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Do *CYP3A* and *ABCB1* genotypes influence the plasma concentration and clinical outcome of donepezil treatment?

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Abstract

Purpose: Aim of our study was to evaluate the impact of *CYP3A4*, *CYP3A5* and *ABCB1* polymorphisms on donepezil disposition and clinical outcome.

Methods: Fifty-four Italian patients diagnosed with probable mild to moderate Alzheimer's disease, treated with donepezil (37 patients 5 mg/day, 17 patients 10 mg/day) were genotyped for *CYP3A4* (*1B, *3 and *4), *CYP3A5* (*2, *3 and *6) and *ABCB1* (3435C>T, 2677G>T/A and 1236C>T) polymorphisms. All patients were evaluated for the degree of cognitive impairment with Mini Mental State Examination (MMSE) screening test at baseline (before treatment) and after at least three months of donepezil treatment at stable dose, when the drug plasma levels were measured.

Results: Three patients carried one detrimental *CYP3A4* allelic variant, and 12 one functional *CYP3A5**1 allele. No statistically significant association was found between *CYP3A4* or *CYP3A5* genotypes and plasma donepezil concentrations, or between genotypes and clinical response (as measured by change in MMSE score). Nine *ABCB1* haplotypes were observed, the most common being 1236C/2677G/3435C (46%) and 1236T/2677T/3435T (41%). Patients homozygous for the T/T/T haplotype had slightly though not significantly lower plasma donepezil concentration to dose ratios than those carrying other genotypes (median (95% CI) 0.18 (0.13-0.45) vs 0.31 (0.30-0.44) mg/l/mg/kg respectively). These patients also showed a slightly better clinical response (as measured by change in MMSE score) than the other genotype groups (median (95% CI) 0 (-1.3-3.3) vs -1.0 (-2.1-0.0), respectively).

Conclusions: Our data suggest that the *CYP3A4* and *CYP3A5* polymorphisms are unlikely to influence donepezil metabolism and/or clinical outcome. On the other hand the *ABCB1* polymorphisms may play a role in donepezil disposition and clinical outcome.

Key words: Donepezil, *CYP3A4*, *CYP3A5*, *ABCB1*, polymorphisms, Alzheimer's disease, Pharmacogenetics

Introduction

Acetylcholinesterase (AChE) inhibitors, such as donepezil, are the current pharmacological class indicated for the symptomatic treatment of moderate to mild Alzheimer's disease (AD) [1]. There is a large variability in the clinical response to donepezil, partially related to inter-individual differences in its pharmacokinetics, caused by factors such as age, concomitant diseases and drug-drug interactions. A role may also be played by genetic polymorphisms affecting drug disposition.

Donepezil is metabolized mainly by the cytochrome P450 enzymes CYP2D6 and 3A [2]. The *CYP2D6* gene is highly polymorphic (<http://www.imm.ki.se/CYPalleles/cyp2d6.htm>) and the population can be divided into phenotypes with deficient (poor metabolizers, PM), normal (extensive metabolizers, EM), or increased (ultrarapid metabolizers, UM) enzymatic activity.

Varsaldi et al. [3] observed a large inter-individual variability in the plasma concentration to dose (C/D) ratios of donepezil among AD patients. This variability was associated with the *CYP2D6* genotype, suggesting a role for this polymorphic enzyme in donepezil kinetics and clinical outcome. Co-administration of substrates or inhibitors of CYP3A (i.e. cimetidine, ketokonazole) has been shown to cause changes in the plasma concentrations of donepezil [4-5], indicating a role for these enzymes in donepezil metabolism as well. A number of allelic variants have been described for the genes encoding CYP3A4 and CYP3A5 (<http://www.imm.ki.se/CYPalleles/cyp3a.htm>). Three *CYP3A4* variants (*CYP3A4*1B*, *CYP3A4*3* and *CYP3A4*4*) have been reported to alter enzyme activity and to affect the in vivo metabolism of commonly used drugs such as simvastatin, tacrolimus and cyclosporine [6-8]. Only subjects carrying at least one *CYP3A5*1* allele (about 10% of the population) express the CYP3A5 enzyme [9]. The main cause of the absence of CYP3A5 among a majority of Caucasians is the *CYP3A5*3* allele [9-10]. *CYP3A5*6* and *CYP3A5*2* are more rare variants coding for a non-functional protein [10].

P-glycoprotein (P-gp) has been recently recognized as an important determinant for the disposition of donepezil, influencing its efflux from the brain to the blood at the blood brain barrier level [11]. The gene encoding this protein (*ABCB1*) is highly polymorphic [12-13]. Three polymorphisms,

1236C>T, *2677G>T/A* and *3435C>T*, that are in linkage disequilibrium [14-15], have been studied extensively with respect to their effects on P-gp function and clinical relevance.

