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## Maintenance therapy with zuclopenthixol decanoate: associations between plasma concentrations, neurological side effects and *CYP2D6* genotype

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**Abstract** *Rationale:* Several antipsychotic drugs are metabolised by the polymorphic cytochrome  $P_{450}$  CYP2D6. The impact of the polymorphism on the plasma levels and the occurrence of side effects have not been clearly established. *Objective:* To investigate the impact of the CYP2D6 polymorphism on the steady-state plasma concentrations of zuclopenthixol and the occurrence of extrapyramidal side effects (EPS) and tardive dyskinesia (TD) during treatment with zuclopenthixol-decanoate. *Methods:* Fifty-two clinically stable schizophrenic outpatients on monotherapy with zuclopenthixol-decanoate (100–400 mg/4 weeks) were genotyped for the CYP2D6 variants CYP2D6\*3 and CYP2D6\*4. Steady-state plasma levels of zuclopenthixol were analysed using high-performance liquid chromatography. Assessments of EPS, TD and psychopathology were performed twice with an 8-week interval using the extrapyramidal symptoms rating scale, the abnormal involuntary movement scale and the brief psychiatric rating scale. *Results:* Thirty-five patients were homozygous extensive metabolisers (EMs), 13 were heterozygous EMs and 4 were poor metabolisers (PMs). While there were no significant genotype-related differences in the doses of zuclopenthixol decanoate, PMs as well as heterozygous EMs had significantly higher steady-state plasma levels of zuclopenthixol

than homozygous EMs (median 9.5 nmol/l; 8.2 nmol/l and 5.9 nmol/l, respectively,  $P < 0.05$ ). The median dose-corrected plasma concentrations were 0.029, 0.038 and 0.048 nmol·l<sup>-1</sup>·mg<sup>-1</sup> in homozygous EMs, heterozygous EMs and PMs, respectively, with statistically significant differences between homozygous EMs and heterozygous EMs ( $P = 0.014$ ) and homozygous EMs and PMs ( $P = 0.03$ ). Patients with neurological side effects were significantly older than patients without ( $P = 0.02$  in case of parkinsonism and  $P = 0.04$  in case of TD). Mutant CYP2D6\*3 and \*4 alleles tended to occur more frequently in patients with neurological side effects. An odds ratio (OR) of 2.3 (95% confidence interval 0.7–6.9) for development of parkinsonism and an OR of 1.7 (95% confidence interval 0.5–4.9) for TD was calculated in an individual with at least one mutated allele. However, the ORs were not statistically significant. *Conclusions:* The higher zuclopenthixol steady-state plasma concentrations in heterozygous EM and PM schizophrenic patients receiving monotherapy with zuclopenthixol-decanoate than in homozygous EMs indicates a significant role of CYP2D6 in the systemic elimination of zuclopenthixol. The tendencies for patients carrying at least one mutated CYP2D6 gene to have an increased risk of parkinsonism and TD are in accordance with previous studies. Age was a significant risk factor for neurological side effects.

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### Introduction

Many traditional antipsychotic agents, including zuclopenthixol, are metabolised by the hepatic cytochrome  $P_{450}$  isozyme CYP2D6 (Dahl et al. 1991; Bertilsson and Dahl 1996). The activity of CYP2D6 is bimodally distributed in the population, and individuals can be classified as either extensive metabolisers (EMs) or poor metaboli-

sers (PMs). The prevalence of the PM phenotype is about 7% in different Caucasian, including Estonian and Russian, populations (Alván et al. 1990; Kiiwet et al. 1993; Marandi et al. 1996, 1997). The most common defect allele associated with the PM phenotype in Caucasian populations is *CYP2D6\*4*, with an allele frequency of about 21%. Other common defect alleles are *CYP2D6\*3* and *CYP2D6\*5*, with allele frequencies of approximately 2% and 5%, respectively (Broly et al. 1991; Dahl et al. 1992). In addition, a large number of other rare defect alleles have been described (Griese et al. 1998). About 1–2% of Swedish subjects carry multiple copies of a functional *CYP2D6* allele and can be classified as ultrarapid metabolisers of *CYP2D6* (Dahl et al. 1995).

In case the elimination of a drug is highly dependent on the polymorphic *CYP2D6*, a lower clearance of this compound is seen in PMs than in EMs. As a consequence, higher steady-state plasma concentrations per given dose would be expected in PMs than in EMs. Thus, PMs might have an increased risk of concentration-dependent side effects at conventionally prescribed dosage regimens (Bertilsson and Dahl 1996).

