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ABCB1 and *cytochrome P450* polymorphisms: clinical pharmacogenetics of clozapine

THESE

préparée sous la direction du Professeur honoraire Pierre Baumann
et avec la collaboration du Dr Chin Bin Eap, Privat-Docent et Maître d'Enseignement et de Recherche

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par

Branka KNEZEVIC - KOVAC

Médecin diplômée de la Faculté de médecine, Université de Sarajevo Bosnie-Herzégovine et diplôme reconnu par la Suisse (MEBEKO).

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ABCB1 and cytochrome P450 polymorphisms: clinical pharmacogenetics of clozapine

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Résumé

Polymorphismes *ABCB1* et *cytochrome P450*: Pharmacogénétique clinique de la clozapine

(*ABCB1* and *cytochrome P450* polymorphisms: clinical pharmacogenetics of clozapine)

Dans le but d'examiner les facteurs génétiques qui influencent la pharmacocinétique de la clozapine in vivo, 75 patients traités avec ce médicament antipsychotique ont été genotypés pour les polymorphismes *CYP* et *ABCB1*, et phénotypés pour l'activité de CYP1A2 et CYP3A. L'activité de CYP1A2 et les taux plasmatiques de clozapine en steady-state corrèlent d'une manière significative ($r=0.61$; $p=1x10^{-6}$), sans influence du génotype de *CYP1A2*1F* ($p=0.38$). Les métaboliseurs déficients C*YP2C19* (génotype **2/*2* genotype) avaient des concentrations de clozapine 2,3 fois ($p=0.036$) plus élevées que les métaboliseurs rapides (non**2/*2*). Chez les patients comédiqués avec la fluvoxamine, un fort inhibiteur de CYP1A2, les concentrations de clozapine et de norclozapine corrèlent siginificativement avec l'activité de CYP3A ($r=0.44$, $p=0.075$; $r=0.63$, $p=0.007$, respectivement). Les porteurs du génotype *ABCB1 3435TT* avaient des concentrations plasmatiques de clozapine 1,6 fois plus élevées que ceux qui ne présentaient pas ce génotype ($p=0.046$). En conclusion, cette étude montre pour la première fois, in vivo, le rôle significatif de CYP2C19 et celui du transporteur P-gp dans la pharmacocinétique de la clozapine. Le CYP1A2 est la forme principale de CYP impliquée dans le métabolisme de clozapine, tandis que le CYP2C19 joue un rôle modéré et que le CYP3A4 n'y contribue que chez les patients qui présentent une activité de CYP1A2 réduite. De plus, le polymorphisme de *ABCB1*, mais pas ceux de C*YP2B6, CYP2C9, CYP2D6, CYP3A5* et *CYP3A7,* influence la pharmacocinétique de la clozapine.

Mots-clé: clozapine; concentration plasmatique; CYP1A2; CYP2C19; CYP3A4; ABCB1

ABCB1 and Cytochrome P450 Polymorphisms

Clinical Pharmacogenetics of Clozapine

Eveline Jaquenoud Sirot, MSc, Branka Knezevic, MD,* Gina Perla Morena, MD,† Sabine Harenberg, PhD,* Beatrice Oneda, PhD,‡ Séverine Crettol, PhD,‡ Nicolas Ansermot, PhD,‡ Pierre Baumann, PhD,‡ and Chin B. Eap, PhD‡§*

Abstract: To examine the genetic factors influencing clozapine kinetics in vivo, 75 patients treated with clozapine were genotyped for CYPs and ABCB1 polymorphisms and phenotyped for CYP1A2 and CYP3A activity. CYP1A2 activity and dose-corrected trough steady-state plasma concentrations of clozapine correlated significantly ($r = -0.61$; $P = 1 \times 10^{-6}$), with no influence of the *CYP1A2*1F* genotype ($P = 0.38$). CYP2C19 poor metabolizers (*2/*2 genotype) had 2.3-fold higher ($P = 0.036$) clozapine concentrations than the extensive metabolizers (non-*2/*2). In patients comedicated with fluvoxamine, a strong CYP1A2 inhibitor, clozapine and norclozapine concentrations correlate with CYP3A activity ($r = 0.44$, $P = 0.075$; $r = 0.63$, $P = 0.007$, respectively). Carriers of the *ABCB1 3435TT* genotype had a 1.6-fold higher clozapine plasma concentrations than noncarriers ($P = 0.046$). In conclusion, this study has shown for the first time a significant in vivo role of CYP2C19 and the P-gp transporter in the pharmacokinetics of clozapine. CYP1A2 is the main CYP isoform involved in clozapine metabolism, with CYP2C19 contributing moderately, and CYP3A4 contributing only in patients with reduced CYP1A2 activity. In addition, *ABCB1*, but not *CYP2B6, CYP2C9, CYP2D6, CYP3A5,* nor *CYP3A7* polymorphisms, influence clozapine pharmacokinetics.