3435C>T is a silent SNP in exon 26, associated with a lower expression and function of the protein [16]. *2677G>T/A* is a tri-allelic polymorphism in exon 21. Both variant alleles (A or T) result in an amino acid change, Ala893Thr and Ala893Ser, respectively, which alter expression and activity of P-gp [17-18]. The studies that have evaluated the role of the *1236C>T* SNP, a silent polymorphism in exon 12, in the pharmacokinetics of drugs such as fexofenadine, have produced discordant results [15, 19-21]. The frequencies of these three *ABCB1* SNPs have also been shown to vary between ethnic groups [22].

The aim of this study was to evaluate the impact of *CYP3A4*, *CYP3A5* and *ABCB1* polymorphisms on the steady-state plasma concentrations and therapeutic outcome of donepezil in a population of AD patients, taking *CYP2D6* genotype status into account.

Methods

Study design and population

Fifty-four Italian AD out-patients of Caucasian ethnicity were enrolled in the study at the Geriatric Clinical Unit “Unità Operativa Autonoma” (UOA), “Ospedale Maggiore della Carità”, Novara, Italy. Most patients (n=42) had participated in a previous study designed to assess the impact of *CYP2D6* polymorphisms on donepezil kinetics and therapeutic outcome [3]. The demographic and clinical characteristics of the patients are shown in Table 1. All participants had been diagnosed with probable mild to moderate AD according to the NINCDS-ADRDA Work Group Criteria for AD diagnosis [23]. They were evaluated for the degree of functional and cognitive impairment with the Mini-Mental State Examination (MMSE) screening test [24] and for daily living with the Clinical Dementia Rating Scale (CDR), the Instrumental Activity of Daily Living (ADL) and the Clinician’s Interview Based Impression of Change-Plus (CIBIC-Plus) scales. The MMSE test is a psychometric test designed to quantitatively estimate the severity of cognitive impairment and to

serially document cognitive changes in domains such as memory, orientation, language and praxis [24]. Its score ranges from 0 to 30, subjects with a score of 24 or more classified as cognitively normal. A negative change in the score reflects a worsening in the cognitive status. The MMSE score was corrected by age and educational level. Physical health, other concomitant drug therapies and adverse effects to donepezil were also recorded. Subjects with cardiac or hepatic impaired functions were excluded from the study. Tests to assess the liver function were performed at baseline (inclusion point) and repeated at the time of the second clinical evaluation. Fifteen patients received concomitant therapies with one or more substrates or weak inhibitors of CYP3A or P-gp (Table 1). The clinical evaluations were performed at baseline, once the patients were diagnosed with AD, before donepezil treatment (first assessment), and at the time of blood sampling for the donepezil concentration measurement (second assessment). All patients started donepezil therapy at 5 mg/day. After 4 weeks of treatment, the treating physicians decided whether maintaining the same dose (37 patients) or increasing it to 10 mg/day (17 patients), based on clinical evaluations. At the second assessment the patients had been treated with a stable dose of donepezil for at least 3 months. The mean treatment time before the second assessment was 9 months (range 3-40 months). The protocol was approved by the Research Ethics Committee at the “Ospedale Maggiore della Carità” of Novara (Italy). Prior to inclusion in the study all patients, or their legal tutors, gave their written informed consent.

Control group

In order to estimate whether the genotype and allele frequencies among the AD patients were consistent with those in the general population, 285 Italian healthy volunteers (Table 1) were also included in the study and genotyped for *CYP3A4*, *CYP3A5* and *ABCB1*.

Determination of donepezil plasma concentrations

Twelve to 15 hours after the last drug administration (i.e. at 8-10 a.m.), a 5 ml blood sample was collected in heparinized tubes. Plasma was separated and stored at -20°C until analysis. Donepezil plasma concentrations were determined by high-performance liquid chromatography (HPLC) with UV absorbance detection, according to Yasui-Furukori et al. [25] with slight modifications, as described by Varsaldi et al. [3]. Mean recoveries were 85–96%, and the intra- and inter-day coefficient of variation were below 4.2 and 5.1%, respectively, at concentrations ranging between 1 and 100 mg/l. The lowest limit of quantification was 1.3 ng/l. No interfering peaks were observed despite the fact that various other drugs were coadministered with donepezil.

Genotyping methods

Genomic DNA was isolated from peripheral leukocytes using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the guidelines of the manufacturer.