Several studies have explored the possible association between the incidence and severity of neurological side effects induced by conventional antipsychotics and impaired metabolic capacity of *CYP2D6* (Arthur et al. 1995). They found a relationship between the metabolic capacity of *CYP2D6* and the severity of extrapyramidal side effects in a pilot study in schizophrenic patients with antipsychotic-induced tardive dyskinesia (TD). Armstrong et al. (1997) found that *CYP2D6* genotype was not a determinant of susceptibility to acute dystonic reactions induced by antipsychotics, but may be a contributing factor in other antipsychotic-induced movement disorders including TD. This finding was in concordance with the results of Andreassen et al. (1997), indicating that genetically impaired *CYP2D6* activity may be a contributing factor for the development of persistent TD. Furthermore, Kapitany et al. (1998) demonstrated that patients who were heterozygous for the functional *CYP2D6\*1* allele had a higher incidence of TD than patients homozygous for this allele.

Benefits of long-term depot treatment of schizophrenia using the lowest effective dose approach are well established (Burnett et al. 1993; Gerlach 1995; Kane et al. 1998). One important aspect in this approach is to minimise disabling side effects of antipsychotic treatment. In this context, the pharmacokinetic and pharmacogenetic properties may be important.

The elimination of zuclopenthixol after single oral dose administration has been shown to be dependent on *CYP2D6* activity (Dahl et al. 1991). Jerling et al. (1996) consequently demonstrated a two-fold difference in zuclopenthixol clearance between EM and PM subjects on continuous oral treatment with zuclopenthixol. No data are available about the role of *CYP2D6* on the clinical pharmacokinetics and effects of depot antipsychotics.

**Table 1** Demographic data of the population studied ( $n=52$ )

Patient characteristics	
Age [years; mean (SD) range]	43 (12) 20–70
Gender (female/male; $n$ )	33/19
Duration of disorder [years; mean (SD) range]	15.4 (9.3) 1–37
Total time on treatment with zuclopenthixol-decanoate at study entry [months; mean (SD) range]	31 (18) 4–62
Zuclopenthixol-decanoate dose per 4 weeks [mg; mean (SD) range]	220 (62) 100–400
Smokers / non – smokers ( $n$ )	15/37
Anticholinergic prescribed/not prescribed ( $n$ )	25/27

The aim of the study was to evaluate the associations between the *CYP2D6* genotype, drug plasma concentrations and neurological side effects in clinically stable schizophrenic outpatients receiving monotherapy with zuclopenthixol-decanoate.

## Materials and methods

### Patients and clinical ratings

Patients were recruited from the population of schizophrenic outpatients already receiving maintenance monotherapy with zuclopenthixol-decanoate (ZD) at Tartu University Psychiatric Clinic and Jämejala Psychiatric Hospital, Estonia. All patients were of Estonian or Russian origin and they were informed of their rights under the Declaration of Helsinki, as well as of the objectives and risks of the study. The Ethics Review Committee of Clinical Research Studies at the University of Tartu and the ethics committee at Huddinge University Hospital approved the study. All patients were included after obtaining the informed consent.

Inclusion criteria were as follows: clinically stabilised outpatients with a diagnosis of schizophrenia (F20) or schizoaffective disorder (F25) according to International Statistical Classification of Diseases and Related Health Problems (ICD-10; World Health Organization 1992), age between 18 years and 70 years and duration of fixed dose ZD treatment of at least 4 months. The stabilised condition was determined by the use of slightly modified (prolonging the requirements for unchanged dose of drug used from 2 months to 4 months) criteria for stabilisation described by Marder et al. (1991) – (a) stability on the psychosis scores (including cluster scores for thought disturbance and paranoia) of the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962) over at least 2 months, with no single item increase by more than one in any consecutive ratings; (b) living in a stable community setting for 2 months; and (c) no change in the dose of ZD during the 4 months before admission to the study. Exclusion criteria were as follows: total duration of the illness less than 1 year, drug or alcohol abuse, pregnancy and/or breast feeding, serious somatic illness and known organic brain disease. Fifty-two patients matched these criteria. Fifteen subjects were smokers, those smoking steadily ten or more cigarettes per day and the remaining 37 were non-smokers. Demographic data of the patient population are presented in Table 1.

No attempts were made to change antipsychotic treatment regimen (ZD dosage and the length of the injection interval). Concomitant treatment with benzodiazepines and the anticholinergic drug trihexyphenidyl was allowed according to the judgement of the psychiatrist currently responsible for the treatment of each patient. Twenty-five of the patients received trihexyphenidyl and ten received benzodiazepines during the study period.

Clinical condition was assessed using different instruments twice during the study, at week 0 (entry into the study) and at

week 8. Rating instruments were – BPRS 18-item version, Abnormal Involuntary Movement Scale (AIMS, US Department of Health, Education and Welfare 1976) and Research Diagnoses for Tardive Dyskinesia (Schooler and Kane 1982), subscale II from Extradyskinesia Rating Scale (ESRS, Chouinard et al. 1980). AIMS was used for assessment of the presence of TD. Subjects with an AIMS score of at least 4 were considered as currently suffering from a movement disorder (Armstrong et al. 1997). Research Diagnoses for Tardive Dyskinesia was used for the evaluation of the presence of persistent TD. Subscale II from ESRS was used for detection of parkinsonian features. Parkinsonism was diagnosed in patients with a total score of more than 3. All ratings were performed by experienced psychiatrists (Peeter Jaanson and Siiri Vään) who were unaware either of the dose of ZD prescribed or the genotype and plasma zuclopenthixol concentrations. Several ratings were performed beforehand to gather inter-rater reliability.