Key Words: clozapine, plasma concentration, CYP1A2, CYP2C19, CYP3A4, ABCB1

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Because of the risk of hematologic adverse effects, clozapine is available as a second-line atypical antipsychotic drug despite its efficacy being considered superior to that of other antipsychotics.[1] However, inadequate response to clozapine is estimated to be as high as 30%.[2] Several studies[3,4] confirmed the existence of a therapeutic window for clozapine and low plasma concentrations, despite adequate dosing, might explain some of the cases of nonresponse. High plasma concentrations are risk factors for side effects such as seizures.[5]

From the *Psychiatrische Dienste Aargau AG, mediQ, Klinik Königsfelden, Brugg; †Section Minkowski, ‡Unité de Biochimie et Psychopharmacologie Clinique, Centre des Neurosciences Psychiatriques, Département de Psychiatrie–Centre Hospitalier Universitaire Vaudois, Université de Lausanne, Hôpital de Cery, Prilly-Lausanne; and §Ecole de Pharmacie, Université de Genève, Université de Lausanne, Genève, Switzerland.
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Reprints: Chin B. Eap, PhD, Unité de Biochimie et Psychopharmacologie Clinique, Centre des Neurosciences Psychiatriques, Site de Cery, CH-1008 Prilly-Lausanne, Switzerland (e-mail: chin.eap@chuv.ch).
E. Jaquenoud Sirot and B. Knezevic contributed equally to this work.
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Genetic and environmental factors contribute to the high interindividual variability in clozapine plasma concentrations.[6–8] In vitro studies suggest that cytochrome P4501A2 (CYP1A2) is the most important CYP isoform contributing to clozapine N-demethylation, leading to the formation of the main active metabolite norclozapine.[9] Because smoking induces CYP1A2,[10] this is in agreement with the lower plasma concentrations of clozapine measured in heavy smokers as compared with nonsmokers. According to in vitro studies, CYP2C19 and CYP3A4 could also be of considerable importance in the metabolism of clozapine.[9] In vivo, CYP3A-inducing drugs such as carbamazepine[11] reduce clozapine plasma concentrations, but it is unclear which CYP3A isoforms (ie, CYP3A4, CYP3A5, and/or CYP3A7) are implicated in clozapine metabolism.

CYP2D6 probably plays a minor role in clozapine metabolism.[9] Indeed, the pharmacokinetics of clozapine was not significantly different between 5 CYP2D6 poor metabolizers (PMs) and 5 extensive metabolizers (EMs) receiving a single oral dose of 10 mg clozapine.[12] Similarly, and in contradiction to in vitro data,[9] clozapine pharmacokinetics was not significantly different between CYP2C19 EMs and PMs receiving a single oral dose of 10 mg clozapine.[12] Finally, an in vitro study suggested that clozapine is a substrate of the P-glycoprotein (P-gp) transporter, encoded by the *ABCB1* gene,[13] a finding that was subsequently contradicted.[14] No in vivo study has yet evaluated whether genetic polymorphisms of the *ABCB1* gene[15] influence clozapine plasma concentrations.

The aim of this study was to examine the in vivo influence of genetic polymorphisms of CYP isoforms (*CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5,* and *CYP3A7*) and *ABCB1* on steady-state plasma concentrations of clozapine. As the activities of CYP1A2 and CYP3A are only partially reflected by genotyping tests, patients were also phenotyped with caffeine (CYP1A2) and midazolam (CYP3A).[16] The impact of environmental factors such as smoking or comedications was investigated. Finally, as weight gain is one of the major side effects of clozapine and a risk factor for metabolic syndrome, and as norclozapine may be implicated in this effect,[17,18] we examined this possible association.

MATERIALS AND METHODS

Patients

Seventy-five inpatients from 2 psychiatric clinics, aged 18 years or older, on stable clozapine treatment and unchanged comedication for at least 2 weeks (4 weeks for fluoxetine) were included in the study. Exclusion criteria were any serious uncontrolled illness, any organic psychiatric illness, or substance dependence. To ensure compliance, patients took their medication under supervision of a nurse for 4 days before blood sampling. The study was approved by the local ethics committees of the 2 participating centers (Königsfelden and

Prilly-Lausanne). Written informed consent was obtained from all patients or their legal representative.

Blood Sampling

On the morning of day 1, before first drug intake, 75 μg oral midazolam was given to the patients for CYP3A phenotyping.[16] A blood sample was taken 30 minutes later for determination of 1′OH-midazolam/midazolam plasma ratio[16] and trough clozapine and norclozapine plasma concentrations. They then received their usual medication, together with 200 mg caffeine for CYP1A2 phenotyping.[19] A second blood sampling was performed 6 hours later for determination of the paraxanthine-caffeine plasma ratio.[19] No caffeine-containing food or beverage was allowed on the test day until after the second blood sampling. Plasma, after centrifugation, and K-EDTA whole blood samples were kept frozen at −20°C until analysis. Measurement of clozapine and norclozapine plasma concentrations was repeated on day 7 to control compliance and exclude within-subject variability. Because there were no significant differences between them (data not shown), results are expressed as the mean of the 2 blood samplings.

Assays of Drugs

Clozapine and norclozapine concentrations were determined by gas chromatography with a nitrogen-phosphorus detector.[20] Fluvoxamine,[21] midazolam, and 1′OH-midazolam[16,22] caffeine and paraxanthine[20] were measured by gas chromatography–mass spectrometry. Measured clozapine and norclozapine plasma concentrations were corrected by clozapine daily dose and hereafter are referred to as plasma concentrations.

