Clinical Chemistry 51:2
376–385 (2005)

Drug Monitoring and Toxicology

Amitriptyline or Not, That Is the Question: Pharmacogenetic Testing of *CYP2D6* and *CYP2C19* Identifies Patients with Low or High Risk for Side Effects in Amitriptyline Therapy

Werner Steimer,[1*] Konstanze Zöpf,[1] Silvia von Amelunxen,[2] Herbert Pfeiffer,[3] Julia Bachofer,[1] Johannes Popp,[1] Barbara Messner,[1] Werner Kissling,[2] and Stefan Leucht[2]

Background: **Amitriptyline has been replaced in many countries by alternative and more expensive drugs based on claims of improved tolerability and toxicity and despite slightly reduced efficacy. Preliminary studies indicate that adverse effects could be linked to polymorphisms of drug-metabolizing enzymes, but information on their clinical impact remains scanty and includes mainly case reports. We conducted a prospective blinded two-center study seeking correlations between *CYP2C19* and *CYP2D6* genotypes, drug concentrations, adverse events, and therapy response.**

Methods: **Fifty Caucasian inpatients with at least medium-grade depressive disorder received amitriptyline at a fixed dose of 75 mg twice a day. Blood samples for concentration monitoring of amitriptyline and nortriptyline were taken weekly until discharge along with evaluations of depression (Hamilton Depression Scale and Clinical Global Impression Scale) and side effect (Dosage Record and Treatment Emergent Symptoms Scale; DOTES) scores.**

Results: **In a ROC analysis, nortriptyline but not amitriptyline concentrations correlated with side effects (DOTES sum score ≥5; area under the curve, 0.733; $P = 0.008$). Carriers of two functional *CYP2D6* alleles had a significantly lower risk of side effects than carriers of only one functional allele (12.1% vs 76.5%; $P = 0.00001$). The lowest risk was observed for carriers of two functional *CYP2D6* alleles combined with only one functional *CYP2C19* allele [0 of 13 (0%) vs 9 of 11 (81.8%) for the high-risk group; $P = 0.00004$]. We found no correlations between drug concentrations or genotypes and therapeutic response.**

Conclusions: **Combined pharmacogenetic testing for *CYP2D6* and *CYP2C19* identifies patients with low risk for side effects in amitriptyline therapy and could possibly be used to individualize antidepressive regimens and reduce treatment cost. Identification of genotypes associated with slightly reduced intermediate metabolism may be more important than currently anticipated. It could also be the key to demonstrating cost-effectiveness for *CYP2D6* genotyping in critical dose drugs.**

Psychiatric disorders contribute significantly to worldwide morbidity and mortality. Forecasts to 2020 rank depression second only to ischemic heart disease *(1)*. Pharmacotherapy is the mainstay of antidepressive treatment, but it is often associated with inadequate response and severe side effects. Identifying patients at risk of adverse drug reactions or nonresponse before initiation of therapy could provide substantial medical and financial benefits.

Tricyclic antidepressants (TCAs)[4] and, in particular,

[1] Institut für Klinische Chemie und Pathobiochemie and [2] Psychiatrische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany.

[3] Bezirkskrankenhaus Haar, Haar, Germany.

*Address correspondence to this author at: Institut für Klinische Chemie und Pathobiochemie, Klinikum rechts der Isar, Technische Universität München, Ismaningerstrasse 22, D-81675 Munich, Germany. Fax 49-89-4140-4875; e-mail W.Steimer@gmx.de.

Presented in part at the 8th International Congress of Therapeutic Drug Monitoring and Clinical Toxicology, September 7–13, 2003, in Basel, Switzerland.

Received August 6, 2004; accepted November 4, 2004.

Previously published online at DOI: 10.1373/clinchem.2004.041327

[4] Nonstandard abbreviations: TCA, tricyclic antidepressant; AT, amitriptyline; NT, nortriptyline; CYP2C19, cytochrome P450 2C19; CYP2D6, cytochrome P450 2D6; PM, poor metabolizer; UM, ultrarapid metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer; HAMD, Hamilton Depression Scale; CGI, Clinical Global Impression Scale; DOTES, Dosage Record and Treatment Emergent Symptoms Scale; AUC, area under the curve; and 95% CI, 95% confidence interval.

amitriptyline (AT) have been the cornerstones of antidepressive therapy for more than three decades. Current treatment guidelines recommend the use of TCAs only in patients with psychotic features and treatment resistance *(2)*. Nevertheless, more than 1 million patients received TCAs in the United States in 2000 *(3)*, and AT is still used extensively in developing countries because of its cost benefits.

The major pathway of AT metabolism is demethylation to nortriptyline (NT), mainly by cytochrome P450 2C19 (CYP2C19) *(4)*. NT is an active compound, which is the reason that the sum of both concentrations is used to guide therapy in therapeutic drug monitoring *(5)*. NT is hydroxylated by cytochrome P450 2D6 (CYP2D6), forming 10-OH-NT, an inactive metabolite.

Both CYP2C19 and CYP2D6 are highly polymorphic, leading to a wide range in enzymatic activity. Whereas CYP2C19 activity is determined mainly by 2 dysfunctional alleles, 68 point mutations and 9 insertions or deletions account for the 44 *CYP2D6* alleles reported to date *(6)*, with many of them affecting expression or activity. Testing for a limited set of *CYP2D6* alleles may predict with close to 100% accuracy the vast majority of Caucasian individuals lacking CYP2D6 activity [poor metabolizers (PMs)] *(7)*. In contrast, only 20% of ultrarapid metabolizers (UMs) can be predicted from the results of genotyping *(8)*.

Because potential benefits were expected to be most pronounced in individuals with extreme pharmacokinetics, studies in clinical patients have concentrated on PMs and UMs. These studies failed to show genotype/response correlations, particularly in newer drugs with wide therapeutic windows *(9)*, but did show nonsignificant trends toward a higher rate of side effects in heterogeneously treated PMs *(10)*. No sufficient prediction of metabolic activity has been possible within the group of normal extensive metabolizers (EMs) *(11)*. Only recently, a new *CYP2D6* mutation (**41*) was reported to account for 60% of phenotypically intermediate metabolizers (IMs) *(8, 12)*, a distinct subgroup of extensive metabolizers (EMs). Consequently, there has been a lack of studies demonstrating clinical utility apart from screening for extremes. Moreover, such studies on older drugs such as TCAs are difficult to finance and perform *(3)*, because these drugs are no longer recommended as a first-line therapy.

