérapeutique destiné à soigner ces maladies, mais ils peuvent induire également des complications métaboliques dont une prise de poids.

L'intensité et la fréquence des effets secondaires liés à l'utilisation de ces psychotropes montrent une grande variabilité interindividuelle. Ce travail de thèse a pour but de déterminer les facteurs cliniques et génétiques prédictifs des effets secondaires et notamment de la prise de poids.
Trois études ont permis le recrutement de 196 patients dans l'Etude prise de poids Genève, 42 patients de différents centres dans l'Etude Risperdal Consta et, à l'heure de la rédaction de ce manuscrit, plus de 500 patients dans l'Etude Suivi métabolique Lausanne.

1. Partie clinique

Nous avons démontré une prévalence de complications métaboliques et de risques cardiovasculaires supérieurs à la population générale dans l'Etude prise de poids Genève. Le type de médicament utilisé, le sexe, un IMC bas et une augmentation d'appétit à l'introduction de la médication, ont été associés à une prise de poids sous psychotropes. Ces résultats confirment les résultats trouvés dans d'autres études publiées [71-73].

Un grand travail de sensibilisation à tous les niveaux du corps médical (médecins, infirmiers, psychologues, réseau de santé) sur les conséquences métaboliques des psychotropes, incluant la formation, la logistique et la transmission des informations, a permis d'initier et d'instaurer dans la pratique clinique du Département de Psychiatrie du CHUV, les recommandations de suivi métabolique décrites dans le manuscrit III. Plus de 1000 patients ont bénéficié de ce programme de santé lors de leur hospitalisation ou lors de leur suivi en ambulatoire, et plus de 500 patients ont accepté de participer à la partie pharmacogénétique à l'heure de la rédaction de ce manuscrit de thèse. L'inclusion des patients est toujours en cours dans l'Etude Suivi métabolique Lausanne.

Nous avons donc contribué à améliorer la sécurité d'utilisation des psychotropes par la détection des patients à risque de complications métaboliques, par la détermination de facteurs prédisposant à

une prise de poids et par l'instauration d'un suivi métabolique avec un contrôle régulier des paramètres métaboliques. Une attention particulière associée à des mesures préventives ciblées (conseils hygiéno-diététiques) peuvent donc être favorisées par le corps médical en collaboration avec le patient en cas d'un risque élevé de complications métaboliques. Ces résultats offrent la possibilité d'une détection rapide de la survenue d'éventuelles complications métaboliques et d'une rapide prise en charge somatique adéquate.

Les trois cohortes, particulièrement celle du Suivi métabolique Lausanne en constante augmentation, constituent une très importante base de données. Dans le futur, les recherches des facteurs pouvant influencer la prise de poids et les complications métaboliques seront poursuivies et étendues sur ce grand collectif.

De plus, le développement des méthodes analytiques fiables et robustes ont permis de mettre à disposition pour le TDM le dosage de onze psychotropes et cinq métabolites. Conjointement à la très importante base de données rendue disponible par le présent travail de thèse, il sera possible d'obtenir des informations cinétiques plus précises sur les psychotropes par des études de pharmacocinétique de population. Ces études permettront également d'améliorer la prise en charge ainsi que l'individualisation du traitement.

2. Partie pharmacogénétique

Les génotypes pour de nombreux SNPs de différents gènes ont été déterminés chez les patients des trois études présentées dans ce travail de recherche. L'activité du CYP3A a été obtenue par phénotypage pour un certain nombre d'entre eux.

Pharmacogenetic study on risperidone long-acting injection: influence of cytochrome P450 2D6 and Pregnane X receptor on risperidone exposure and drug-induced side-effects

L'étude Risperdal Consta est constituée de 42 patients et fait l'objet du manuscrit V. Cette étude a permis d'améliorer la compréhension de la pharmacogénétique de la risperidone sous forme injectable.

Concernant la pharmacocinétique, nous avons montré que les polymorphismes des gènes *CYP2D6* et *NR1I2 (rs7643645A>G*), ce dernier codant pour le récepteur nucléaire PXR, influencent les taux

plasmatiques normalisés par la dose de risperidone (RIS), de son métabolite le 9-hydroxy-rispéridone (OHRIS) ou de leur somme (RIS+OHRIS) qui est considérée comme la fraction active. Les métaboliseurs rapides du CYP2D6 ont des taux significativement inférieurs de RIS comparés aux métaboliseurs intermédiaires confirmant les résultats observés sous risperidone orale. A notre connaissance, ceci est la première fois qu'il est démontré que les individus porteurs du génotype *NR1I2 rs7643645GG*, associé à une activité *in vitro* diminuée, ont un taux significativement inférieur de métabolite OHRIS et de RIS+OHRIS. De plus, l'influence du génotype *NR1I2 rs7643645GG* sur les taux d'OHRIS et OHRIS+RIS a pu être confirmée dans une cohorte indépendante composée d'individus sous Risperdal Consta provenant des Etudes Prise de poids Genève et Suivi métabolique Lausanne (n=20). La mesure de l'activité du CYP3A par un test de phénotypage nous a permis de démontrer que le CYP3A n'a pas, ou seulement un faible rôle dans le métabolisme de la risperidone sous forme injectable. De plus, aucune association entre les polymorphismes des gènes *CYP3A* et *ABCB1* n'a été observée avec les taux de RIS, OHRIS et OHRIS+RIS.

Concernant la pharmacodynamie, nous avons montré une association entre l'intensité des effets extrapyramidaux, les taux de prolactine et le polymorphisme *rs2472677C>T* du gène *NR1I2.* Les individus porteurs du génotype *rs2472677CC* préalablement montré comme étant associé à une activité diminuée dans des études *in vitro*, présentaient un taux de prolactine plus bas et un nmobre d'effets secondaires extrapyramidaux plus élevés que les autres génotypes.

Nos résultats suggèrent également un rôle de l'activité du CYP3A dans la régulation de la lipémie avec une corrélation positive entre le cholestérol-HDL et l'activité CYP3A. Ces résultats sont en accord avec des observations décrites dans des analyses *in vitro* [136] et dans des modèles animaux [137]. Il sera nécessaire de confirmer ces résultats dans d'autres études avec un nombre plus élevé de patients. Les résultats sur l'association positive entre un polymorphisme du *NR1I2* et les taux de RIS et OHRIS, même si déjà confirmé sur une deuxième cohorte, devront également être répliqués avec une cohorte plus importante.

Il sera également intéressant d'analyser l'influence des SNPs du *NR1I2* sur les taux plasmatiques des autres psychotropes substrats des CYP3A ou de la P-gp chez les patients inclus dans les deux autres cohortes de ce présent travail de thèse.

Influence of *CRTC1* polymorphisms on body mass index in patients with psychotropic treatments

Le travail de recherche présenté dans le manuscrit VI se focalise sur l'influence du gène *CRTC1* sur l'IMC pour les patients traités par des psychotropes. Les souris *KO* pour le gène *CRTC1* ont été récemment montrées comme étant hyperphagiques et développant une obésité sur le long terme. L'influence du CRTC1 sur l'appétence serait liée à son activation par la leptine. Au total, 152 patients de l'Etude Prise de Poids Genève et 174 patients de l'Etude Suivi métabolique Lausanne ont été inclus dans cette analyse. Jusqu'à maintenant, aucun SNP du *CRTC1* n'a été associé à l'obésité, à une prise de poids ou à une activité fonctionnelle. Pour ce travail, trois SNPs du *CRTC1* pouvant potentiellement influencer son activité ont donc été sélectionnés de façon exploratoire.

Nous avons montré que le SNP *rs3746266A>G* du gène *CRTC1*, conduisant à une modification de la séquence d'acides aminés, est significativement associé avec la valeur de l'IMC. Ce résultat a été montré dans la première cohorte, répliqué dans la deuxième cohorte et confirmé lorsque les cohortes ont été combinées. Les porteurs de l'allèle mutant *G* ont un IMC plus faible que les individus *AA*. Dans la cohorte combinée, cet effet protecteur est particulièrement fort chez les femmes pré-ménopausées. La différence entre les individus porteurs de l'allèle G et les individus AA atteint 2.9 kg/m^2, ce qui est considéré comme cliniquement important. Notre hypothèse est que la régulation de l'énergie et de l'appétence par le CRTC1 intervient par le biais de neuropetides (CART, BDNF) et par le biais du système endocrinien. Par contre, les SNPs *rs10402536* et *rs8104411* du *CRTC1* n'ont pas été associés à l'IMC et aucune association avec la prise de poids induite par le traitement psychotrope en cours lors de leurs inclusions n'a été observée. Les deux cohortes étudiées sont composées majoritairement de patients au long cours qui ont déjà reçu un certain nombre de traitements. Il est possible que l'influence du CRTC1 sur l'IMC soit le reflet d'une prise de poids dans le passé. A notre connaissance, ceci est la première fois que le rôle d'un polymorphisme génétique du *CRTC1* sur l'IMC de patients traités par psychotropes est démontré.

Une nouvelle étape dans la compréhension de la régulation de l'appétit et de la prise de poids a été initiée par ce travail. Les résultats prometteurs du *CRTC1* poussent à étendre les recherches sur ce gène. Il serait intéressant de pouvoir tester le mécanisme d'action du *CRTC1* dans la régulation de l'appétence, l'éventuelle activité fonctionnelle du SNP *rs3746266* (notamment *in vitro* grâce à des expériences de transfection incluant un gène rapporteur associé à la voie de signalisation de la

leptine pour la régulation de la prise alimentaire) ainsi que d'approfondir les recherches entre les liens possibles du CRTC1 avec le système endocrinien.

Les conséquences fonctionnelles du SNP *rs3746266* sont en cours d'investigation. Grâce aux échantillons récoltés durant le présent travail de recherche, des études supplémentaires mesurant la concentration du *brain-derived neurotrophic factor* (BDNF), protéine dont l'expression est sous le contrôle du CRTC1, vont être effectuées prochainement dans le plasma de patients porteurs des différents génotypes du *CRTC1*.
L'influence du *CRTC1* est également en cours d'investigation dans une population générale non psychiatrique. Le génotype de plus de 6000 individus de Lausanne (l'étude CoLaus [138]) a été déterminé pour le SNP *rs6510997*, un SNP associé au *rs3746266* (r^2=0.7), ce dernier n'étant pas disponible dans cette population. Les individus porteurs de l'allèle muté présentent une quantité de masse grasse significativement inférieure en comparaison des individus porteurs de l'autre allèle. Cet effet protecteur est spécialement marqué chez les femmes préménopausées, ce qui corrobore les résultats obtenus chez les populations psychiatriques. L'article intégrant l'ensemble des données incluant les deux populations psychiatriques et cette population générale est en cours de préparation.

De plus, dans ce travail de thèse, des échantillons particuliers permettant le dosage du mRNA (PAXgene Blood RNA Tube, PreAnalytix, Qiagen, Suisse) ont été récoltés avant l'introduction du traitement psychotrope et un mois plus tard pour un certain nombre de patients. Il sera intéressant d'analyser les changements d'expression du mRNA de certaines protéines cibles. Ces informations pourraient permettre l'identification de nouveaux gènes impliqués dans les mécanismes patho-physiologiques de la prise de poids. L'influence de polymorphismes de gènes identifiés par ce biais serait ensuite analysée sur l'ensemble des populations psychiatriques disponibles.

Il sera également intéressant d'analyser, grâce à cette base de données de patients sous psychotropes, d'autres facteurs influençant les complications métaboliques. Au niveau clinique, par l'analyse des facteurs prédictifs dans l'étude Suivi métabolique Lausanne et au niveau génétique, par une recherche exhaustive des gènes impliqués dans les risques cardiovasculaires sur des patients sélectionnés dans les trois populations psychiatriques. L'utilisation d'une puce *Illumina iSelect Cardio-Metabo-Chip genotyping array* (Illumina Inc., USA) contenant 190'000 marqueurs génétiques associés à des risques cardiovasculaires est en préparation. D'autre part, certains gènes associés à

l'obésité ont été dernièrement analysés. Ainsi, l'influence des SNPs des gènes du *LEPR, LEP, MC4R, $5HT_{2C}$, FTO, UCP2* sur l'IMC et la prise de poids a été étudiée sur deux cohortes caucasiennes provenant de l'Etude prise de poids Genève (N=156) et l'Etude Suivi métabolique Lausanne (N=292). Un manuscrit sur ce sujet sera prochainement réalisé.

Des grandes difficultés limitent l'intégration des résultats pharmacogénétiques dans la pratique clinique, notamment en psychiatrie où l'établissement d'un diagnostic précis est parfois difficile. De plus, la survenue d'un effet secondaire métabolique est complexe et multifactorielle. Néanmoins, l'utilisation future d'une puce intégrant les principaux gènes de susceptibilité de la prise de poids, identifiés grâce à nos populations psychiatriques et d'autres études, permettrait de dépasser ces difficultés et faciliterait l'implémentation dans la pratique clinique de la prise en compte du patrimoine génétique du patient. Une optimisation d'un traitement individualisé sera alors favorisée.

