Factors affecting interindividual differences in clozapine response: a review and case report

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Objective Clozapine is the most powerful new-generation antipsychotic. Although this drug leads to great therapeutic benefits, two types of undesirable conditions frequently occur with its use: side effects and resistance to treatment. Therapeutic drug monitoring of clozapine would be very useful to avoid both these situations. The necessity of monitoring the therapy is the result of a wide interindividual variability in the metabolism of clozapine. In this review, we highlight all the conditions underlying this variability, analyzing them one by one.

Methods Relevant literature was identified through a search of MEDLINE and PubMed. In addition, the case of a treatment-resistant patient with accelerated metabolism of clozapine is reported as representative of utility of therapeutic drug monitoring in terms of clozapine dose adjustment.

Results Genetic polymorphisms of cytochrome P450 enzymes and of neurotransmitter receptors; drug interactions; interactions of clozapine with other substances such as food and drink; smoking; and nonmodifiable variables such as age, ethnicity, and gender have been examined in relation to the existing scientific literature. The laboratory techniques that clinicians could use to identify these variables and adequate therapies are also reviewed. Copyright © 2011 John Wiley & Sons, Ltd.

key words — clozapine metabolism; therapeutic drug monitoring; CYP450 polymorphisms; drug–drug interactions

INTRODUCTION

Clozapine

Clozapine is classified as an atypical antipsychotic drug because of its capacity of binding serotonin as well as dopamine receptors (Naheed and Green, 2001); its effects on various dopamine-mediated behaviors also differ from those exhibited by more typical antipsychotics. In particular, clozapine interferes to a lower extent with the binding of dopamine at D₁, D₂, D₃, and D₅ receptors and has a high affinity for the D₄ receptor, but it does not induce catalepsy nor inhibit apomorphine-induced stereotypy in animal models as is seen with conventional neuroleptics. This evidence suggests that clozapine is preferentially more active at limbic than at striatal dopamine receptors and may explain the relative freedom of clozapine from extrapyramidal side effects together with strong anticholinergic activity.

Clozapine was first introduced in Europe in 1971 but was voluntarily withdrawn by the manufacturer in 1975 after it was shown to cause agranulocytosis. In 1989, after studies demonstrated that it was more effective than any other antipsychotic for treating schizophrenia, the US Food and Drug Administration approved clozapine use but only for treatment-resistant schizophrenia. In 2002, the Food and Drug Administration approved clozapine for reducing the risk of suicidal behavior for patients with schizophrenia and schizoaffective disorder.

Metabolism, pharmacokinetics, and pharmacodynamics

Several metabolites of clozapine exhibit binding profiles similar to clozapine. Norclozapine (N-desmethylclozapine) may contribute significantly to the atypical effects of clozapine treatment. Norclozapine acts as an agonist and/or partial agonist at D₂, D₃, δ-opioid, M₁, M₂, M₃, M₄, and M₅ receptors and as an antagonist/inverse agonist at 5-HT2A and 5-HT2C receptors. Clozapine is also a partial agonist at the 5-HT1A receptor and is supposed to improve depression, anxiety, and negative and cognitive symptoms. Clozapine is also a strong
antagonist at different subtypes of adrenergic, cholinergic, and histaminergic receptors, the last two being predominantly responsible for its side-effect profile. More details about the pharmacodynamics of clozapine are in the section “Genetic polymorphisms of neurotransmitter receptors.”

The absorption of clozapine is almost complete, but the oral bioavailability is only 60–70% because of first-pass metabolism. The time to peak concentration after oral dosing is about 2.5 h, and food does not appear to affect the bioavailability of clozapine. The elimination half-life of clozapine is about 14 h at steady-state conditions (varying with daily dose).

Clozapine is extensively metabolized in the liver, by the cytochrome P450 (CYP450) system, to polar metabolites suitable for elimination in the urine and feces. After the first-pass metabolism, the two metabolites norclozapine and N-oxide are formed. The major metabolite, norclozapine, is pharmacologically active. Norclozapine has special pharmacological activities: it increases the release of dopamine and acetylcholine in the prefrontal cortex and hippocampus; it enhances the activity of N-methyl-D-aspartate receptors in the hippocampus (Li et al., 2005), and it is the only available M1 agonist antipsychotic that may improve cognitive symptoms in clozapine-treated patients.

The cytochrome P450 isoenzyme 1A2 (CYP1A2) is primarily responsible for clozapine metabolism, but 2C, 2D6, and 3A4 appear to play roles as well. Agents that induce or inhibit CYP1A2 may increase or decrease, respectively, the metabolism of clozapine. Approximately 50% of the administered dose of clozapine is excreted in the urine and 30% in the feces. Excretion of the two clozapine metabolites (N-oxide and norclozapine) depends on the activity of a transmembrane transporter expressed in the liver and the kidneys named ABCB1/p-glycoprotein. The activity of ABCB1 may strongly affect drug pharmacokinetics during absorption and distribution (Consoli et al., 2009). More details about the pharmacokinetics of clozapine are in the section “Genetic polymorphisms related to clozapine metabolism.”