We conducted a prospective blinded study in a Caucasian population of depressive inpatients treated with AT. We determined the *CYP2C19* and *CYP2D6* genotypes and measured serum concentrations of AT and NT and sought correlations with adverse events and therapy response.

Here we report genotype and concentration outcome relationships. The influence of *CYP2D6* and *CYP2C19* on the pharmacokinetics of AT and NT in this population is discussed elsewhere *(13)*.

Materials and Methods

PATIENTS

Over a period of 12 months, a total of 50 patients were included in the study. The study was approved by the Institutional Review Boards (Technische Universität München and Bezirkskrankenhaus Haar) and followed the principles of the Helsinki Declaration. Patients were informed of the aims and design of the study and gave written consent.

The following criteria had to be met: at least medium-grade depressive disorder according to ICD-10 criteria and a Hamilton Depression Scale (HAMD; total of 21 items) of 16 or higher. On admission to hospital and weekly thereafter (plus on day 18), the Clinical Global Impression Scale (CGI), the HAMD, and the Dosage Record and Treatment Emergent Symptoms Scale (DOTES) *(14)* were performed by the treating physician, who was blind to genotypes and serum concentrations until day 21. The DOTES scale includes 30 single items with a rating of slight (score value of 1), moderate (2), or strong effect (3) and is organized in five clusters.

Exclusion criteria were drug or alcohol abuse, clinically relevant laboratory abnormalities, severe illness not allowing the use of TCAs (e.g., severe epilepsy, glaucoma, or cardiovascular disease), other relevant psychiatric diseases (e.g., dementia or schizophrenia), and pregnancy. For the baseline characteristics of the patient population, see Table 1.

DOSING

The AT dose was increased over the first 2 days and was then given at a fixed dose of 150 mg/day (75 mg twice a day; 12-h dosing interval) for the first 3 weeks of treatment. In five patients, the dose was changed at the treating psychiatrist's discretion during the first 3 weeks (75 mg/day, n = 1; 100 mg/day, n = 3; 125 mg/day, n = 1). Patients stayed in the hospital for the entire study period and took their medication under supervision to reduce noncompliance. Accompanying medication was allowed, but substances interfering with CYP2D6 or CYP2C19 metabolism were avoided whenever possible according to the judgment of the attending physicians.

BLOOD SAMPLING AND SERUM CONCENTRATIONS

Blood samples were taken immediately before the morning dose at ~0830 on days 0, 7, 14, 18, and 21. Serum concentrations were measured with the Emit® immunoassay specific for AT and NT (Syva; center 1) or a commercial HPLC assay (Bio-Rad; center 2, and center 1 for confirmatory measurements). Accuracy was ensured for both centers by participation in an international proficiency testing scheme (Heathcontrol).

GENOTYPING

Each patient gave 2.7 mL of EDTA-blood. Genotyping of the dysfunctional *CYP2C19* alleles **2*, **3*, and **4* was performed according to published methods *(15–17)*.

Table 1. Baseline characteristics of the 50 depressive patients included in the study.

Variable	
Age, years	
Mean (SD)	50.6 (12.1)
Range	22–74
Weight, kg	
Mean (SD)	69.3 (12.0)
Range	47–99
Male, %	44
Depression according to ICD-10, n	
Medium-grade episode	20
Severe episode	25
Severe depressive episode within a bipolar affective disorder	5
Time of current episode, months	
Mean (SD)	4.3 (3.6)
Range	0.5–12.5
Psychotropic comedication, n	
None	6
Benzodiazepines	40
Low-potency antipsychotics	4
High-potency antipsychotics	6
Atypical antipsychotics	23
Mood stabilizers	13
Other antidepressants	
Selective serotonin re-uptake inhibitors	8
Other new antidepressants	8
Others	5

CYP2D6 genotype was determined by real-time and conventional PCR *(18, 19)*. The most important alleles in a Caucasian population were assessed: fully functional alleles (**1* and **2*); completely dysfunctional alleles (**3*–**8*); alleles with reduced function (**9*, **10*, and **41*); and duplicated alleles with enhanced function.

STATISTICS

Statistical calculations were performed with SPSS 11.5. We analyzed differences of the mean with the Kruskal–Wallis *H*-test and the Mann–Whitney nonparametric *U*-test. The Fisher exact test was used for prevalence comparisons among the groups. The *P* values in this report are always two-tailed. For the ROC analysis, we used predefined response criteria relying on the HAMD scores and a DOTES side effects score of 5 as cutoff (next score value above the mean of all patients). The ROC analysis describes the power of a diagnostic test (drug concentrations) to differentiate between two different outcomes (e.g., above-average side effects or below-average side effects).

Results

PATIENTS

Five of the original 55 patients could not be analyzed for the following reasons: bipolar disease with rapid change to manic phase (n = 1); withdrawal of consent (n = 2); increased hepatic enzymes (n = 1); or missed sampling for genotyping (n = 1). Of the remaining 50 patients (Table 1), 45 reached day 21 of the study. One patient developed a total right bundle branch block and had to be released from the study on day 9. Two patients discontinued the study on days 14 and 18, respectively, because of lack of improvement and intolerable side effects. On days 14 and 18, respectively, two other patients discontinued participation in the study and left the hospital against medical advice, in complete remission but suffering from side effects. For these five patients, we carried the last observations forward to day 21. The overall clinical response, as measured by HAMD, Beck Depression Inventory, and CGI scores, was as expected after 3 weeks of treatment in this population of patients (Table 2).

SERUM CONCENTRATIONS AND CLINICAL OUTCOMES

On day 7, mean AT and NT concentrations reached 95% of the concentrations observed by day 21. The mean CV of serum concentrations (AT + NT) between days 7 and 21 was 14%, indicating that steady state had been achieved. ROC analysis showed that the mean concentrations for each patient between days 7 and 21 [AT, NT, and AT +N T (sum of both concentrations)] and the NT/AT ratio could predict neither full response nor complete nonresponse to therapy [areas under curves (AUCs), 0.380–0.599; all *P* values >0.2]. When we repeated the analysis for substantial side effects, NT but not AT concentrations were associated with DOTES scores ≥5 [AUC_{NT} = 0.733; 95% confidence interval (CI), 0.577–0.888; $P = 0.008$; $AUC_{AT} = 0.547$; 95% CI, 0.384–0.711; $P = 0.587$]. When a cutoff of 66 μg/L (251 nmol/L) NT was chosen, the sensitivity for substantial side effects was 70.6% with a specificity of 69.7%.