### Blood sampling

Drug monitoring was based on the minimum plasma concentration during the administration interval. Blood samples for determination of zuclopenthixol plasma levels were obtained on week 0 and week 8, just before the next injection of ZD, into heparinised vacutainer tubes. Samples were centrifuged for 10 min at 400 g; plasma was separated and kept frozen at  $-20^{\circ}\text{C}$  until the analysis.

Blood samples for the determination of the *CYP2D6* genotype were collected at week 0 into vacutainer tubes containing ethylene diamine tetraacetic acid (EDTA) and kept frozen at  $-20^{\circ}\text{C}$  until determination of the genotype.

### Determination of zuclopenthixol plasma concentrations

Plasma concentrations of zuclopenthixol were analysed using high-performance liquid chromatography. A 1-ml sample of plasma, mixed with 50  $\mu\text{l}$  internal standard (0.7  $\mu\text{mol}\cdot\text{l}^{-1}$  2,8-dichloroperphenazine) and 0.5 ml 0.5  $\text{mol}\cdot\text{l}^{-1}$  sodium hydroxide in a 14-ml polypropylene tube, was extracted with 3 ml 3% isoamyl alcohol in heptane. After extraction by rotation for 10 min, and centrifugation for 10 min at 3000 g, the water phase was frozen in ethanol/water bath at  $-30^{\circ}\text{C}$ , and the organic phase was transferred to another polypropylene tube containing 100  $\mu\text{l}$  25  $\text{mmol}\cdot\text{l}^{-1}$  formic acid. After extraction for 5 min and centrifugation for 5 min, the organic phase was sucked off. Heptane (0.5 ml) was added and the tube was whirled for 10 s. The heptane phase was sucked off completely, and 25  $\mu\text{l}$  of the acid phase was injected onto a 100 $\times$ 2.0-mm Phenomenex Luna C18 column with 3- $\mu\text{m}$  particles. The eluent was a 40- $\text{mmol}\cdot\text{l}^{-1}$  phosphate buffer (pH 7.5) containing 48% acetonitrile. The flow rate was 0.3 ml/min and the temperature was  $40^{\circ}\text{C}$ . Ultraviolet detection was carried out at a wavelength of 258 nm. Retention times were 7.12 min for zuclopenthixol and 11.6 min for internal standard. Linear standard curves with correlation coefficients better than 0.99 were obtained in the range 0–60  $\text{nmol}\cdot\text{l}^{-1}$ . The limit of detection was 1.0  $\text{nmol}\cdot\text{l}^{-1}$ . A coefficient of variation (within day) of 5.1% was obtained at 5.0  $\text{nmol}\cdot\text{l}^{-1}$ .

### Genotyping

Genomic DNA was isolated from peripheral leukocytes by Qiagen Blood and Cell Culture DNA Kit (Qiagen, Hilden, Germany). The *CYP2D6* genotype was determined in leukocyte DNA by analysis of the two mutated alleles, *CYP2D6*\*3 and \*4, by means of allele-specific polymerase chain reaction (PCR) according to Heim and Meyer (1990). Alleles where neither the \*3- nor \*4-specific mutations could be identified were classified as the functional *CYP2D6*\*1 alleles.

### Statistical analysis

The within-individual differences in consecutive clinical ratings were compared using the Friedman analysis of variance (ANOVA)

method, and the within-individual differences in plasma zuclopenthixol concentrations were compared using *t*-tests for dependent samples. The Kruskal-Wallis ANOVA was used for inter-group comparisons. The relationship between the presence of parkinsonism or TD and the demographic variables was evaluated using the logistic regression method. In this model the presence of parkinsonism or TD was the dependent variable. Gender, age, duration of illness, plasma zuclopenthixol concentration and *CYP2D6* genotype were included as independent variables. Multiple regression analysis was performed to relate the dose-corrected plasma zuclopenthixol (C/D) concentrations (dependent variable) with age, gender, presence or absence of smoking, duration of illness, BPRS score and *CYP2D6* genotype (independent variables). Statistical significance of allele frequencies was assessed using the  $\chi^2$  test for 2 $\times$ 2 tables. *P* values of 0.05 or less were considered as significant.