Therapeutic drug monitoring of clozapine

Therapeutic drug monitoring of psychotropic medication is an important component of modern psychiatric management. It may be used to assist in monitoring compliance, maximizing the efficacy of medications with variable metabolism, avoiding toxicity, minimizing adverse effects, and diagnosing overdose. Clozapine and norclozapine plasma levels may also be monitored, though they show a significant degree of variation; for example, they are higher in women and increase with age (Naheed and Green, 2001). Monitoring of plasma levels of clozapine and norclozapine has been shown to be useful in assessment of compliance, metabolic status, prevention of toxicity and dose optimization. Despite the introduction of newer drugs, clozapine remains the most effective drug in psychotic patients who are resistant to treatment with other antipsychotic drugs, typical or atypical. Optimal therapeutic responses to clozapine have been reported with serum concentrations between 350 and 1000 ng/ml (Raedler et al., 2008).

METHODS

Relevant literature was identified through a search of MEDLINE and PubMed. Search terms included clozapine, clozapine metabolism, clozapine interactions, CYP450 polymorphisms, CYP3A4, CYP1A2, clozapine drug–drug interactions, clozapine therapeutic drug monitoring, and clozapine resistance. References of select citations were searched manually to retrieve articles not found in the electronic database search. We included in our study several clinical trials, reviews, and meta-analyses.

CASE REPORT

The patient is a 23-year-old white man, celibate and unemployed. He has been hospitalized in our institute after a delusional autolesionist act (he cut the angles of his lips to enlarge his mouth).

The patient had a diagnosis of disorganized schizophrenia, and his psychopathological onset was at the age of 16 years, with a psychotic symptomatology characterized by a strong dysmorphic component associated with various autolesionist acts such as setting himself on fire to make his skin hairless, stripping his own skin off to remove moles, and pulling his genitals to make them bigger. His toxicological anamnesis was positive for a strong abuse of substances (cannabis, cocaine, ketamine, lysergic acid diethylamide) before the psychopathological onset.

During the 5 years following the psychopathological onset, he was treated with clozapine, haloperidol, benzodiazepines, and amisulpride at unknown dosages and in a ragged way.

For the next 2 years, he was hospitalized several times in different psychiatric units and nursing homes.

In April 2010, he was hospitalized at our institute (the psychiatric unit of the University Hospital Policlinico Umberto I) in the condition of mandatory psychiatric hold.

At admission, he was vigilant and space–time oriented; he had a shabby appearance and neglected personal hygiene. He was cooperative with the clinical...
staff. He presented disorganized thought and disorganized speech characterized by bizarre content with dysmorphic orientation. He also had a structured delusion centered on sentimental issues. Because of the severity of his clinical condition and the low response to pharmacological therapy, there was a suspicion of psycho-organic syndrome, and we organized the following diagnostic assessment: cerebral magnetic resonance imaging, cerebral single-photon emission computed tomography (SPECT) and neuropsychological exams. Cerebral magnetic resonance imaging both with and without spectroscopy did not show significant abnormalities. Cerebral SPECT showed a diffuse irregularity of the cortical vascular rolation, where the prevalence of perfusion defects was linked to focal abnormally hyperactive zones. Neuropsychological exams evidenced deficits in mnemonic, executive, and attentive functions involving mostly fronto-subcortical circuits.

During the hospitalization, the patient received the following therapy: clozapine up to 900 mg/day, amisulpride up to 1200 mg/day, haloperidol up to 10 mg/day, valproic acid 500 mg/day, clonazepam 4.5 mg/day, diazepam 18 mg/day, risperidone 6 mg/day, aripiprazole up to 30 mg/day, amoxicillin 2 g/day, betamethasone 1 mg/day, and acetylcysteine 300 mg/day.

In June 2010, clozapine was gradually withdrawn because of minimal clinical effects, and it was replaced with risperidone. This change led to a dramatic worsening of his psychopathological status: new episodes of self-injurious behavior occurred; speech and thought became much more disorganized and accelerated, with a worsening of dysmorphic ideation. Daily, the patient drank 6–7 cups of coffee and smoked 15–20 cigarettes.

Despite arriving at up to the maximum indicated dosages of clozapine, the patient did not have a significant clinical improvement during his hospitalization, nor did he present any side effect related to this drug. In October 2010, the patient was transferred to a therapeutic community, while he was assuming the following therapy: clozapine 800 mg/day, risperidone 4 mg/day, valproic acid 500 mg/day, and diazepam 4.5 mg/day.