The actual DOTES sum score correlated with mean NT but not AT concentrations: day 21, Pearson correlation coefficients, 0.51 for NT ($P = 0.00008$) and 0.15 for AT ($P = 0.144$).

GENOTYPES AND OUTCOME

No dysfunctional *CYP2C19* alleles other than **2* were detected, which corresponds with previous publications *(20, 21)*. One CYP2C19 PM, 18 heterozygous IMs, and 30 homozygous EMs were identified, whereas in 1 patient, *CYP2C19* genotyping was not successful. According to current literature *(8, 11)*, the *CYP2D6* genotypes found in our population had to be classified (predicted phenotype) as 0 PMs (two completely dysfunctional alleles), 3 IMs (one completely dysfunctional allele and **41* or **10*), 46 EMs (any combination including at least one fully functional allele), and 1 UM (duplication of fully functional alleles), which conformed to the expected range for a population of that size *(22)* (Table 2).

In contrast to the above classification and based on previously reported genotype/concentration correlations *(13)*, we split our population into two groups: those

Table 2. Clinical outcomes, serum concentrations, and genotyping results.

	n	Median	Mean (SD)	Range
Clinical scores				
HAMD on day 0		23	24.7 (7.2)	16–47
HAMD on day 21		9	9.8 (6.4)	0–27
BDI[a] on day 0		26	25.9 (8.9)	8–46
BDI on day 21		14	13.9 (9.3)	1–40
CGI on day 0		5	5.4 (0.6)	4–7
CGI on day 21		4	3.7 (1.2)	1–6
Response by day 21				
Full response (HAMD ≤8 and improvement >30%)	24			
Partial nonresponse (HAMD ≤16 and improvement >30%)	15			
Complete nonresponse (HAMD ≥16 or improvement <30%)	11			
Side effect score				
DOTES on day 0		0	2.0 (3.6)	0–16
DOTES on day 21		4	4.1 (3.4)	0–15
Cluster a (mental side effects)		0	0.72 (1.31)	0–5
Cluster b (neuromuscular symptoms)		0	0.20 (0.57)	0–2
Cluster c (anticholinergic/gastrointestinal symptoms)		2	2.74 (2.26)	0–11
Cluster d (cardiovascular symptoms)		0	0.28 (0.76)	0–3
Cluster e (other symptoms)		0	0.30 (0.71)	0–3
Substantial side effects on day 21 (DOTES ≥5)[b]		17		
Serum concentrations, μg/L				
AT[c] day 7		76	85 (37)	28–188
AT[c] day 21		81	91 (42)	24–208
NT[d] day 7		65	72 (38)	21–193
NT[d] day 21		60	75 (44)	24–190
CYP2C19 genotypes				
**1/*1* (EM)	30			
**1/*2* (IM)	18			
**2/*2* (PM)	1			
Genotyping not successful	1			
Possible CYP2C19-relevant comedication[e]	6			
CYP2D6 genotypes				
**2/*1xn* (UM)	1			
**1/*1* (EM)	8			
**1/*2* (EM)	7			
**2/*2* (EM)	6			
**1/*41* (EM)	4			
**1/*10* (EM)	2			
**2/*41* (EM)	5			
**1/*4* (EM)	11			
**2/*4* (EM)	2			
**2/*5* (EM)	1			
**41/*4* (IM)	2			
**10/*4* (IM)	1			
Possible CYP2D6-relevant comedication[f]	13			

[a] BDI, Beck Depression Inventory.
[b] Next full score above mean.
[c] To convert AT values to nmol/L, multiply by 3.61.
[d] To convert NT values to nmol/L, multiply by 3.80.
[e] Citalopram, diazepam, and omeprazole.
[f] Flupentixol, haloperidol, metoprolol, risperidone, sertraline, venlafaxine, and yohimbine.

carrying at least one completely dysfunctional allele (*CYP2D6* gene dose = 1; 2D6-1; n = 17); or those carrying only functional alleles (2D6-2; n = 33; **1*, **2*, and **10* or **41*). The same was done for *CYP2C19* (2C19-1 or 2C19-2; n = 19 and 30, respectively). We observed no significant differences between the groups regarding response on day 21 for either *CYP2C19* or *CYP2D6* (Fisher exact test in all cases, $P > 0.4$). The prevalence of substantial side effects

(DOTES ≥5) was 21.1% (4 of 19) in the 2C19-1 compared with 40% (12 of 30) in the 2C19-2 group ($P = 0.219$).

When we analyzed *CYP2D6*, we found a significant difference between genotype groups regarding adverse effects ($P = 0.00001$). Carriers of a dysfunctional allele (2D6-1) had a higher risk [13 of 17 (76.5%; 95% CI, 50.1–93.2%)] for side effects (DOTES ≥5) than individuals exclusively harboring functional alleles [2D6-2; 4 of 33 (12.1%; 95% CI, 3.4–28.2%)]. When we considered only patients not receiving CYP2D6-relevant comedications, the difference remained highly significant ($P = 0.00005$): for 2D6-1, 9 of 13 (69.2%; 95% CI, 38.6–90.1%); for 2D6-2, 1 of 24 (4.2%; 95% CI, 0–21.1%).

On the basis of our observation that NT rather than AT concentrations correlated with side effects, we concluded that the subgroup of slower CYP2C19 metabolizers (2C19-1) who are also faster metabolizers regarding CYP2D6 (2D6-2) should display the lowest risk of adverse events. In fact, none of the 13 patients in this group developed substantial side effects (Fig. 1) as opposed to 81.8% (9 of 11) in the high-risk group (2C19-2/2D6-1; $P = 0.00004$). The overall trend over the four groups was highly significant ($P = 0.000006$). On the basis of that finding, we labeled the groups as low, medium-low, medium-high, and high risk (risk classifications 1–4; Fig. 1).

In contrast to our previous report on this population *(13)*, in this report we analyzed absolute serum concentrations not corrected for dose and body weight, aiming primarily at clinical outcome. We found significant differences of AT, NT, and AT + NT serum concentrations and the NT/AT ratio across the four risk groups. NT concentrations and the NT/AT ratio increased with increasing risk, from the low- to the high-risk groups. NT concentrations and the DOTES total sum score on day 21 yielded significant differences for all comparisons between risk groups with different *CYP2D6* status ($P < 0.05$). This was not the case when the only change was *CYP2C19* status. However, when we compared the two biggest groups, which differed only in their *CYP2C19* status (low and medium-low risk), we observed a trend toward significant differences for both the DOTES total sum score and NT concentrations ($P < 0.1$). These findings highlight the possible clinical importance of combined slight differences in the activity of two metabolically involved enzymes.

