Impact of *CYP1A2* and *CYP2D6* Polymorphisms on Drug Metabolism and on Insulin and Lipid Elevations and Insulin Resistance in Clozapine-Treated Patients

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Objective: Adverse metabolic effects of atypical antipsychotics have increasingly been recognized. Recently, we found that levels of insulin and triglycerides increased by increasing serum clozapine concentration in clozapine-treated patients. As these insulin and triglyceride elevations probably are drug concentration–dependent, they also would be expected to be drug metabolism–related. The genetically polymorphic cytochromes P450 CYP1A2 and CYP2D6 catalyze the metabolism of clozapine. The aim of this study was to evaluate the impact of *CYP1A2* and *CYP2D6* polymorphisms on serum drug and metabolite levels and on insulin and triglyceride elevations and insulin resistance in patients receiving clozapine.

Method: Seventeen clozapine-treated patients were genotyped for *CYP1A2* and *CYP2D6* by polymerase chain reaction–based methods. Serum concentrations of clozapine and its N-desmethylmetabolite, blood glucose, and serum levels of insulin, C-peptide, triglycerides, and cholesterol were analyzed, and homeostasis model assessment index for insulin resistance (HOMA-IR) was determined.

Results: Clozapine and N-desmethylclozapine concentration-to-dose (C/D) ratios were significantly higher in patients carrying 2 *CYP1A2* single nucleotide polymorphisms (SNPs), previously suggested to cause low enzyme activity, compared to those with no such SNPs ($p < .05$). In contrast, clozapine and N-desmethylclozapine C/D ratios were not related to the *CYP2D6* genotype. Furthermore, patients with elevated insulin levels more frequently carried *CYP1A2*1C* and/or **1D* alleles, had higher clozapine and N-desmethylclozapine C/D ratios, and had higher lipid levels and HOMA-IR, compared to patients with normal insulin levels ($p < .05$).

Conclusion: *CYP1A2* variants **1C* and **1D* seem to be associated with higher serum clozapine concentrations and an increased risk of developing insulin and lipid elevations and insulin resistance on a given dose of clozapine.

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The atypical antipsychotic drug clozapine has been shown to have an antipsychotic effect that is as good as or even better than that of both classical and other atypical antipsychotics, and clozapine is often used in the treatment of patients with psychosis who are unresponsive to classical or other atypical agents.[1–4] However, adverse metabolic effects such as excessive weight gain, lipid abnormalities, and diabetes mellitus have increasingly been recognized with the use of clozapine.[5–7] Results from clinical studies also show that clozapine treatment is associated with elevated levels of insulin, leptin, and lipids and insulin resistance.[8–10] Interestingly, we recently found that levels of insulin and triglycerides increased by increasing serum clozapine concentration in clozapine-treated patients.[9,11] Consequently, as these insulin and triglyceride elevations probably are drug concentration–dependent, they would also be expected to be drug metabolism–related. Thus, exploring whether a genetically impaired drug metabolic capacity is related to insulin and triglyceride elevations and even to insulin resistance in patients receiving clozapine would be worthwhile.

Clozapine is metabolized in the liver, predominantly to N-desmethylclozapine and clozapine-N-oxide. In vitro, the cytochrome P450 (CYP) 1A2 enzyme and, to a lesser extent, CYP2D6, CYP2C19, and CYP3A4 have been demonstrated to mediate the N-demethylation of clozapine, and CYP3A4 and the flavin-containing monooxygenase enzyme FMO3, the N-oxidation.[12,13] Additionally,

glucuronidation, catalyzed by the UDP-glucuronosyltransferase 1A4 enzyme, is reported to be an important metabolic pathway for clozapine.[14]

CYP1A2 activity, measured by a caffeine test, has been found to make an important contribution to steady-state serum concentrations of clozapine.[15,16] CYP1A2 activity is influenced by genetic as well as environmental factors and shows large interindividual variation.[17] Potent inhibitors of CYP1A2, such as fluvoxamine and ciprofloxacin, have been reported to cause a significant increase in clozapine steady-state level, sometimes with parallel development of side effects.[18] On the other hand, inducers of CYP1A2, such as tobacco smoke, carbamazepine, phenytoin, and rifampicin may accelerate the metabolism of clozapine, resulting in lower serum concentrations and impaired antipsychotic effect.[18] The role of CYP2D6 in the clinical pharmacokinetics of clozapine in vivo has been suggested by some studies,[19,20] but questioned by others.[21,22]