## Results

### Stability of clinical condition and zuclopenthixol plasma concentrations throughout the study

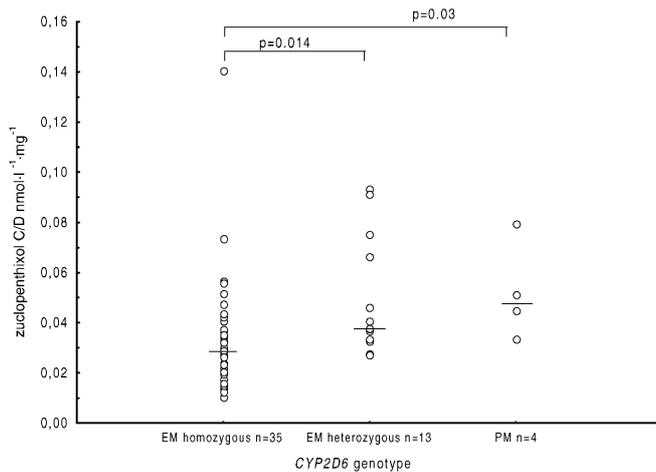
The clinical ratings of the population studied remained stable throughout the study ( $P=0.58$  for parkinsonism,  $P=0.36$  for TD,  $P=0.39$  for BPRS score). Neither were there any significant differences in the zuclopenthixol plasma concentrations between week 0 and week 8 ( $P=0.51$ ). Thus, the results of the clinical ratings and zuclopenthixol plasma concentrations from the baseline visit were considered appropriate for further analysis.

### *CYP2D6* genotypes

Four patients (8%) were genotyped as PMs (*CYP2D6*\*3/\*4) and 13 (25%) as heterozygous EMs (*CYP2D6*\*1/\*4). The remaining 35 (67%) were homozygous EMs (*CYP2D6*\*1/\*1). The allele frequencies of *CYP2D6*\*1, \*3 and \*4 were thus 0.80, 0.04 and 0.16, respectively.

### Steady-state plasma concentrations of zuclopenthixol and *CYP2D6* genotype

The patients received a dose of ZD ranging from 100 mg to 400 mg per 4 weeks. There was no difference between the three genotype groups with respect to duration of treatment with ZD (31.7 months in homozygous EMs and heterozygous EM groups and 32.2 months in the PM group;  $P=0.9$ ). Dose of ZD in the three genotype groups did not differ significantly (212.4 mg/4 weeks in homozygous EMs, 246 mg/4 weeks in heterozygous EMs and 200 mg/4 weeks in PMs; Kruskal-Wallis ANOVA  $P=0.2$ ). The median absolute plasma concentrations were 5.9 nmol/l (range 2.1–28 nmol/l); 8.2 nmol/l (range 5.6–36.4 nmol/l) and 9.5 nmol/l (range 6.8–16 nmol/l) in homozygous EMs, heterozygous EMs and PMs, respectively. Statistically significant differences were found between homozygous EMs and heterozygous EMs (Kruskal-Wallis ANOVA  $P=0.02$ ) and also between homozygous EMs and PMs ( $P=0.03$ ). The concentrations



**Fig. 1** The plasma concentration-to-dose (C/D) ratios of zuclopenthixol in relation to the *CYP2D6* genotype in 52 schizophrenic patients on maintenance treatment with zuclopenthixol decanoate. The median in each group is indicated by a horizontal line

did not differ significantly between heterozygous EMs and PMs ( $P=0.09$ ).

Due to individual dose variation, the measured concentrations of zuclopenthixol were corrected for the dose. Ratios between steady-state concentration and dose of ZD per 4 weeks are expressed as  $\text{nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$ . The distributions of the concentration-to-dose (C/D) ratios in the three genotype groups are shown in Fig. 1. The median C/D ratios of zuclopenthixol were  $0.029 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$  (range  $0.01\text{--}0.14 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$ );  $0.038 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$  (range  $0.02\text{--}0.09 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$ ) and  $0.048 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$  (range  $0.03\text{--}0.08 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$ ) in homozygous EMs, heterozygous EMs and PMs, respectively. Statistically significant differences were found between homozygous EMs and heterozygous EMs (Kruskal-Wallis ANOVA  $P=0.014$ ) and also between homozygous EMs and PMs (Kruskal-Wallis ANOVA  $P=0.03$ ). Differences of C/D ratios between the heterozygous EMs and PMs were not statistically significant ( $P=0.2$ ).

Multiple regression analysis was performed to evaluate the contribution of age, gender, duration of illness, *CYP2D6* genotype and smoking to the C/D ratio. Only the variable "genotype" remained in the equation ( $P=0.029$ ). To evaluate the demographic characteristics (age, gender, smoking habits, duration of illness and BPRS score) between the three genotype groups, Kruskal-

Wallis ANOVA was performed. The three groups of patients, separated according to the genotype, did not differ significantly (age  $P=0.94$ ; gender  $P=0.74$ ; smoking  $P=0.14$ ; duration of illness  $P=0.78$  and BPRS score  $P=0.42$ ).