*CYP3A4*3*, *CYP3A4*4*, *CYP3A5*2*, *CYP3A5*6* and *ABCB1* polymorphisms were identified by real time PCR with TaqMan kits purchased from Applied Biosystems (Warrington, UK), according to the guidelines of the manufacturer (for *CYP3A4*3*, Assay ID: C__27535825_20, for *CYP3A4*4*, Assay ID: C__30634211_30, for *CYP3A5*2*, Assay ID: C__30633862_10, for *CYP3A5*6*, Assay ID: C__30203950_10; for *ABCB1 1236C>T*, Assay ID: C__7586662_10, for *ABCB1 3435C>T*, Assay ID: C__7586657_1_, and for *ABCB1 2677G>A/T*, Forward Primer GTA AGC AGT AGG GAG TAA CAA AAT AAC ACT, Reverse Primer GAC AAG CAC TGA AAG ATA AGA AAG AAC T, 2677G probe VIC-CCT TCC CAG CAC CT, 2677A probe FAM-CTT CCC AGT ACC TTC, 2677T probe FAM-CTT CCC AGA ACC TT). The *CYP3A5*3* was identified by real time PCR according to Mirghani et al. [26]. The *CYP3A4*1B* (-290A>G) was analysed by allele-specific PCR followed by digestion with restriction enzyme according to van Schaik et al. [27].

The *CYP2D6* genotype was available for most patients from the previous study [3]. The newly included patients were genotyped with the same methods.

Statistical analysis

Data were analyzed by Kruskal-Wallis non-parametric rank analysis of variance to establish an overall difference between the genotypes, and by the Mann-Whitney T-test for comparisons of two groups. When allele and genotype frequencies between AD patients and controls were compared, the Chi-square test was used. Statistical analysis was performed using the GraphPad Prism 4 software (San Diego, CA, U.S.A). A *p* value of 0.05 or lower was regarded as statistically significant.

Results

Genotypes

Three AD patients (5.6%) and twenty-four (8.4%) volunteers carried one detrimental *CYP3A4* allele (AD patients: *CYP3A4**1/*1*B* n=2 and *CYP3A4**1/*3 n=1; Volunteers: *CYP3A4**1/*1*B* n=20, and *CYP3A4**1/*3 n=4).

Twelve AD patients (22.2%) were heterozygous for *CYP3A5**1, while all the others (77.8%) were homozygous for the *CYP3A5**3 allele. Among volunteers, 246 (86.3%) were homozygous for *CYP3A5**3 and four (1.4%) carried the *CYP3A5**3/*6 genotype. Five volunteers (1.7%) were homozygous and thirty (10.5%) were heterozygous for *CYP3A5**1. No AD patient or volunteer carried *CYP3A4**4 or *CYP3A5**2 alleles. The frequencies of the allelic variants of *CYP3A4* and *CYP3A5* did not differ between patients and volunteers, and were similar to those reported in other Caucasian populations (Table 2). The allele and genotype frequencies among both volunteers and patients were in equilibrium with the Hardy-Weinberg equation.

The allele frequencies of individual *ABCB1* polymorphisms are given in Table 2. Nine haplotypes for *ABCB1* were observed. The most common *ABCB1* haplotypes among AD patients and

volunteers were *1236C/2677G/3435C* (with frequencies of 46% and 45% respectively) and *1236T/2677T/3435T* (41% and 42%, respectively, Table 3).

Fourteen (25.9%) AD patients could be classified as heterozygous *CYP2D6* EM (*CYP2D6**1/*3 n=2, *CYP2D6**1/*4 n=10, *CYP2D6**1/*5 n=1, *CYP2D6**1/*6 n=1). Two (3.7%) patients carried two detrimental alleles (*CYP2D6**3/*6 n=1, *CYP2D6**5/*5 n=1) and were classified as PM, while 2 (3.7%) were found to carry extra copies of a functional *CYP2D6* allele and were thus classified as UM.

Donepezil concentrations and the *CYP3A4/3A5/ABCB1* genotypes

The plasma concentrations of donepezil ranged from 0.078 to 0.654 mg/l among patients receiving a donepezil dose of 5 mg/day and from 0.024 to 0.537 mg/l among those receiving 10 mg/day. Since the pharmacokinetics of donepezil is linear [28-29], and there was a large variation in body weight (39-90 kg), the measured plasma concentrations were corrected for the dose and the body weight (C/D).

The C/D ratios were not associated with the *CYP3A4* or *CYP3A5* genotypes ($p>0.05$), and a considerable overlap was observed in C/D ratios between subjects homozygous and heterozygous for both *CYP3A4**1 and *CYP3A5**3. The median C/D ratio among the 15 patients receiving concomitant *CYP3A4* and/or P-gp substrates/weak inhibitors was slightly, not significantly ($p=0.21$), higher than that among patients not receiving any *CYP3A4*- or P-gp-interacting drugs (median 0.41 (95% CI 0.29-0.51) (range 0.17-0.91) vs 0.28 (95% CI 0.26-0.42) (range 0.02-0.95) mg/l/mg/kg, respectively).