The *CYP1A2* gene, located on chromosome 15, contains 7 exons and 6 introns and has possible transcriptional regulatory regions in its 5′-flanking region and intron 1.[23] A number of single nucleotide polymorphisms (SNPs) in the *CYP1A2* gene have been reported (www.cypalleles.ki.se). However, the impact of these mutations on the enzyme activity is still largely unknown. The –3860G→A exchange (*CYP1A2*1C*) in the 5′-flanking region has been associated with decreased, and the –163C→A (*CYP1A2*1F*) in intron 1, with increased inducibility of the enzyme, while the –2467T→delT (*CYP1A2*1D*) in the 5′-flanking region and the –739T→G (*CYP1A2*1E*) in intron 1 have not yet been functionally characterized.[24–27] In some recent case reports, treatment resistance to clozapine has been suggested to be associated with the *CYP1A2* –163 A/A genotype that may confer high inducibility of the CYP1A2 enzyme in smoking patients.[28,29] However, 2 clinical studies showed no association between this polymorphism and clozapine clearance in schizophrenic patients, neither in smoking nor nonsmoking patients.[27,30] Two additional variants were recently described, *CYP1A2*1J* (characterized by the combination of –163A and –739G) and *CYP1A2*1K* (i.e., –163A in combination with –739G and –729T).[31] CYP1A2 enzyme activity was reported to be significantly reduced in nonsmokers with the *CYP1A2*1K* haplotype, as compared with those carrying *CYP1A2*1A, CYP1A2*1F*, or *CYP1A2*1J*.[31]

The large interindividual variation in *CYP2D6* activity, from complete lack of enzyme activity to ultrarapid activity, is largely genetically determined with little environmental influence.[13] The *CYP2D6* gene is located on chromosome 22, and to date, more than 70 allelic variants of the gene have been reported (www.cypalleles.ki.se). The most common alleles associated with complete lack of CYP2D6 activity in so-called poor metabolizers are *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, and *CYP2D6*6*. In the other extreme, alleles with duplication or multiduplication of a functional *CYP2D6* gene cause extremely high CYP2D6 activity (ultrarapid metabolizer).[13,32] In contrast to CYP1A2, CYP2D6 in the liver is not inducible.[33]

The aim of this study was to evaluate the impact of *CYP1A2* and *CYP2D6* genetic polymorphisms on serum drug concentrations and on levels of insulin and lipids and insulin resistance in patients treated with clozapine.

METHOD

Patients

Consecutive outpatients on therapy with clozapine were asked to participate in this study. The study was approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden, and all patients gave their informed consent. Patients who had a substance-related disorder, known diabetes mellitus, other physical illness, or drugs that could influence the glucose and lipid metabolism were excluded. In addition, all patients in this study were Caucasian individuals. Taken together, 17 patients, 12 men and 5 women, diagnosed with either schizophrenia (N = 16) or schizoaffective disorder (N = 1) according to the DSM-IV criteria,[34] were included.

The median age of the patients was 41 years (range, 29–63 years), and their median body mass index was 29 kg/m^2 (range, 21–47 kg/m^2). The duration of disease ranged from 8 to 42 years (median = 20 years). Six (35%) of the patients were smokers (of whom 2 patients smoked 1–10 cigarettes/day, 3 patients 11–20 cigarettes/day, and 1 patient > 20 cigarettes/day).

At assessment, the patients had been on treatment with clozapine for at least 0.7 years, with a median treatment time of 6.9 years (range, 0.7–16.3 years). The median daily dose of clozapine was 400 mg (range, 100–600 mg), and the only concomitant medications used were benzodiazepines (in 4 patients) and/or levomepromazine 25–50 mg daily (in 3 patients) and/or lithium 84 mg daily (in 1 patient).

Collection of Blood Samples

Fasting blood samples were collected in the morning, 12–14 hours after drug intake. Serum and whole blood samples were stored at –20°C until analysis.