Genotyping

Genomic DNA was extracted from EDTA blood samples with the FlexiGene DNA Kit (Qiagen, Hombrechtikon, Switzerland). All the single-nucleotide polymorphisms (SNPs), with the exception of *CYP2D6*5* and *CYP2D6*xN*, were detected by real-time PCR with 5′-nuclease allelic discrimination assays (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland) with primers and probes obtained from Applied Biosystems. The *CYP1A2*1F*, *CYP2B6*4*, *CYP2B6*5*, *CYP2B6*6*, *CYP2B6*7*, *CYP2B6*9*, *CYP2C9*2*, *CYP2C9*3*, *CYP2C19*2*, *CYP2C19*3*, *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*6*, *CYP3A4*1B*, *CYP3A5*3*, *ABCB1 61A>G*, *2677G>T*, and *3435C>T* SNPs were analyzed as previously described.[20,23] *CYP2D6* gene deletion (allele **5*) and duplication/multiduplication (allele **xN*) were analyzed by quantitative real-time polymerase chain reaction (PCR) and long PCR, respectively.[23]

*CYP3A7*1C* (*−262T>A* and *−270T>G*) allele was determined as previously described.[24] *CYP2C19*17* (*−806C>T*) allele was determined using the following primers, GTTTG GAAGTTGTTTTGTTTTGCTAA (forward), CATCGTGGCG CATTATCTCTT (reverse), and labeled probes, 6-FAM-TTCTCAAAGcATCTCT-MGBNFQ, and VIC–TTCTGTTCT CAAAGtATCT-MGBNFQ. The 25 μL PCR mixture contained 12.5 μL TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM of each primer, 200 nM of each TaqMan minor groove binder nonfluorescent quencher probe, and 40 ng (100 ng for *CYP2C19*17*) of genomic DNA. After an activation step comprising AmpErase (50°C for 2 minutes) and AmpliTaq Gold enzyme activation (95°C for 10 minutes), 60 PCR cycles (50 cycles for *CYP2C19*17*) were performed with 15 seconds at 92°C and 1 minute at 58°C (1.5 minutes at 60°C for *CYP2C19*17*). *CYP3A4 rs4646437C>T* was analyzed with commercial TaqMan Drug Metabolism Genotyping Assays according to the manufacturer's instructions (Assay Ids C_32306227_10; Applied Biosystems).

Clinical Assessments

Routine clinical chemistry and hematologic parameters were measured at baseline. All patients underwent a physical examination at screening; their medical history was recorded, and psychiatric and somatic diagnoses were confirmed. On days 1 and 7, vital signs, weight, spontaneously reported adverse events, and lifestyle factors (smoking, caffeine, and grapefruit intake) were noted. Weight gain data were collected retrospectively from the patient's medical files.

Statistical Analysis

Clozapine and norclozapine blood concentrations were compared between different genotypes by nonparametric analyses (Kruskal-Wallis test for >2, Mann-Whitney *U* test for 2 groups). Correlations between plasma concentrations and CYP1A2 or CYP3A activity were assessed by Spearman test, and multivariate analyses were performed using linear regression (backward method). A $P < 0.05$ was considered to indicate statistical significance. All statistical tests were performed in the whole group of patients and in the 2 subgroups with and without fluvoxamine as inhibition by fluvoxamine could mask the potential influence of other factors. Statistical analyses were performed using SPSS version 15.0 (SPSS, Inc, Chicago, Ill). For *ABCB1* polymorphisms, Hardy-Weinberg equilibrium was tested, and linkage disequilibrium (Lewontin's D'coefficient) was estimated with STATA (version 10; Stata Corporation, College Station, Tex). Haplotypes were inferred using the haplo.em function in R (http://www.r-project.org/), which uses expectation-maximization algorithm. As none of the inferred haplotypes had a posterior probability below 98%, haplotype uncertainty can be considered as minimal. Genetic association studies were conducted using the haplo.score function in R (which uses generalized linear models and takes haplotype uncertainty into account) with an additive effect and a Gaussian distribution for the trait.

RESULTS

Patient Characteristics

Seventy-five patients (39 men and 36 women; 73 white, 1 Asian, and 1 black African) participated in the study. Their median age was 44 years (mean, 48 years; SD, 17 years; range, 20–90 years). The majority were diagnosed with schizophrenic disorders (n = 73), one with bipolar disorder, and one with dementia of unknown etiology. Thirty-three patients presented with 1 or more somatic comorbidities, including 12 who experienced arterial hypertension. Four patients developed diabetes during clozapine treatment, and 4 were diabetic before taking clozapine for the first time. Treatment was generally well tolerated; the most frequent complaints were hypersalivation and weight gain.

The median weight at entry to the study was 79 kg (range, 52–128 kg; 74.5 kg and 83 kg, for women and men, respectively). The median body mass index was 27.4 kg/m^2 (range, 19.1–36.6 kg/m^2). Thirty-two patients (43%) gained 10% or more of their starting body weight during the course of clozapine treatment, with the maximum increase being 97% for 15 years for a male aged 32 years with a body mass index of 36.6 kg/m^2. Three patients lost weight, 25 remained stable, and 13 increased their weight slightly to moderately (<10% of body weight); for 2 patients, the initial body weight was unknown.