The positive predictive values for substantial side effects (DOTES ≥5) were 81.8% for the group of patients classified as high risk and 76.5% for the medium-high- and high-risk groups combined. The negative predictive values for the absence of substantial side effects for the low-risk group were 100% and 90.6% for the combined medium-low- and low-risk groups.

Anticholinergic/gastrointestinal and mental side ef-

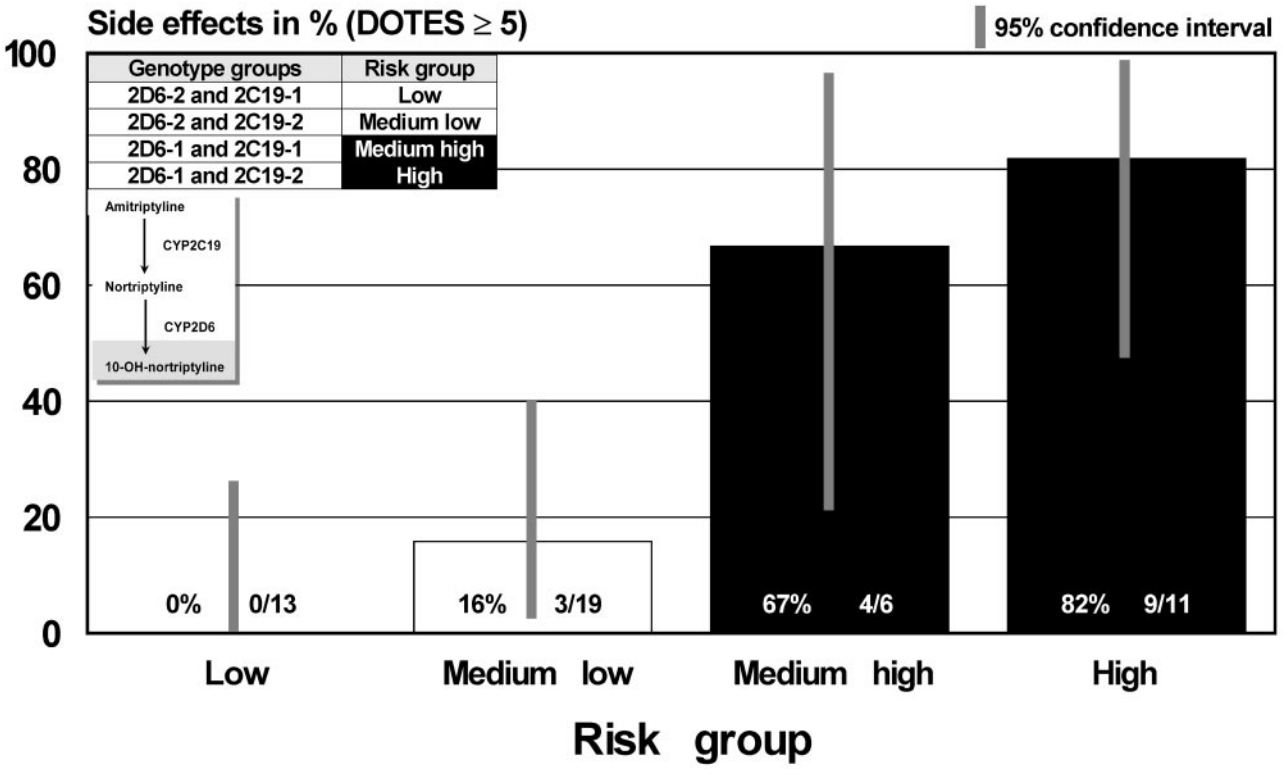

Fig. 1. Risk of side effects in relation to the combined *CYP2D6/CYP2C19* genotype and major metabolic pathway of AT.

2D6-1 and *2C19-1* indicate carriers of at least one dysfunctional allele; *2D6-2* and *2C19-2* indicate carriers of at least two functional alleles. The overall difference is highly significant ($P = 0.000006$, Fisher exact test).

Table 3. Combinations of *CYP2D6* and *CYP2C19* genotypes and their effects on adverse drug reactions, clinical response, and serum concentrations of AT and NT (n = 49).

	Risk group				Overall significance (*P*)[a]
	Low	Medium low	Medium high	High	
CYP2D6/CYP2C19 genotype	2D6-2/2C19-1	2D6-2/2C19-2	2D6-1/2C19-1	2D6-1/2C19-2	
Prevalence, n (%)	13 (27)	19 (39)	6 (12)	11 (22)	
Risk of side effects (DOTES sum score ≥5 on day 21)					0.000006[b]
n/total	0/13	3/19	4/6	9/11	
% (95% CI)	0 (0–24.7)	15.8 (3.4–39.6)	66.7 (22.3–95.7)	81.8 (48.2–97.7)	
Mean DOTES scores on day 21					
Overall severity of adverse effects, 1–5					
Rated by the physician	1.77	2.16	2.5	3.18	0.0004[c]
Rated by the patient	1.85	2.11	2.67	3.18	0.0014[c]
Cluster a (mental side effects)	0.15	0.21	2.17	1.36	0.0036[c]
Cluster b (neuromuscular symptoms)	0.00	0.05	0.67	0.27	0.0554[c]
Cluster c (anticholinergic/GI[d] symptoms)	1.46	2.42	4.17	4.00	0.0257[c]
Cluster d (cardiovascular symptoms)	0.00	0.11	0.50	0.82	0.0168[c]
Cluster e (other symptoms)	0.15	0.26	0.33	0.55	0.3960[c]
Total sum score	1.77	2.95	7.83	6.64	0.0004[c]
P, Mann–Whitney *U*-test					
vs low-risk group		0.0979	0.0220	0.0002	
vs medium-low-risk group			0.0389	0.0007	
vs medium-high-risk group				0.5786	
Response					0.9313[b]
Complete nonresponse[e]					
n/total	2/13	4/19	1/6	4/11	
%	15.4	21.1	16.7	36.4	
Full response[f]					
n/total	6/13	10/19	3/6	4/11	
%	46.2	52.6	50.0	36.4	
Serum concentration (mean of day 7 to day 21)					
NT,[g] μg/L	49.0	65.0	101.2	108.4	0.0005[c]
P, Mann–Whitney *U*-test					
vs low-risk group		0.0709	0.0066	0.0003	
vs medium-low-risk group			0.0208	0.0061	
vs medium-high-risk group				1.0	
AT,[h] μg/L	105.8	70.5	100.8	93.5	0.0197[c]
P, Mann–Whitney *U*-test					
vs low-risk group		0.0059	0.8983	0.6085	
vs medium-low-risk group			0.0361	0.0428	
vs medium-high-risk group				0.7325	
AT + NT, μg/L	154.8	134.7	202.0	201.9	0.0156[c]
NT/AT ratio	0.48	0.98	1.00	1.23	0.0002[c]
Only patients without CYP2D6-relevant comedication					0.000051[b]
Total, n	10	14	4	8	
Side effects (DOTES sum score ≥5 on day 21)					
n/total	0/10	1/14	3/4	6/8	
% (95% CI)	0 (0–30.8)	7.1 (0.2–33.9)	75.0 (19.4–99.4)	75.0 (34.9–96.8)	