#### Smoking and plasma zuclopenthixol concentration

Fifteen of the 52 patients were smokers. There were thirteen smokers (87% of all smokers) in the homozygous EM group and two (13% of all smokers) in the heterozygous EM group; all PMs were non-smokers. Median zuclopenthixol C/D ratios in smokers and non-smokers were  $0.029 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$  (range  $0.01\text{--}0.06 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$ ) and  $0.037 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$  (range  $0.01\text{--}0.14 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$ ), respectively, with a statistically significant difference ( $P=0.049$ ) between smokers and non-smokers. Due to the overrepresentation of smokers in the homozygous EM group, this subgroup was analysed separately with respect to the effect of smoking. The median zuclopenthixol C/D ratios of smokers and non-smokers in the homozygous EM group were  $0.029 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$  (range  $0.01\text{--}0.06 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$ ) and  $0.033 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$  (range  $0.01\text{--}0.14 \text{ nmol}\cdot\text{l}^{-1}\cdot\text{mg}^{-1}$ ), respectively. This difference in zuclopenthixol C/D ratios between homozygous EM smokers and non-smokers was not statistically significant (Kruskal-Wallis ANOVA  $P=0.36$ ).

#### *CYP2D6* genotype, plasma zuclopenthixol concentrations and side effects

The prevalence of neurological side effects is shown in Table 2. Of the 52 patients, 11 (21%) had TD according to the research criteria for TD (Schooler and Kane 1982) and 32 (62%) had parkinsonism as defined by Simpson and Angus criteria (1970). Nineteen patients (37%) did not suffer from any movement disorder. Co-existence of TD and parkinsonism was seen in ten patients (19%), of whom five (50%) were homozygous EMs, four (40%) heterozygous EMs and one (10%) PM.

Patients with parkinsonism or TD were significantly older than patients without these syndromes (Kruskal-Wallis ANOVA  $P=0.02$  in case of parkinsonism and  $P=0.04$  in case of TD) but the difference in duration of illness between these groups was not significant. Plasma zuclopenthixol concentrations were not significantly

**Table 2** Age, duration of illness and *CYP2D6* genotype in relation to occurrence of movement disorders

	Tardive dyskinesia		Parkinsonism		All patients
	Yes	No	Yes	No	
Patients ( <i>n</i> )	11	41	32	20	52
Age [years; mean (SD)]	50.3 (13.8)*	41.9 (11.1)	47.8 (10.0)*	37.5 (12.5)	43.7(12.1)
Duration of illness [years; mean (SD)]	14.7 (4.6)	15.6 (10.2)	16.9 (8.6)	12.9 (10.1)	15.4 (9.3)
Homozygous EMs ( <i>n</i> , %)	6 (55%)	29 (71%)	20 (63%)	15 (75%)	35 (67%)
Heterozygous EMs ( <i>n</i> , %)	4 (36%)	9 (22%)	8 (25%)	5 (25%)	13 (25%)
PMs ( <i>n</i> , %)	1 (9%)	3 (7%)	4 (12%)	0	4 (8%)

\* $P<0.05$ ; Kruskal-Wallis ANOVA

**Table 3** Movement disorders in relation to *CYP2D6* alleles

Movement disorder	Number (frequency) of alleles		<i>P</i> value; $\chi^2$ ; OR (95% CI)
	<i>CYP2D6</i> *1	<i>CYP2D6</i> *3or*4	
Parkinsonism yes	48 (0.75)	16 (0.25)	<i>P</i> =0.12; $\chi^2$ =2.4 OR 2.3 (95%CI 0.7–6.9)
Parkinsonism no	35 (0.88)	5 (0.12)	
TD yes	16 (0.73)	6 (0.27)	<i>P</i> =0.35; $\chi^2$ =0.9 OR 1.7 (95%CI 0.5–4.9)
TD no	67 (0.82)	15 (0.18)	

different between patients with and without TD or parkinsonism (Kruskal-Wallis ANOVA  $P=0.56$  for parkinsonism and  $P=0.73$  for TD).

There was a tendency for heterozygous EM and PM patients to have more severe parkinsonism than homozygous EMs, but this difference did not reach statistical significance (Kruskal-Wallis ANOVA  $P=0.067$ ). Logistic regression analysis was performed where the presence of TD was the dependent variable and age, gender, duration of illness, plasma zuclopenthixol concentration and *CYP2D6* genotype were independent variables. Neither *CYP2D6* genotype, duration of illness nor plasma zuclopenthixol concentration contributed to the presence of TD. Similarly, when the presence of parkinsonism was the dependent variable, neither *CYP2D6* genotype, duration of illness nor plasma zuclopenthixol concentration contributed to the presence of parkinsonism. Age was defined as a risk factor for parkinsonism ( $P=0.008$ , OR 1.08 95%CI 1.03–1.16) and TD ( $P=0.016$ , OR 1.09 95%CI 1.02–1.19).