3. Conclusion générale

Durant son évolution, l'espèce humaine a été confrontée à un milieu où la nourriture était peu abondante, conduisant à une sélection naturelle vis-à-vis des individus ayant les gènes permettant de conserver l'énergie [101]. Cet élément vital est devenu dans le mode de vie occidental un facteur de risque induisant une prise de poids dont les conséquences les plus dramatiques sont observées dans l'explosion de l'obésité rencontrée ces dernières décennies.

Dans le cadre d'un problème multifactoriel et complexe conduisant à une prise de poids, les résultats obtenus permettent de contribuer à la compréhension des facteurs cliniques et génétiques impliqués dans ce phénomène. La variabilité interindividuelle dans la disposition du médicament et dans la réponse au traitement ont montré l'importance du bagage génétique dans ces phénotypes.

Les résultats de cette thèse ainsi que les autres travaux effectués dans ce domaine pourront permettre de mieux identifier les patients à risque d'une prise de poids importante lors de la prescription de psychotropes. Ceci pourrait aider le médecin et/ou le patient à être sensibilisé à ces effets secondaires métaboliques et à intervenir dès le début du traitement. Nous répondons ainsi en partie à la question posée au début de ce travail. Ces résultats peuvent contribuer au développement futur d'une médecine personnalisée en psychiatrie.

Liste de références

Reference List

1. De Hert M, Dekker JM, Wood D et al. Cardiovascular disease and diabetes in people with severe mental illness position statement from the European Psychiatric Association (EPA), supported by the European Association for the Study of Diabetes (EASD) and the European Society of Cardiology (ESC). Eur Psychiatry 2009; 24(6):412-424.
2. Compendium Suisse de Médicaments®. 31e ed. Bâle (Suisse): Documed S.A., 2010.
3. Casey DE. Dyslipidemia and atypical antipsychotic drugs. J Clin Psychiatry 2004; 65 (Suppl. 18):27-35.
4. De Hert M, van Winkel R, Van Eyck D et al. Prevalence of diabetes, metabolic syndrome and metabolic abnormalities in schizophrenia over the course of the illness: a cross-sectional study. Clin Pract Epidemol Ment Health 2006; 2:14.
5. Heiskanen T, Niskanen L, Lyytikainen R et al. Metabolic syndrome in patients with schizophrenia. J Clin Psychiatry 2003; 63:575-579.
6. American Diabetes Association, American Psyhiatric Association, American Association of Clinical Endocrinologists et al. Consensus development conference on antipsychotic drugs and obesity and diabetes. Diabetes Care 2004; 27(2):596-601.
7. Fontaine KR, Heo M, Harrigan EP et al. Estimating the consequences of anti-psychotic induced weight gain on health and mortality rate. Psychiatry Research 2001; 101(3):277-288.
8. Henderson DC. Clozapine: diabetes mellitus, weight gain, and lipid abnormalities. J Clin Psychiatry 2001; 62 (Suppl. 23):39-44.
9. Vieweg WVR, Levy JR, Fredrickson SK et al. Psychotropic drug considerations in depressed patients with metabolic disturbances. Am J Med 2008; 121(8):647-655.
10. Lander ES, Linton LM, Birren B et al. Initial sequencing and analysis of the human genome. Nature 2001; 409(6822):860-921.
11. Guttmacher AE, Collins FS. Genomic medicine--a primer. N Engl J Med 2002; 347(19):1512-1520.
12. The International HapMap Project. Nature 2003; 426(6968):789-796.
13. Meyer UA. Pharmacogenetics - five decades of therapeutic lessons from genetic diversity. Nat Rev Genet 2004; 5(9):669-676.
14. Nebert DW, Zhang G, Vesell ES. From human genetics and genomics to pharmacogenetics and pharmacogenomics: past lessons, future directions. Drug Metab Rev 2008; 40(2):187-224.
15. Garrod AE, Oxon MD, Lond FRCP. The incidence of alkaptonuria: a study in chemical individuality. Lancet 1902;1616-1620.
16. Carson PE, Flanagan CL, Ickes CE et al. Enzymatic deficiency in primaquine-sensitive erythrocytes. Science 1956; 124:484-485.
17. Lehmann H, Ryan E. The familial incidence of low pseudocholinesterase level. Lancet 1956; 271(6934):124.
18. Bonicke R, Reif W. Enzymatische Inaktivierung von Isonicotinsaurehydrazid im menschlichen und tierischen Organismus. [Enzymatic inactivation of isonicotinic acid hydrizide in human and animal organism]. Naunyn Schmiedebergs Arch Exp Pathol Pharmakol 1953; 220(4):321-323.
19. Hugues HB. On the metabolic fate of isoniazid. The Journal of pharmacology and experimental therapeutics 1953; 109(4):444-452.
20. Vogel F. Moderne Probleme der Humangenetik. Ergebn Inn Med Kinderheilkd 1959; 12:52-125.
21. McCarthy MI, Abecasis GR, Cardon LR et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008; 9(5):356-369.
22. Stahl SM. Stahl's Essential Psychopharmacology - Neuroscientific Basis and Practical Applications. Thrid edition ed. New York: Cambridge University Press, 2008.
23. van Os J, Kapur S. Schizophrenia. Lancet 2009; 374(9690):635-645.
24. Correll CU, Malhotra AK, Kaushik S et al. Early prediction of antipsychotic response in schizophrenia. Am J Psychiatry 2003; 160(11):2063-2065.

25. Evans WE, Mcleod HL. Pharmacogenomics - Drug disposition, drug targets, and side effects. New Engl J Med 2003; 348(6):538-549.
26. Mauri MC, Volonteri LS, Colasanti A et al. Clinical pharmacokinetics of atypical antipsychotics: a critical review of the relationship between plasma concentrations and clinical response. Clin Pharmacokinet 2007; 46(5):359-388.
27. Benet LZ, Kroetz DL, Sheiner LB. Pharmacokinetics: The dynamics of drug absorption, distribution, and elimination. In: Goodman and Gilman's, editor. The Pharmacological basis of therapeutics. 1996: 3-28.
28. Guengerich FP. Cytochrome P450s and other enzymes in drug metabolism and toxicity. AAPS J 2006; 8(1):E101-E111.
29. Crettol S, Petrovic N, Murray M. Pharmacogenetics of phase I and phase II drug metabolism. Curr Pharm Des 2010; 16(2):204-219.
30. Service de pharmacologie et toxicologie clinique, Hôpitaux Universitaires Gèneve. Interactions medicamenteuses et cytochrome P450. 2010. Available at: http://www.pharmacoclin.ch/_library/pdf/cytp450.pdf. Accessed May 8, 2011.
31. Bender S, Eap CB. Very high cytochrome P4501A2 activity and nonresponse to clozapine. Arch Gen Psychiatry 1998; 55:1048-1050.
32. Eap CB, Bender S, Jaquenoud Sirot E et al. Nonresponse to clozapine and ultrarapid CYP1A2 activity: Clinical data and analysis of the CYP1A2 gene. J Clin Psychopharmacol 2004; 24:214-219.
33. Melkersson KI, Scordo MG, Gunes A et al. Impact of CYP1A2 and CYP2D6 polymorphisms on drug metabolism and on insulin and lipid elevations and insulin resistance in clozapine-treated patients. J Clin Psychiatry 2007; 68(5):697-704.
34. Dobrinas M, Cornuz J, Oneda B et al. Impact of smoking, smoking cessation, and genetic polymorphisms on CYP1A2 activity and inducibility. Clin Pharmacol Ther 2011; 90(1):117-125.
35. Ieiri I, Takane H, Otsubo K. The MDR1 (ABCB1) gene polymorphism and its clinical implications. Clin Pharmacokinet 2004; 43(9):553-576.
36. Boulton DW, DeVane CL, Liston HL et al. In vitro P-glycoprotein affinity for atypical and conventional antipsychotics. Life Sci 2002; 71:163-169.
37. Sun J, He ZG, Cheng G et al. Multidrug resistance P-glycoprotein: crucial significance in drug disposition and interaction. Med Sci Monit 2004; 10(1):RA5-14.
38. Marzolini C, Paus E, Buclin T et al. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther 2004; 75(1):13-33.
39. Wang JS, Ruan Y, Taylor RM et al. The brain entry of risperidone and 9-hydroxyrisperidone is greatly limited by P-glycoprotein. Int J Neuropsychopharmacol 2004; 7(4):415-419.
40. Doran A, Obach RS, Smith BJ et al. The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. Drug Metab Dispos 2005; 33(1):165-174.
41. Schmitt U, Abou El-Ela A, Guo LJ et al. Cyclosporine A (CsA) affects the pharmacodynamics and pharmacokinetics of the atypical antipsychotic amisulpride probably via inhibition of P-glycoprotein (P-gp). J Neural Transm 2006; 113(7):787-801.
42. Wang JS, Zhu HJ, Donovan JL et al. Aripiprazole brain concentration is altered in P-glycoprotein deficient mice. Schizophr Res 2009; 110(1-3):90-94.
43. Lin YC, Ellingrod VL, Bishop JR et al. The relationship between P-glycoprotein (PGP) polymorphisms and response to olanzapine treatment in schizophrenia. Ther Drug Monit 2006; 28(5):668-672.
44. Xing Q, Gao R, Li H et al. Polymorphisms of the ABCB1 gene are associated with the therapeutic response to risperidone in Chinese schizophrenia patients. Pharmacogenomics 2006; 7(7):987-993.
45. Zhang B, Xie W, Krasowski MD. PXR: a xenobiotic receptor of diverse function implicated in pharmacogenetics. Pharmacogenomics 2008; 9(11):1695-1709.

46. Synold TW, Dussault I, Forman BM. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. Nat Med 2001; 7(5):584-590.
47. Kliewer SA, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr Rev 2002; 23(5):687-702.
48. Francis GA, Fayard E, Picard F et al. Nuclear receptors and the control of metabolism. Annu Rev Physiol 2003; 65:261-311.
49. Lamba J, Lamba V, Strom S et al. Novel single nucleotide polymorphisms in the promoter and intron 1 of human pregnane X receptor/NR1I2 and their association with CYP3A4 expression. Drug Metab Dispos 2008; 36(1):169-181.
50. Siccardi M, D'Avolio A, Baietto L et al. Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C-->T) with reduced concentrations of unboosted atazanavir. Clin Infect Dis 2008; 47:1222-1225.
51. Nasrallah HA. Atypical antipsychotic-induced metabolic side effects: insights from receptor-binding profiles. Mol Psychiatry 2008; 13(1):27-35.
52. Casey DE, Zorn SH. The pharmacology of weight gain with antipsychotics. J Clin Psychiatry 2001; 62 (Suppl 7):4-10.
53. Szekeres G, Keri S, Juhasz A et al. Role of dopamine D3 receptor (DRD3) and dopamine transporter (DAT) polymorphism in cognitive dysfunctions and therapeutic response to atypical antipsychotics in patients with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2004; 124B:1-5.
54. Jonsson EG, Flyckt L, Burgert E et al. Dopamine D3 receptor gene Ser9Gly variant and schizophrenia: association study and meta-analysis. Psychiatr Genet 2003; 13(1):1-12.
55. Lane HY, Hsu SK, Liu YC et al. Dopamine D3 receptor Ser9Gly polymorphism and risperidone response. J Clin Psychopharmacol 2005; 25(1):6-11.
56. Zhang JP, Malhotra AK. Pharmacogenetics and antipsychotics: therapeutic efficacy and side effects prediction. Expert Opin Drug Metab Toxicol 2011; 7(1):9-37.
57. Bostwick JR, Guthrie SK, Ellingrod VL. Antipsychotic-induced hyperprolactinemia. Pharmacotherapy 2009; 29(1):64-73.
58. Holzer L, Eap CB. Risperidone-induced symptomatic hyperprolactinemia in adolescents. J Clin Psychopharmacol 2006; 26(2):167-171.
59. Meaney AM, Smith S, Howes OD et al. Effects of long-term prolactin-raising antipsychotic medication on bone mineral density in patients with schizophrenia. Br J Psychiatry 2004; 184:503-508.
60. Kleinberg DL, Davis JM, De Coster R et al. Prolactin levels and adverse events in patients treated with risperidone. J Clin Psychopharmacol 1999; 19(1):57-61.
61. Spina E, Avenoso A, Facciolà G et al. Relationship between plasma risperidone and 9-hydroxyrisperidone concentrations and clinical response in patients with schizophrenia. Psychopharmacology 2001; 153:238-243.
62. Meyer JM. Strategies for the long-term treatment of schizophrenia: real-world lessons from the CATIE trial. J Clin Psychiatry 2007; 68 Suppl 1:28-33.
63. McIntyre RS, Mancini DA, Basile VS. Mechanisms of antipsychotic-induced weight gain. J Clin Psychiatry 2001; 62 (Suppl. 23):23-29.
64. Shirdazi AA, Ghaemi SN. Side efffects of atypical antispychotics: extrapyramidal symptoms and the metabolic syndrome. Harv Rev Psychiatry 2006; 14:152-164.
65. Barnett AH, Mackin P, Chaudhry I et al. Minimising metabolic and cardiovascular risk in schizophrenia: diabetes, obesity and dyslipidaemia. J Psychopharmacol 2007; 21(4):357-373.
66. Michelsen JW, Meyer JM. Cardiovascular effects of antipsychotics. Expert Rev Neurother 2007; 7(7):829-839.
67. Chagnon YC. Susceptibility genes for the side effect of antipsychotics on body weight and obesity. Current Drug Targets 2006; 7:1681-1695.
68. Taylor DM, McAskill R. Atypical antipsychotics and weight gain - a systematic review. Acta Psychiatr Scand 2000; 101:416-432.