Genotyping Methods

DNA was isolated from peripheral leukocytes by QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the guidelines of the manufacturer. *CYP1A2* genotyping was performed by allele-specific polymerase chain reaction (PCR) followed by digestion with restriction enzymes. The –3860G→A (*CYP1A2*1C*) and the –2467T→delT (*CYP1A2*1D*)

Table 1. Frequencies of *CYP1A2* Single Nucleotide Polymorphisms (SNPs) in the 17 Patients Investigated, Grouped According to Expected Influence on Enzyme Activity[a]

	Normal/Enhanced Enzyme Activity		Reduced Enzyme Activity	
Nucleotide Change (allele)		Number (Frequency)		Number (Frequency)
5'-Flanking region				
–3860G→A (*CYP1A2*1C*)	–3860G	33 (97.1%)	–3860A	1 (2.9%)
–2467T→delT (*CYP1A2*1D*)	–2467T	29 (85.3%)	–2467delT	5 (14.7%)
Intron 1				
–163C→A (*CYP1A2*1F*)	–163A	27 (79.4%)	–163C	7 (20.6%)
–739T→G (*CYP1A2*1E*)	–739T	33 (97.1%)	–739G	1 (2.9%)
–729C→T	–729C	33 (97.1%)	–729T	1 (2.9%)

[a]Nomenclature according to www.cypalleles.ki.se.

SNPs were analyzed according to Chida et al.[24] The –163C→A (*CYP1A2*1F*) SNP was identified with the method described by Sachse et al.,[35] while the –739T→G (*CYP1A2*1E*) and –729C→T SNPs were investigated according to Aklillu et al.[31] Regarding *CYP2D6* genotypes, the *CYP2D6*3* and *CYP2D6*4* alleles were identified by allele-specific PCR followed by digestion with restriction enzymes.[36] The *CYP2D6*5* allele, with deletion of the entire *CYP2D6* gene, was investigated by long-PCR analysis, and the *CYP2D6*6* allele by tetra-primer PCR, as described by Hersberger et al.[37] Alleles with neither *CYP2D6*3, *4*, nor **6* specific mutations or identified as *CYP2D6*5* were classified as functional alleles. All samples were further analyzed by long-PCR for the duplicated/multiduplicated *CYP2D6* gene.[38,39] The patients were classified as those carrying 0, 1, 2, or more than 2 functional *CYP2D6* alleles.

Analysis of Serum Clozapine and N-Desmethylclozapine Concentrations

Serum concentrations of clozapine and its N-desmethyl metabolite were analyzed using an unpublished high-performance liquid chromatography (HPLC) method (U. Bondesson, Ph.D., personal communication). In brief, serum was alkalized and clozapine and its metabolite were extracted with hexan-dichlor-methan. Propyl clozapine was used as internal standard. The organic layer was evaporated to dryness, and the residue was dissolved in 50% methanol solution. The sample was then injected into an HPLC system (column LiChrospher 60 RP-Select B, 75 × 4.6 mm [5 μm], Merck, Darmstadt, Germany) with a UV detector set to 240 nm. The limit of quantification was 150 nmol/L for both clozapine and N-desmethylclozapine. To get indirect measures of the metabolic rate of clozapine, the concentration-to-dose (C/D) ratios of clozapine and N-desmethylclozapine were also calculated.

Analysis of B-Glucose, Hormones, and Lipids

B-glucose levels were determined by a glucose-oxidase method using the 950 Immunologic-Rate-Colorimetric system (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, N.Y.). Insulin and C-peptide were measured using commercial kits consisting of fluoroimmunometric assays (Delfia insulin and Delfia C-peptide, Wallac, Inc., Turku, Finland). Triglyceride concentrations were determined by an enzymatic method as described by Spayd et al.,[40] and cholesterol by an enzymatic method similar to that proposed by Allain et al.[41]

Determination of HOMA Index for Insulin Resistance

The Homeostasis Model Assessment index for insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin concentration (μU/mL) × fasting glucose concentration (mmol/L)/22.5.[42,43]

Statistics

As the different variables were assumed not to be normally distributed, nonparametric statistical methods were used. Data are described as median and range. The Kruskal-Wallis analysis of variance on ranks was used to evaluate differences when 3 groups were compared. When a significant difference among 3 groups was detected, pair-wise comparisons were performed by means of Dunn's test. To evaluate differences between 2 groups, the Mann-Whitney test was employed, and to compare frequencies of variables between groups, the Fisher exact test was used. In addition, the strength of the linear relationship between 2 parameters was calculated using the Spearman rank correlation coefficient (r_s). A p value of less than .05 was considered statistically significant. All calculations were made with the statistical program GraphPad Prism 4 (GraphPad Software, Inc., San Diego, Calif.).