Comparison of *CYP2D6* allele frequencies in relation to the presence of side effects revealed that the frequency of mutated alleles tended to be higher in patients suffering from movement disorders than among those without but this difference did not reach statistical significance (Table 3).

#### Concomitant medication

The anticholinergic drug trihexyphenidyl up to 8 mg per day did not influence the plasma concentrations of zuclopenthixol (Kruskal-Wallis ANOVA  $P=0.64$ ). Of patients with parkinsonism, 17 (53%) did receive the anticholinergic agent. Eight (73%) of the patients with TD and eight (40%) without any signs of parkinsonism were receiving anticholinergic treatment. There was a strong tendency (Kruskal-Wallis ANOVA  $P=0.07$  that anticholinergic medication reduced the parkinsonian rating scores. No differences in the plasma levels of zuclopenthixol were found between patients with and without concomitant benzodiazepine medication ( $P=0.48$ ).

#### Discussion

Higher absolute and dose-corrected zuclopenthixol steady-state plasma concentrations in heterozygous EMs and

PMs than in homozygous EM patients was one of the main findings of the study (Fig. 1). No significant differences were found between heterozygous EM and PM groups. This suggests that a significant decrease in the metabolism of zuclopenthixol occurs in patients with at least one *CYP2D6*-inactivating mutation. *CYP2D6* is of high-affinity and low-capacity character, and saturation of the isozyme may occur in EMs during repeated dosing, decreasing the quantitative differences in the metabolic capacity between EMs and PMs. It is of interest to note that differences in zuclopenthixol concentrations between genotypes were apparent during treatment with an intramuscular depot preparation. The previous documentation for the role of *CYP2D6* in zuclopenthixol metabolism was derived from a study using single oral doses (Dahl et al. 1991) and from patients on continuous oral treatment (Jerling et al. 1996). During depot administration, the first-pass metabolism in gut and liver is avoided, and the pharmacokinetics might be expected to show less inter-individual variations than during oral treatment. The difference in the median concentrations (and C/Ds) between PM and homozygous EM in this study was 1.6-fold, similar to that found by Jerling et al. (1996). The results of the present study thus indicate a quantitatively important role of *CYP2D6* also in the systemic elimination of zuclopenthixol. It is to be pointed out that there was a marked inter-individual variability in the C/D also within each genotype group and significant overlap between the genotype groups (Fig. 1). These data are similar to those reported for other antipsychotics including risperidone (Scordo et al. 1999).

Interestingly, there were no differences in the doses of zuclopenthixol decanoate between the genotype groups, indicating that the clinicians had not adjusted the dosage because of possibly increased side effects in PM. Therapeutic drug monitoring of antipsychotic drugs is not available in Estonia, excluding the use of this tool for dose titration. The common use of a 4-week dosage interval is not optimal due to the short apparent half-life of zuclopenthixol in the compound viscoleo (Poulsen et al. 1994). However, this was common routine in Estonia at the time of the study.

It is well established that smoking can influence drug disposition in humans, mainly due to induction of hepatic isozyme CYP1A2 but probably also other CYP enzymes. Reviewing data about the influence of smoking on plasma concentrations of a number of antipsychotics, ZumBrunnen and Jann (1998) concluded that the effects

of smoking on antipsychotic drug disposition tend to be consistent, with smokers having lower plasma drug concentrations. Our finding of lower plasma concentrations of zuclopenthixol in smokers than in non-smokers is confounded by the overrepresentation of smokers in the homozygous EM group. However, the finding that zuclopenthixol plasma concentrations of smokers and non-smokers within the homozygous EM group did not differ significantly is of interest. More prospective studies are needed to evaluate the impact of smoking on plasma zuclopenthixol concentrations in different *CYP2D6* genotype groups.

The allele frequencies of *CYP2D6*\*3 and \*4 in the patients studied were similar to those found in Estonian and Russian healthy subjects (Marandi et al. 1996, 1997). As other rare inactivating mutations in the *CYP2D6* are missed by the genotyping method used, some of the patients could have been misclassified. Especially, as the *CYP2D6*\*5 allele with deletion of the active *CYP2D6* gene was not analysed, some of the subjects classified as homozygous for \*1 might in fact have been heterozygous for \*1 and \*5. We have previously shown that genotyping for *CYP2D6*\*3 and \*4 allowed prediction of the PM phenotype with high accuracy in the Estonian population (Marandi et al. 1996); while, in a Russian population living in Estonia, some phenotypically PM subjects were classified as EM by genotype analysis (Marandi et al. 1997). Analysis of the *CYP2D6*\*6 allele did not explain this discrepancy (unpublished data). Whether this discordance in the Russian population was of genetic or environmental origin remains unknown.