69. Jones B, Basson BR, Walker DJ et al. Weight change and atypical antipsychotic treatment in patients with schizophrenia. J Clin Psychiatry 2001; 62 (Suppl. 2):41-44.
70. Baptista T. Body weight gain induced by antipsychotic drugs: mechanisms and management. Acta Psychiatr Scand 1999; 100:3-16.
71. Zimmermann U, Kraus T, Himmerich H et al. Epidemiology, implications and mechanisms underlying drug-induced weight gain in psychiatric patients. J Psychiatr Res 2003; 37(3):193-220.
72. Basson BR, Kinon BJ, Taylor CC et al. Factors influencing acute weight change in patients with schizophrenia treated with olanzapine, haloperidol, or risperidone. J Clin Psychiatry 2001; 62(4):231-238.
73. Gebhardt S, Haberhausen M, Heinzel-Gutenbrunner M et al. Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. J Psychiatr Res 2009; 43(6):620-626.
74. Harrison PJ. Weight gain with antipsychotic drugs: the role of the 5-HT_{2C} receptor (HTR2C) and other genes. Pharmacogenetics and Genomics 2005; 15:193-194.
75. Reynolds GP, Zhang ZJ, Zhang XB. Polymorphism of the promoter region of the serotonin 5-HT_{2C} receptor gene and clozapine-induced weight gain. Am J Psychiatry 2003; 160(4):677-679.
76. Miller DD, Ellingrod VL, Holman TL et al. Clozapine-induced weight gain associated with the 5HT2C receptor -759C/T polymorphism. Am J Med Genet B Neuropsychiatr Genet 2005; 133(1):97-100.
77. Templeman LA, Reynolds GP, Arranz B et al. Polymorphism of the 5-HT2C receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis. Pharmacogenetics and Genomics 2005; 15:195-200.
78. Ellingrod VL, Perry PJ, Ringold JC et al. Weight gain associated with the -759C/T polymorphism of the 5HT2C receptor and olanzapine. Am J Med Genet B Neuropsychiatr Genet 2005; 134(1):76-78.
79. Yuan X, Yamada K, Ishiyama-Shigemoto S et al. Identification of polymorphic loci in the promoter region of the serotonin 5-HT2C receptor gene and their association with obesity and type II diabetes. Diabetologia 2000; 43(3):373-376.
80. Pooley EC, Fairburn CG, Cooper Z et al. A 5-HT2C receptor promoter polymorphism (HTR2C -759C/T9) is associated with obesity in women, and with resistance to weigt loss in heterozygotes. Am J Med Genet B Neuropsychiatr Genet 2004; 126B(1):124-127.
81. Loktionov A. Common gene polymorphisms and nutrition: emerging links with pathogenesis of multifactorial chronic diseases (review). J Nutr Biochem 2003; 14(8):426-451.
82. Abraham KI, Ieni JR, Meyerson LR. Purificaton and properties of human plasma endogenous modulator for the platelet tricyclic binding/serotonin transport complex. Biochim Biophys Acta 1987; 923:8-21.
83. Lima JJ, Feng H, Duckworth L et al. Association analyses of adrenergic receptor polymorphisms with obesity and metabolic alterations. Metabolism 2007; 56(6):757-765.
84. Muller DJ, Klempan TA, De L, V et al. The SNAP-25 gene may be associated with clinical response and weight gain in antipsychotic treatment of schizophrenia. Neurosci Lett 2005; 379(2):81-89.
85. Basile VS, Masellis M, McIntyre RS et al. Genetic dissection of atypical antipsychotic-induced weight gain: novel preliminary data on the pharmacogenetic puzzle. J Clin Psychiatry 2001; 62 (Suppl. 23):45-66.
86. Gunes A, Melkersson KI, Scordo MG et al. Association between HTR2C and HTR2A polymorphisms and metabolic abnormalities in patients treated with olanzapine or clozapine. J Clin Psychopharmacol 2009; 29(1):65-68.
87. Park JY, Kim KA, Park PW et al. Effect of CYP3A5*3 genotype on the pharmacokinetics and pharmacodynamics of alprazolam in healthy subjects. Clin Pharmacol Ther 2006; 79(6):590-599.

88. Zhang ZJ, Yao ZJ, Zhang XB et al. No association of antipsychotic agent-induced weight gain with a DA receptor gene polymorphism and therapeutic response. Acta Pharmacol Sin 2003; 24(3):235-240.
89. Rietschel M, Naber D, Oberlander H et al. Efficacy and side-effects of clozapine: testing for association with allelic variation in the dopamine D_4 receptor gene. Neuropsychopharmacology 1996; 15(5):491-496.
90. Dahlman I, Arner P. Obesity and polymorphisms in genes regulating human adipose tissue. Int J Obes (Lond) 2007; 31(11):1629-1641.
91. Reynolds GP, Kirk SL. Metabolic side effects of antipsychotic drug treatment--pharmacological mechanisms. Pharmacol Ther 2010; 125(1):169-179.
92. Farooqi IS, O'Rahilly S. Genetics of obesity in humans. Endocr Rev 2006; 27(7):710-718.
93. Martinez-Hernandez A, Enriquez L, Moreno-Moreno MJ et al. Genetics of obesity. Public Health Nutr 2007; 10(10A):1138-1144.
94. Sentissi O, Epelbaum J, Olie JP, Poirier MF. Leptin and Ghrelin Levels in Patients With Schizophrenia During Different Antipsychotics Treatment: A Review. Schizophr Bull. In press.
95. Zhang Y, Proenca R, Maffei M et al. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372(6505):425-432.
96. Ellingrod VL, Bishop JR, Moline J et al. Leptin and leptin receptor gene polymorphisms and increases in body mass index (BMI) from olanzapine treatment in persons with schizophrenia. Psychopharmacol Bull 2007; 40(1):57-62.
97. Ruaño G, Goethe JW, Caley C et al. Physiogenomic comparison of weight profiles of olanzapine- and risperidone-treated patients. Mol Psychiatry 2007; 12(5):474-482.
98. Zhang XY, Tan YL, Zhou DF et al. Association of clozapine-induced weight gain with a polymorphism in the leptin promoter region in patients with chronic schizophrenia in a Chinese population. J Clin Psychopharmacol 2007; 27(3):246-251.
99. Friedman JM. Causes and control of excess body fat. Nature 2009; 459(7245):340-342.
100. Morris DL, Rui L. Recent advances in understanding leptin signaling and leptin resistance. Am J Physiol Endocrinol Metab 2009; 297(6):E1247-E1259.
101. Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. Cell 2004; 116(2):337-350.
102. Altarejos JY, Goebel N, Conkright MD et al. The Creb1 coactivator Crtc1 is required for energy balance and fertility. Nat Med 2008; 14(10):1112-1117.
103. Screaton RA, Conkright MD, Katoh Y et al. The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. Cell 2004; 119(1):61-74.
104. Bittinger MA, McWhinnie E, Meltzer J et al. Activation of cAMP response element-mediated gene expression by regulated nuclear transport of TORC proteins. Curr Biol 2004; 14(23):2156-2161.
105. Breuillaud L, Halfon O, Magistretti PJ et al. Mouse fertility is not dependent on the CREB coactivator Crtc1. Nat Med 2009; 15(9):989-990.
106. Rance KA, Johnstone AM, Murison S et al. Plasma leptin levels are related to body composition, sex, insulin levels and the A55V polymorphism of the UCP2 gene. International Journal of Obesity 2007; 31(8):1311-1318.
107. Jun HS, Kim IK, Lee HJ et al. Effects of UCP2 and UCP3 variants on the manifestation of overweight in Korean children. Obesity 2009; 17(2):355-362.
108. Kosuge K, Soma M, Nakayama T et al. Human uncoupling protein 2 and 3 genes are associated with obesity in Japanese. Endocrine 2008; 34(1-3):87-95.
109. Thorleifsson G, Walters GB, Gudbjartsson DF et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 2009; 41(1):18-24.
110. Scuteri A, Sanna S, Chen WM et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet 2007; 3(7):1200-1210.

111. Meyre D, Delplanque J, Chevre JC et al. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. Nat Genet 2009; 41(2):157-159.
112. Renstrom F, Payne F, Nordstrom A et al. Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. Hum Mol Genet 2009; 18(8):1489-1496.
113. Cunnington MS, Mayosi BM, Hall DH et al. Novel genetic variants linked to coronary artery disease by genome-wide association are not associated with carotid artery intima-media thickness or intermediate risk phenotypes. Atherosclerosis 2009; 203(1):41-44.
114. Nijman IJ, Kuipers S, Verheul M et al. A genome-wide SNP panel for mapping and association studies in the rat. BMC Genomics 2008; 9:95.
115. Gerken T, Girard CA, Tung YC et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007; 318(5855):1469-1472.
116. Farooqi IS, Keogh JM, Yeo GS et al. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med 2003; 348(12):1085-1095.
117. Yeo GS, Farooqi IS, Challis BG et al. The role of melanocortin signalling in the control of body weight: evidence from human and murine genetic models. QJM 2000; 93(1):7-14.
118. Zhiqin Li, Yingjiang Zhou, Christin Carter-Su et al. SH2B1 Enhances Leptin Signaling by Both Janus Kinase 2 Tyr813 Phopsphorylation-Dependent and -Independent Mechanism. Mol Endocrinol 2007; 21 (9):2270-2281.
119. Willer CJ, Speliotes EK, Loos RJ et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 2009; 41(1):25-34.
120. Neal M. Pharmacologie médicale. 2° édition française ed. de boeck, 2003.
121. Baumann P, Hiemke C, Ulrich S et al. The AGNP-TDM expert group concensus guidelines: therapeutic drug monitoring in psychiatry. Pharmacopsychiatry 2004; 37:243-265.
122. Hiemke C, Dragicevic A, Gründer G et al. Therapeutic monitoring of new antipsychotic drugs. Ther Drug Monit 2004; 26(2):156-160.
123. Thompson C, Peveler RC, Stephenson D et al. Compliance with antidepressant medication in the treatment of major depressive disorder in primary care: A randomized comparison of fluoxetine and a tricyclic antidepressant. Am J Psychiatry 2000; 157:338-343.
124. Kim S. Survey of medication adherence in patients with schizophrenia-Korean ADHES data. Hum Psychopharmacol Clin Exp 2006; 21:533-537.
125. Misdrahi D. Compliance in schizophrenia: predictive factors, therapeutic considerations and research implications. L'Encéphale 2002; 28(3):266-272.
126. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005; 353(5):487-497.
127. FDA FaDA. Guidance for industry: Bioanalytical method validation. U S Department of Health and Human Services,FDA,Centre for drug evaluation and research 2001.
128. Hubert P, Nguyen-Huu JJ, Boulanger B et al. Validation of quantitative analytical procedure. Harmonization of approaches. STP Pharma Pract 2003; 13(3):101-138.
129. Hubert P, Nguyen-Huu JJ, Boulanger B et al. Harmonization of strategies for the validation of quantitative analytical procedures: A SFSTP proposal Part IV. Examples of application. J Pharm Biomed Anal 2008; 48(3):760-771.
130. Osby U, Correia N, Brandt L. Mortality and causes of death in schizophrenia on Stockholm Country, Sweden. Schizophr Res 200; 45:21-28.
131. Mortensen PB, Juel K. Mortality and causes of death in first admitted schizophrenic patients. Br J Psychiatry 1993; 163:183-189.
132. Casey DE, Hansen. Excessive mortality and morbidity in schizophrenia. In: Meyer JM, Nasrallah HA, editors. Medical illness and Schizophrenia. Washington DC: American Psychiatric Publishing, 2003: 13-14.
133. Marder, et al. Physical health monitoring of patients with schizophrenia. Am J Psychiatry 2004; 161(8):133.