We requested the dosage of serum clozapine levels 1 week before his transfer, and we had the result after his transfer. The dosage reported a plasma clozapine concentration of 168.03 ng/ml, which was much lower than the therapeutic threshold (350 ng/ml).

Although the patient was correctly treated in relation both to the drug doses and to the duration of treatment, his metabolic status induced a failure in the achievement of effective clozapine plasma levels.

**FACTORS AFFECTING SERUM CLOZAPINE LEVELS**

As mentioned in the “Introduction,” the concentration of clozapine in plasma varies with the level of efficiency of its metabolism. It seems evident that an increased metabolism of this molecule leads to a low clozapine plasma level and therefore to the need of increasing the dosage of the drug to obtain a good therapeutic response, whereas a decreased metabolism of the molecule will lead to a high clozapine plasma level and therefore to the need of reducing the dosage of the drug to avoid side effects.

Now, we give an overview of all the known factors that may affect serum clozapine levels.

**Genetic polymorphisms of neurotransmitter receptors**

The interindividual variability in response to clozapine poses limitations with respect to the therapeutic use of this drug. It is, therefore, important to determine sources of interindividual variation in response, as these may lead to improvements in the therapeutic use of clozapine.

Variables including early age at onset of illness and female gender were found to be predictors of poor response, whereas predictors of good response included the presence of extrapyramidal symptoms (EPS) during previous treatment with conventional antipsychotics and a diagnosis of paranoid schizophrenia (Lieberman et al., 1994). Although these variables are useful for determining outcome once patients have started the treatment, pharmacogenetics can be employed to determine the genetic basis of variability among patients to drug response prior to the initiation of treatment.

Clozapine has affinity, ranging from low to high, for many receptors of multiple neurotransmitter systems. These include the dopamine D1–D5, serotonin 5-HT1A, 5-HT2A, 5-HT2C, 5-HT3, 5-HT6, 5-HT7, alpha1-adrenergic, muscarinic cholinergic M1–M5, and histaminergic H1 and H3 receptors. The low affinity of clozapine for dopamine D2 receptors (Nordstrom et al., 1995) and high affinity for muscarinic and 5-HT2A receptors (Miller and Hiley 1974) have been implicated in its low propensity to induce EPS. Blockade of D1 receptors by clozapine has been hypothesized to contribute to its efficiency against negative symptoms as well as its ability to prevent tardive dyskinesias (TD) (Kinon and Lieberman, 1996). The low EPS and other advantages of clozapine have been attributed to its D4 blocking properties based on the observation that clozapine has a 10-fold higher affinity for the D4 receptor than for D2 and D3 receptors (Sanyal and Van Tol, 1997). Meltzer and his colleagues have hypothesized that the clinical advantages of clozapine...
and other atypical antipsychotics, especially those related to EPS and negative symptoms, may be due to their significantly higher affinity for 5-HT2A receptors relative to their affinity at D2 receptors (Meltzer and Nash, 1991). In vivo studies, using positron emission tomography, demonstrate that all atypical antipsychotics share this particular pharmacodynamic feature (Kapur, 1998). Currently, this is the most widely accepted hypothesis that can explain many of the unique clinical attributes of clozapine. Polymorphism in neurotransmitter receptors for which clozapine has affinity may contribute, in part, to the variability among individuals in response.

Dopamine receptors. D1-like. Among D1-like receptors, D5 receptor does not display significant affinity for clozapine or appear to contribute to the efficacy of the drug (Mancama et al., 2002).

On the other side, Hwang et al. investigated the effect of four single-nucleotide polymorphisms (SNPs) in dopamine D1 receptor gene on clozapine response in two distinct schizophrenic populations (white and African American) refractory or intolerant to conventional antipsychotics. They have obtained data which may support a minor role for the dopamine D1 receptor gene rs4532 heterozygote genotype in poor response to clozapine in either whites or African Americans. An SNP (rs265976) was also associated with response in African Americans (Hwang et al., 2007).

D2-like. Several studies have focused on the potential influence of polymorphisms within genes encoding the D2-like receptors, namely D2, D3, and D4, because these subtypes may be integral to the antipsychotic action of clozapine.

D2. A high presence of polymorphisms has been demonstrated within the D2 receptor (Wong et al., 2000), including a mutation that alters structure (Ser311 → Cys) and a polymorphism (−141 Ins/Del) that alters function. However (Malhotra et al., 1999), while a single positive association has been reported between the −141 Ins/Del polymorphism and response, previous findings largely do not support the influence of D2 polymorphisms on clozapine response (Shaikh et al., 1994).

D3. Findings for the D3 receptor are currently less clear. A structural polymorphism (Ser9 → Gly) has been found to be associated with clozapine response (Scharfetter et al., 1998). However, because several further studies have not been able to confirm this association, the potential involvement of variants in this receptor remains the subject of further investigation.