Donepezil C/D was not significantly influenced by *ABCB1* polymorphisms ($p>0.05$) (Table 4). On the other hand, there was a trend for subjects homozygous for the T-variants at all three sites to have lower donepezil C/Ds compared to other genotype groups (Table 4). Consistently, subjects homozygous for the haplotype *1236T/2677T/3435T* had slightly, though not significantly ($p=0.176$),

lower plasma C/D of donepezil (median 0.18 (95% CI 0.13-0.45) (range 0.07-0.85) mg/l/mg/kg) compared to the other genotypes (median 0.31 (95% CI 0.30-0.44) (range 0.02-0.95) mg/l/mg/kg) (Fig. 1).

Clinical Outcome and the *CYP3A4/3A5* and *ABCB1* genotypes

No statistically significant association was found between *CYP3A4* or *CYP3A5* genotypes and clinical response, as measured by the CIBIC-plus score or change in the MMSE score. However, patients carrying one *CYP3A5**1 allele showed a slightly worse clinical response, measured as MMSE change from the first to the second assessment, compared to the *CYP3A5**3/*3 group (median -1.8 (95% CI -3.9 – 1.8) (range -8.0 – 8.4) vs -0.05 (-1.5 – 0.5) (-8.6 – 6.0) respectively, $p=0.519$). No impact of concomitant *CYP3A4* and/or P-gp substrates/weak inhibitors was seen on the clinical outcome, as measured by in the change in MMSE score (data not shown).

Subjects with *ABCB1* T/T genotypes showed a trend towards a better clinical response, as measured by the change in the MMSE score, compared with other genotypes (Table 4). Consistently, the median change in MMSE score among patients homozygous for the *ABCB1* 1236T/2677T/3435T haplotype was 0 (95% CI -1.3 – 3.3) (range -3.0 – 8.4) compared to -1.0 (95% CI -2.1 – 0.0) (range -8.6 – 6.0) among those with other genotypes ($p=0.138$) (Fig. 2).

***CYP2D6* genotype, donepezil plasma concentrations and clinical outcome**

The median C/D ratios of donepezil in UMs, homozygous EMs, heterozygous EMs and PMs were 0.13 (95% CI -0.57 – 0.82) (range 0.07-0.18), 0.31 (95% CI 0.29 – 0.43) (range 0.07-0.91), 0.30 (95% CI 0.21 – 0.49) (range 0.02-0.85) and 0.51 (95% CI -5.1 – 6.1) (range 0.07-0.95) mg/l/mg/kg, respectively. The heterozygous EM showed a significantly better clinical response to the therapy than homozygous EM as measured by change in the MMSE score (median 1.20 vs -1.00, respectively; $p=0.03$). The two UMs and the two PMs were among the subjects who experienced a

most marked worsening in their cognitive functions (UMs change in MMSE score -4.4 and -2.0, PMs change in MMSE score -3.3 and -1.8, respectively).

Discussion

To our knowledge this is the first study to evaluate the impact of *CYP3A4* and *ABCB1* genotypes on the clinical outcome in donepezil therapy. The frequencies of *CYP3A4*, *CYP3A5* and *ABCB1* allelic variants detected in this study were similar among the two groups studied and consistent with earlier data on Caucasian populations (Table 2). No statistically significant differences in allele or genotype frequencies were detected between AD patients and volunteers, suggesting that *CYP3A4/3A5* and *ABCB1* polymorphisms are unlikely to represent a risk factor for susceptibility to Alzheimer's disease. No AD patient or volunteer carried the *CYP3A4*4* allele, indicating that this variant has a frequency <0.5%, and could therefore represent a rare single gene defect, rather than an actual polymorphism. Consistently, so far there are no data regarding this allele's frequency in Caucasians. The *CYP3A5*2* allele was detected in a previous study with a frequency of 1% [10], but in our study it was not found either among AD patients or volunteers, suggesting that this is also a very rare variant among Italians.

Kuehl et al. [30] have reported that the *CYP3A4*1B* and *CYP3A5*1* alleles are in linkage disequilibrium in African Americans, and suggested that this linkage disequilibrium might influence CYP3A activity towards exogenous and endogenous compounds. Since the frequency of *CYP3A4*1B* is lower in Caucasians than in African Americans, larger populations would be required to demonstrate whether *CYP3A4*1B* and *CYP3A5*1* are in linkage disequilibrium in Caucasians [31]. Furthermore, Ingelman-Sundberg et al. [32] suggested that the presence of a *CYP3A5*1* allele, leading to expression of CYP3A5, could compensate for the reduction in CYP3A4 activity caused by the *CYP3A4*1B* allele (that causes decreased transcription [33]) and result in total CYP3A activity similar to that of *CYP3A4*1A/CYP3A5*3* carriers. Previous studies in Germans [34] and Spaniards [35] reported frequencies of 4% and 5.4%, respectively, for the

*CYP3A4*1B/CYP3A5*1* allele combination. Consistently, in the present study, we found the allele combination *CYP3A4*1B/CYP3A5*1* with a frequency of 3.7% among AD patients and 4.9% among volunteers (two out of two AD patients and fourteen out of twenty volunteers with *CYP3A4*1B* also carried *CYP3A5*1*).