134. Goff DC, Cather C, Evins AE et al. Medical Morbidity and mortality in schizophrenia: guidelines for psychiatrists. J Clin Psychiatry 2005; 66(2):183-194.
135. Cohn TA, Remington G. Risk factors for coronary heart disease in longer term patients on antipsychotic medication: high prevalence of syndrome X. Schizophr Res 2003; 60:353-354.
136. Luoma PV, Sotaniemi EA, Pelkonen RO et al. Plasma high-density lipoprotein cholesterol and hepatic cytochrome P-450 concentrations in epileptics undergoing anticonvulsant treatment. Scand J Clin Lab Invest 1980; 40(2):163-167.
137. Bachmann K, Patel H, Batayneh Z et al. PXR and the regulation of apoA1 and HDL-cholesterol in rodents. Pharmacol Res 2004; 50(3):237-246.
138. Firmann M, Mayor V, Vidal PM et al. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc Disord 2008; 8:6.

Annexes

Review

Expert Opinion

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The permeability P-glycoprotein: a focus on enantioselectivity and brain distribution

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Importance of the field: The permeability glycoprotein (P-gp) is an important protein transporter involved in the disposition of many drugs with different chemical structures, but few studies have examined a possible stereoselectivity in its activity. P-gp can have a major impact on the distribution of drugs in selected organs, including the brain. Polymorphisms of the *ABCB1* gene, which encodes for P-gp, can influence the kinetics of several drugs.
Areas covered in this review: A search including publications from 1990 up to 2009 was performed on P-gp stereoselectivity and on the impact of *ABCB1* polymorphisms on enantiomer brain distribution.
What the reader will gain: Despite stereoselectivity not being expected because of the large variability of chemical structures of P-gp substrates, structure–activity relationships suggest different P-gp-binding sites for enantiomers. Enantioselectivity in the activity of P-gp has been demonstrated by *in vitro* studies and in animal models (preferential transport of one enantiomer or different inhibitory potencies towards P-gp activity between enantiomers). There is also *in vivo* evidence of an enantioselective drug transport at the human blood-brain barrier.
Take home message: The significant enantioselective activity of P-gp might be clinically relevant and must be taken into account in future studies.

Keywords: ABCB1, BBB, chirality, drug disposition, drug transporters, enantiomers, genetic polymorphism, isomer, P-glycoprotein

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1. Introduction

Permeability glycoprotein (P-gp) is a transport protein involved not only in the detoxification of cells by exporting a large number of chemically unrelated toxins but also in the multidrug resistance phenomenon occurring, among others, with anticancer agents such as the anthracyclines, vinca alkaloids and taxanes [1]. The gene coding for P-gp is the ATP-binding cassette 1 (*ABCB1*) gene, which shares many characteristics with bacterial and eucaryotic ABC transporter genes, given that this family has been highly conserved throughout phylogenetic evolution [2]. P-gp is a 170 kDa plasma membrane protein and is one of the 48 members of the ABC transporters. It consists of 1280 amino acids that are organized into two similar halves (43% amino-acid identity) and are joined by a linker domain. Each half exhibits six transmembrane domains (TMD) followed by a hydrophilic nucleotide-binding domain (NBD) (**Figure 1**) [3]. Each NBD contains two consensus motifs, namely Walker A and Walker B motifs, and a signature C motif, unique to the ABC transporter family. These motifs play a critical role in ATP binding and hydrolysis, which converts ATP to ADP, producing the energy needed by the transporter to pump the substrates across the membrane. The binding of ATP on the NBDs also induces conformational changes in the TMDs, which form the pathway through which the substrates pass the membrane [3].

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Article highlights.

- Present knowledge on permeability glycoprotein (P-gp) structure and localization in critical organs shows its key role in the distribution of numerous drugs. Functional activity and recent binding information allow a better understanding of the interaction with its substrates. This highlights the clinical impact of P-gp from a therapeutic perspective.
- P-gp can expel its substrates from membranes at different levels, namely absorption, distribution and excretion. Thus, P-gp has an influence on the pharmacokinetics of its substrates.
- Many compounds with a wide range of structures were reported to be substrates of P-gp. Structure–activity relationships were found. Recent reviews have even shown different P-gp-binding sites for isomers or enantiomers.
- Several therapeutic classes of drugs are P-gp substrate and their transport by or inhibition of P-gp can differ between enantiomers or stereoisomers.
- Different polymorphisms in the *ABCB1* gene can lead to a change in its expression or in its activity. The polymorphisms' occurrence can be different depending on the ethnic population.
- Some *ABCB1* polymorphisms were shown to have clinical side effects or therapeutic response related consequences at the CNS. Some reports included some examples of chiral drugs.

This box summarizes key points contained in the article.

Among the structural information on P-gp published up to present, an important understanding is given by the publication of the 3.8 Å crystal structure of mouse P-gp that shows a large internal cavity of ~ 6000 Å^3, and a 30 Å separation of the two NBDs [4]. Despite the size of the cavity, the structure of P-gp with the QZ59 molecule, a cyclic hexapeptide with SSS- and RRR-isomers, showed that P-gp can distinguish between stereoisomers mainly because they have different binding modes. These structures are open to the cytoplasm and the inner leaflet, corresponding to the inward-facing conformation of P-gp. Entrance of hydrophobic molecules directly from the membrane could be possible through the portals formed by the TMD 4/6 and 10/12 [4].

In another report, the 3D structure of mouse P-gp previously reported [4] was used to describe a model of human P-gp for the inward-facing conformation of the protein [5]. This model confirms the role of TMDs 4, 6, 10 and 12 as entrance gates to the internal cavity and describes differences in their functions [5]. Furthermore, the analysis of human P-gp interactions with the QZ59 stereoisomers confirms the multi-specific binding of P-gp with its ligands [5]. For a detailed review on structures, functions and binding characteristics of ABC transporters, see reference [6].

P-gp is expressed in the apical membrane of epithelial cells from the colon, small intestine, kidney, liver, placenta, adrenal glands and the blood-brain barrier (BBB) [7]. P-gp transports a wide variety of substrates very different in terms of size, function and structure. These include many classes of drugs, such as immunosuppressants, calcium-channel blockers, opioids, anti-HIV drugs, chemotherapeutics and drugs acting at the central nervous system (CNS) level. Exhaustive lists of P-gp substrates, inhibitors and inductors are reported elsewhere [3,7,8].

For drugs that have to reach the CNS to exert their effect, the distribution into the brain is essential. At this level, two physiological barriers are present: the BBB, which isolates the brain interstitial fluid from endothelial cells in brain capillaries, and the blood–cerebrospinal fluid barrier (BCSFB), which separates the cerebrospinal fluid (CSF) from the vascular choroid plexus and from the arachnoid membrane and controls the exchange of water, ions and substances between blood and CSF [2].

These two barriers express on their surface transport proteins from the family of the ABC transporters, which belong mainly to three subfamilies: ABCB (P-gp/*ABCB1*), ABCC (multidrug resistance-associated protein 1 (MRP1)/*ABCC1* gene coding for MRP1 protein) and ABCG (BCRP/*ACG2* gene coding for breast cancer protein) [9]. These proteins play a role in protecting the brain from toxic effects of xenobiotics, by pumping them out of the brain endothelial cells, but also reduce drug penetration into other organs and into tumors, producing the multidrug resistance phenotype [10]. Even if the ABC transporters are present at both the BBB and the BCSFB, their relative distribution between the two barriers is not yet completely elucidated. A different localization of P-gp and Mrp1 proteins was described in rat brain, with P-gp being predominantly localized in the BBB and Mrp1 in the BCSFB, respectively [11]. The same pattern of localization was observed in humans. This different localization could be explained by the characteristics of substrates of the two transporters. While P-gp substrates are rather lipophilic compounds, MRP1 transports more amphiphilic substances, such as organic anions including conjugated molecules. Consequently, the localization of P-gp in the BBB would prevent the accumulation of lipid toxins into the brain parenchyma (of lipid-rich nature), while the role of the MRP1 at the BCSFB may be to limit the access of amphiphilic compounds to the CSF and to contribute to the detoxification made by the choroidal tissue [11].

Molecular chirality is fundamental in biological processes because enantiomers of endogenous substrates or xenobiotics can interact and bind differently with enzymes, transporters or receptors. Enantiomers can display different pharmacological and toxicological profiles: the two enantiomers might be equally pharmacologically active, or only one enantiomer can have the main therapeutic activity while the other is inactive, or can produce a different/opposite or even toxic effect [12-14]. Studies on the enantiomers of chiral drugs are thus essential to understand their therapeutic and toxic profiles. The present paper reviews the role of P-gp on the pharmacokinetics of drugs, in particular its implication on the blood to brain distribution. A special emphasis is also placed

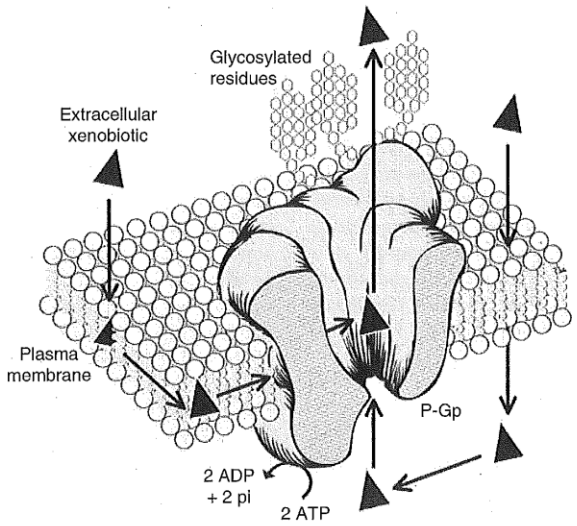

Figure 1. P-gp function. This model shows that P-gp-mediated efflux transport of drug substrates can occur at the level of the plasma membrane or from the intracellular compartment.
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P-gp: Permeability glycoprotein.

on data suggesting a possible stereoselective activity of this transporter, with their potential clinical implications.

1.1 Research strategy for writing this review

The strategy used to identify relevant studies consisted in a search of the Medline and Pubmed database, using the following keyword combination:

- P-glycoprotein, ABCB1, blood brain barrier, drug transporters, drug disposition, ABCB1 polymorphism, brain concentration

with

- Chirality, enantioselectivity, enantiomer, isomer, stereoselectivity

This search was limited to studies in relation with humans, drug components and to reports published after 1990. In a second step, some selected publications appearing in the reference lists of the identified studies were also examined.

2. P-gp and pharmacokinetics

2.1 Absorption

In the intestine, P-gp pumps the drugs out of the mucosa cells back into the lumen, therefore, reducing their absorption and bioavailability [9]. For example, the absorption of anti-HIV protease inhibitors indinavir, nelfinavir and saquinavir, which is generally low, was found to be two to five times higher in *abcb1a* knockout mice than in wild-type mice [15]. Similarly, the bioavailability of tacrolimus after oral administration was three times higher in knockout mice compared to wild-type mice [16].

Interestingly, absorption of some drugs depends on the dose and on the site of absorption in the intestine. This has been illustrated for the β-blocker drug talinolol in rats: at a low perfusate concentration of 0.025 mmol/l, P-gp-mediated transport is important, thus, limiting the absorption of the drug [17]. On the contrary, at a higher concentration of 0.5 mmol/l, P-gp transport is saturated, and so drug absorption is enhanced. Moreover, talinolol absorption has been shown to decrease along the intestine (jejunum-ileum-colon), in agreement with the parallel increase of P-gp mRNA levels and expression [17].

2.2 Distribution

At the BBB, P-gp is involved in the excretion of drugs and their metabolites, thus, protecting the brain from the potential toxic effects of xenobiotics [2]. This has been demonstrated in *abcb1* knockout mice, in which brain penetration was increased for several drugs: ivermectin, dexamethasone, morphine, vinblastin, digoxin, quinolones, cyclosporin A or HIV-protease inhibitors [18]. Thus, the antiparasitic ivermectin produced an almost 100-fold higher neurotoxicity in the *abcb1a* knockout than the wild-type mice [19]. Similarly, the *abcb1a/1b* knockout mice showed a 27-fold increase in

the brain and 3.3-fold increase in the CSF of amprenavir (a HIV-protease inhibitor) concentration [20]. The role of P-gp in brain distribution is also emphasized by the antihistaminic H_1 drugs. First generation antihistamines are known to produce side effects such as sedation, caused by their brain distribution, given that they do not present affinity for P-gp. On the contrary, second generation antihistamines are usually non-sedating, because they are recognized by P-gp and, therefore, their brain distribution is limited [21]. Transport of drugs also occurs in other blood–tissue barriers. In placenta, P-gp is expressed on the maternal side of placental cells and not on the fetal side, thus, restricting the transport of xenobiotics from mother to fetus and protecting the fetus from their potential toxicity [22].