D4. The D4 receptor, which possesses the highest dopamine receptor affinity for clozapine, is known to contain several functionally important polymorphisms. Included among these is a 13-base-pair deletion that leads to a truncated, nonfunctional receptor and a 48-base-pair repeat polymorphism that alters the affinity of the receptor for clozapine (Sanyal and Van Tol, 1997).

Despite the functional nature of these polymorphisms, no evidence has yet been found to support their involvement in treatment response.

In contrast, the third D4 polymorphism, constituting a (G)n mononucleotide repeat, has also been recently investigated and found to be moderately associated with response (Ozdemir et al., 1999). However, as with the other associations, which have been demonstrated between polymorphisms in this system and response, further verification through additional independent studies is still required.

Overall therefore, there is as yet insufficient evidence to support a significant contribution of genetic variation within dopamine receptor genes to clozapine response. In the meantime, investigation of novel polymorphisms in the promoter region of the D4 gene may help to clarify the current picture; this is also the case for the D3 receptor, for which possession of particular haplotypes is significantly associated with increased susceptibility to schizophrenia (Ishiguro et al., 2000). At the same time, it is clear that the collection of larger, more extensively characterized patient samples might improve the likelihood of establishing individual associations between polymorphic variants and particular symptoms or adverse effects related to treatment. This approach has been applied and its potential tentatively demonstrated for antipsychotic-induced TD, for which homozygosity for the glycine variant of the D3 Ser9 → Gly polymorphism has been found to be associated with increased susceptibility towards TD (Basile et al., 1999). However, other studies of similar design have not been able to confirm this, and further work is required to clarify these early findings (Mancama et al., 2002).

Serotonin receptors. Clozapine has been shown to potently antagonize 5-HT2A, 5-HT2C, and 5-HT6 receptors, making the genes that encode them strong candidates through which alterations might significantly influence drug response. In spite of clozapine
role in 5HT1A partial antagonism, we have not found any study investigating genetic alterations of 5HT1A receptor and their influence on clozapine response.

5-HT2A. In the T102C polymorphism of the 5HT2a receptor gene, the base in nucleotide position 102 may be thymine (T) or cytosine (C), with three possible genotypes TT, TC, or CC. This mutation does not result in any change in the amino acid sequence of the 5HT2a receptor, as both alleles encode for a serine in codon 34 (Warren et al., 1993).

In 1995, Arranz and colleagues identified a significant association between the silent 102-T/C polymorphism in the 5-HT2A gene and clozapine response. However, several subsequent studies were unable to confirm the reported association (Nothen et al., 1995; Malhotra et al., 1996), prompting further investigation by meta-analysis of combined data from these studies (Arranz et al., 1998).

The results demonstrated the presence of an overall trend towards association between the 102-T/C polymorphism and response, supporting the original findings (Arranz et al., 1995).

A structural polymorphism (His452→Tyr) has also been identified in the 5-HT2A receptor, for which the 452Tyr variant demonstrates association with poor response to clozapine (Arranz et al., 1996). Although two subsequent studies (Nothen et al., 1995; Malhotra et al. 1996) did not lead to statistically significant findings, their results suggested the presence of a similar underlying trend. This trend was subsequently confirmed through meta-analysis of the four studies and inclusion of a further independent study, where significant association was observed between the 452Tyr allele and poor response to treatment (Arranz et al., 1998). Concurrent findings (Masellis et al., 1998) have provided some support for a moderate association between this polymorphism and treatment response.

5-HT2C. Relatively less support exists for an influence of 5-HT2C receptor polymorphisms on clozapine response, in spite of the potency of the drug at this site. Although an association was initially reported between a nonfunctional 5-HT2C Cys23→Ser polymorphism and response (Sodhi et al., 1995), subsequent studies do not support this (Masellis et al., 1998).

5-HT3A and 5-HT5A. Of the remaining serotonin receptors, polymorphisms within the 5-HT3A and 5-HT5A receptors do not appear to confer a significant influence on response (Birkett et al., 2000).

5-HT6. Yu et al. tested the hypothesis that clinical response to clozapine in patients refractory to typical antipsychotic treatment is related to the genetic variant (C267T) of the 5HT6 receptors. Ninety-nine schizophrenic patients with a history of nonresponse to typical antipsychotics were included in the study. The results demonstrated a modest but significant relationship between the presence of the variant of the 5HT6 receptors and the response to clozapine in these patients. Patients with homogenous 267 T/T genotype had a better response than other patients. Although replication is required, these results suggest that the 5HT6 receptor C267T polymorphism may be involved in clozapine response (Yu et al., 1999). In contrast, 2 years later, Masellis et al. report a lack of association between a C267T polymorphism and clozapine response (Masellis et al., 2001).