Varsaldi et al. [3] showed that *CYP2D6* polymorphism does influence donepezil kinetics and therapeutic outcome. In the present study we confirmed these results in a somewhat larger population. Heterozygous EM had a better clinical response than homozygous EM as measured by change in the MMSE score ($p=0.03$). UM subjects had the lowest median C/D ratio and PMs had the highest. However, only two patients with these genotypes were included in the study, and the data are thus very uncertain with respect to these groups.

In accordance with previous interaction studies [4-5, 36], the concurrent administration of *CYP3A4* and/or P-gp substrates and/or weak inhibitors was found to be associated with slightly, but not significantly, higher C/D ratios compared with patients with no concomitant interacting therapies.

The large inter-individual variability in the C/D ratio of donepezil among AD patients was not related to the *CYP3A* genotype and a considerable overlap was observed in C/D ratios between subjects homo- and heterozygous for both *CYP3A4*1* and *CYP3A5*3*. Furthermore, the low frequency of *CYP3A4* allelic variants in the Italian population suggests that polymorphisms in this gene are unlikely to play a fundamental role for donepezil.

In previous studies, reduced P-gp activity and higher exposure levels of drug substrates such as digoxin were associated with homozygosity for the three variant alleles of *ABCB1*, *3435T*, *2677T* and *1236T* [15, 37-38]. Accordingly in our study, AD patients homozygous for the haplotype *1236T/2677T/3435T* showed a tendency towards a better clinical response, measured by change in MMSE score, although the results did not reach statistical significance. Concurrently, these patients had slightly lower C/Ds of donepezil. The same trend was also seen when the three SNPs were analysed separately (Table 4), so suggesting that this haplotype might play a certain role in the clinical outcome and plasma levels of donepezil.

The low C/D in patients with tendency for better response, in *T/T/T* homozygous subjects was somewhat surprising. However, Lamba et al. [39] showed that subjects homozygous for the *ABCB1* 2677T allele had higher CYP3A4 expression than subjects homozygous for the 2677G allele, suggesting a link between *ABCB1* and CYP3A4 regulation. This would be in agreement with our findings of low C/Ds of donepezil among *T/T/T* homozygous subjects. Furthermore, these patients showed a tendency towards a better clinical response. A possible explanation could be that the SNPs in the *ABCB1* gene, associated with decreased P-gp activity, may lead to increased donepezil CNS levels due to a reduced efflux of the drug from the CNS to the blood compartment. Such a phenomenon has been earlier suggested for other P-gp substrates such as risperidone [40].

Limitations of this study are the small number of patients and the lack of a placebo control-group. Thus, larger and preferably prospective studies are necessary to clarify the clinical relevance of the *CYP3A4*, *CYP3A5*, and *ABCB1* genotypes on donepezil plasma concentrations and clinical outcome among AD patients.

Conclusions

Our results suggest that the *CYP3A4* and *CYP3A5* allele variants considered in the present study do not play a pivotal role in the variability in donepezil metabolism. Conversely, *ABCB1* polymorphisms may contribute to the variability in donepezil disposition and clinical outcome.

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The study protocol was approved by the Research Ethics Committee at the “Ospedale Maggiore della Carità” of Novara (Italy) in accordance with the ethical standards laid down in the Declaration of Helsinki.

References

1. Scarpini E, Scheltens P, Feldman H (2003) Treatment of Alzheimer's disease: current status and new perspectives. *Lancet Neurol* 2(9):539-547
2. Barner EL, Gray SL (1998) Donepezil use in Alzheimer's disease. *Ann Pharmacother* 32(1):70-77
3. Varsaldi F, Miglio G, Scordo MG, Dahl ML, Villa LM, Biolcati A, Lombardi G (2006) Impact of the CYP2D6 polymorphism on steady-state plasma concentrations and clinical outcome of donepezil in Alzheimer's disease patients. *Eur J Clin Pharmacol* 62(9): 721-726
4. Tiseo PJ, Perdomo CA, Friedhoff LT (1998) Concurrent administration of donepezil HCL and cimetidine: assessment of pharmacokinetic changes following single and multiple doses. *Br J Clin Pharmacol* 46(Suppl 1):25-29
5. Tiseo PJ, Perdomo CA, Friedhoff LT (1998) Concurrent administration of donepezil HCL and ketoconazole: assessment of pharmacokinetic changes following single and multiple doses. *Br J Clin Pharmacol* 46(Suppl 1):30-34
6. Wang A, Yu BN, Luo CH, Tan ZR, Zhou G, Wang LS, Zhang W, Li Z, Liu J, Zhou HH (2005) Ile118Val genetic polymorphism of CYP3A4 and its effects on lipid-lowering efficacy of simvastatin in Chinese hyperlipidemic patients. *Eur J Clin Pharmacol* 60(12):843-848
7. Hesselink DA, van Shaik RHN, van der Heiden IP, et al (2003) Genetic polymorphisms of the *CYP3A4*, *CYP3A5*, and *MDR-1* genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 74:245-54
8. Hesselink DA, van Gelder T, van Schaik RH, Balk AH, van der Heiden IP, van Dam T, van der Werf M, Weimar W, Mathot RA (2004) Population pharmacokinetics of cyclosporine in kidney and heart transplant recipients and the influence of ethnicity and genetic polymorphisms in the *MDR-1*, *CYP3A4*, and *CYP3A5* genes. *Clin Pharmacol Ther* 76(6):545-556
9. Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, Nuessler AC, Neuhaus P, Klattig J, Eiselt R, Koch I, Zibat A, Brockmüller J, Halpert JR, Zanger UM, Wojnowski L (2001) The genetic determinants of the CYP3A5 polymorphism. *Pharmacogenetics* 11(9):733-9