2.3 Excretion

Some drugs that are excreted by active secretion into the bile, such as grepafloxacin, have their transport mediated by P-gp, and P-gp inhibitors (such as doxorubicin, cyclosporin A or erythromicin) can strongly inhibit their biliary secretion [23]. Similarly, because renal secretion of digoxin is mainly mediated by P-gp, a P-gp inhibitor such as clarithromycin might diminish its renal excretion, resulting in increased plasma concentration and consequent side effects [24]. Albendazole, an antihelmintic drug widely used in human and veterinary medicine, undergoes intestinal and hepatic metabolism to produce albendazole sulfoxide as the active metabolite. The study of intestinal elimination in rat showed a significant interaction between albendazole metabolites and drug efflux inhibitors and also a stereoselective intestinal elimination of (-) albendazole sulfoxide enantiomer [25].

3. P-gp substrates, structure–activity relationship and stereoselectivity

Given the heterogeneity of P-gp substrates, the structural requirements for a substance to be a P-gp substrate are hardly defined. Hundreds of P-gp substrates have been identified, with different physico-chemical properties, but a typical substrate compound could be considered a large molecule (Mr > 400 kDa), hydrophobic, amphipathic, with a planar mainly aromatic ring system, and usually positively charged at physiological pH [26]. But P-gp transports also neutral compounds (digoxin, cyclosporin A), substances with negatively charged carboxyl groups (fexofenadin, atorvastatin), hydrophilic drugs (methotrexate, peptides) or drugs weighing < 400 kDa (cimetidin, ranitidin) [27].

Quantitative structure–activity relationships as well as computational models have increasingly been used in order to produce more efficient screening techniques for identification of molecular properties necessary to bind to a pharmacophore. These techniques can be used in order to identify interactions with drug transporters such as P-gp. Different pharmacophore models have been used that can discriminate between P-gp substrate or non-substrate based on monolayer efflux classification [3,28-30]. Some other models have suggested the discrimination being due to physicochemical properties such as lipophilicity, hydrogen-molecular mass or bonding ability, with recent models being able to predict P-gp substrate with accuracy up to 90% [3]. A 3D pharmacophore approach combined with statistical analysis to develop a method that could differentiate P-gp substrates from non-substrates has also been used [31]. It was shown that important characteristics for substrate affinity might be hydrogen-bond acceptors, aromatic rings, hydrophobic groups and a positive ionizable group [31]. The importance of these 3D criteria was also recently demonstrated for the binding of a series of flavonoids to the ATP-binding site [32].

Interestingly, when reviewing P-gp pharmacophore modeling, it was reported that the presence or absence of an m-nitrophenyl group on a drug with dihydropyridine structure can change enantioselective interaction with P-gp (calculated with the EC_{50} of the intracellular accumulation of vinblastin) [30]. By listing enantiomers of PET-tracers, a stereoselective transport across the BBB by human and other mammalian P-gp was shown, corroborating the enantioselectivity hypothesis of P-gp transport [33]. Moreover, a report suggested some differences for the two enantiomers in the P-gp-binding sites recognition [30]. Competition between cyclosporin A and mefloquine has been described with isomers of mefloquine binding to multiple sites on P-gp molecules. Interestingly, the (+)-mefloquine binds not only to its site, but also onto the site where cyclosporin A equally binds. This is not the case with the (-)-mefloquine enantiomer where no competition to the cyclosporin A-binding site was observed [30].

Finally, more data are available on stereoselective differences for diasteroisomers and geometrical isomers. In a recent report of a crystal structure of mouse P-gp, which is 87% identical to human P-gp, it was shown that this transporter can distinguish between the two stereoisomers of QZ59, a cyclic hexapeptide as previously described (see Section 1) [4]. While QZ59-RRR binds at the center of P-gp protein in one site, the QZ59-SSS binds at two sites of P-gp on an upper position with an overlap. This report illustrates the possible different binding sites of isomers, and thus the possibility of discrimination between stereoisomers by P-gp [4]. In another study using a cell model, *cis* and *trans*-isomers of flupentixol inhibit P-gp-mediated transport [30]. The pharmacological potency of doxorubicin, a P-gp substrate, was increased by 15-fold by *trans*-flupentixol as compared to *cis*-flupentixol. Finally, a P-gp stereoselectivity was also demonstrated *in vitro* between quinine and quinidine, two diastereoisomers [34].

4. Chiral drugs and P-gp transport

4.1 Cardiovascular drugs

The stereoisomers of several calcium antagonists were studied for their potencies as calcium channel blockers and as P-gp inhibitors [35,36]. The inhibition of P-gp was tested by their

capacity to increase intracellular accumulation of [3H]-vinblastin in a doxorubicin-resistant cell line [37]. For the substances with phenylalkylamine structures such as (+/-)-verapamil, and with dihydropyridine structures such as (+/-)-felodipin or (+/-)-niguldipine, there were marked differences in their potencies as calcium blockers, but similar inhibitory activity of P-gp. Thus, both verapamil and niguldipine enantiomers showed different potencies as calcium blockers: (+)-verapamil was 10-fold less potent than the (-)-isomer, (-)-niguldipine was 45-fold less potent than the (+)-form, while the enantiomers had similar activities as P-gp inhibitors. In another study on the uptake of daunomycin into rat liver by P-gp, no difference in the inhibitory effects between the enantiomers of verapamil was found [35]. No stereoselectivity in the transport and on the distribution of (R)- and (S)-[^{11}C]verapamil was found *in vivo* in *abcb1a/1b* double knockout or wild-type mice, in the BBB and in testis and no stereoselectivity was observed either in the *in vitro* experiments [36]. A stereoselective pharmacokinetics of verapamil can thus be explained by the higher metabolism rate for (S)-verapamil, with a bioavailability of 20 and 50% for the (S)- and (R)-enantiomer, respectively, after its oral administration in healthy volunteers [38]. Another dihydropyridine analogue, telupidine, showed a similar P-gp inhibitory activity for its R- and S-enantiomers in two daunorubicin resistance cell lines. Given that (+)-verapamil, (-)-niguldipine and (R)-telupidine have low affinity to calcium channels, it has been proposed that these compounds could be used together with chemotherapy to reverse multiple drug resistance in cancer patients [37,39]. However, more specific P-gp inhibitors with less side effects have been recently developed such as elacridar, valspodar and zosuquidar [8,40,41].

The antiarrhythmic agent propafenone and its metabolites (5-hydroxypropafenone and N-desalkylpropafenone) were studied in a Caco-2 cell line model in order to assess their potential as P-gp substrates or inhibitors [42]. Only 5-hydroxypropafenone was shown to be a P-gp substrate, while both metabolites and propafenone were inhibitors of P-gp, as shown by the digoxin transport model in the cell monolayer. Interestingly, (R)-propafenone had a more pronounced effect on digoxin translocation, suggesting that propafenone enantiomers interact in a stereoselective manner with P-gp [42]. Similar results were obtained in another study, which showed a statistically significant higher inhibitory activity of (R)-propafenone as compared to the (S)-enantiomer [43]. The GP-88, a propafenone-type P-gp-modulator, was also synthesized and the four isomers ((R,R), (R,S), (S,R) and (S,S)) were studied. It was shown that the (R,R) and (S,R)-stereoisomers exhibited an almost twice higher inhibition on the P-gp-mediated daunomycin transport than the (R,S) and (S,S)-forms [43].

Propranolol demonstrated a stereospecific inhibition of daunomycin transport, which was significant for (R)-(+)- and (R,S)-(+,-)-propranolol, while no inhibition was observed with (S)-(-)-propranolol [35]. Finally, a marginal stereoselectivity in the disposition of talinolol was found in healthy volunteers, but this was considered most likely to be due to the presystemic biotransformation by the cytochrome P4503A, rather than a stereoselective transport by P-gp [44].

4.2 Opioids

Methadone is a synthetic opioid used for the treatment of opioid addiction and pain. It is usually administered as a racemic mixture, but (R)-methadone is mainly responsible for the opioid effect [45]. Methadone is a substrate and an inhibitor of P-gp, which has been demonstrated in several *in vitro* models [46,47]. The enantiomers of methadone were tested with regards to their transport by P-gp and it was concluded that both (R)- and (S)-enantiomers are substrates of P-gp [48]. A stereoselective activity of P-gp for (S)-methadone was suggested, given that the brain concentrations of (R)-methadone were significantly higher in the *abcb1* knockout mice than in wild-type mice. Another study, using human *ABCB1*-transfected cells confirmed that methadone and another opioid with a similar chemical structure, namely L-α-acetylmethadol, are substrates of human P-gp [49]. The authors also reported a weak stereoselectivity in methadone transport towards the (S)-form, at high incubation concentrations.

The distribution of *trans*-tramadol and its metabolite (*trans-O*-demethyltramadol) in the CNS was studied in rats. The distribution of both substances was stereoselective, as higher concentrations of (+)-*trans*-tramadol and of (-)-*trans-O*-demethyltramadol were found in several brain areas [50]. The authors suggested that distribution of the enantiomers of these substances could be produced by active and/or facilitated transport, but no contribution of P-gp was mentioned, and this was even recently excluded [51].

4.3 Other chiral drugs

Butaclamol, an antipsychotic drug, was shown to stereoselectively inhibit P-gp activity, with (-)-butaclamol having a more potent inhibitory activity in an *in vitro* cell model than the (+) enantiomer [52]. No data are however available on a possible stereoselective transport of the two enantiomers by P-gp *in vivo*. The transport of daunorubicin and its enantiomers in multi-drug resistant cells overexpressing P-gp or MRP1 was also studied to evaluate if chirality plays a role in the multidrug resistance to daunorubicin [53]. Both P-gp and MRP1 transported the two enantiomers equally, and so the authors concluded a lack of stereoselectivity in the transport of daunorubicin by the two proteins.

The inhibitory effect of itraconazole, a P-gp inhibitor, on the stereoselective pharmacokinetics of fexofenadine was also studied in healthy volunteers [54]. Itraconazole affected the pharmacokinetic parameters of (S)-fexofenadine to a greater extent than those of (R)-fexofenadine. The results suggested that the stereoselective pharmacokinetics of fexofenadine could be associated with P-gp-mediated transport.

5. Pharmacogenetics

ABCB1 gene is composed of 29 exons encoded by a 209-kb gene located on chromosome 7 at q21.12, with to date > 1750 variants being identified, among which about 40 single nucleotide polymorphisms (SNPs) are missense (last NCBI database search on October 2009, NCBI Reference Sequence: NT_007933.14). The three most common SNPs described are *C1236T*, *G2677T* and *C3435T*, located in exons 12, 21 and 26, respectively (**Figure 2**) [3,7,55-58]. Allelic frequencies were reported to vary with ethnicity. In Afro-Americans and in Caucasians, homozygote *3435C* was reported in 61 and 26% of subjects, respectively [59,60]. It has been suggested that haplotypes, that is, combinations of SNPs, are better correlated with phenotype than single SNPs, and the frequency of the two major haplotypes (*ABCB1*1* and *ABCB1*13*) also differed between ethnicity [57]. Thus, the Caucasian population has a twofold higher *ABCB1*13* frequency than the *ABCB1*1*, whereas in the Afro-American population, *ABCB1*1* is the most common haplotype. Therefore, special interest should be given to ethnicity when studying response to treatment for drugs which are P-gp substrates.

The impact of *ABCB1* polymorphisms on P-gp expression and function as well as on the kinetics of several P-gp substrates yielded equivocal results [7]. The synonymous *3435C > T* SNP, which is in linkage disequilibrium with the non-synonymous *2677 G > T* SNP, was the first to be associated with lower duodenal P-gp expression [61], possibly due to a decrease in mRNA stability [62]. Another study found similar mRNA and protein levels in the variant and wild-type proteins, but observed an altered conformation of the variant protein, thereby, affecting the structure of substrate and inhibitor interaction sites [63]. After the initial study of Hoffmeyer *et al.* suggesting that higher digoxin plasma levels in carriers of the *3435T* allele are caused by a twofold reduction of P-gp expression in the intestine [61], controversial results were subsequently published in both Caucasian and Asian populations [7]. However, although few clinical trials have described a clear clinical outcome of *ABCB1* polymorphisms, some studies have shown that functional relevant *ABCB1* polymorphisms may influence intracerebral drug concentration and thus the clinical response in terms of effectiveness or side effects [64]. For instance, a clinical study genotyped depressed patients under nortriptyline (n = 78), which is P-gp substrate. Although nortriptyline dose and plasma concentration did not differ between genotype groups, the risk of developing postural hypotension was higher in the *3435T* allele carriers [65].