SERT. The serotonin transporter gene, involved primarily in the removal of serotonin from the synaptic cleft and its transportation into the presynaptic terminal, has also been the subject of recent investigation. However, of the polymorphisms investigated to date, none appears to be an important determinant of clozapine response.

Other receptors. H1 and H2. Although functional polymorphisms have been identified within the H1 receptor, none of these have been found to influence response to clozapine (Mancama et al., 2000).

However, a novel promoter polymorphism (−1018-G/A) has been identified in the H2 receptor, which displays a minor association with response (Arranz et al., 2000), although this currently awaits independent confirmation.

M1, M3, and M4. Mancama et al. investigated novel polymorphisms in the M1, M3, and M4 receptor genes but have not identified any that might be important regarding clozapine response.

Alfa1–Alfa2. At present, there is no evidence to support any participation of alfa1 and alfa2-receptor polymorphisms in treatment response.

Genetic polymorphisms related to clozapine metabolism

Most drugs are metabolized through reactions of phase 1 and phase 2. The primary role of phase 1 reactions is the addition of functional groups (e.g., –OH, –COOH, etc) often terminating biologic activity, whether, as written before, clozapine represents an exception because norclozapine (biologically active) is formed.
in this phase. Phase 2 reactions serve to increase water solubility and increase elimination of a drug facilitating its renal elimination. The oxidative reactions are among the most important phase 1 reactions; they are often mediated by the CYP enzyme family. Nowadays, more than 17 genetic families coding for CYP have been identified in mammals and more than 30 human gene products. CYP enzymes showing a homology in the DNA sequence greater than or equal to 40% are classified in the same family, those with homology greater than 55% in the same subfamily. Thus, for example, CYP3A4 is located in the CYP enzyme family number 3, subfamily A, genetic product 4. A drug may be the substrate of one or more different CYP. Several kinds of CYP are present in the population, and this is the explanation of the considerable interindividual variability in the metabolism of different substances. According to the variants of CYP expressed, people can be classified as slow metabolizers, rapid metabolizers and ultrarapid metabolizers.

Even transmembrane transporter ABCB1 polymorphisms in the kidneys and liver can affect the plasma concentrations of clozapine.

We now describe the details of the role of the major cytochromes and proteins involved in the metabolism of clozapine.

CYP1A2. CYP1A2 gene is coded by a 7.8-kb gene, consisting of seven exons and six introns, mapped to chromosome 15. A considerable interindvidual variability, because of both genetic mutations and environmental factors, was observed in the elimination of drugs metabolized by CYP1A2. CYP1A2 is an enzyme highly inducible both in terms of enzyme activity and in terms of its genetic expression (Shu-Feng et al., 2009). It is estimated that this interindvidual variability is linked to genetic factors in a proportion roughly ranging between 35% and 75% (Rasmussen et al., 2002). The identification of genetic, epigenetic, and environmental factors regulating the expression and activity of CYP1A2 may be helpful in choosing the best dosage of all drugs metabolized by CYP1A2. So far, 36 allelic variants have been identified (from 1B* to *16) (http://www.cypalleles.ki.se/, update 12/11/2010) (Table 1).

- CYP1A2*1A1 is considered as the wild-type variant.
- The variant allele CYP1A2*1C contains the SNP 3860G>A located in the flanking region in the 5' of the CYP1A2 gene. The expression of this allele seems to be associated with reduced enzyme activity, probably linked to a reduced inducibility of the enzyme and a consequent reduced expression of it (Nakajima et al., 1999).

• The expression of CYP1A2*1K allele is associated with a significant reduction of enzyme activity compared with the expression of the wild-type allele. The SNP 729C>T leads to the disappearance of a binding site for nuclear factor Ets, leading to a significant reduction in the expression of CYP1A2 (Aklillu et al., 2003). Higher concentrations of clozapine and its metabolite N-desmethylclozapine (norclozapine) were found in patients presenting two allelic variants of CYP1A2 (*1C, *1K) associated with reduced enzymatic activity (Mekkerson et al., 2007).
• CYP1A2*1F allele (163C>A, intron 1) is quite common in the general population (Chida et al., 1999). The mutation 163C>A appears to be associated with an increase in enzyme activity (Shimizu et al., 1991).

Several clinical studies have been conducted to examine the impact of CYP1A2 polymorphisms on the clearance of drugs and on the clinical response related to them. Resistance to clozapine associated with low plasma levels of the drug has been reported in smoker schizophrenic patients carrying the CYP1A2*1F (Eap et al., 2004).

The caffeine test is very useful to have a rapid and reliable evaluation of CYP1A2 activity (Fuhr et al., 1996). It is assessed that caffeine is metabolized by CYP1A2. The test consists in giving to the patients 150 mg of caffeine in tablet and analyzing the urines of caffeine concentration in the following 24 h.