[a] The overall significance of differences between the groups was computed over all four groups depicted.
[b] Fisher exact test.
[c] Kruskal–Wallis test.
[d] GI, gastrointestinal.
[e] Complete nonresponse was defined as a value ≥16 or an improvement <30% on the HAMD scale by day 21.
[f] Full response was defined as a value ≤8 and an improvement >30% on the HAMD scale by day 21.
[g] To convert NT values to nmol/L, multiply by 3.80.
[h] To convert AT values to nmol/L, multiply by 3.61.

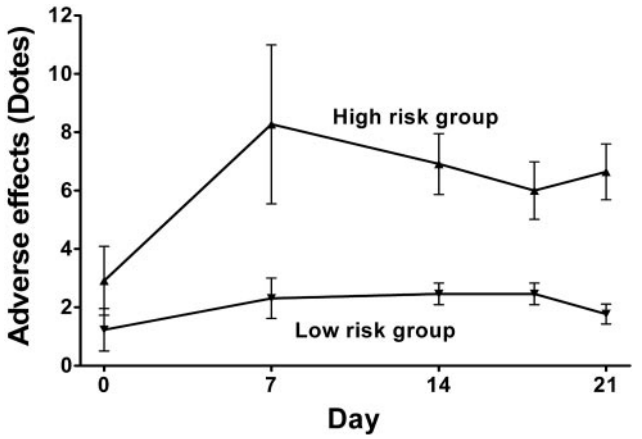

Fig. 2. Development of adverse effects between day 0 and day 21.

Depicted are the DOTES total sum scores. Only side effects related to TCAs were considered in the DOTES rating, which includes 30 single items with a rating of slight (score of 1), moderate (2), or strong effect (3) and is organized in five clusters (mental side effects, neuromuscular symptoms, anticholinergic/gastrointestinal symptoms, cardiovascular symptoms, and other symptoms). The total rating therefore theoretically ranges between 0 and 90. Depicted are mean (SE; indicated by *error bars*) total scores. For clarity, only the two extreme groups, low and high risk, are shown. The differences in the DOTES total sum score between these two groups were significant on all days except day 0: Mann–Whitney *U*-test (day 0 to day 21), $P = 0.3308$, 0.0340, 0.0018, 0.0055, and 0.0002. This was the same when a Kruskal–Wallis test including all four groups (low, low-medium, high-medium, and high risk) was performed (day 0 to day 21, $P = 0.7376$, 0.0361, 0.0100, 0.0130, and 0.0004).

fects contributed 84% to the total sum score. For all five side effect clusters and the overall severity of side effects, there was a uniform pattern of increasing scores from low to high risk with only three exceptions between the medium-high- and high-risk groups (Table 3).

The high-risk group developed adverse effects early in the course of therapy, and the risk remained high, whereas the low-risk group did not display substantial side effects throughout the whole study period (Fig. 2). All five patients who terminated the study prematurely before day 21 were either in the high- (n = 4) or the medium-high-risk group (mean DOTES total sum score, 6.4; range, 5–9). When we compared the risk classification of patients who were finally discharged on AT with those discharged on other drugs, there was a nonsignificant trend toward higher risk in the group that had changed to alternative drugs (Mann–Whitney *U*-test, $P = 0.097$; mean risk classification, 2.12 vs 2.73).

Discussion

Despite the well-documented pharmacokinetic consequences of *CYP2D6* polymorphisms, reports on the clinical impact of *CYP2D6 (11)* and *CYP2C19 (23)* testing remain scanty and include mainly case reports *(24–27)*. Several studies have investigated the relationship between *CYP2D6* genotype and side effects of psychoactive medication. The results have been inconsistent but tend to show a slight overrepresentation of dysfunctional *CYP2D6* alleles, e.g., in patients with neuroleptic-induced movement disorders *(11)*. Two studies *(10, 28)* reported a nonsignificant trend toward an increased frequency of dysfunctional *CYP2D6* alleles in heterogeneously treated depressed patients with adverse drug reactions. Recently, however, Murphy et al. *(9)* reported that *CYP2D6* genotype failed to predict adverse events in depressed patients treated with two newer antidepressants, paroxetine and mirtazapine. Both drugs display a wide therapeutic window, and dosing is, therefore, usually not guided by drug concentration as is recommended for TCAs. Additionally, the saturable metabolism of paroxetine in CYP2D6 EMs produces a maximum twofold difference in drug concentrations between PMs and EMs in chronic dosing *(29)*. Both facts may explain why an influence of CYP2D6 on adverse effects could not be detected. TCAs and NT, in particular, have been studied extensively concerning the pharmacokinetic consequences of CYP2D6 polymorphisms. Therapeutic ranges reported for concentration monitoring of TCAs have been derived with considerable difficulty and mainly with regard to response *(5, 30)*. It is, however, well known that serious toxic events at concentrations above the therapeutic range are to be expected *(31)*. Nevertheless, there is a complete lack of published studies investigating the direct impact of *CYP2D6* and *CYP2C19* genotype on the adverse effects of TCA in clinically treated patients.

