Cytochrome P4502D6 genotype does not determine response to clozapine

M. J. ARRANZ, E. DAWSON, S. SHAIKH, P. SHAM, T. SHARMA, K. AITCHISON, M. A. CROCQ¹, M. GILL², R. KERWIN & D. A. COLLIER

Departments of Neuropathology and Psychological Medicine, The Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, ¹Centre Hospitalier, FORENAP, 68250 Rouffach, France and ²Trinity Centre for Health Sciences, Department of Psychiatry, Dublin, Ireland

- 1 The atypical antipsychotic drug clozapine, used in the treatment of resistant schizophrenia, is metabolized partly by the hepatic cytochrome P450 enzyme CYP2D6. Two phenotypes with respect to the activity of the enzyme are recognized (extensive metabolisers (EM) and poor metabolisers (PM)), resulting from allelic variation in the gene, *CYP2D6*.
- 2 Genotype was determined in 123 schizophrenic patients currently being treated with clozapine, in order to determine if EM or PM status influences response to this drug. Patients were divided into responders and non-responders using the Global Assessment Scale, and genotyped for the A and B poor metaboliser mutations by digesting PCR products with *HpaII* or *BstNI*.
- 3 Fifty-nine patients were heterozygous for allele B and for allele A. Eight patients were determined as poor metabolisers since they were homozygous either for A and B. Poor metabolisers were equally distributed between responders and non-responders and no correlation between *CYP2D6* alleles and response to clozapine was found.
- 4 The results are consistent with recent findings showing that CYP1A2, rather than CYP2D6, is the major enzyme responsible for the metabolism of clozapine.

Keywords schizophrenia genetics *CYP2D6* neuroleptic

Introduction

Clozapine is an atypical antipsychotic drug with a 60% success rate in schizophrenic patients refractory to treatment with at least three other classes of drugs [1]. The precise mechanism of action of clozapine and the reasons some patients fail to respond are not understood. The primary site of action is not thought to reside at the dopamine D₂ receptor since relatively low occupancy has been observed in PET scanning studies of responding patients [2]. Instead, action at multiple receptors has been proposed [3–6]. It has been postulated that the major site of action of clozapine is the dopamine D₄ receptor [4], which exists in at least nine polymorphic forms differing in their affinity for neuroleptics [7]. Alternatively, response to clozapine may be determined by its rate of metabolism. The drug

is metabolized to clozapine-*N*-oxide, *N*-desmethyl-clozapine and a phenolic *N*-desmethyl compound in humans [8, 9]. The hydroxylation and *N*-demethylation reactions are carried out by the cytochrome P450 family of enzymes. Fischer *et al.* [10] showed that hepatic cytochrome P4502D6 (CYP2D6) catalyses the metabolism of clozapine *in vitro*, and it has therefore been proposed as a major metabolising enzyme *in vivo*. However, recent studies by Dahl *et al.* [11] and Jerling *et al.* [12] have found evidence that CYP2D6 may not be responsible for the major metabolic pathway of clozapine. CYP2D6 is a widely studied cytochrome characterised by debrisoquine/sparteine metabolism, which is a polymorphic trait in humans. Five to seven percent of Caucasians and 1–2% of black

Americans and Orientals do not metabolise debrisoquine/ sparteine well [13–15] and are thus called poor metabolisers (PM), in contrast to the normal extensive metabolisers (EM). Poor metabolisers attain high plasma concentrations of a variety of psychotropic drugs, including clomipramine, desipramine and nortriptyline, and may experience adverse reactions even on low doses [16]. Mutations of the *CYP2D6* gene causing a deficient or absent protein are responsible for the poor metaboliser phenotype and several methods for their detection, including PCR methods, have been described [17–22]. Known mutations of *CYP2D6* might influence response to treatment with clozapine. Consequently we have examined the common *CYP2D6* mutations in a group of 123 clozapine treated schizophrenic patients classified as responders and non-responders, and attempted to correlate drug response with EM and PM genotypes.

Methods

Patient sample

Blood samples from 123 unrelated schizophrenic patients undergoing clozapine treatment for a minimum of 2 months were obtained from a variety of sources (Burnley, Cambridge, Camberwell, London and France). All patients included in the study had a DSM-III-R (Diagnostic and statistical manual of mental disorder, revised 3rd edition) [23] diagnosis of schizophrenia. They were all Caucasians and had previously been treated with at least two different neuroleptics at a dose equivalent to 500 mg chlorpromazine daily, and failed to respond to treatment. Clozapine doses were chosen on the basis of optimization of clinical response and varied between 125 mg and 600 mg day⁻¹. Clozapine response was determined using the Global Assessment Scale (GAS) [24] before and after clozapine treatment by observers blind to laboratory results using a 20-point improvement in GAS scores as a cut-off point for response. Seventy-seven patients were classified as responders and 46 as non-responders.