CYP2C. Compared with CYP1A2, few data are available regarding the role of CYP2C subfamily in the metabolism of clozapine. In vitro studies have demonstrated the ability of CYP2C9 to demethylate clozapine; nevertheless, from a clinical point of view, its role seems to be minor. CYP2C19 has the same

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characteristics of CYP2C9 (Linnet and Olesen, 1997; Fang et al., 1998).

The role of CYP2C8 is controversial. According to some authors, it would not be involved in the metabolism of clozapine (Eiermann et al., 1997); others recognize a role of this enzyme in the N-demethylation of clozapine and in the formation of N-oxide (Fang et al. 1998).

CYP2D6. The role of CYP2D6 in the metabolism of clozapine is contradictory. Several studies carried out in vitro indicate an active role of this enzyme in the catalysis of clozapine (Linnet and Olesen, 1997; Fang et al., 1998; Tugnait et al., 1999). There are no correlations between CYP2D6 phenotype and alterations in the catabolism of clozapine in vivo (Pirmohamed et al., 1995).

CYP3A4. While the CYP1A2 seems to play an important role in the clozapine N-demethylation, the CYP3A4 is mainly involved in its N-oxidation (Tugnait et al., 1999).

Several in vitro studies have underlined the role played by CYP3A4 in the metabolism of clozapine (Linnet and Olesen, 1997; Fang et al., 1998; Tugnait et al., 1999). A study of in vitro stimulation (Linnet and Olesen, 1997) suggests that this may be responsible for about 35% of the metabolism of clozapine in vivo. However, more recent evidence seems to refute this fact, giving less importance to CYP3A4 in the metabolism of clozapine.

ABCB1. Consoli et al. documented the existence of two principal alleles for this protein that can generate three different phenotypical expressions: CC, CT, and TT.

In this study, CC patients required higher clozapine doses to achieve the same plasma concentrations as CT or TT patients (Consoli et al., 2009).

Ethnicity

There are significant ethnic differences in the distribution of common and rare CYP1A2 alleles. CYP1A2*1C is less prevalent in whites than in Asians (21–25%) (Chida et al., 1999). The frequency of *1C was significantly lower in the Turkish population (4%) (Bilgen et al., 2008) than in the Japanese (21.0%) (Chida et al., 1999) and the Chinese (25%), whereas the frequency of *1C was relatively equal in the Turkish and Egyptian populations (7%) (Hamdy et al., 2003). The frequency of CYP1A2*1D allele is lower in whites compared with Asians and Africans. In the Turkish, the frequency of *1D is very high (92%) (Bilgen et al., 2008). CYP1A2*1E and *1G were frequent in Ethiopians (10%) (63), Saudi Arabians (9.6%) (Aklilllu et al., 2003), and Japanese (8.2%) (Chida et al., 1999). However, British (Sachse et al., 2003), German–white (Skarke et al., 2005), Spaniard (Aklilllu et al., 2003), Turkish (Bilgen et al., 2008), and Egyptian (Hamdy et al., 2003) populations had a low frequency for this allele (0.4%, 1.6%, 1.7%, 1.0%, and 3.0%, respectively).

Drugs

Clozapine is frequently combined with other drugs to enhance efficacy and reduce adverse reactions, but pharmacokinetic interactions can have a significant impact on drug response.

In fact, all drugs that inhibit the activity of enzymes that metabolize clozapine (see previous section) potentially lead to an increase in plasma clozapine concentration and to an increased risk of dose-related side effects such as drowsiness, sedation, dizziness, headache, seizures, dry mouth, sialorrhea, gastrointestinal disturbances, hepatotoxicity, metabolic syndrome, visual disturbances, sweating, tachycardia, orthostatic hypotension, myocarditis, leukopenia, agranulocytosis, and neuroleptic malignant syndrome. On the other hand, drugs that induce the activity of these enzymes lead to a reduction of plasma clozapine levels and to a reduced therapeutic response.

Table 2 shows the drugs that most strongly modulate the metabolism of clozapine. Most of these drugs are inhibitors or inducers of CYP450 system.

Numerous studies have demonstrated the interaction between these molecules and specific isoforms of CYP450: Fluvoxamine inhibits both CYP1A2 and

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<td>Fluvoxamine</td>
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CYP3A4 (Jerling et al., 1994); fluoxetine and paroxetine inhibit CYP2D6 (Gossen et al., 2002), as well as quinidine. Macrolides, especially erythromycin and clarithromycin, are inhibitors of CYP3A4 (Pai et al., 2000), as well as ritonavir, nefazodone, and ketoconazole (Dresser et al., 2000). Isoniazid inhibits both CYP3A4 and CYP2C19 (Wen et al., 2002), as well as chloramphenicol. Cimetidine inhibits CYP2C6 and CYP2C11. Ciprofloxacain reduces the biosynthesis of all the enzymes of the CYP3A class (Toda et al., 2009).