It has been a paradigm from early studies that individuals are classified as poor, intermediate, extensive, or ultrarapid CYP2D6 metabolizers. This was based on metabolic activity in relation to specific test drugs and has been transferred to the genotyping era without being reconsidered *(13)*. This concept does not differentiate between carriers of two or only one functional allele. Both are classified as EMs (predicted phenotype from genotype), despite the demonstration of a gene-dose effect in several studies *(32–35)* and the generally accepted notion of enhanced function as a result of duplicated alleles *(35)*.

Here we show that major effects on therapeutic outcomes may remain undetected by this practice. The results reported here are in complete congruence with a previous report from this patient population showing a close and highly significant relationship between *CYP2D6* and *CYP2C19* gene dose and AT/NT concentrations within the group of patients conventionally termed as EMs (predicted from genotype) *(13)*. The findings suggest that gene dose is a much better predictor because it allows not only the detection of extreme outliers (PMs and UMs) but correlates with adverse effects in the two largest groups, carriers of two or only one functional allele.

This may have a major impact on cost/benefit estimations of pretherapeutic *CYP2D6* genotyping and ultimately on the wider adoption of these methods for clinical purposes. Currently, there is no defined clinical situation in which pretherapeutic *CYP2D6* genotyping is accepted as a standard procedure before drug selection or for individualizing drug therapy. Despite demonstration of severe adverse events in PMs *(10, 36)* and a lack of response in UMs *(37, 38)*, it has been difficult to demonstrate the cost-effectiveness of screening for these poly-

morphisms *(39, 40)*. To detect one PM or UM in a Caucasian population, 12 patients have to be genotyped, and the extra cost these patients generate is ill-defined at present *(41)*. Consequently, only a few institutions currently perform pretherapeutic *CYP2D6* genotyping *(42)*. This could change dramatically if clinical situations are defined in which therapeutic decisions based on genotyping results are made for every patient.

Our results suggest that a genetically distinct large subgroup of patients (65%), i.e., low-risk and perhaps medium-low-risk patients, tolerate standard-dosage AT therapy well with very few adverse events and without apparent loss of efficacy. This could also enhance the regularly observed lack of compliance and is, hence, of particular interest in outpatients as well.

AT has been replaced by newer drugs as first-line therapies based solely on claims of improved tolerability, with uncertain clinical significance *(43)*, rather than improved efficacy *(44)*. Pretherapeutic genotyping of *CYP2D6* and perhaps *CYP2C19* may therefore form the basis for a revival of this well-established drug.

There are two reasons that AT might be preferable to newer drugs, presuming that the burden of side effects is reduced. Recent metaanalyses reported a slight, but not significant, advantage of AT regarding efficacy *(43, 44)*, and the cost of AT therapy is substantially lower than that for new drugs. Currently, in Germany, 1 year of AT therapy costs approximately €245 as opposed to €1550 for Venlafaxin. These potential savings easily cover the extra cost for genotyping, which can be estimated as approximately €100–150 per patient *(41)*. Two of three patients could receive standard doses of AT, whereas "high-risk" patients could be treated with newer, more expensive drugs, preferably not metabolized by CYP2D6, or receive modified doses of AT *(45)*. The detection of PMs and UMs, who are very likely at an even higher risk for side effects or therapeutic failure, provides an extra benefit in this situation and may not need to serve as primary economic justification for genotyping.

This scenario describes one of the first applications for the clinical use of pretherapeutic genotyping of metabolizing enzymes to individualize therapy for all patients rather than just screening for extreme outliers.

Another interesting new finding with potential consequences was the observation that NT but not AT concentrations correlated significantly with adverse events. Contrary to the situation in CYP2D6, this led to the idea that slower CYP2C19 metabolizers would suffer less from side effects than faster metabolizers. In our data, there was a uniform trend to confirm that notion, but statistical significance was not reached when we analyzed it on its own. The effect on NT concentrations and side effects was much weaker than that of CYP2D6, but our population with diminished CYP2C19 function consisted almost exclusively of IMs. The effect might be more pronounced in Asian populations, in which the number of CYP2C19 PMs is higher *(15, 20, 23)*.

One could also speculate that cotherapy with inducers of CYP2D6 and/or more readily available inhibitors of CYP2C19 might increase the tolerability of AT therapy, allowing higher, possibly more effective doses without intolerable adverse effects. Ideally, such an inhibitor would be another antidepressant that possibly creates synergistic effects (e.g., citalopram). Particularly in CYP2C19 IMs, low doses of such an inhibitor might be sufficient to obtain the desired effect without the risk of extra side effects.

Lastly, this study also has implications for therapeutic drug monitoring because it appears that assessing the risk of side effects is best done by NT concentrations rather than the sum of NT + AT as is current practice.

Some limitations of this study have to be considered. Side effects and response were assessed after 3 weeks of therapy, and no prediction can be made about long-term therapy. However, most side effects surfaced early in the course of therapy. Noncompliance as an indicator of side effects was not evaluated because the study was designed to avoid noncompliance. Comedication and even CYP2D6-relevant comedication could not be completely avoided, which could have had an impact on both response and side effects. One could argue, however, that detecting effects under these "clinical" conditions supports their clinical relevance *(41)*. The study also does not confirm that the reduced rate of AT-related side effects in low-risk groups is comparable to or lower than the rate for alternative drugs, but reported results from metaanalyses comparing adverse events for AT and other compounds tend to support such a conclusion *(43, 44)*. The results cannot be extended to UMs or PMs, and in other ethnic groups, completely dysfunctional *CYP2D6* alleles are observed less frequently than in Caucasians. However, alleles that lead to diminished function (**10* in Asians and **17* in Africans) are very frequent and may, particularly when present in the homozygous state, change metabolic activity to a comparable extent *(13, 22)*. Finally, the correlations observed here are probably relevant to critical-dose drugs only and may not be relevant to drugs with a wide therapeutic window *(9)*.

Because of the potentially large economic and medical benefits, this report could pave the way for larger studies otherwise difficult to finance and perform. If our results are confirmed, pretherapeutic genotyping of *CYP2D6* and *CYP2C19* may allow individualization of antidepressant therapy in the future. This prospective study demonstrates, for the first time, a statistically significant correlation between *CYP2D6* genotype and adverse effects of antidepressive medication.

Beyond the immediate implications for antidepressive therapy, this study highlights how combined slight differences in genetically determined enzymatic activity can lead to the accumulation of intermediate metabolites and have a significant impact on clinical outcome.