10. van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J (2002) CYP3A5 variant allele frequencies in Dutch Caucasians. *Clin Chem* 48(10):1668-1671
11. Ishiwata K, Kawamura K, Yanai K, Hendrikse NH (2007) In vivo evaluation of P-glycoprotein modulation of 8 PET radioligands used clinically. *J Nucl Med* 48(1):81-87
12. Kroetz DL, Pauli-Magnus C, Hodges LM, Huang CC, Kawamoto M, Johns SJ, Stryke D, Ferrin TE, DeYoung J, Taylor T, Carlson EJ, Herskowitz I, Giacomini KM, Clark AG; Pharmacogenetics of Membrane Transporters Investigators (2003) Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics* 13(8):481-94
13. Ito S, Ieiri I, Tanabe M, Suzuki A, Higuchi S, Otsubo K (2001) Polymorphism of the ABC transporter genes, MDR1, MRP1 and MRP2/Cmoat, in healthy Japanese subjects. *Pharmacogenetics* 11(2):175-84
14. Horinouchi M, Sakaeda T, Nakamura T, Morita Y, Tamura T, Aoyama N, Kasuga M, Okumura K (2002) Significant genetic linkage of MDR1 polymorphisms at position 3435 and 2677: functional relevance to pharmacokinetics of digoxin. *Pharm Res* 19(10):1581-5
15. Johne A, Köpke K, Gerloff T, Mai I, Rietbrock S, Meisel C, Hoffmeyer S, Kerb R, Fromm MF, Brinkmann U, Eichelbaum M, Brockmöller J, Cascorbi I, Roots I (2002) Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. *Clin Pharmacol Ther* 72(5):584-94
16. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmöller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U (2000) Functional polymorphisms of the human multidrug resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Soc USA* 97(7):3473-3478
17. Verstuyft C, Schwab M, Schaeffeler E, Kerb R, Brinkmann U, Jaillon P, Funck-Brentano C, Becquemont L (2003) Digoxin pharmacokinetics and MDR1 genetic polymorphisms. *Eur J Clin Pharmacol* 58(12):809-12
18. Morita Y, Sakaeda T, Horinouchi M, Nakamura T, Kuroda K, Miki I, Yoshimura K, Sakai T, Shirasaka D, Tamura T, Aoyama N, Kasuga M, Okumura K (2003) MDR1 genotype related duodenal absorption rate of digoxin in healthy Japanese subjects. *Pharm Res* 20(4): 552-6
19. Illmer T, Schuler US, Thiede C, Schwarz UI, Kim RB, Gotthard S, Freund D, Schäkel U, Ehninger G, Schaich M (2002) MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res* 62(1):4955-4962
20. Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, Lan LB, Schuetz JD, Schuetz EG, Wilkinson GR (2001) Identification of

functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 70(2):189-199

21. Mathijssen RH, Marsh S, Karlsson MO, Xie R, Baker SD, Verweij J, Sparreboom A, McLeod HL (2003) Irinotecan pathway genotype analysis to predict pharmacokinetics. *Clin Cancer Res* 9(9): 3246-3253
22. Ozawa S, Soyama A, Saeki M, Fukushima-Uesaka H, Itoda M, Koyano S, Sai K, Ohno Y, Saito Y, Sawada J (2004) Ethnic differences in genetic polymorphisms of CYP2D6, CYP2C19, CYP3As and MDR1/ABCB1. *Drug Metab Pharmacokinet* 19(2):83-95
23. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 34(7):939-944
24. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-Mental State". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr Res* 12(3):669-677
25. Yasui-Furukori N, Furuya R, Takahata T, Tateishi T (2002) Determination of donepezil, an acetylcholinesterase inhibitor, in human plasma by high-performance liquid chromatography with ultraviolet absorbance detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 768(2):261-265
26. Mirghani RA, Sayi J, Aklillu E, Allqvist A, Jande M, Wennerholm A, Eriksen J, Herben VM, Jones BC, Gustafsson LL, Bertilsson L (2006) CYP3A5 genotype has significant effect on quinine 3-hydroxylation in Tanzanians, who have lower total CYP3A activity compared to a Swedish population. *Pharmacogenet Genomics* 16(9):637-45
27. van Schaik RH, de Wildt SN, van Iperen NM, Uitterlinden AG, van den Anker JN, Lindemans J (2000) CYP3A4-V Polymorphism detection by PCR-Restriction Fragment Length Polymorphism Analysis and its allelic frequency among 199 Dutch Caucasians. *Clin Chem* 46(11): 1834-36
28. Rogers SL, Friedhoff LT (1998) Pharmacokinetic and pharmacodynamic profile of donepezil HCl following single oral doses. *Br J Clin Pharmacol* 46[suppl 1]:1-6
29. Rogers SL, Cooper NM, Sukovaty R, Pederson JE, Lee JN, Friedhoff LT (1998) Pharmacokinetic and pharmacodynamic profile of donepezil HCl following multiple oral doses. *Br J Clin Pharmacol* 46[suppl 1]:7-12
30. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K,

- Boguski MS, Schuetz E (2001) Sequence diversity in CYP3A4 promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 27(4):383-391
31. Lamba JK, Lin YS, Schuetz EG, Thummel KE (2002) Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 54(10):1271-1294
 32. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C (2007) Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetics, pharmacoepigenetic and clinical aspects. *Pharmacol Ther* 116(3):496-526
 33. Rodriguez-Antona C, Sayi JG, Gustafsson LL, Bertilsson L, Ingelman-Sundberg M (2005) Phenotype-genotype variability in the human CYP3A locus as assessed by the probe drug quinine and analyses of variant CYP3A4 alleles. *Biochem Biophys Res Commun* 338(1):299-305
 34. Dally H, Bartsch H, Jäger B, Edler L, Schmezer P, Spiegelhalder B, Dienemann H, Drings P, Kayser K, Schulz V, Risch A (2004) Genotype relationship in the CYP3A locus in Caucasians. *Cancer Lett* 207(1):95-99
 35. Sinues B, Vicente J, Fanlo A, Vasquez P, Medina JC, Mayayo E, Conde B, Arenaz I, Martinez-Jarreta B (2007) CYP3A5*3 AND CYP3A4*1B allele distribution and genotype combinations: differences between Spaniards and Central Americans. *Ther Drug Monit* 29(4):412-416
 36. Nagy CF, Kumar D, Perdomo CA, Wason S, Cullen EI, Pratt RD (2004) Concurrent administration of donepezil HCl and sertraline HCl in healthy volunteers : assessment of pharmacokinetic changes and safety following single and multiple oral doses. *Br J Clin Pharm* 58(Suppl 1):25-33
 37. Chowbay B, Cumaraswamy S, Cheung YB, Zhou Q, Lee EJ (2003) Genetic polymorphisms in MDR1 and CYP3A4 genes in Asians and the influence of MDR1 haplotypes on cyclosporine disposition in heart transplant recipients. *Pharmacogenetics* 13(2): 89-95
 38. Kurata Y, Ieiri I, Kimura M, Morita T, Irie S, Urae A, Ohdo S, Ohtani H, Sawada Y, Higuchi S, Otsubo K (2002) Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin Pharmacol Ther* 72(2):209-19
 39. Lamba J, Strom S, Venkataramanan R, Thummel KE, Lin YS, Liu W, Cheng C, Lamba V, Watkins PB, Schuetz E (2006) MDR1 genotype is associated with hepatic cytochrome P450 3A4 basal and induction phenotype. *Clin Pharmacol. Ther* 79(4):325-338
 40. Gunes A, Spina E, Dahl M-L, Scordo MG (2008) ABCB1 polymorphisms influence steady-state plasma levels of 9-hydroxyrisperidone and risperidone active moiety. *Ther Drug Monit* 30(5):628-633

41. Sata F, Sapone A, Elizondo G, Stocker P Miller VP, Zheng W, Raunio H, Crespi CL, Gonzalez FJ (2000) CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. *Clin Pharmacol Ther* 67(1):48-56
42. van Schaik RH, de Wildt SN, Brosens R, van Fessem M, van den Anker JN, Lindemans J (2001) The CYP3A4*3 allele: is it really rare? *Clin Chem* 47(6):1104-1106
43. Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, Schaeffeler E, Eichelbaum M, Brinkmann U, Roots I (2001) Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter *MDR1* gene in white subjects. *Clin Pharmacol Ther* 69(3):169-174

Figure Legends

Figure 1. Relationship between the *ABCB1* genotypes and plasma concentration-to-dose (C/D) ratio of donepezil. *Horizontal bars* represent median values.