Determination of *CYP2D6* genotype

We used the PCR method of Smith *et al.* [22], with some modifications, to detect the two most frequent mutations, *CYP2D6-A* and *CYP2D6-B*, which are responsible for the poor metaboliser phenotype in greater than 90% of cases. Rarer mutations, which in general occur at a frequency of less than 1%, and deletion of the gene itself which is responsible for the PM mutation in <2% of cases, were not analyzed. For some analyses, *CYP2D6-A* and *CYP2D6-B* results were combined (*CYP2D6-A,B*) as both determine the PM phenotype.

Correlation between *CYP2D6* genotype and response to clozapine was analyzed using Pearson's coefficient as part of the SPSS [25] package of programs. Regression analysis was performed using GAS score differences with the A and B alleles as interacting factor.

Results

Table 1 shows the genotype frequencies in the patients. Mutant B was found with a frequency of 20%, and mutant A with a frequency of 2%, these values being within the range found in other studies [17–22]. None of the patients analysed showed both mutations. The number of homozygous mutants (or PMs) was eight (seven homozygous for the B mutation and one homozygous for the A mutation). However, they were randomly distributed between responders (four) and non-responders (four).

No correlation between the *CYP2D6* alleles and the response to clozapine was found using Pearson's coefficient, since correlations were all $< \pm 0.1$. Regression analysis of the response to clozapine using differences in pre- and post treatment GAS scores against the presence of A and B alleles did not show any significant effect ($P > 0.10$ in all cases).

Discussion

We have found no correlation between the A and B alleles at *CYP2D6* and response to clozapine as four poor metabolisers responded to treatment and four poor metabolisers did not show any improvement (OR = 1.74, chi-squared = 0.58). In addition, linear regression failed to show any correlation between the response to clozapine and the *CYP2D6* genotype. Although the genotyping method we have employed detects > 90% of PM mutations in *CYP2D6*, it is possible that additional undetected PM homozygotes existed in our sample. On the basis of probability we would expect one PM in the sample to be misclassified as an EM, which would not have a significant effect on the conclusions of this study.

Our findings are consistent with those of Dahl *et al.* [11] who measured metabolites of clozapine in 15 normal individuals of whom five were poor metabolisers of debrisoquine, five poor metabolisers of

Table 1 Genotype frequencies of the common *CYP2D6* mutations in Caucasian schizophrenic patients being treated with clozapine

	PM	EM	Statistics
Genotype			
AA	1	—	frequency = 0.008
BB	7	—	0.06
Aabb	—	3	0.02
aaBb	—	35	0.28
aabb	—	77	0.63
Responders	4	73	chi-squared = 0.58 $P = 0.45$
Non-responders	4	42	OR = 1.74 95% CI = 0.31–9.80
GAS difference			
mean	23.33	20.32	$F = 0.28$
s.e.	6.79	1.37	$P = 0.60$ 95% CI for mean difference = -8.18 to 14.20

S-mephenytoin and five extensive metabolisers of both drugs. They found no correlation between the metabolism of clozapine and the PM or EM phenotype. Although the power of their study was relatively low, they concluded that clozapine metabolism is not associated strongly with polymorphic variations in *CYP2D6*. The most likely explanation for the findings of the present study and that of Dahl *et al.* [11] is that other cytochrome P450 enzymes are involved in clozapine disposition. Recently, Jerling *et al.* [12] have shown that the concentration of clozapine increased by a factor of 5–10, when fluvoxamine was added to treatment with clozapine. As fluvoxamine is a potent inhibitor of CYP1A2, this enzyme seems to be the major enzyme involved in clozapine disposition.

PMs of debrisoquine were not significantly over-represented in our group of schizophrenic patients being treated with clozapine in comparison with general population rates. An excess of PMs in clozapine patients would not have been surprising since the possibility of intolerance of PMs of debrisoquine to other antipsychotic medications may have lead to enrolment on a clozapine treatment programme.

Alleles at *CYP2D6* have also been examined directly for linkage and association with schizophrenia. Vallada *et al.* [26] reported no evidence for linkage between the *CYP2D6* locus on chromosome 22 and schizophrenia in 23 multiply affected families. Dawson *et al.* [27] report no evidence for alleles association between the A and B alleles of *CYP2D6*

and schizophrenia using a sample of 200 unrelated affected individuals, since alleles frequencies did not deviate significantly from those observed in control populations. This indicates that there is no hidden stratification of allele frequencies in schizophrenic populations which might alter our conclusions.

It is still possible that polymorphism at *CYP2D6* can influence response to clozapine, either through the generation of toxic or clinically active metabolites, or through the effect of less common allelic variants of the gene. Also, psychiatric patients who have ultrarapid hydroxylation of debrisoquine have been described [28, 29] in whom even high doses of drugs failed to achieve therapeutic plasma concentrations. The ultrarapid metaboliser phenotype appears to result from a gene duplication or amplification of a functionally active *CYP2D6* gene leading to increased levels of enzyme which is inherited in a Mendelian manner [29]. The frequency of this gene in the general population is not known, although it appears to be rare. There are no simple methods for its detection at present.

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