Plasma Concentrations of Clozapine and/or Norclozapine, Comedications and Clinical Variables

The median clozapine daily dose was 250 mg (range, 25–800 mg). Six patients received clozapine monotherapy; 17 patients (23%) had comedication with the strong CYP1A2 and moderate CYP3A and 2C19 inhibitor fluvoxamine (dose range, 25–300 mg/d).[25,26] The median trough plasma concentrations of clozapine and norclozapine were 1.14 ng/mL × mg (range, 0.15–6.24 ng/mL × mg) and 0.60 ng/mL × mg (range, 0.04–2.36 ng/mL × mg) in the whole group of patients and 0.99 ng/mL × mg (range, 0.15–2.88 ng/mL × mg) and 0.49 ng/mL × mg (range, 0.04–1.28 ng/mL × mg) in the group of patients without fluvoxamine, respectively. The median clozapine, norclozapine, and clozapine + norclozapine plasma concentrations were 3.5-, 2.4-, and 3.3-fold higher, respectively, in the group with fluvoxamine as compared with the group without fluvoxamine ($P = 4.9 \times 10^{-7}$, $P = 1.3 \times 10^{-5}$, and $P = 1.1 \times 10^{-6}$, respectively). Correlations (logarithmic regressions) were observed between fluvoxamine plasma concentrations and clozapine ($r^2 = 0.65$), norclozapine ($r^2 = 0.11$), and clozapine + norclozapine ($r^2 = 0.52$) plasma concentrations (Fig. 1). In addition, this figure suggests saturation of inhibition in the range of 50 to 100 ng/mL of fluvoxamine. In agreement with a strong inhibition of CYP1A2 activity by fluvoxamine, the median paraxanthine-caffeine ratios were 0.72 (range, 0.19–3.12) and 0.33 (range, 0.08–3.49) in the groups of patients without and with fluvoxamine, respectively. Flattening of the correlation curve (power regression, $r^2 = 0.71$) between fluvoxamine plasma concentrations and paraxanthine-caffeine ratios suggests saturation of the inhibition of CYP1A2 activity with increasing fluvoxamine plasma concentrations (Fig. 2).

A group of patients was identified with other possibly relevant comedications (maximal dose; number of patients): sertraline[27] (150 mg/d; 6), paroxetine[28] (40 mg/d; 3), fluoxetine[29] (20 mg/d; 1), levomepromazine[30] (150 mg/d; 3), amlodipine (10 mg/d; 2), phenytoin[31] (300 mg/d; 1), and omeprazole[32] (20 mg/d; 1). There was no significant effect of these comedications on clozapine ($P > 0.3$), norclozapine ($P > 0.9$), or clozapine + norclozapine ($P > 0.6$) concentrations when considered individually or as a group. Sex and age in the total study population did not seem to influence clozapine plasma concentrations ($P = 0.34$ and $P = 0.43$, respectively; data not shown). However, when excluding patients taking fluvoxamine, women had significantly higher clozapine but not norclozapine ($P = 0.12$, data not shown) plasma concentrations (median, 1.11 [range, 0.18–2.88] ng/mL × mg vs 0.61 [range, 0.15–2.72] ng/mL × mg, in women and men, respectively, $P = 0.027$).

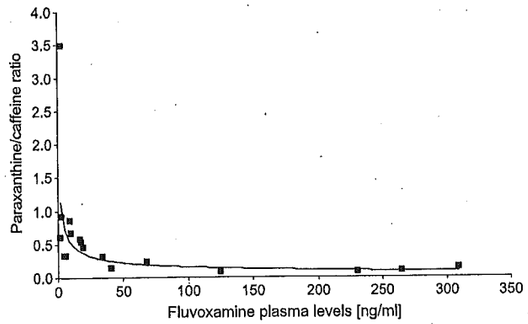

FIGURE 2. Correlation between fluvoxamine plasma levels and CYP1A2 activity measured by the paraxanthine-caffeine ratio (power regression: $y = 1.62\ x^{-0.51}$; $r^2 = 0.71$). The outlier corresponds to a patient with a CYP2D6 ultrarapid metabolizer polymorphism with very low fluvoxamine plasma levels.

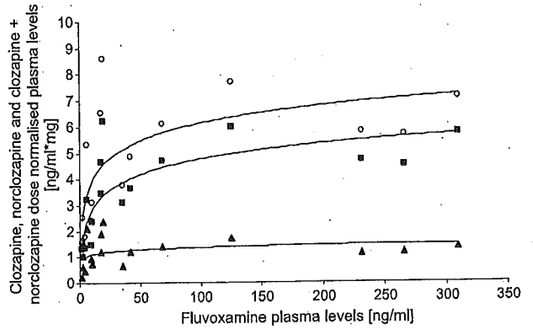

FIGURE 1. Correlations (logarithmic regressions) between fluvoxamine plasma levels and (■) clozapine (y = 0.84Ln(x) + 0.88; $r^2 = 0.65$), (▲) norclozapine (y = 0.11Ln(x) + 0.85; $r^2 = 0.11$) and (○) clozapine + norclozapine (y = 0.96Ln(x) + 1.73; $r^2 = 0.52$) dose-normalized plasma levels.

Forty-five patients were smokers (26 men and 19 women), and 30 were nonsmokers (13 men and 17 women). The number of cigarettes smoked per day ranged from 1 to 60 (median, 20). Smoking induces CYP1A2 as shown by the 1.5-fold higher median paraxanthine-caffeine ratio ($P = 0.031$) in smokers (0.74 [range, 0.08–3.49]) compared with nonsmokers (0.50 [range, 0.09–1.15]). Lower norclozapine (median, 0.49 ng/mL × mg vs 0.67 ng/mL × mg; $P = 0.039$), but not clozapine (1.03 ng/mL × mg vs 1.30 ng/mL × mg; $P = 0.175$), plasma concentrations were measured in smokers compared with nonsmokers. As expected, this effect was more pronounced in the group without fluvoxamine, where the influence of smoking was also significant on clozapine plasma concentrations (median, 0.72 ng/mL × mg vs 1.21 ng/mL × mg, in smokers and nonsmokers, respectively, $P = 0.011$). The effect of smoking on clozapine or norclozapine plasma concentrations was not related to the number (>20, 11–20, 6–10, ≤5) of cigarettes smoked per day (data not shown).