The pharmacodynamic effects of intermediate metabolites determine how genetic variations of metabolizing

enzymes influence adverse events and therapeutic response. Decreased elimination of an active metabolite may enhance therapeutic response, whereas decreased formation may antagonize the desired therapeutic effects. The opposite holds true for a predominantly toxic metabolite. Diminished metabolism may, therefore, be detrimental or beneficial, depending on the metabolic pathway of a particular drug. This means that variable sequences of normal and diminished metabolism in involved enzymes may pose a higher risk for adverse events or promise increased response. In addition, slightly diminished metabolism, as observed in IMs, may amount to clinically relevant effects if they are present in several enzymes that are sequentially involved in the metabolism of a drug. Therefore, detailed knowledge of metabolism is necessary to predict the effects of genetic variation in drug-metabolizing enzymes for a particular drug.

In conclusion, this study shows how the combination of normal (fast) CYP2C19 function and slightly diminished CYP2D6 function leads to high concentrations of a toxic intermediate metabolite (NT) and a high risk for adverse events. It therefore supports the concept that fast formation and reduced elimination of active or toxic intermediate metabolites increase the importance of genotyping for reasons other than identifying individuals with the most extreme phenotypes (PM and UM). This may be of major relevance to current and future drugs, and AT may serve as a model drug to study sequential effects of the *CYP2C19* and *CYP2D6* genotypes on adverse events and response to therapy.

We are indebted to C. Müller, B. Eber, and K. Siegmann for performing the drug concentration analyses at center 1 and ensuring that scheduled blood samples were taken. We also thank B. Schoppek for drug concentration analyses at center 2, K. Steimer and G. Mössmer for proofreading the manuscript, and all of our colleagues from both hospitals who helped to collect the clinical data. This study was initiated by Werner Steimer and Stefan Leucht. Responsible for the design were Werner Steimer, Stefan Leucht, Herbert Pfeiffer, and Werner Kissling. Clinical data acquisition and patient safety were supervised by Werner Kissling and Stefan Leucht at center 1 and Herbert Pfeiffer at center 2. Konstanze Zöpf, Julia Bachofer, Johannes Popp, and Barbara Messner participated in the genotyping. Data analyses were performed by Werner Steimer, Konstanze Zöpf, and Silvia von Amelunxen. Werner Steimer was responsible for coordination, supervised all laboratory work and data acquisition, wrote the manuscript, and was responsible for the final version. All authors critically revised the manuscript and have seen and approved the final version. Werner Steimer reports having received lecture fees and/or travel grants from Dade-Behring, Abbott, and Roche and financial support for a previous publication from Bio-Rad. Herbert Pfeiffer received travel grants to attend scientific meetings and/or to organize scientific meetings from Janssen-Cilag, SmithKline Beecham, Ciba-Geigy, Bristol-Myers-Squibb, Eli Lilly, Wyeth-Pharma, AstraZeneca, Bayer AG Pharma, Lundbeck, and Sanofi-Synthélabo. Stefan Leucht and Werner Kissling received lecture honoraria and/or travel grants to attend scientific meetings from Janssen-Cilag, Bristol-Myers-Squibb, Eli Lilly, Lundbeck, Pfizer, Sanofi-Synthélabo, and AstraZeneca. They also received financial support for a randomized trial from Eli Lilly and for metaanalyses from Sanofi-Synthélabo.

References

1. Ustun TB. The global burden of mental disorders. Am J Public Health 1999;89:1315–8.
2. Crismon ML, Trivedi M, Pigott TA, Rush AJ, Hirschfeld RM, Kahn DA, et al. The Texas Medication Algorithm Project: report of the Texas Consensus Conference Panel on Medication Treatment of Major Depressive Disorder. J Clin Psychiatry 1999;60:142–56.
3. Tucker GT. Advances in understanding drug metabolism and its contribution to variability in patient response. Ther Drug Monit 2000;22:110–3.
4. Venkatakrishnan K, Greenblatt DJ, von Moltke LL, Schmider J, Harmatz JS, Shader RI. Five distinct human cytochromes mediate amitriptyline *N*-demethylation in vitro: dominance of CYP 2C19 and 3A4. J Clin Pharmacol 1998;38:112–21.
5. Perry PJ, Zeilmann C, Arndt S. Tricyclic antidepressant concentrations in plasma: an estimate of their sensitivity and specificity as a predictor of response. J Clin Psychopharmacol 1994;14:230–40.
6. Ingelman-Sundberg M, Daly AK, Nebert DW. Home Page of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee. http://www.imm.ki.se/CYPalleles/ (accessed November 2003).
7. Chou W-H, Yan F-X, Robbins-Weilert DK, Ryder TB, Liu WW, Perbost C, et al. Comparison of two CYP2D6 genotyping methods and assessment of genotype-phenotype relationships. Clin Chem 2003;49:542–51.
8. Zanger UM, Fischer J, Raimundo S, Stuven T, Evert BO, Schwab M, et al. Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. Pharmacogenetics 2001;11:573–85.
9. Murphy GM Jr, Kremer C, Rodrigues HE, Schatzberg AF. Pharmacogenetics of antidepressant medication intolerance. Am J Psychiatry 2003;160:1830–5.
10. Chou WH, Yan FX, de Leon J, Barnhill J, Rogers T, Cronin M, et al. Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness. J Clin Psychopharmacol 2000;20:246–51.
11. Bertilsson L, Dahl ML, Dalen P, Al-Shurbaji A. Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. Br J Clin Pharmacol 2002;53:111–22.
12. Raimundo S, Fischer J, Eichelbaum M, Griese EU, Schwab M, Zanger UM. Elucidation of the genetic basis of the common 'intermediate metabolizer' phenotype for drug oxidation by CYP2D6. Pharmacogenetics 2000;10:577–81.
13. Steimer W, Zopf K, Von Amelunxen S, Pfeiffer H, Bachofer J, Popp J, et al. Allele-specific change of concentration and functional gene dose for the prediction of steady-state serum concentrations of amitriptyline and nortriptyline in CYP2C19 and CYP2D6 extensive and intermediate metabolizers. Clin Chem 2004;50:1623–33.
14. Guy W. Dosage Record and Treatment Emergent Symptoms scale (DOTES). In: ECDEU assessment manual for psychopharma-

cology—revised. DHEW Publication No. ADM 76-338. Rockville, MD: US Department of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, NIMH Psychopharmacology Research Branch, Division of Extramural Research Programs, 1976:223–44.