RESULTS

CYP1A2 and *CYP2D6* Genetic Polymorphisms

The frequencies of the *CYP1A2* SNPs analyzed in the study, and grouped according to expected influence on enzyme activity, are given in Table 1. Among the 17 patients, 4 carried 2 *CYP1A2* SNPs related to expectedly reduced enzyme activity, 7 carried 1, and 6 carried no such SNPs.

Concerning *CYP2D6*, 1 patient (5.9%) carried 2 detrimental *CYP2D6* alleles, 5 (29.4%) carried 1, and the

Table 2. Serum Clozapine and Metabolite Concentrations, Their Concentration-to-Dose Ratios and Clozapine-to–N-Desmethylclozapine Concentration Ratio, as Well as B-Glucose, Hormone, and Lipid Levels and Homeostasis Model Assessment Index for Insulin Resistance (HOMA-IR) in the 17 Patients Investigated

	Reference Range		Median (range)	
Variable	SI Units	Metric Units	SI Units	Metric Units
Serum clozapine concentration	nmol/L	ng/mL	1200 (210–2810)	392 (69–918)
Serum N-desmethylclozapine concentration	nmol/L	ng/mL	920 (280–2050)	288 (88–641)
Clozapine concentration-to-dose ratio	nmol/L/mg	ng/mL/mg	3.2 (1.1–16.2)	1.0 (0.4–5.3)
N-desmethylclozapine concentration-to-dose ratio	nmol/L/mg	ng/mL/mg	2.8 (1.0–8.0)	0.9 (0.3–2.5)
Clozapine-to–N-desmethylclozapine concentration ratio			1.2 (0.8–2.0)	
B-glucose	3.5–6.4 mmol/L	63.1–115.3 mg/dL	5.5 (4.5–6.9)	99.1 (81.1–124.3)
Insulin	< 79 pmol/L	< 11 μU/mL	90 (22–163)	13 (3–23)
C-peptide	0.22–0.68 nmol/L	0.66–2.05 ng/mL	0.81 (0.29–1.36)	2.45 (0.88–4.11)
Triglyceride			1.9 (0.9–4.8)	168.3 (79.7–425.2)
≤ 50 y	0.3–1.8 mmol/L	26.6–159.4 mg/dL		
> 50 y	0.4–2.2 mmol/L	35.4–194.9 mg/dL		
Cholesterol			5.5 (3.6–7.4)	212.7 (139.2–286.2)
< 40 y	< 5.5 mmol/L	< 212.7 mg/dL		
40–59 y	< 6.0 mmol/L	< 232.0 mg/dL		
≥ 60 y				
Men	< 6.0 mmol/L	< 232.0 mg/dL		
Women	< 6.5 mmol/L	< 251.4 mg/dL		
HOMA–IR	μU/mL		2.79 (0.72–6.46)	

remaining 11 (64.7%) carried 2 functional alleles. No patient carried more than 2 functional alleles.

Serum Clozapine and Metabolite Concentrations

The median and range of serum clozapine and N-desmethylclozapine concentrations, their concentration-to-dose (C/D) ratios, and clozapine-to–N-desmethylclozapine concentration ratio in the patients are described in Table 2. A large interindividual variation was seen in all these parameters. The serum clozapine concentration did not correlate with the clozapine dose. However, a high correlation ($r_s = 0.96$, $p < .0001$) was found between the N-desmethylclozapine and clozapine concentrations. No significant differences were found in clozapine and N-desmethylclozapine C/D ratios or in clozapine-to–N-desmethylclozapine concentration ratio between smokers (N = 6) and nonsmokers (N = 11) (data not shown).