Because only 3 patients drank grapefruit juice, and all but 2 had regular caffeine intake, the effect of grapefruit and caffeine on clozapine plasma concentrations could not be determined. In contrast to 2 previous studies,[17,18] there was no significant correlation between norclozapine plasma levels (not corrected by dose) and weight gain ($r = 0.11$, $P = 0.38$) nor after subgroup analysis of nonsmokers ($r = 0.28$, $P = 0.14$) and smokers ($r = -0.07$, $P = 0.65$).

CYP and ABCB1 Genotyping

The observed genotype frequencies of *CYP1A2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, CYP3A4, *CYP3A5*, *CYP3A7*, and *ABCB1* are presented in Table 1. They are similar to those previously described in white populations (http://www.cypalleles.ki.se)[33,34] and all the SNPs are in Hardy-Weinberg equilibrium for the white subsample (n = 73). All 3 SNPs of the *ABCB1* genes are in strong linkage disequilibrium, as previously reported.[15]

In the whole patient group (n = 75) *CYP2C19* genotypes significantly influenced clozapine ($P = 0.036$) but not

TABLE 1. Frequency of *CYP1A2*1F, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP3A7,* and *ABCB1* Genotypes in 73 White Patients Treated With Clozapine

Genotype	n	Frequency	95% Confidence Interval (%)
*CYP1A2*1F*			
**1/*1*	8	10.9	4.8–20.5
**1/*1F*	31	42.5	31.0–54.6
**1F/*1F*	34	46.6	34.8–58.6
CYP2B6			
**1/*1*	30	41.1	29.7–53.2
**1/*4*	1	1.4	0.03–7.4
**1/*5*	8	10.9	4.8–20.5
**1/*6*	20	27.4	17.6–39.1
**1/*7*	4	5.5	1.5–13.4
**5/*5*	2	2.7	0.3–9.5
**6/*6*	8	10.9	4.8–20.5
CYP2C9			
**1/*1*	51	69.9	58.0–80.1
**1/*2*	11	15.1	7.8–25.4
**1/*3*	8	10.9	4.8–20.5
**2/*2*	1	1.4	0.03–7.4
**2/*3*	2	2.7	0.3–9.5
CYP2C19			
**1/*1*	24	32.9	22.3–44.9
**1/*2*	17	23.3	14.2–34.6
**1/*17*	18	24.6	15.3–36.1
**2/*2*	4	5.5	1.5–13.4
**2/*17*	4	5.5	1.5–13.4
**17/*17*	6	8.2	3.1–17.0
CYP2D6			
**1/*1*	40	54.8	42.7–66.5
**1/*3*	4	5.5	1.5–13.4
**1/*4*	16	21.9	13.1–33.1
**1/*5*	3	4.1	0.9–11.5
**1/*6*	1	1.4	0.03–7.4
**1/*xN*	4	5.5	1.5–13.4
**4/*4*	4	5.5	1.5–13.4
**4/*xN*	1	1.4	0.03–7.4
CYP3A			
*CYP3A5*3*			
**1/*1*	1	1.4	0.03–7.4
**1/*3*	8	10.9	4.8–20.5
**3/*3*	64	87.7	77.9–94.2
*CYP3A7*1C*			
**1/*1*	66	90.4	81.2–96.1
**1/*1C*	6	8.2	3.1–17.0
**1C*1C*	1	1.4	0.03–7.4
CYP3A4 rs4646437 (intron 7)			
CC	58	79.4	68.4–88.0
CT	14	19.2	10.9–30.1
TT	1	1.4	0.03–7.4
ABCB1			
61A>G			
AA	71	97.3	90.5–99.7
AG	2	2.7	0.3–9.5
2677G>T (exon 21)			
GG	25	34.2	23.5–46.3
GT	38	52.1	40.0–63.9
TT	10	13.7	6.8–23.8
3435C>T (exon 26)			
CC	18	24.6	15.3–36.1
CT	40	54.8	42.7–66.5
TT	15	20.5	12.0–31.6

norclozapine ($P = 0.185$) plasma concentrations (Figs. 3A, B), with a 2.3-fold higher median clozapine concentrations in PMs (**2/*2* genotype, n = 5, 2.58 ng/mL × mg [1.10–5.98]) than in extensive metabolizers (non-**2/*2* genotypes, 1.11 ng/mL × mg [0.15–6.24]) and 1.9-fold ($P = 0.057$) higher clozapine + norclozapine levels. Similarly, between carriers of the **17* allele associated with an increased CYP2C19 activity (**17/*17, *1/*17*) and PMs, the differences were 2.3-, 1.9-, and 1.6-fold, respectively, for clozapine ($P = 0.033$), clozapine + norclozapine ($P = 0.039$), and norclozapine ($P = 0.112$). On the other hand, no significant differences in clozapine ($P = 0.558$), norclozapine ($P = 0.186$), and clozapine + norclozapine ($P = 0.407$) plasma levels were found between the carriers of the **17* allele (**17/*17, *1/*17*) and extensive metabolizers (**1/*1, *1/*2, *2/*17*; data not shown). In the smaller group of patients without fluvoxamine, significant differences were observed between *CYP2C19 *1/*1, *1/*17 or *17/*17 and *2/*17, *1/*2 or *2/*2* individuals for clozapine ($P = 0.027$), norclozapine ($P = 0.074$), and the sum of both ($P = 0.042$).