The finding that age is a risk factor for parkinsonism is in accordance with previous evidence (Morgenstern and Glazer 1993; Miller et al. 1995). Also, the co-existence of parkinsonism and TD has been reported previously (Richardson and Craig 1982). In our study we did not find a contributory role of the duration of illness to the frequency of parkinsonism or TD.

There was a non-significant trend for subjects carrying at least one mutated *CYP2D6* allele to have an increased risk of movement disorders. This finding is in agreement with previous studies (Andreassen et al. 1997; Armstrong et al. 1997) and suggests that genetically impaired activity of *CYP2D6* may be a cofactor in the development of antipsychotic drug-induced parkinsonism. Tendency for PM and heterozygous EM subjects to have more severe movement disorders is in accordance with Arthur et al. (1995); Andreassen et al. (1997); Armstrong et al. (1997) and Kapitany et al. (1998), who found a relationship between the degree of impairment of *CYP2D6* activity and severity of neurological side effects.

The effects of trihexyphenidyl medication on the disposition of zuclopenthixol are not well established. Our results suggesting that the anticholinergic did not influence the average plasma concentrations of depot zuclopenthixol are in accordance with previous data (ZumBrunnen and Jann 1998). However, regardless of this finding, anticholinergics are indicated for only short-

term treatment of parkinsonian side effects because they can induce delirium or memory deficits and abuse (Fleischhacker and Hummer 1997). The finding that 40% of the patients not having parkinsonism and 73% of patients that had TD were receiving an anticholinergic drug is alarming. Use of anticholinergics in patients with TD is known to contribute to the worsening of TD (Mussettola et al. 1993) and is thus contraindicated. Our findings thus demonstrate that, in routine maintenance therapy with antipsychotics, careful assessment of neurological side effects and re-evaluation of the need for anticholinergic medication are essential.

In conclusion, the present study demonstrates for the first time the relationship between the *CYP2D6* genotype and the steady-state plasma levels of zuclopenthixol when administered as depot formulation. The tendency for an increased risk for parkinsonism in patients carrying mutated *CYP2D6* genes is in line with previous studies. Larger studies with careful side-effect registration and assessment are, however, required to fully establish such a relationship.

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## References

- Alvan G, Bechtel P, Iselius L, Gundert-Remy U (1990) Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. *Eur J Clin Pharmacol* 39:533–537
- Andreassen OA, MacEwan T, Gulbrandsen AK, McCreddie RG, Steen VM (1997) Non-functional *CYP2D6* alleles and risk for neuroleptic-induced movement disorders in schizophrenic patients. *Psychopharmacology* 131:174–179
- Armstrong M, Daly AK, Blennerhassett R, Ferrier N, Idle JR (1997) Antipsychotic drug-induced movement disorders in schizophrenics in relation to *CYP2D6* genotype. *Br J Psychiatry* 170:23–26
- Arthur H, Dahl ML, Siwers B, Sjöqvist F (1995) Polymorphic drug metabolism in schizophrenic patients with tardive dyskinesia. *J Clin Psychopharmacol* 15:211–216
- Bertilsson L, Dahl ML (1996) Polymorphic drug oxidation: relevance to the treatment of psychiatric disorders. *CNS Drugs* 3:200–223
- Broly F, Gaedigk A, Heim M, Eichelbaum M, Morike K, Meyer UA (1991) Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of *CYP2D6* in a European population. *DNA Cell Biol* 10:545–558
- Burnett PL, Galletly CA, Moyle RJ, Clark CR (1993) Low-dose depot medication in schizophrenia. *Schizophr Bull* 19:155–164
- Chouinard G, Ross-Chouinard A, Annable L, Jones BD (1980) Extrapramidal symptom rating scale. *Can J Neurosci* 8:233
- Dahl ML, Ekqvist B, Widen J, Bertilsson L (1991) Disposition of the neuroleptic zuclopenthixol cosegregates with the polymorphic hydroxylation of debrisoquine in humans. *Acta Psychiatr Scand* 84:99–102