B-Glucose, Hormones, Lipids, and HOMA-IR

The median and range of B-glucose, insulin, C-peptide, triglycerides, cholesterol, and HOMA-IR are given in Table 2. Elevated levels of B-glucose (> 6.4 mmol/L; > 115.3 mg/dL) were found in 2 (12%), elevated levels of insulin (≥ 79 pmol/L; ≥ 11 μU/mL) in 10 (59%), and elevated levels of C-peptide (> 0.68 nmol/L; > 2.05 ng/mL) in 13 (76%) of the patients. Furthermore, 8 (47%) of the patients had elevated triglyceride levels, and 6 (35%) had elevated cholesterol values. Levels of insulin an C-peptide correlated positively to the serum clozapine concentration ($r_s = 0.53$, $p = .03$ and $r_s = 0.51$, $p = .04$, respectively), and between triglyceride levels and the serum clozapine concentration, there was a tendency toward a correlation ($r_s = 0.46$, $p = .06$). However, no correlation was found between cholesterol and the serum clozapine concentration. Neither did the hormone or lipid levels significantly correlate to the serum N-desmethylclozapine concentration.

Impact of *CYP1A2* and *CYP2D6* Genetic Polymorphisms on Clozapine Kinetics

There were significant differences in clozapine and N-desmethylclozapine C/D ratios between the patients carrying 2, 1, or no *CYP1A2* SNPs related to expectedly reduced enzyme activity (as defined in Table 1) (Figure 1). The C/D ratios of both clozapine and N-desmethylclozapine were higher in the patients with 2 such SNPs compared to those with none ($p < .05$; Figure 1). However, no differences were found in the clozapine-to–N-desmethylclozapine concentration ratio in relation to *CYP1A2* genotypes.

No differences were found in either clozapine or N-desmethylclozapine C/D ratios or in clozapine-to–N-desmethylclozapine concentration ratio between the patients with 1 or 2 *CYP2D6* detrimental alleles (N = 6) and those with 2 functional *CYP2D6* alleles (N = 11).

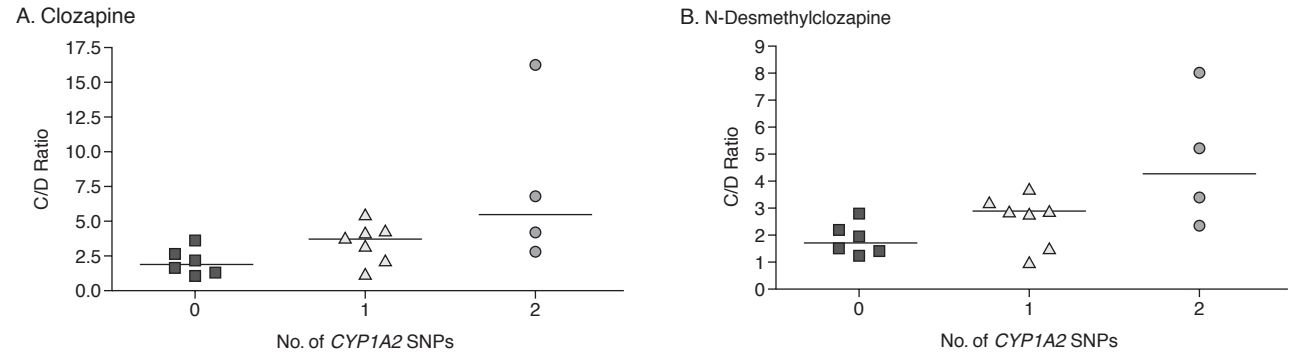
Impact of *CYP1A2* and *CYP2D6* Genetic Polymorphisms on Hormones, Lipids, and HOMA-IR

No significant differences were found in insulin, C-peptide, triglyceride, or cholesterol levels or in HOMA-IR among the patients carrying 2, 1, or no *CYP1A2* SNPs related to expectedly reduced enzyme activity.

Neither were there any significant differences in insulin, C-peptide, triglyceride, or cholesterol levels or in HOMA-IR between the patients with 1 or 2 *CYP2D6* detrimental alleles (N = 6) and those with 2 functional *CYP2D6* alleles (N = 11).

However, when the patients were divided according to insulin levels (elevated or normal), it was revealed that

Figure 1. Concentration-to-Dose (C/D) Ratios for Clozapine (A) and N-Desmethylclozapine (B) in Patients Carrying 0, 1, or 2 *CYP1A2* Single Nucleotide Polymorphisms (SNPs), Related to Expectedly Reduced Enzyme Activity (–3860A, –2467delT, –163C, –739G, and/or –729T)[a]



[a]Median C/D ratios in each group of patients (horizontal lines) are indicated.