Interestingly, a stereoselectivity of *ABCB1* gene regulation has been suggested by examining P-gp expression in Caco-2 cells treated with cetirizine enantiomers [66]. While (R)-cetirizine promoted overexpression of P-gp, the (S)-enantiomer led to downexpression, both in a concentration-dependent manner. Thus, Caco-2 cells pretreated with (S)-cetirizine displayed a higher sensitivity to paclitaxel, as determined by the IC_{50} value, and a lower rate of transport of P-gp substrates (rhodamine-123 and doxorubicin). These results were confirmed by measuring the quantity of *ABCB1* mRNA (by RT-PCR) and the content of P-gp (by flow cytometry) in Caco-2 cells, with increased levels of *ABCB1* mRNA and P-gp in cells pre-incubated with (R)-cetirizine and decreased levels with (S)-cetirizine [66].

Transcription of *ABCB1* gene can be modulated by exposure to mediators of inflammation or cell stress [2]. Thus, inflammation which occurs in many CNS diseases produces several inflammatory mediators such as cytokines, which can reduce P-gp activity. On the other hand, reactive oxygen species, which are produced during cellular stress or heat stress, enhance the expression of P-gp [67]. Another important modulator of P-gp expression consists of gene coregulators. *ABCB1* gene expression is regulated by several nuclear receptors, such as nuclear pregnane X receptor (PXR) [68] and other membrane transporters such as organic anion transporter (OATPB1) [69]. PXR and orphan nuclear steroid X receptor have been identified for being responsible for the PXR-mediated induction of the *ABCB1* gene [68-70]. PXR coregulates drug metabolism and transport through the *CYP3A* and *ABCB1* genes in liver and intestine [69,71], and P-gp expression can also be regulated by different xenobiotics, such as dietary compounds, drugs or toxins [67]. Moreover, regulation of P-gp expression was also described at the BBB. Dexamethasone, a PXR-ligand, increased P-gp expression in rat brain capillaries at the BBB [68]. As several polymorphisms were described for the *PXR* gene, the clinical relevance of *PXR* polymorphisms on P-gp expression remains to be assessed.

6. Effect of ABCB1 gene polymorphisms on central therapeutic effects

6.1 Fexofenadine

Fexofenadine is a selective H1-receptor antagonist administered as a racemic mixture. A study examined the pharmacokinetics of fexofenadine in the presence and in the absence of itraconazole, a P-gp inhibitor, in healthy subjects with the *ABCB1* wild-type haplotype *2677GG/3435CC* (G/C, n = 7) and mutated haplotype *2677TT/3435TT* (T/T, n = 7) [72]. Fexofenadine pharmacokinetics was similar between haplotypes with placebo, while itraconazole produced a significant increase of the AUC of fexofenadine, depending on the genotypes. Interestingly, the peripheral anti-histamine effects (skin challenge test) of fexofenadine were significantly stronger with itraconazole than with placebo. Moreover, the mean fexofenadine AUC for the T/T haplotype was significantly ($p = 0.007$) higher than for the G/C haplotype (**Figure 3**). Even if the haplotypes have a moderate effect on fexofenadine, when a P-gp inhibitor such as itraconazole is taken along with fexofenadine, the peripheral effects of the drug are significantly different between haplotypes [72].

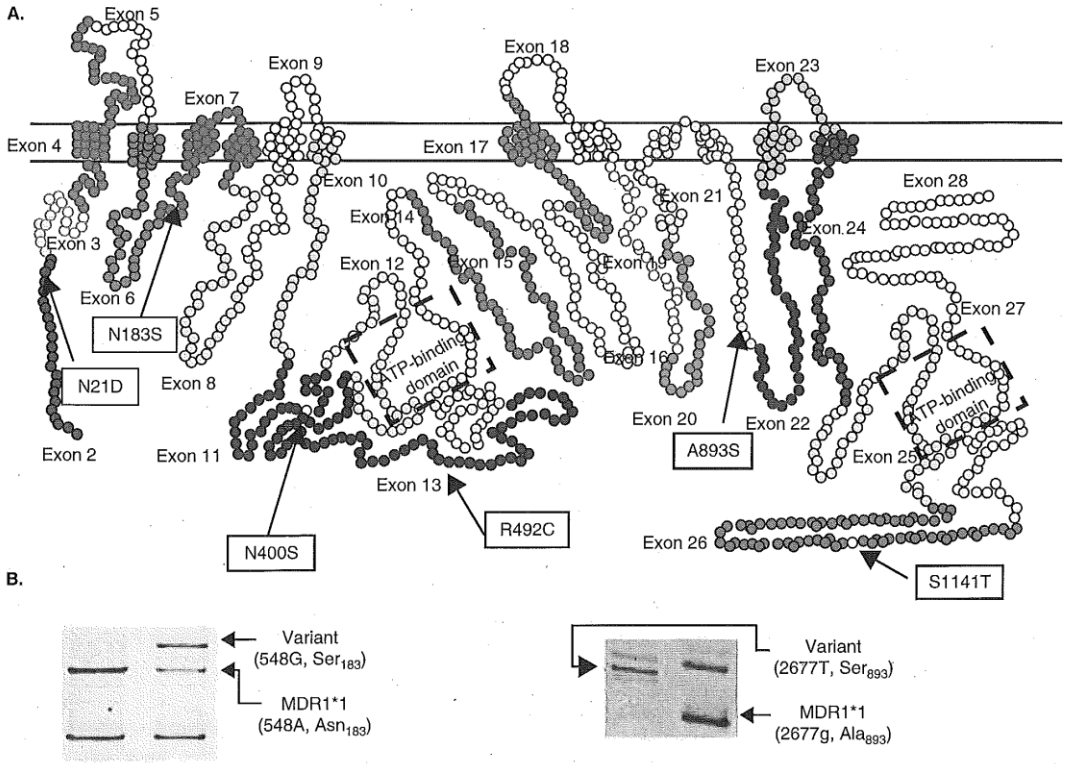

Figure 2. Schematic drawing of human P-glycoprotein transporter; exons encoding regions of P-glycoprotein are indicated in different colors, whereas nonsynonymous nucleotide substitutions identified in this study are indicated by arrows. A and B represent SSCP patterns of two identified MDR1 variants. The variant bands were sequence verified.

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KIM RB: Identification of functionally variant MDR1 alleles among European Americans and African Americans; MDR1: Multidrug resistance 1.

The stereoselective transport of fexofenadine by P-gp was suggested by a higher P-gp affinity for (S)(-)- fexofenadine than for the (R)(+)- enantiomer as shown by a study with six healthy volunteers [73]. This is in agreement with a study with 13 healthy Japanese volunteers showing that verapamil (a P-gp inhibitor) produced a greater effect on the pharmacokinetics of (S)(-)- fexofenadine compared to (R)(+)- fexofenadine [74]. Indeed, with verapamil, the AUC of the (S)(-)- and of the (R)(+)-enantiomer was 3.5- and 2.2-fold higher, respectively. Although *ABCB1* haplotype effects on fexofenadine pharmacokinetics were studied, the role of haplotypes on the two enantiomers of fexofenadine remains to be further assessed [74].

6.2 Citalopram

ABCB1 G2677T polymorphism was found to significantly influence (R,S)-citalopram plasma and CSF concentrations in 15 patients treated with this drug but no stereoselectivity in the activity of P-gp could be demonstrated [75]. This lack of stereoselectivity for citalopram is consistent with the results of an *in vitro* study with cells isolated from bovine brain microvessels endothelial [76]. In another study, several antidepressants (citalopram, venlafaxine and mirtazapine) were injected subcutaneously in knockout mice lacking the human P-gp and in mice transfected with human P-gp [77]. The knockout mice showed higher intracerebral (but not plasma) antidepressant concentration than the wild-type littermate for citalopram and venlafaxine but not for mirtazapine, suggesting that the two former drugs, but not the latter, were P-gp substrates. The same antidepressants were further investigated in humans with depressive disease [77]. The association of *ABCB1* polymorphism with the number of weeks before remission was assessed. Different SNPs were associated with the phenotype of remission, and carriers of the C allele for the *rs2032583* genotype had higher odds ratio for remission (7.72, 95% CI 2.8 – 21.3) at 4 weeks

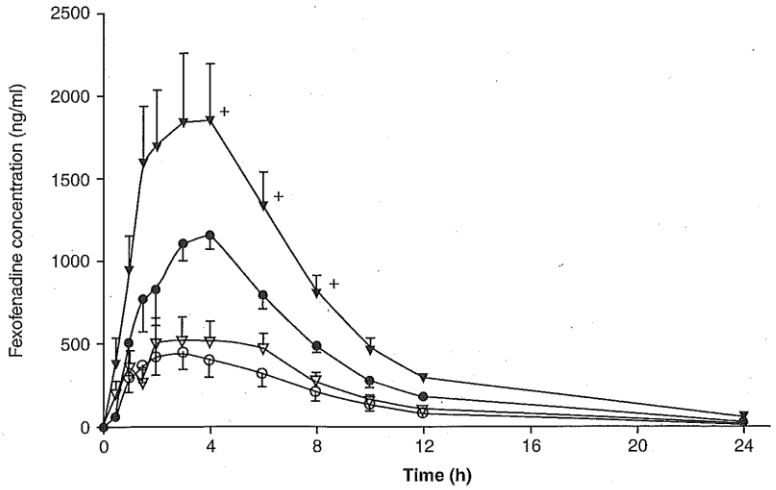

Figure 3. Mean plasma concentration (± s.e.) of fexofenadine after oral administration of 180 mg single dose when pretreated with placebo (open symbols) or 200 mg itraconazole (solid symbols) in MDR1 gene 2677G/3435C (circles) and 2677T/3435T (triangles) haplotype groups. Plus sign, $p < 0.05$, rank sum test between MDR1 haplotype groups.
Reprinted by permission from Macmillan Publishers Ltd [72].
MDR1: Multidrug resistance 1; SHON: Effect of itraconazole on the pharmacokinetics and pharmacodynamics of fexofenadine in relation to the MDR1 genetic polymorphism.

compared to T-carriers when treated with P-gp substrate antidepressants (citalopram, paroxetine, venlafaxine, amitriptyline). This correlation was even stronger when restricted to patients with unipolar depression, with an odds ratio of 9.4 (95% CI 3 – 29.4). Interestingly, that association was not found for non-P-gp substrates such as mirtazapine [77].

6.3 Mefloquine

The antimalaric mefloquine is known for its neuropsychiatric side effects. The association between some polymorphisms in the *ABCB1* gene and the frequency of neuropsychiatric side effects has been investigated in a prospective study with 89 healthy Caucasians taking mefloquine [78]. Higher risks for psychiatric side effects were significantly detected for the women double mutated (TT) for each of the three SNPs C1236T (rs1128503), G2677T (rs2032582), and C3435T (rs1045642) than the women in the wild-type group. Moreover, the TTT haplotype led to an even higher risk of neurotoxicity with an odds ratio of 4.5 (95% CI 1.3 – 16). No association was found either with male subjects or with plasma concentration [78]. The latter finding could be explained by the stronger influence of P-gp at the target organs (i.e., brain) than in the blood. It has been hypothesized that a higher serum concentration of mefloquine in combination with a lower basal expression of *ABCB1* makes women more susceptible to the effect of *ABCB1* polymorphism [78]. In another study, a higher brain:plasma ratio of (+)-mefloquine than (-)-mefloquine was determined in human cerebral biopsies in two patients receiving mefloquine treatment, suggesting a stereoselective brain uptake [79]. A stereoselective transport of mefloquine across the BBB was confirmed in a mice study as well, with (+)-mefloquine being excreted faster than (-)-mefloquine, after a single intraperitoneal administration of the racemic mixture [41]. Interestingly, the higher brain concentration of (-)-mefloquine was replicated in another rat study, suggesting the inversed stereoselectivity of mefloquine brain penetration between human and rodent [80]. Moreover, following a treatment with elacridar, a potent P-gp inhibitor, the excretion rate in mice decreased for the two enantiomers, with a more pronounced effect for (+)-mefloquine than for (-)-mefloquine [41].

6.4 Loperamide

Along with studies on P-gp inhibition resulting in drug-related central side effects, the potential respiratory depression following potent μ-opioid receptor agonist treatment was tested. Loperamide, an anti-diarrheal treatment which, under normal conditions presents no central side effect, was prescribed randomly in a double-blind study with either placebo or quinidine, a P-gp inhibitor [81]. Respiratory depression was significantly ($p < 0.001$) observed in the 8 healthy white volunteers when quinidine was present [81]. Based on this result and the hypotension side effect occurring during nortriptyline treatment for *3435TT* genotype [65], another study examined the effect of loperamide on respiratory depression in healthy white volunteers with *3435TT* (n = 8) and *3435CC* (n = 8)

Table 1. Consequences of *ABCB1* polymorphisms on function of P-gp at the BBB.