In the whole patient group (n = 75) *ABCB1 3435 G>T* polymorphism significantly influenced clozapine plasma concentrations ($P = 0.046$), with a 1.6-fold higher median clozapine concentrations in *3435TT* genotype (n = 16; median, 1.6 ng/mL × mg [range, 0.27–5.98 ng/mL × mg] in *TT* genotypes; n = 59; median, 1.1 ng/mL × mg [range, 0.15–6.24 ng/mL × mg] in *CC/CT* genotypes). Statistical analysis on the *61 A>G* polymorphism was not performed because of the low observed genetic variability (Table 1). No significant influence of the *2677 G>T* polymorphism on clozapine plasma concentration was observed (data not shown). In addition, norclozapine and clozapine + norclozapine plasma concentrations did not differ significantly between different genotypes (*2677G>T* and *3435C>T*) (data not shown). Haplotype analysis revealed a trend toward higher clozapine concentration for carriers of *2677G-3435T* haplotype (global score, 0.1; haplotype specific score, 0.01). Because of the small sample size when considering haplotypes, we also computed permutation tests (global empirical $P = 0.10$ haplotype-specific empirical $P = 0.01$), which are in very close agreement with the asymptotic P based on a χ^2 distribution. Similar results were obtained after adjusting for sex and age (data not shown).

Finally, other genetic polymorphisms were without influence on clozapine, norclozapine, or clozapine + norclozapine plasma levels: *CYP1A2* ($P = 0.386$, 0.632, and 0.533), *CYP2B6* ($P = 0.664$, 0.540, and 0.522), *CYP2C9* ($P = 0.252$, 0.344, and 0.370), *CYP2D6* ($P = 0.464$, 0.696, and 0.718), *CYP3A4* ($P = 0.355$, 0.341, and 0.444), *CYP3A5* ($P = 0.865$, 0.206, and 0.627), and *CYP3A7* ($P = 0.586$, 0.384, and 0.493), in the whole group (and in the patients without fluvoxamine [data not shown]).

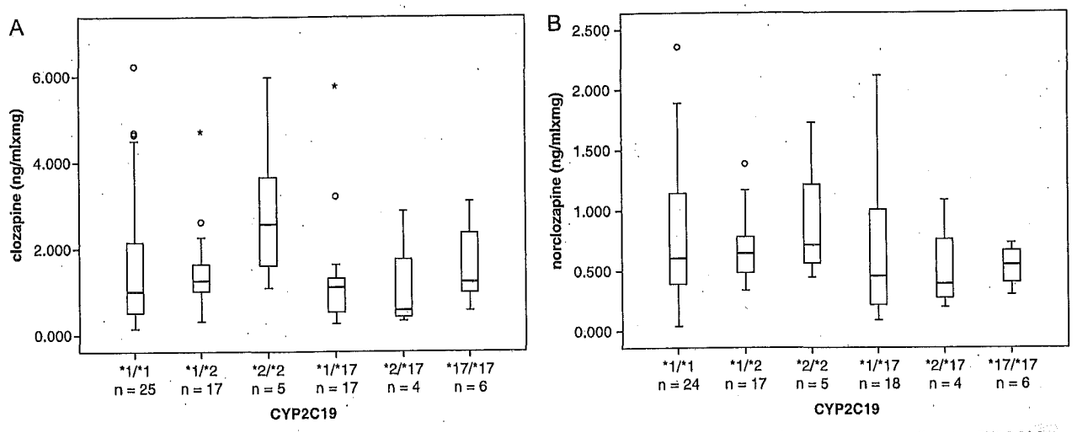

FIGURE 3. A, Boxplot with median and interquartile range of clozapine plasma concentration (ng/mL × mg) according to *CYP2C19* genotypes. Clozapine plasma level of 1 patient with the *CYP2C19*1/*17* genotype was not detected. B, Boxplot with median and interquartile range of norclozapine plasma concentration (ng/mL × mg) according to *CYP2C19* genotypes. Norclozapine plasma level of 1 patient with the *CYP2C19*1/*1* genotype was not detected.

CYP1A2 and CYP3A Phenotyping and Clozapine Plasma Concentrations

A strong correlation was observed between CYP1A2 activity and plasma concentrations of clozapine, norclozapine, and clozapine + norclozapine in the whole population ($r = -0.61$, $P = 1{\cdot}10^{-6}$; $r = -0.48$, $P = 2{\cdot}10^{-5}$; $r = -0.59$, $P = 1{\cdot}10^{-6}$), in the subgroup without fluvoxamine (n = 58) ($r = -0.51$, $P = 5{\cdot}10^{-5}$; $r = -0.41$, $P = 0.001$; $r = -0.50$, $P = 1{\cdot}10^{-4}$), and in the fluvoxamine subgroup (n = 17) ($r = -0.69$, $P = 0.002$; $r = -0.39$, $P = 0.12$; $r = -0.64$, $P = 0.006$).