Table 3. *CYP1A2* Genetic Polymorphisms, Clozapine Metabolism, C-Peptide, Lipids, and HOMA-IR in the 17 Patients Investigated When Subgrouped by Elevated or Normal Insulin Levels[a]

Variable	Elevated Insulin Levels (≥ 79 pmol/L)	Normal Insulin Levels (< 79 pmol/L)	p Value
Patients, N	10	7	
Insulin, pmol/L	130 (86–163)	50 (22–74)	.0001
Smokers, N	4[b]	2[c]	NS
Patients carrying –3860A and/or –2467delT *CYP1A2* variants, N	5	0	.04
Patients carrying –163C and/ or –739G and/or –729T *CYP1A2* variants, N	5	3	NS
Serum clozapine concentration, nmol/L	1525 (320–2810)	780 (210–2370)	NS (.07)
Clozapine C/D ratio	4.0 (2.2–16.2)	1.6 (1.1–6.8)	.02
N-desmethylclozapine C/D ratio	2.9 (2.0–8.0)	1.5 (1.0–5.2)	.02
Clozapine-to–N-desmethylclozapine concentration ratio	1.2 (1.1–2.0)	1.3 (0.8–1.5)	NS
C-peptide, nmol/L	1.11 (0.70–1.36)	0.66 (0.29–0.85)	.003
Triglycerides, mmol/l	2.8 (1.6–4.8)	1.0 (0.9–1.9)	.0007
Cholesterol, mmol/L	6.6 (4.0–7.4)	5.0 (3.6–5.6)	.02
HOMA-IR	4.98 (2.40–6.46)	1.87 (0.72–2.43)	.0002

[a]Values are given as median (range) unless otherwise specified.
[b]Of whom 1 patient smoked 1–10 cigarettes/day, 2 patients smoked 11–20 cigarettes/day, and 1 patient smoked > 20 cigarettes/day.
[c]Of whom 1 patient smoked 1–10 cigarettes/day, and 1 patient smoked 11–20 cigarettes/day.
Abbreviations: C/D = concentration-to-dose, HOMA-IR = homeostasis model assessment index for insulin resistance, NS = not significant.

the patients with elevated insulin levels carried the –3860A and/or –2467delT *CYP1A2* variants more frequently, compared to the patients with normal insulin levels (p = .04; Table 3). In contrast, there were no differences in the number of patients carrying –163C and/or –739G and/or –729T *CYP1A2* variants (Table 3), or different *CYP2D6* genotypes, between the 2 subgroups. Moreover, clozapine and N-desmethylclozapine C/D ratios, levels of C-peptide, triglycerides, and cholesterol and HOMA-IR were higher in the patients with elevated insulin levels compared to the patients with normal insulin levels (Table 3).

DISCUSSION

In this study, clozapine-treated patients carrying *CYP1A2* SNPs, reported to be associated with expectedly reduced enzyme activity, had higher clozapine and N-desmethylclozapine C/D ratios, compared with patients not carrying these variants, suggesting genetically determined variation in clozapine metabolism via CYP1A2. In contrast, *CYP2D6* genotype did not influence either clozapine or N-desmethylclozapine C/D ratios. The results are, thus, in line with previous in vivo findings, indicating a major role for CYP1A2 in the metabolism of clozapine, in contrast to a minor or no involvement of CYP2D6.[15,16,21,22]

With respect to the metabolic parameters insulin, C-peptide, triglycerides, cholesterol, and HOMA-IR, no differences were found between clozapine-treated patients carrying 2, 1, or no *CYP1A2* SNPs associated with expectedly reduced enzyme activity, or different *CYP2D6* genotypes. These findings may point to a lack of a direct

relationship between the *CYP1A2* or *CYP2D6* polymorphisms studied and elevations of insulin and lipids and insulin resistance in clozapine-treated patients.