Study	Population	SNP	Functional outcome
Roberts *et al.* [65]	78 Depressive patients with nortriptylin	C3435T	Increased risk of postural hypotension
Yamauchi *et al.* [83]	17 Hepatic grafted patients with tacrolimus	G2677T	Increased risk of neurotoxicity
		C3435T	Decreased risk of neurotoxicity
Aarnoudse *et al.* [78]	89 Patients with mefloquine	C1236T	Increased risk of neuropsychiatric side effects
Uhr *et al.* [77]	443 Depressive patients with different antidepressants (citalopram, paroxetine, venlafaxin, amitriptyline)	G2677T/A	Increased rate of remission after 4 weeks of treatment

P-gp: Permeability glycoprotein; SNP: Single nucleotide polymorphism.

genotypes [82]. No influence of *ABCB1* genotypes could be found, possibly due to overlapping substrate specificity between P-pg, cytochrome P450 or other brain transporters such as OATP, as well as a limited number of participants [82].

6.5 Tacrolimus

Tacrolimus, an immunosuppressant, is known to induce neurotoxicity in patients treated with this drug following transplantation. In a study on liver transplantation, patients with (n = 6) and without neurotoxic events (n = 11) were genotyped for *ABCB1* polymorphisms [83]. The *G2677T/A* SNP in exon 21 was found to be a significant predictor for the development of neurotoxicity, while the polymorphism *C3435T* in exon 26 contributed negatively, despite the close linkage (84.6%) between the two SNPs. Even if further studies with higher sample size are needed to confirm these findings, the authors suggested that *ABCB1* genotype could be a predictor of tacrolimus-induced neurotoxicity, together with high tacrolimus concentration and liver dysfunction [83].

6.6 Ivermectin

Ivermectin is a potent antiparasitic drug from the avermectin group of compounds. Intravenous injection in mice leads to about 90-fold higher brain concentration in P-gp knockout mice than in wild type, while plasma concentration does not change much (threefold) [19]. Moreover, the neurotoxic side effects of ivermectin were more strongly observed in knockout mice, which compared with wild-type mice required a 100-fold lower dose of ivermectin for getting similar LD_{50} [19]. The neurotoxicity of avermectin is associated with its brain concentration and depends on P-gp function at the BBB in mice [84]. The neurologic toxicity is equally described in humans [85]. However, its neurotoxicity related to P-gp activity remains unclear, but it can be the consequence of a loss and/or a diminished function linked to some *ABCB1* haplotypes. Based on the lack of subjects with a total absence of P-gp activity, it has been concluded that neurotoxicity is likely to be rare in patients treated with avermectin or with other structurally related drugs such as avermectin pesticides [85]. On the other hand, such neurotoxicity could occur in the presence of drugs and/or xenobiotics which are P-gp inhibitors.

Table 1 lists the clinical impact of *ABCB1* polymorphisms on P-gp transport activity at the BBB. Consequences of *ABCB1* polymorphisms on pharmacokinetics of substrate drugs can be found elsewhere [7,59].

7. Conclusion

Enantioselectivity in the activity of P-gp has been shown for several drugs, either by a preferential transport of one enantiomer or by different inhibitory potencies towards P-gp activity between enantiomers. This can result in different brain concentrations of one enantiomer, influencing the central effect of chiral drugs. Several *ABCB1* polymorphisms have been associated with different responses to drugs. However the potential influence of *ABCB1* polymorphism on enantioselectivity of P-gp activity and on enantiomer brain distribution remains to be determined, making studies on this topic a challenge in future clinical research.

8. Expert opinion

P-gp has a wide range of substrates with broad chemical structures and a chiral discrimination by this transporter is not expected. However, some studies have shown a significant enantioselective activity of this protein which might be clinically relevant and must be taken into account in future studies. In addition, chirality of P-gp substrates might also be important in the reversal of multidrug resistance for anticancer drugs if it can be coupled with a large enantioselectivity in therapeutic and/or toxic effects (i.e., use of pharmacologically inactive enantiomers to block P-gp activity). So far, in clinical studies, much attention has been generally paid to the racemate and less attention to the enantiomers. The need to develop chiral analytical methods could contribute, in part, to the paucity of studies on enantiomers but the increasing simplicity of chiral methods might shift the focus towards a broader and more comprehensive vision. Concerning the pharmacogenetics of P-gp, the clinical importance of *ABCB1* gene polymorphisms is limited in humans by the lack of subjects with absence of P-gp activity, such genotypes being found naturally in other species (e.g., in dogs). However, because P-gp can play a major role in the availability at target organs, in particular in the

brain, even moderate differences in the variability of P-gp activity can produce clinically relevant differences in the therapeutic and/or unwanted side effects. Further studies are, therefore, needed to examine a stereoselectivity in the activity of P-gp, the influence of *ABCB1* genetic polymorphisms, with the analysis of haplotypes, and their clinical impact *in vivo*, also taking into account the ethnicity of the studied populations. Such studies will contribute to the build up of knowledge necessary for a future drug prescription tailored to the genetic basis of the patient.

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Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Goda K, Bacso Z, Szabo G. Multidrug resistance through the spectacle of P-glycoprotein. Curr Cancer Drug Targets 2009;9:281-97
2. Scherrmann JM. Expression and function of multidrug resistance transporters at the blood-brain barriers. Expert Opin Drug Metab Toxicol 2005;1:233-46
3. Zhou SF. Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. Xenobiotica 2008;38:802-32
4. Aller SG, Yu J, Ward A, et al. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science 2009;323:1718-22
5. Pajeva IK, Globisch C, Wiese M. Comparison of the inward- and outward-open homology models and ligand binding of human P-glycoprotein. FEBS J 2009;276:7016-26
6. Eckford PD, Sharom FJ. ABC efflux pump-based resistance to chemotherapy drugs. Chem Rev 2009;109:2989-3011
7. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther 2004;75:13-33

•• **Reviews clinical impact of ABCB1 polymorphisms.**

8. Kim RB. Drugs as P-glycoprotein substrates, inhibitors, and inducers. Drug Metabolism Reviews 2002;34:47-54
9. Sharom FJ. ABC multidrug transporters: structure, function and role in chemoresistance. Pharmacogenomics 2008;9:105-27
10. Scherrmann JM. The biology and function of transporters in comprehensive medicinal chemistry II. In: Taylor JB, Triggle DJ, editors, ADME- Tox Approaches. Elsevier Science Ltd, Oxford, UK, 2006. p. 51-85
11. Gazzin S, Strazielle N, Schmitt C, et al. Differential expression of the multidrug resistance-related proteins ABCb1 and ABCc1 between blood-brain interfaces. J Comp Neurol 2008;510:497-507
12. Lu H. Stereoselectivity in drug metabolism. Expert Opin Drug Metab Toxicol 2007;3:149-58
13. Brocks DR, Mehvar R. Stereoselectivity in the pharmacodynamics and pharmacokinetics of the chiral antimalarial drugs. Clin Pharmacokinet 2003;42:1359-82
14. Soudijn W, Van Wijnagaarden I, Ijzerman AP. Stereoselectivity of drug-receptor interaction. Idrugs 2003;6:43-56
15. Kim RB, Fromm MF, Wandel C, et al. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J Clin Invest 1998;101:289-94
16. Yokogawa K, Takahashi M, Tamai I, et al. P-glycoprotein-dependent disposition kinetics of tacrolimus: studies in mdr 1a knockout mice. Pharm Res 1999;16:1213-8
17. Wagner D, Spahn-Langguth H, Hanafy A, et al. Intestinal drug efflux: formulation and food effects. Adv Drug Deliv Rev 2001;50(Suppl 1):S13-31
18. Schinkel AH. P-Glycoprotein, a gatekeeper in the blood-brain barrier. Adv Drug Deliv Rev 1999;36:179-94

• **Reviews effects of xenobiotics at the CNS on P-gp knockout mice.**

19. Schinkel AH, Smit JJ, Van Tellingen O, et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 1994;77:491-502
20. Polli JW, Jarrett JL, Studenberg SD, et al. Role of P-glycoprotein on the CNS disposition of amprenavir (141W94), an HIV protease inhibitor. Pharm Res 1999;16:1206-12
21. Chishty M, Reichel A, Siva J, et al. Affinity for the P-glycoprotein efflux pump at the blood-brain barrier may explain the lack of CNS side-effects of modern antihistamines. J Drug Target 2001;9:223-8
22. Ushigome F, Takanaga H, Matsuo H, et al. Human placental transport of vinblastine, vincristine, digoxin and progesterone: contribution of P-glycoprotein. Eur J Pharmacol 2000;408:1-10
23. Zhao YL, Cai SH, Wang L, et al. Possible involvement of P-glycoprotein in the biliary excretion of grepafloxacin. Clin Exp Pharmacol Physiol 2002;29:167-72
24. Wakasugi H, Yano I, Ito T, et al. Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. Clin Pharmacol Ther 1998;64:123-8
25. Merino G, Molina AJ, Garcia JL, et al. Intestinal elimination of albendazole sulfoxide: pharmacokinetic effects of inhibitors. Int J Pharm 2003;263:123-32
26. Sharom FJ. The P-glycoprotein efflux pump: how does it transport drugs? J Membr Biol 1997;160:161-75
27. Sun J, He ZG, Cheng G, et al. Multidrug resistance P-glycoprotein: crucial significance in drug disposition

and interaction. Med Sci Monit 2004;10:RA5-14

28. Ekins S, Kim RB, Leake BF, et al. Three-dimensional quantitative structure-activity relationships of inhibitors of P-glycoprotein. Mol Pharmacol 2002;61:964-73

29. Ekins S, Kim RB, Leake BF, et al. Application of three-dimensional quantitative structure-activity relationships of P-glycoprotein inhibitors and substrates. Mol Pharmacol 2002;61:974-81

• **Summarizes the features required for a substance to be a P-gp subtrate.**

30. Bhatia P, Kolinski M, Moaddel R, et al. Determination and modelling of stereoselective interactions of ligands with drug transporters: a key dimension in the understanding of drug disposition. Xenobiotica 2008;38:656-75

•• **Approach of the relationship between P-gp 3D structure and chiral drug affinity.**

31. Li WX, Li L, Eksterowicz J, et al. Significance analysis and multiple pharmacophore models for differentiating P-glycoprotein substrates. J Chem Inf Model 2007;47:2429-38

32. Boccard J, Bajot F, Di PA, et al. A 3D linear solvation energy model to quantify the affinity of flavonoid derivatives toward P-glycoprotein. Eur J Pharm Sci 2009;36:254-64

33. Luurtsema G, Molthoff CF, Windhorst AD, et al. (R)- and (S)-[11C] verapamil as PET-tracers for measuring P-glycoprotein function: in vitro and in vivo evaluation. Nucl Med Biol 2003;30:747-51

34. Hooiveld GJ, Heegsma J, van Montfoort JE, et al. Stereoselective transport of hydrophilic quaternary drugs by human MDR1 and rat Mdr1b P-glycoproteins. Br J Pharmacol 2002;135:1685-94

35. Kwon Y, Kamath AV, Morris ME. Inhibitors of P-glycoprotein-mediated daunomycin transport in rat liver canalicular membrane vesicles. J Pharm Sci 1996;85:935-9

36. Luurtsema G, de Lange EC, Lammertsma AA, Franssen EJ. Transport across the blood-brain barrier: stereoselectivity and PET-tracers. Mol Imaging Biol 2004;6:306-18

37. Hollt V, Kouba M, Dietel M, Vogt G. Stereoisomers of calcium antagonists which differ markedly in their potencies as calcium blockers are equally effective in modulating drug transport by P-glycoprotein. Biochem Pharmacol 1992;43:2601-8

38. Vogelgesang B, Echizen H, Schmidt E, Eichelbaum M. Stereoselective first-pass metabolism of highly cleared drugs: studies of the bioavailability of L- and D-verapamil examined with a stable isotope technique. 1984. Br J Clin Pharmacol 2004;58:S796-803

39. Tolomeo M, Gancitano RA, Musso M, et al. Effects of R-enantiomer (GR66234A) and L-enantiomer (GR66235A) of telupidine, a new dihydropyridine derivative, on cell lines displaying the multidrug resistant phenotype. Haematologica 1994;79:328-33

40. Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Adv Drug Deliv Rev 2003;55:3-29

41. Barraud de Lagerie S, Comets E, Gautrand C, et al. Cerebral uptake of mefloquine enantiomers with and without the P-gp inhibitor elacridar (GF1210918) in mice. Br J Pharmacol 2004;141:1214-22

42. Bachmakov I, Rekersbrink S, Hofmann U, et al. Characterisation of (R/S)-propafenone and its metabolites as substrates and inhibitors of P-glycoprotein. Naunyn Schmiedebergs Arch Pharmacol 2005;371:195-201