No correlation was found between clozapine ($r = -0.16$, $P = 0.16$), norclozapine ($r = -0.07$, $P = 0.58$), and clozapine + norclozapine ($r = -0.161$, $P = 0.172$) plasma concentrations and CYP3A activity in the whole group. In the fluvoxamine subgroup, however, a weak correlation was found between CYP3A activity and clozapine + norclozapine ($r = 0.51$, $P = 0.038$), a moderate correlation with norclozapine ($r = 0.63$, $P = 0.007$), and a trend with clozapine concentrations ($r = 0.44$, $P = 0.075$).

Multivariate Analyses

Multivariate analyses between clozapine, norclozapine, and clozapine + norclozapine plasma concentrations and the main factors potentially influencing their kinetics yielded the following models in the whole group of patients. For clozapine, presence of fluvoxamine ($P < 10^{-8}$), high fluvoxamine concentrations ($P = 0.0001$), low CYP1A2 activity ($P = 0.0001$), and absence of *CYP2C19 *17*17* or **17/*1* genotype ($P = 0.008$) were predictive of higher plasma concentrations ($r = 0.84$, $P < 10^{-17}$). Other variables such as fluvoxamine dose ($P = 0.88$), sex ($P = 0.19$), smoking ($P = 0.29$), CYP3A activity ($P = 0.67$), *CYP3A4 rs4646437* allele T ($P = 0.69$), *CYP1A2*1F/1F* genotype ($P = 0.32$), *ABCB1 2677TT* genotype ($P = 0.22$), and *ABCB1 3435TT* genotype ($P = 0.17$) did not significantly contribute to the model. For norclozapine, presence of fluvoxamine ($P < 10^{-8}$), nonsmoking ($P = 0.004$), low CYP1A2 activity ($P = 0.025$), and absence of *CYP2C19 *17*17* or **17/*1* genotype ($P = 0.036$) were predictive of higher plasma concentrations ($r = 0.72$, $P < 10^{-9}$). For clozapine + norclozapine, presence of fluvoxamine ($P < 10^{-8}$), high fluvoxamine concentrations ($P = 0.004$), low CYP1A2 activity ($P = 0.0001$), and absence of *CYP2C19 *17*17* or **17/*1* genotype ($P = 0.012$) were predictive of higher plasma concentrations ($r = 0.82$, $P < 10^{-15}$). Similar models can be built including presence of *CYP2C19 *2/*2* or **2/*1* genotype instead of absence of *CYP2C19 *17*17* or **17/*1* genotype as a significant covariate for higher clozapine ($P = 0.017$) and clozapine + norclozapine ($P = 0.030$) plasma concentrations.

DISCUSSION

The measured trough plasma concentrations of clozapine, norclozapine, and clozapine + norclozapine corrected by daily dose presented a very wide interindividual variability, with a 41-, 59-, and 23-fold variation, respectively. The determination of genetic and environmental factors contributing to this variation is therefore of clinical relevance considering the existence of a narrow therapeutic window for clozapine (350–600 ng/mL),[3] with plasma levels more than 800 to 1000 ng/mL being associated with increased risk of side effects such as convulsions.[5] Previous in vitro and in vivo studies suggested that the main CYP isoform mediating the metabolism of clozapine is CYP1A2.[8,35,36] Therefore, modulation of CYP1A2 activity will have a major influence on clozapine plasma levels and effect. We examined 4 factors believed to have a relevant influence on CYP1A2 activity: *CYP1A2*1F* polymorphism, the effect of smoking and caffeine consumption, and comedication with fluvoxamine.

*CYP1A2*1F* has been associated with increased CYP1A2 activity in smokers, possibly because of increased inducibility.[20,37] In contrast to 2 previous studies,[20,37] but in agreement with 2 others,[38,39] we could not confirm any influence of *CYP1A2*1F* polymorphism on clozapine plasma concentrations or CYP1A2 activity, in the whole group and in the group of smokers; a strong influence of this polymorphism on clozapine plasma concentrations seems therefore unlikely. On the other hand, the important inducing effect of smoking on CYP1A2 activity and clozapine metabolism[40] was confirmed in our study by the 1.5-fold higher CYP1A2 activity in smokers compared

with nonsmokers in all patients and those without fluvoxamine comedication. Measured clozapine and norclozapine plasma levels in smokers compared with nonsmokers were thus 93% (not significant) and 77% ($P = 0.039$) in the whole group, and 67% ($P = 0.011$) and 64% ($P = 0.003$) in the group without fluvoxamine. Interestingly, the number of cigarettes smoked seemed to be of little relevance. Such a decrease in clozapine plasma concentrations in smokers is in agreement with most other studies.[39–42] Considering the narrow therapeutic window of clozapine, therapeutic drug monitoring is recommended when smoking habits are changed, as cessation of smoking can lead to a significant rise in clozapine concentrations and risk of overdosage.[43]