A subgroup analysis, however, revealed that patients with elevated fasting insulin levels had more frequently *CYP1A2* SNPs associated with reduced enzyme activity (–3860A, –2467delT), higher clozapine and N-desmethylclozapine C/D ratios, and higher lipid levels and HOMA-IR, compared to the patients with normal insulin levels. Our findings are in accord with previous studies, showing an association between the –3860A *CYP1A2* variant (*1C) and decreased inducibility (implying reduced activity) of the CYP1A2 enzyme, but no association between the *CYP1A2* polymorphism **1F* and clozapine clearance.[24,25,27,30,35] Thus, patients with genetic polymorphisms in the *CYP1A2* gene, especially those having *CYP1A2* **1C* and/ or **1D,* seem to have higher serum clozapine concentrations, and thereby an increased risk of developing insulin and lipid elevations and insulin resistance on a given dose of clozapine. To our best knowledge, metabolic adverse effects of clozapine such as insulin and lipid elevations and insulin resistance have not earlier been studied in relation to impaired CYP1A2 enzyme activity or *CYP1A2* genotypes.

Interestingly, an earlier in vitro study has demonstrated that insulin suppresses CYP1A2 mRNA expression in hepatocytes dose-dependently.[44] Furthermore, animal studies have shown that streptozocin-induced or spontaneous insulin-dependent diabetes mellitus may cause induction of CYP P450 enzymes that restore when insulin is supplied.[45–47] On the contrary, the activities of CYP1A2 and other CYP enzymes seem not to be influenced by non–insulin-dependent diabetes mellitus when studied in humans or in the ob/ob mouse model.[48,49] In our study, although insulin resistance was found in several of the patients and 2 patients were diagnosed with a prediabetic state, none of the patients had manifest diabetes mellitus. Therefore, there is no reason to believe that the current metabolic state in our clozapine-treated patients would have influenced their CYP1A2 and CYP2D6 enzyme activities.

Occurrence of *CYP1A2* polymorphisms in clozapine-treated patients may explain why some patients, but not others, get higher serum clozapine concentrations and develop insulin and lipid elevations and insulin resistance on a given dose of the drug. In this context, the findings in this study underline the importance of determination of serum clozapine concentration together with regular monitoring of fasting B-glucose and fasting serum insulin and lipid levels in patients receiving clozapine, in order to optimize the clozapine dosage and thereby decrease the risk of adverse metabolic effects in this patient group.[10] These measures seem to be of main clinical concern, given that a high prevalence of the metabolic syndrome (i.e., insulin resistance and hyperlipidemia and/or obesity) recently has been established among clozapine-treated patients[50] and that this syndrome may increase the risk not only of diabetes mellitus, but also of cardiovascular disease in these patients, especially in the long term.[6,7,51]

Although tobacco smoke is a potent inducer of CYP1A2 enzyme activity,[52–54] no significant difference was found in clozapine C/D ratio between smokers and nonsmokers in our study. This may be explained by both the limited numbers of smokers respective to nonsmokers in the subgroups compared, as well as the fact that only 4 of the 17 patients in the study smoked more than 10 cigarettes daily. Nevertheless, it is unlikely that the smoking behavior of the patients confounded our results. Neither did the patients take any medications that are known to induce or inhibit CYP1A2 and/or CYP2D6 enzyme activities.[33,55–58] In addition, the frequencies of *CYP1A2* and *CYP2D6* genotypes in the present study are close to those reported earlier in other Caucasian populations,[13,31,35,59] indicating that the patient group is representative in this respect.

In conclusion, although the limited number of patients included in our study does not allow definite conclusions to be drawn, our results indicate that *CYP1A2*, but not *CYP2D6*, genetic polymorphisms are associated with reduced clozapine metabolism in patients on continuous treatment with clozapine. Moreover, patients carrying *CYP1A2* SNPs associated with expectedly low enzyme activity seem to have an increased risk for developing higher serum clozapine and N-desmethylclozapine concentrations, insulin and lipid elevations, and insulin resistance on a given dose of clozapine. Finally, our results underline the clinical importance of controlling for both serum clozapine concentrations and metabolic parameters in patients receiving clozapine.

Drug names: carbamazepine (Carbatrol, Tegretol, and others), ciprofloxacin (Cipro, Proquin XR, and others), clozapine (FazaClo, Clozaril, and others), lithium (Eskalith, Lithobid, and others), phenytoin (Dilantin and others), streptozocin (Zanosar).

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