The possibility of a drug interaction between clozapine and oral contraceptives has been recently suggested based on the case of a 47-year-old woman on a long-term oral contraceptive treatment with norethindrone (0.5 mg)/ethinyl-estradiol (0.035 mg) also treated with 550 mg/day of clozapine (Gabbay et al., 2002). After discontinuation of oral contraceptives, a decrease by about 50% in plasma concentrations of clozapine was observed and attributed to the inhibition of CYP1A2, CYP2C19, and CYP3A4 by oral contraceptives (Spina et al., 2007). That suggests that the assumption of oral contraceptives is related to an increase in clozapine plasma levels.

There are conflicting findings concerning the effect of valproic acid on clozapine metabolism. In fact, plasma concentrations of clozapine and its metabolites have been reported to be either decreased or increased slightly after the addition of valproic acid (Spina et al., 2007).

Other drugs such as warfarin compete with the binding to plasma proteins and can lower plasma levels of clozapine. Omeprazole induces CYP1A2, which is the greatest metabolizer of clozapine, and reduces clozapine plasma concentration (Bondolfi et al., 2005).

Even the induction of CYP3A4 increases the metabolism of clozapine and reduces its plasma levels. Phenobarbital (Facciolà et al., 1998), carbamazepine (Jerling et al., 1994), phenytoin, and rifampicin induce this enzyme.

Nicotine

Tobacco smoking is associated with induction of CYP1A2 and, possibly, uridine diphosphate-glucuronosyltransferase because of its by-products, in particular the polycyclic aromatic hydrocarbons. As a consequence, smoking influences the elimination of those antipsychotics, such as clozapine and olanzapine, whose metabolism is mainly dependent on CYP1A2 and UDP-glucuronosyltransferase. Different studies have shown that plasma concentrations of clozapine and olanzapine are lower, at the same dose, in smokers as compared with nonsmokers (Hasegawa et al., 1993). Concerning clozapine, the inducing effect of smoking was more evident in men than in women (Hasegawa et al., 1993). Smoking cessation, if not accompanied by a dosage decrease, may be associated with increased plasma concentrations of these antipsychotics, possibly resulting in dose-related toxic effects. Regarding this, McCarthy (1994) described the case of a patient on a stable clozapine dose that developed a myoclonic seizure and a generalized crisis a few weeks following a sudden smoking cessation. Meyer (2001) has documented a mean increase of 72% in clozapine concentration in 11 patients following smoking withdrawal, with occurrence of unwanted effects in the patient showing the highest increase. For these reasons, after cessation of smoking, plasma clozapine levels must be monitored closely, and adjustments in dosage may be considered.

Caffeine

Caffeine may significantly inhibit clozapine metabolism, when taken in amounts between 400 and 1000 mg/day (Hagg et al., 2000). Caffeine is metabolized by CYP1A2, primarily responsible for clozapine biotransformation. It is assumed that caffeine and clozapine compete for the same enzyme: clozapine elimination is generally decreased when caffeine is taken in moderate to elevated amounts (Hagg et al., 2000). This interaction was first documented by Vainer and Chouinard (1994), who described a patient with side effects on clozapine after the addition of caffeine. A controlled study in patients with schizophrenia has documented an approximate 50% reduction of plasma clozapine concentrations after the removal of caffeine from the diet (Carrillo et al., 1998).

Food and other substances

Many other substances besides those mentioned can interfere with the metabolism of clozapine. Among these, some are able to activate an important transcription factor of CYP1A2 called aryl hydrocarbon receptor (AHR). AHR ligands can be divided into two categories: natural and synthetic. Among the synthetic ligands, there are halogenated aromatic hydrocarbons (dibenzo-dioxins, dibenzofurans, polychlorinated biphenyls) and polycyclic aromatic hydrocarbons (3-methylcholanthrene, benzo-alpha-pyrene, benzoanthracene) (Denison and Nagy, 2003); among the natural ligands, there are some tryptophan derivatives, indigo, indirubin (Adachi et al., 2001), the tetrapyrroles such as bilirubin, arachidonic acid metabolites, and carotenoids. AHR is a member of the family of basic helix-loop-helix transcription factors. The AHR is normally inactive, and it is bound to different chaperones in the cytosol. As a result of interaction with one of its ligands,
the chaperones dissociate allowing the translocation of AHR factor inside the nucleus, where it dimerizes with AHR nuclear translocator, inducing the transcription of the gene for CYP1A2.