In the present study, 23% of the patients were comedicated with the antidepressant fluvoxamine. Such a high proportion is explained by the fact that in 1 study center (Königsfelden), patients not responding and/or intolerant to high doses of clozapine are switched to a combination of low-dose clozapine and fluvoxamine, with therapeutic drug monitoring to adapt clozapine doses.[17,20,44] Fluvoxamine is a strong CYP1A2 inhibitor, which is confirmed by the 2.2-fold higher paraxanthine-caffeine ratios determined in the patients without fluvoxamine compared with those with fluvoxamine. Accordingly, fluvoxamine markedly increases clozapine (3.5-fold) and norclozapine plasma concentrations (2.4-fold), indicating that it blocks the metabolism of both clozapine and norclozapine. The question arises whether the blocking effect of fluvoxamine on CYP1A2 is dose dependent or is saturable at low doses. We investigated this in an earlier case series and concluded that comedication with 150 mg/d fluvoxamine has the same blocking effect as 300 mg/d.[45] This is confirmed by the relationship between fluvoxamine, clozapine, and norclozapine plasma concentrations (Fig. 1), suggesting saturation of inhibition at low fluvoxamine plasma levels (approximately 50–100 ng/mL). Thus, a daily dose of approximately 100 mg fluvoxamine[46] would be sufficient to have a major blocking effect on the metabolic pathways of clozapine and norclozapine. Saturation of the inhibitory effect on CYP1A2 activity is also observed with paraxanthine-caffeine ratios at approximately 50 ng/mL fluvoxamine (Fig. 2). Finally, studies have suggested that caffeine consumption, in particular, when consumption fluctuates over time, can influence clozapine plasma concentrations, possibly by inhibition of CYP1A2.[47] In the present study, as all but 2 patients had regular intake of caffeine, the influence of caffeine on clozapine plasma concentrations could not be verified.

Conflicting results have been published on the implication and relative importance of other CYP isoforms besides CYP1A2 in the metabolism of clozapine.[9,12,48] We found no evidence of an effect of CYP2B6, CY2C9, CYP2D6, CYP3A5, or CYP3A7 on the steady-state kinetics of clozapine or norclozapine. On the other hand, this seems to be the first study to demonstrate a significant in vivo involvement of CYP2C19 in the pharmacokinetics of clozapine, previously suggested by an in vitro study[9] but challenged by an in vivo study with a single oral low-dose of clozapine.[12] Thus, *CYP2C19* PMs had 2.3-fold higher plasma concentrations of clozapine than patients with other *CYP2C19* genotypes. The absence of a significant influence of the *CYP2C19*17* allele could be attributed to its limited effect especially when present in 1 copy only.[49] A possible explanation for the negative results observed in the single-dose (10 mg) study is that, with such a low oral dose,[12] only CYP1A2 was responsible for the metabolism of clozapine.

The effect of CYP3A4 has been previously examined in interaction studies with CYP3A4 inhibitors and inducers.[11,48] Based on in vitro affinity constants, it has been suggested that its role becomes increasingly relevant with higher doses of clozapine.[9] In our study, the dose ranged from 25 to 800 mg/d, with a median of 250 mg/d. In the whole study population, there was no correlation between CYP3A activity and clozapine or norclozapine plasma concentrations. On the other hand, the observed correlation between 1-OH-midazolam–midazolam ratios and clozapine plasma concentrations in the fluvoxamine comedication group probably reflects the increasing importance of CYP3A4 in patients with blocked CYP1A2 activity. The very strong inhibition of clozapine metabolism by fluvoxamine can be explained by the fact that fluvoxamine is not only a strong CYP1A2 inhibitor but also a moderate inhibitor of CYP3A4 and CYP2C19. Finally, the present study is the first, to our knowledge, to suggest that clozapine plasma concentration is significantly influenced by the genetic polymorphism of the *ABCB1* gene, with higher concentrations measured in the *3435TT* genotype, a genotype previously associated with lower P-gp expression.[15] No conclusive results could be driven from the haplotype analysis because of the small number of patients in the haplotype groups.

No serious adverse drug reactions were reported, but hypersalivation and weight gain were frequently reported to be troublesome and difficult to manage. Weight gain is considered one of the major side effects of clozapine and is a risk factor for developing metabolic syndrome. Forty-three percent of patients gained 10% or more body weight during clozapine treatment. Some authors found a reduced risk for weight gain when combining fluvoxamine with clozapine.[17] Another group found a correlation between norclozapine plasma concentrations and weight gain in nonsmoking patients.[18] These results could not be confirmed in our study probably because of the small number of nonsmokers included in the present study. Another limitation is that the duration of clozapine treatment and the nature of the pretreatment could not be determined for all patients and that some patients were comedicated with valproic acid and lithium, which are also associated with weight gain. Because of the important clinical problems associated with weight gain in patients treated with atypical antipsychotics,[50] this should be examined further in prospective longitudinal studies. Finally, because of the limitation of the sample size, the results of the present study should be replicated by another study with a larger number of patients.

In conclusion, this study examined thoroughly the in vivo implication of drug metabolizing enzymes and transporters in clozapine kinetics to explain its large interindividual variability. CYP1A2 is the major CYP isoform involved in clozapine metabolism in vivo, with CYP2C19 contributing to a moderate extent and CYP3A4 contributing in the presence of comedications that induce activity of this isozyme or when CYP1A2 is blocked by drugs such as fluvoxamine. *ABCB1* genetic polymorphism also contributes to clozapine pharmacokinetic variability. To our knowledge, this is the first study showing a significant in vivo role of CYP2C19 and the P-gp transporter in the clozapine kinetics. Besides these genetic factors, environmental factors such as smoking or comedications (eg, fluvoxamine) markedly influence the kinetics of clozapine. Considering the narrow therapeutic range, therapeutic drug monitoring of clozapine, in particular in the presence of nonresponse and/or side effects, is strongly recommended.

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AUTHOR DISCLOSURE INFORMATION

The authors declare no conflict of interest.

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