15. de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of *S*-mephenytoin metabolism in humans. J Biol Chem 1994;269:15419–22.
16. De Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K, Goldstein JA. Identification of a new genetic defect responsible for the polymorphism of (*S*)-mephenytoin metabolism in Japanese. Mol Pharmacol 1994;46:594–8.
17. Ferguson RJ, De Morais SM, Benhamou S, Bouchardy C, Blaisdell J, Ibeanu G, et al. A new genetic defect in human CYP2C19: mutation of the initiation codon is responsible for poor metabolism of *S*-mephenytoin. J Pharmacol Exp Ther 1998;284:356–61.
18. Muller B, Zopf K, Bachofer J, Steimer W. Optimized strategy for rapid cytochrome P450 2D6 genotyping by real-time long PCR. Clin Chem 2003;49:1624–31.
19. Ji L, Pan S, Marti-Jaun J, Hanseler E, Rentsch K, Hersberger M. Single-step assays to analyze CYP2D6 gene polymorphisms in Asians: allele frequencies and a novel *14B allele in mainland Chinese. Clin Chem 2002;48:983–8.
20. Bertilsson L. Geographical/interracial differences in polymorphic drug oxidation. Current state of knowledge of cytochromes P450 (CYP) 2D6 and 2C19. Clin Pharmacokinet 1995;29:192–209.
21. Rodrigues AD, Rushmore TH. Cytochrome P450 pharmacogenetics in drug development: in vitro studies and clinical consequences. Curr Drug Metab 2002;3:289–309.
22. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. Pharmacogenomics 2002;3:229–43.
23. Wedlund PJ. The CYP2C19 enzyme polymorphism. Pharmacology 2000;61:174–83.
24. Bertilsson L, Aberg-Wistedt A, Gustafsson LL, Nordin C. Extremely rapid hydroxylation of debrisoquine: a case report with implication for treatment with nortriptyline and other tricyclic antidepressants. Ther Drug Monit 1985;7:478–80.
25. Bertilsson L, Mellstrom B, Sjokvist F, Martenson B, Asberg M. Slow hydroxylation of nortriptyline and concomitant poor debrisoquine hydroxylation: clinical implications. Lancet 1981;1:560–1.
26. Sallee FR, DeVane CL, Ferrell RE. Fluoxetine-related death in a child with cytochrome P-450 2D6 genetic deficiency. J Child Adolesc Psychopharmacol 2000;10:27–34.
27. Swanson JR, Jones GR, Krasselt W, Denmark LN, Ratti F. Death of two subjects due to imipramine and desipramine metabolite accumulation during chronic therapy: a review of the literature and possible mechanisms. J Forensic Sci 1997;42:335–9.
28. Chen S, Chou WH, Blouin RA, Mao Z, Humphries LL, Meek QC, et al. The cytochrome P450 2D6 (CYP2D6) enzyme polymorphism: screening costs and influence on clinical outcomes in psychiatry. Clin Pharmacol Ther 1996;60:522–34.
29. Sindrup SH, Brosen K, Gram LF, Hallas J, Skjelbo E, Allen A, et al. The relationship between paroxetine and the sparteine oxidation polymorphism. Clin Pharmacol Ther 1992;51:278–87.
30. Ulrich S, Northoff G, Wurthmann C, Partscht G, Pester U, Herscu H, et al. Serum levels of amitriptyline and therapeutic effect in non-delusional moderately to severely depressed in-patients: a therapeutic window relationship. Pharmacopsychiatry 2001;34: 33–40.
31. Preskorn SH, Burke MJ, Fast GA. Therapeutic drug monitoring. Principles and practice. Psychiatr Clin North Am 1993;16:611–45.
32. Kvist EE, Al-Shurbaji A, Dahl ML, Nordin C, Alvan G, Stahle L. Quantitative pharmacogenetics of nortriptyline: a novel approach. Clin Pharmacokinet 2001;40:869–77.
33. Murphy GM Jr, Pollock BG, Kirshner MA, Pascoe N, Cheuk W, Mulsant BH, et al. CYP2D6 genotyping with oligonucleotide microarrays and nortriptyline concentrations in geriatric depression. Neuropsychopharmacology 2001;25:737–43.
34. Shimoda K, Someya T, Yokono A, Morita S, Hirokane G, Takahashi S, et al. Impact of CYP2C19 and CYP2D6 genotypes on metabolism of amitriptyline in Japanese psychiatric patients. J Clin Psychopharmacol 2002;22:371–8.
35. Dalen P, Dahl ML, Ruiz ML, Nordin J, Bertilsson L. 10-Hydroxylation of nortriptyline in white persons with 0, 1, 2, 3, and 13 functional CYP2D6 genes. Clin Pharmacol Ther 1998;63:444–52.
36. Spina E, Gitto C, Avenoso A, Campo GM, Caputi AP, Perucca E. Relationship between plasma desipramine levels, CYP2D6 phenotype and clinical response to desipramine: a prospective study. Eur J Clin Pharmacol 1997;51:395–8.
37. Baumann P, Broly F, Kosel M, Eap CB. Ultrarapid metabolism of clomipramine in a therapy-resistant depressive patient, as confirmed by CYP2 D6 genotyping. Pharmacopsychiatry 1998;31:72.
38. Bertilsson L, Dahl ML, Sjoqvist F, Aberg-Wistedt A, Humble M, Johansson I, et al. Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine. Lancet 1993;341:63.
39. Wedlund PJ, de Leon J. Pharmacogenomic testing: the cost factor. Pharmacogenomics J 2001;1:171–4.
40. Steimer W, Potter JM. Pharmacogenetic screening and therapeutic drugs. Clin Chim Acta 2002;315:137–55.
41. Wedlund PJ. Practical considerations for pharmacogenetic testing. MLO Med Lab Obs 2001;33:16–21, 23, quiz 24–5.
42. Wolf CR, Smith G, Smith RL. Science, medicine, and the future: pharmacogenetics. BMJ 2000;320:987–90.
43. Anderson IM. SSRIS versus tricyclic antidepressants in depressed inpatients: a meta-analysis of efficacy and tolerability. Depress Anxiety 1998;7(Suppl 1):11–7.
44. Guaiana G, Barbui C, Hotopf M. Amitriptyline versus other types of pharmacotherapy for depression [Review]. Cochrane Database Syst Rev 2003;2:CD004186.
45. Kirchheiner J, Brosen K, Dahl ML, Gram LF, Kasper S, Roots I, et al. CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages. Acta Psychiatr Scand 2001;104:173–92.