- Dahl ML, Johansson I, Palmertz MP, Ingelman-Sundberg M, Sjöqvist F (1992) Analysis of the CYP2D6 gene in relation to debrisoquin and desipramine hydroxylation in a Swedish population. *Clin Pharmacol Ther* 51:12–17
- Dahl ML, Johansson I, Bertilsson L, Ingelman-Sundberg M, Sjöqvist F (1995) Ultrarapid hydroxylation of debrisoquine in a Swedish population. Analysis of the molecular genetic basis. *JPET* 274:515–520
- Fleischhacker WW, Hummer M (1997) Drug treatment of schizophrenia in the 1990s: achievements and future possibilities in optimising outcomes. *Drugs* 53:915–929
- Gerlach J (1995) Depot neuroleptics in relapse prevention: advantages and disadvantages. *Int Clin Psychopharmacol* 9[Suppl 5]:17–20
- Griese EU, Zanger UM, Brudermanns U, Gaedigk A, Mikus G, Mörike K, Stuvén T, Eichelbaum M (1998) Assessment of the predictive power of genotypes for the in-vivo catalytic function of CYP2D6 in a German population. *Pharmacogenetics* 8: 15–26
- Heim M, Meyer UA (1990) Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. *Lancet* 336: 529–532
- Jerling M, Dahl ML, Åberg-Wistedt A, Liljenberg B, Landell NE, Bertilsson L, Sjöqvist F (1996) The CYP2D6 genotype predicts the oral clearance of the neuroleptic agents perphenazine and zuclopenthixol. *Clin Pharmacol Ther* 59:423–428
- Kane JM, Aguglia E, Altamura AC, Gutierrez JLA, Brunello N, Fleischhacker WW, Gaebel W, Gerlach J, Guelfi JD, Kissling W, Lapiere YD, Lindström E, Mendlewicz J, Racagni G, Carulla LS, Schooler NR (1998) Guidelines for depot antipsychotic treatment in schizophrenia. *Eur Neuropsychopharm* 8:55–66
- Kapitany T, Meszaros K, Lenzinger E, Schindler SD, Barnas C, Fuchs K, Sieghart W, Aschauer HN, Kasper S (1998) Genetic polymorphism for drug metabolism (CYP2D6) and tardive dyskinesia in schizophrenia. *Schizophr Res* 32:101–106
- Kiivet RA, Svensson JO, Bertilsson L, Sjöqvist F (1993). Polymorphism of debrisoquine and mephenytoin hydroxylations among Estonians. *Pharmacol Toxicol* 72:113–115
- Marandi T, Dahl ML, Kiivet RA, Rågo L, Sjöqvist F (1996) Debrisoquin and S-mephenytoin hydroxylation phenotypes and CYP2D6 genotypes in an Estonian population. *Pharmacol Toxicol* 78:303–307
- Marandi T, Dahl ML, Rågo L, Kiivet RA, Sjöqvist F (1997) Debrisoquin and S-mephenytoin hydroxylation polymorphisms in a Russian population living in Estonia. *Eur J Clin Pharmacol* 53:257–260
- Marder SR, Midha KK, van Putten T, Aravagiri M, Hawes EM, Hubbard JW, McKay G, Mintz J (1991) Plasma levels of fluphenazine decanoate: relationship to clinical response. *Br J Psychiatry* 158:658–665
- Miller CH, Simioni M, Oberbauer H, Schwitzer J, Barnas C, Kulhanek F, Boissel KE, Meise U, Hinterhuber H, Fleischhacker WW (1995) Tardive dyskinesia prevalence rates during a ten-year follow-up. *J Nerv Ment Dis* 183:404–407
- Morgenstern H, Glazer WM (1993) Identifying risk factors for tardive dyskinesia among long-term outpatients maintained with neuroleptic medications. *Arch Gen Psychiatry* 50:723–733
- Muscettola G, Pampallona S, Barbato G, Casiello M, Bollini P (1993) Persistent tardive dyskinesia: demographic and pharmacological risk factors. *Acta Psychiatr Scand* 87:29–36
- Overall JE, Gorham DR (1962) The brief psychiatric rating scale. *Psychol Rep* 10:799–812
- Poulsen JH, Olesen OV, Larsen NE (1994) Fluctuation of serum zuclopenthixol concentrations in patients treated with zuclopenthixol decanoate in viscoleo. *Ther Drug Monit* 16:155–159
- Richardson MA, Craig TJ (1982) The coexistence of parkinsonism-like symptoms and tardive dyskinesia. *Am J Psychiatry* 139: 341–343
- Schooler NR, Kane JM (1982) Research diagnoses for tardive dyskinesia. *Arch Gen Psychiatry* 39:486–487
- Scordo MG, Spina E, Facciola G, Avenoso A, Johansson I, Dahl ML (1999) Cytochrome P450 2D6 genotype and steady state plasma levels of risperidone and 9-hydroxyrisperidone. *Psychopharmacology* 147:300–305
- Simpson GM, Angus JWS (1970) A rating scale for extrapyramidal side effects. *Acta Psychiatr Scand* 212[Suppl]:11–19
- US Department of Health, Education & Welfare (1976) Abnormal involuntary movements scale. In: Guy W (ed) ECDEU assessment manual. US Department of Health, Education & Welfare, Rockville, Md.
- World Health Organization (1992) International Statistical Classification of Diseases and Related Health Problems (ICD-10). World Health Organization, Geneva, Switzerland
- ZumBrunnen TL, Jann MW (1998) Drug interactions with antipsychotic agents: incidence and therapeutic implications. *CNS Drugs* 9:381–401