As mentioned in Table 3, many foods contain high doses of different ligands of AHR. Coconut oil, peanut oil, corn oil, soybean oil, paprika, celery, smoked tea, marjoram, oregano, thyme, cereal and its derivatives (bread, pasta, and pizza), smoked chicken, grilled duck, lettuce, apples, smoked trout, and broiled fish are some of the foods that contain high concentrations of benzo-alpha-pyrene (Dennis et al., 1991) and should therefore be sparingly used in patients treated with clozapine. Even food particularly rich in tryptophan (sea lion meat, salted cod, pumpkin seeds, parmesan cheese, and soy flour) or carotenoids (carrots, tomato, salmon, and shrimp) may induce clozapine metabolism with the same mechanism.

Finally, grapefruit juice can inhibit the activity of CYP3A4 in the intestine and in the liver and may elevate plasma concentrations of its substrates (Vandel et al., 2000).

Age and gender

Lane et al. reported a 34.9% higher clozapine concentration in women compared with men and found that increasing age was related to an increase in serum clozapine levels (Lane et al. 1999).

Physical diseases

Clozapine should be used with caution in patients with pre-existing liver or kidney diseases. There have been a number of case reports of very high clozapine serum levels in patients with these diseases (Uges et al., 2000).

DISCUSSION

The clinical case reported is the clear example of the difficult therapeutic management of a patient treated with clozapine. The patient met several factors increasing or decreasing clozapine plasma levels: he was male and young (section “Age and gender”), he had an inappropriate diet (section “Food and other substances”), he daily smoked 15–20 cigarettes (section “Nicotine”), he took valproic acid (section “Drugs”), and he daily drank six to seven cups of coffee (section “Caffeine”).

The large number of factors that interfere with clozapine metabolism requires the continuous drug monitoring of blood concentrations of the drug because a consistent relation between milligrams and plasma concentrations is not guaranteed. The continuous monitoring of clozapine plasma levels allows us to adapt the drug dosages to the genetic, epigenetic, and metabolic individual characteristics: it would be evidence-based medicine. On the other hand, our case report shows that in absence of side effects, for some patients, the maximum dose recommended by the guidelines (900 mg) may be largely insufficient to achieve the minimum blood levels (350 ng/ml) necessary to ensure the real therapeutic benefits. Despite the fact that blood monitoring of clozapine is not widely diffused, we believe that this laboratory test should become a routine procedure for all patients treated with clozapine, as is usually done with lithium and valproic acid. This would help both to reduce the risk of dose-dependent side effects and to improve the therapeutic response and the quality of life of patients. If we consider the low cost of this laboratory technique (13.40 euro in our reference laboratory), we have to underline that the benefit/cost ratio is very high.

We also want to emphasize that other factors may be considered in addition to the clozapine plasma concentration in order to better manage the therapy. In addition to the well-known polymorphisms of CYP that should be further investigated, Consoli et al. assessed that the ABCB1 genotyping should be considered as a novel strategy to improve the drug management.

Even though variants in most of transmitter systems do not appear to be predictor variables of clozapine response, they do, however, remain strong candidates for the investigation of adverse effects related to such treatment. Knowledge of the genes that influence variability to the adverse effects of clozapine would undoubtedly be useful for identifying susceptible patients prior to medication, thereby minimizing the prevalence of these potential difficulties.

Even the caffeine test for the assessment of CYP1A2 activity, which is nowadays used almost exclusively for research, is extremely useful, inexpensive and easy to use; all clinicians should take it into consideration as an aid in the management of a patient treated with clozapine.

Table 3. Foods that may affect clozapine metabolism by inhibiting CYP3A4 or inducing CYP1A2

<table>
<thead>
<tr>
<th>Foods that may increase clozapine levels</th>
<th>Foods that may decrease clozapine levels</th>
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<tbody>
<tr>
<td>Grapefruit juice, coffee</td>
<td>Coconut oil, peanut oil, corn oil, soybean oil, paprika, celery, smoked tea, marjoram, oregano, thyme, cereal, bread, pasta, pizza, smoked chicken, grilled duck, lettuce, apples, smoked trout, broiled fish, sea lion meat, salted cod, pumpkin seeds, parmesan cheese, soy flour, carrots, tomato, salmon, and shrimp</td>
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However, according to the previous studies and with this case experience, we think that for these patients treated with clozapine with low clinical improvements, increasing clozapine dosages is more effective than switching to another newer atypical antipsychotic (McEvoy et al., 2006).

Finally, because of the high variability of the metabolism of clozapine, the possibility of a direct administration of norclozapine might be considered (Table 4).

CONFLICT OF INTEREST
None of the authors has any commercial interest in increasing or decreasing the prescription and use of clozapine.

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INTERINDIVIDUAL DIFFERENCES IN CLOZAPINE RESPONSE