Figure 2. Relationship between the *ABCB1* genotypes and change in Mini-Mental State Examination (MMSE) screening test score. *Horizontal bars* represent median values.

Table 1. Demographic and clinical characteristics of patients with Alzheimer’s disease (AD) and volunteers

	AD patients (n=54)	Volunteers (n=285)
Age ^a (years)	61-93 (79±6)	19-52 (30±9)
Male/Female	14/40	155/130
Body weight ^a (kg)	39-90 (63±12)	
Smokers/No-Smokers	1 (less than 10 cig per day)/53	
MMSE at the baseline ^b	19.1 (3-28.3)	
MMSE at the steady-state ^b	18.5 (7.7-30)	
MMSE change	-0.6 (-8.6-8.4)	
Co-administered drugs	Alprazolam (2), risperidone (2), trazodone (4), atorvastatin (1) , simvastatin (4), felodipine (1), lecanidipine (1), nicardipine (1), verapamil (2), amiodarone (1)	

^aData are expressed as range (mean± SD)

^bData are expressed as median (range)

Table 2. *CYP3A* and *ABCB1* allele frequencies in patients with Alzheimer’s disease (AD) and in volunteers

Allelic Variant	Allele frequencies		95% Confidence interval ^a	χ^2	Allele frequencies in other Caucasian populations
	AD patients	Volunteers			
CYP3A4*1B (rs 2740574)	0.019	0.036	0.021-0.051	ns	0.045 [41]
CYP3A4*3 (rs 4986910)	0.009	0.007	0.007-0.021	ns	0.011 [42]
CYP3A4*4	Not detected	Not detected	-	-	No data
CYP3A5*3 (rs 776746)	0.889	0.933	0.912-0.954	ns	0.90 [10]
CYP3A5*6 (rs 10264272)	Not detected	0.007	0.000-0.014	-	0.001 [10]
CYP3A5*2 (rs 28365083)	Not detected	Not detected	-	-	0.01 [10]
ABCB1 1236T	0.472	0.458	0.417-0.499	ns	0.41 [43]
ABCB1 3435T	0.481	0.502	0.461-0.543	ns	0.539 [43]
ABCB1 2677T	0.435	0.458	0.417-0.499	ns	0.416 [43]
ABCB1 2677A	0.009	0.024	0.011-0.037	ns	0.019 [43]

^a Based on volunteer frequencies

Table 3. *ABCB1* haplotype frequencies among patients with Alzheimer’s disease (AD) and volunteers (%)

C1236T	G2677T/A	C3435T	AD patients	Volunteers
C	G	C	46	45
T	T	T	41	42
C	G	T	5	6
T	T	C	2	2
T	G	C	4	1
C	T	T	0.9	1
T	G	T	0.9	0
C	A	T	0.9	0.2
C	A	C	0	2

Table 4. Dose and weight adjusted donepezil plasma concentrations (C/D, mg/l/mg/kg) and change in MMSE score in relation to *ABCB1* genotypes. The data are given as median (95% Confidence Interval) and (range). The number of patients in each genotype group is given in parenthesis.

<i>Polymorphisms</i>					<i>p-value*</i>
C1236T		C/C (17)	C/T (23)	T/T (14)	
	Donepezil C/D	0.31 (0.23 – 0.44) (0.02 – 0.69)	0.31 (0.28 – 0.50) (0.05 – 0.95)	0.21 (0.19 – 0.48) (0.07 – 0.85)	0.684
	Change in MMSE score	-1.0 (-2.57 – 0.61) (-6.0 – 3.70)	-1.0 (-2.82 – 0.30) (-8.6 – 5.0)	0.0 (-1.32 – 2.90) (-3.0 – 8.4)	0.394
G2677T/A		G/G (19)	G/T-A (22)	T/T (13)	
	Donepezil C/D	0.39 (0.24 – 0.44) (0.02 – 0.69)	0.31 (0.28 – 0.49) (0.05 – 0.95)	0.2 (0.16 – 0.49) (0.07 – 0.85)	0.507
	Change in MMSE score	-2.0 (-3.35 – -0.24) (-8.0 – 3.7)	-0.05 (-2.09 – 1.15) (-8.6 – 6.0)	0.0 (-1.26 – 2.77) (-3.0 – 8.4)	0.147
C3435T		C/C (15)	C/T (26)	T/T (13)	
	Donepezil C/D	0.27 (0.20 – 0.43) (0.02 – 0.66)	0.31 (0.30 – 0.49) (0.07 – 0.95)	0.18 (0.14 – 0.48) (0.05 – 0.85)	0.229
	Change in MMSE score	-3.0 (-3.77 – 0.16) (-8.0 – 3.7)	-1.0 (-2.21 – 0.58) (-8.6 – 6.0)	1.0 (-0.83 – 2.95) (-3.0 – 8.4)	0.111

* the p-value refers to the three group comparison by Kruskal-Wallis test

Figure 1.