43. Chiba P, Rebitzer S, Richter E, et al. Synthesis and pharmacological activity of the stereoisomers of GP-88, a propafenone-type modulator of multidrug resistance. Bioorg Med Chem Lett 1998;8:829-32

44. Zschiesche M, Lemma GL, Klebingat KJ, et al. Stereoselective disposition of talinolol in man. J Pharm. Sci 2002;91:303-11

45. Eap CB, Buclin T, Baumann P. Interindividual variability of the clinical pharmacokinetics of methadone: Implications for the treatment of opioid dependence. Clin Pharmacokinet 2002;41:1153-93

46. Störmer E, Perloff MD, von Moltke LL, Greenblatt DJ. Methadone inhibits rhodamine123 transport in caco-2 cells. Drug Metab Dispos 2001;29:954-6

47. Bouer R, Barthe L, Philibert C, et al. The roles of P-glycoprotein and intracellullar metabolism in the intestinal absorption of methadone: in vitro studies using the rat everted intestinal sac. Fundam Clin Pharmacol 1999;13:494-500

48. Wang JS, Ruan Y, Taylor RM, et al. Brain penetration of methadone (R)- and (S)-enantiomers is greatly increased by P-glycoprotein deficiency in the blood-brain barrier of Abcb1a gene knockout mice. Psychopharmacology 2004;173:132-8

49. Crettol S, Digon P, Golay KP, et al. In vitro P-glycoprotein-mediated transport of (R)-, (S)-, (R,S)-methadone, LAAM and their main metabolites. Pharmacology 2007;80:304-11

• **P-gp enantioselectivity in an *in vitro* model.**

50. Liu HC, Wang N, Liu CS, et al. Distribution of enantiomers of trans-tramadol and trans-O-demethyltramadol in central nervous system of rats. Acta Pharmacol Sin 2001;22:871-5

51. Kanaan M, Daali Y, Dayer P, Desmeules J. Uptake/efflux transport of tramadol enantiomers and O-desmethyl-tramadol: focus on P-glycoprotein. Basic Clin Pharmacol Toxicol 2009;105:199-206

52. Szabo D, Molnar J. The role of stereoselectivity of chemosensitizers in the reversal of multidrug resistance of mouse lymphoma cells. Anticancer Res. 1998;18:3039-44

53. Loetchutinat C, Heywang C, Priebe W, Garnier-Suillerot A. The absence of stereoselective P-glycoprotein- and multidrug resistance-associated protein-mediated transport of daunorubicin. Biochem Pharmacol 2001;62:561-7

54. Tateishi T, Miura M, Suzuki T, Uno T. The different effects of itraconazole on the pharmacokinetics of fexofenadine enantiomers. Br J Clin Pharmacol 2008;65:693-700

55. Ieiri I, Takane H, Hirota T, et al. Genetic polymorphisms of drug transporters: pharmacokinetic and

pharmacodynamic consequences in pharmacotherapy. Expert Opin Drug Metab Toxicol 2006;2:651-74

56. Schwab M, Eichelbaum M, Fromm MF. Genetic polymorphisms of the human MDR1 drug transporter. Annual Review of Pharmacology and Toxicology 2003;43:285-307

57. Kroetz DL, Pauli-Magnus C, Hodges LM, et al. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. Pharmacogenetics 2003;13:481-94

•• **Describes ABCB1 gene polymorphisms and haplotypes in different ethnic populations.**

58. Tanabe M, Ieiri I, Nagata N, et al. Expression of P-glycoprotein in human placenta: Relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J Pharmacol Exp Ther 2001;297:1137-43

59. Fromm MF. The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans. Adv Drug Deliv Rev 2002;54:1295-310

60. Schaeffeler E, Eichelbaum M, Brinkmann U, et al. Frequency of C3435T polymorphism of MDR1 gene in African people. Lancet 2001;358:383-4

61. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistant gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 2000;97:3473-8

62. Wang D, Johnson AD, Papp AC, et al. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C > T affects mRNA stability. Pharmacogenet Genomics 2005;15:693-704

63. Kimchi-Sarfaty C, Oh JM, Kim IW, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315:525-8

• **An ABCB1 polymorphism might change P-gp 3D structure.**

64. Eichelbaum M, Fromm MF, Schwab M. Clinical aspects of the MDR1 (ABCB1) gene polymorphism. Ther Drug Monit 2004;26:180-5

65. Roberts RL, Joyce PR, Mulder RT, et al. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotensin in patients treated for major depression. Pharmacogenomics J 2002;2:191-6

66. Shen S, He Y, Zeng S. Stereoselective regulation of MDR1 expression in Caco-2 cells by cetirizine enantiomers. Chirality 2007;19:485-90

67. Bauer B, Hartz AM, Fricker G, Miller DS. Modulation of p-glycoprotein transport function at the blood-brain barrier. Exp Biol Med 2005;230:118-27

68. Bauer B, Hartz AM, Fricker G, Miller DS. Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood-brain barrier. Mol Pharmacol 2004;66:413-9

69. Kerb R. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. Cancer Lett 2006;234:4-33

70. Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. J Biol Chem 2001;276:14581-7

71. Synold TW, Dussault I, Forman BM. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. Nat Med 2001;7:584-90

72. Shon JH, Yoon YR, Hong WS, et al. Effect of itraconazole on the pharmacokinetics and pharmacodynamics of fexofenadine in relation to the MDR1 genetic polymorphism. Clin Pharmacol Ther 2005;78:191-201

73. Miura M, Uno T, Tateishi T, Suzuki T. Pharmacokinetics of fexofenadine enantiomers in healthy subjects. Chirality 2007;19:223-7

74. Sakugawa T, Miura M, Hokama N, et al. Enantioselective disposition of fexofenadine with the P-glycoprotein inhibitor verapamil. Br J Clin Pharmacol 2009;67:535-40

75. Nikisch G, Eap CB, Baumann P. Citalopram enantiomers in plasma and cerebrospinal fluid of ABCB1 genotyped depressive patients and clinical response: a pilot study. Pharmacol Res 2008;58:344-7

• **Relationship between ABCB1 genotype and clinical outcome of a chiral drug.**

76. Rochat B, Baumann P, Audus KL. Transport mechanisms for the antidepressant citalopram in brain microvessel endothelium. Brain Res 1999;831:229-36

77. Uhr M, Tontsch A, Namendorf C, et al. Polymorphisms in the drug transporter gene ABCB1 predict antidepressant treatment response in depression. Neuron 2008;57:203-9

78. Aarnoudse AL, van Schaik RH, Dieleman J, et al. MDR1 gene polymorphisms are associated with neuropsychiatric adverse effects of mefloquine. Clin Pharmacol Ther 2006;80:367-74

• **Describes side effects at the CNS linked to ABCB1 polymorphisms for a chiral drug.**

79. Pham YT, Nosten F, Farinotti R, et al. Cerebral uptake of mefloquine enantiomers in fatal cerebral malaria. Int J Clin Pharmacol Ther 1999;37:58-61

80. Baudry S, Pham YT, Baune B, et al. Stereoselective passage of mefloquine through the blood-brain barrier in the rat. J Pharm Pharmacol 1997;49:1086-90

81. Sadeque AJM, Wandel C, He H, et al. Increased drug delivery to the brain by P-glycoprotein inhibition. Clin Pharmacol Ther 2000;68:231-7

82. Pauli-Magnus C, Feiner J, Brett C, et al. No effect of MDR1 C3435T variant on loperamide disposition and central nervous system effects. Clin Pharmacol Ther 2003;74:487-98

83. Yamauchi A, Ieiri I, Kataoka Y, et al. Neurotoxicity induced by tacrolimus after liver transplantation: Relation to genetic polymorphisms of the ABCB1 (MDR1) gene. Transplantation 2002;74:571-3

84. Lankas GR, Cartwright ME, Umbenhauer D. P-glycoprotein deficiency in a subpopulation of CF-1 mice enhances avermectin-induced neurotoxicity. Toxicol Appl Pharmacol 1997;143:357-65

85. Macdonald N, Gledhill A. Potential impact of ABCB1 (p-glycoprotein)

polymorphisms on avermectin toxicity in humans. Arch Toxicol 2007;81:553-63

86. Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 2001;70(2):189-99

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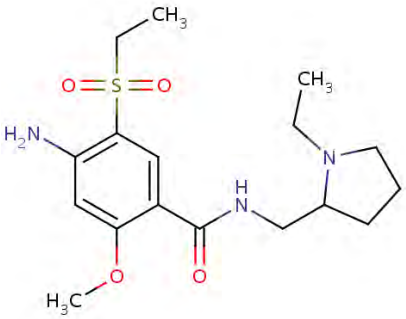
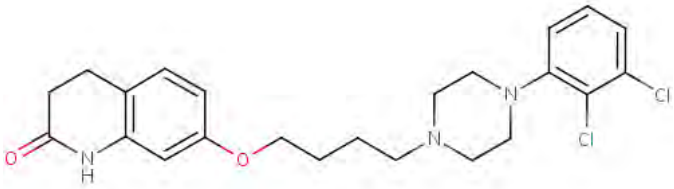
Structures chimiques des antipsychotiques atypiques et stabiliseurs de l'humeur étudiés

I. Antipsychotiques atypiques

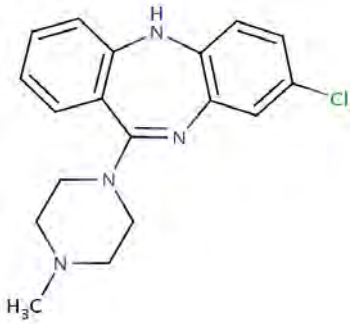
Amisulpride



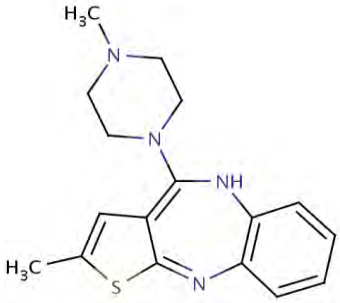
Aripiprazole



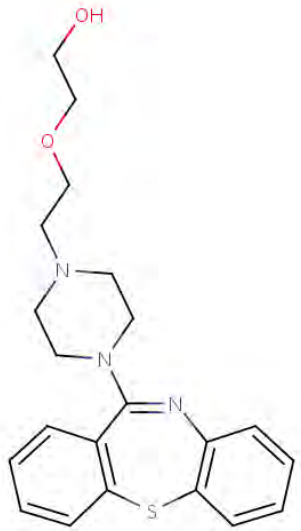
Clozapine



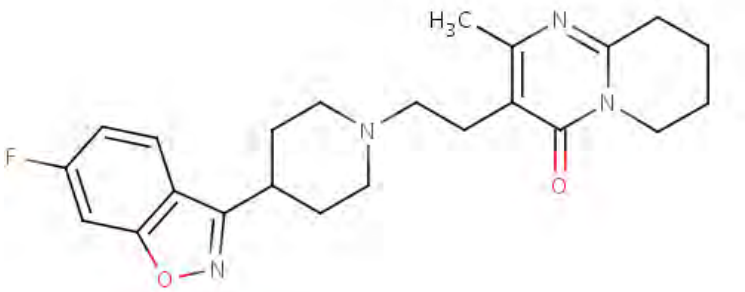
Olanzapine



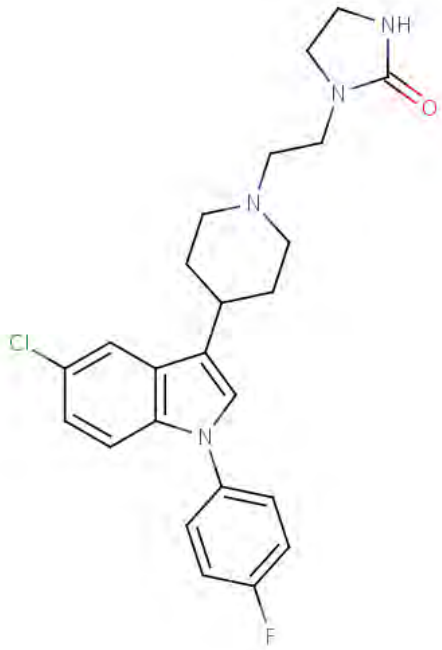
Quétiapine



Rispéridone

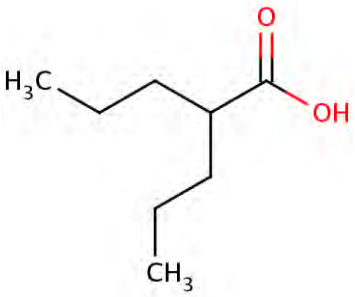


Sertindole



II. Stabiliseurs de l'humeur

Acide valproique



Lithium

Référence :

Drug Bank. Available at: http://www.drugbank.ca/ . Access on June 30, 2011.