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# Clinical Response to Donepezil in Mild and Moderate Dementia: Relationship to Drug Plasma Concentration and CYP2D6 and APOE Genetic Polymorphisms

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**Abstract**. The clinical response to donepezil in patients with mild and moderate dementia was investigated in relation to the drug plasma concentration and *APOE* and *CYP2D6* polymorphisms. In a prospective naturalistic observational study, 42 patients with Alzheimer's disease (AD) and AD with cerebrovascular disease who took donepezil (10 mg) for 12 months were evaluated. Their DNA was genotyped, and the donepezil plasma concentrations were measured after 3, 6, and 12 months. Good responders scored $\geq -1$ on the Mini-Mental State Examination at 12 months in comparison to the baseline score. The study results indicated the good response pattern was influenced by the concentration of donepezil, but not by *APOE* and *CYP2D6* polymorphisms.

Keywords: Alzheimer's disease, APOE, CYP2D6, donepezil, genetics, naturalistic study

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## INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia worldwide, accounting for 60–70% of all dementias [1–4]. AD is frequently associated with neuropsychiatric symptoms, which can lead to progressive cognitive and functional impairment [1, 5].

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Pharmacological treatment of AD is currently based on cholinesterase inhibitors (ChEI) and memantine, which have been shown to lead to modest, although effective, clinical benefits. Donepezil is a ChEI metabolized through the cytochrome P (CYP) 450, primarily by the 3A4 and 2D6 isoforms. The *CYP2D6* gene presents polymorphisms that can alter its expression [6, 7]. The plasma therapeutic level ranges from 30 to 75 ng/mL, and 50% of acetylcholinesterase inhibition is achieved when the concentration reaches 15.6 ng/mL [8, 9]. An optimal plasma level is greater than 50 ng/mL [10].

Several variant alleles of *CYP2D6* gene can enhance (UM, ultrafast metabolizer, several copies of alleles), attenuate (IM, intermediate metabolizer, one null; i.e., not functional; and one allele with reduced function are normally found), cancel (PM, poor metabolizer, two defective alleles), or maintain (EM, extensive metabolizer) the function of the protein [11]. Therefore, the polymorphisms may influence the individual's response to treatment with donepezil [6] and the concentration of the drug in AD patients, without achieving the desired effect. However, most of the individuals are EM, i.e., the metabolism of the drug occurs according to the expected kinetics [7] and is associated with the presence of one or two wild-type alleles.

Among these alleles, the frequency varies according to the population and representative sample studied. Zhou et al. [11] observed that the most common alleles in the Caucasian population are *3, *4, *5, and *6, corresponding to 97% of the PM phenotype in this group [12]. However, Menoyo et al. [12] found that the most frequent allelic distribution in Caucasian population are *1, *2, *4, and *5, while allele *10 is mainly represented in the Asian population study [13]. The *CYP2D6**10 polymorphism is associated with the IM profile because this allele decreases the protein function [14]. Therefore, donepezil metabolized by *CYP2D6* in patients who are PM could have an elevated plasma concentration and, consequently, more side-effects [15].

The *Apolipoprotein E* gene (*APOE*) presents three alleles, namely, $\varepsilon 2$, which is associated with a protective effect against AD, $\varepsilon 3$ and allele $\varepsilon 4$, both of which confer risk to the development of the disease [16].

The aims of this study were to investigate the pattern of clinical response to donepezil in a group of patients with AD and AD with cerebrovascular disease (CVD) in relation to the plasmatic concentration of donepezil and polymorphisms of the *CYP2D6* and *APOE* genes.

## PATIENTS AND METHODS

This is an observational, longitudinal, naturalistic study, conducted in the Geriatric and Neurology outpatient clinics of the Hospital das Clínicas of the Federal University of Minas Gerais (UFMG), a public-affiliated university hospital in Belo Horizonte, Brazil. These clinics are reference units for diagnosis and treatment of patients with cognitive impairment and dementia in the city.

### *Study design and drug treatment*

Patients underwent a screening visit to ascertain cognitive, functional, and behavioral impairment. The first consultation has been done by the treating physician from the Geriatric or Neurology outpatient clinics. Subsequently, patients were scheduled to be evaluated by the investigator (LFJRM) after a one-week interval, always accompanied by a caregiver. Patients were reassessed at $3 \pm 1$, $6 \pm 1$, and $12 \pm 1$ months of treatment.

Overall, 97 patients fulfilling the inclusion criteria were evaluated. The choice of the ChEI prescribed was made by the treating physician. Only patients who received donepezil were analyzed in the present study, and the target effect dosage considered for treatment was 10 mg. We divided the patients in two groups: good responders, defined as those who scored $\geq -1$ in the Mini-Mental State of Examination (MMSE) at 12 months in comparison to baseline; and bad responders, who lost 2 or more points in the MMSE at the final visit in comparison to baseline. We classified the patients into the two groups based on articles published in the medical literature; the first group could be also referred to as slow decliners and the second as fast decliners [17–19]. Additional cognitive and functional tests were applied (Table 4), without additional differences between the two groups.

### *Inclusion criteria for entry in the study*

In order to participate in the study, patients had to fulfill the diagnostic criteria of dementia due to AD from the National Institute on Aging and the Alzheimer's Association Workgroup [20] or the diagnosis of AD with CVD, according to NINDS-AIREN criteria [21], with mild or moderate dementia (Clinical Dementia Rating (CDR) 1 or 2, respectively).

*Exclusion criteria*

Patients were excluded from the study if they presented with mild cognitive impairment; other types of dementia, such as pure vascular dementia, frontotemporal dementia, dementia with Lewy bodies, corticobasal degeneration; severe dementia (CDR 3); or if were previous users of ChEI. Finally, disagreement between the first investigator and the treating physician regarding the diagnosis resulted in the patients' exclusion from the study.

*Clinical and laboratory assessments*

The clinical and genetic (*APOE* and *CYP2D6*) protocols for the study have been described in detail previously [22].

Briefly, patients taking donepezil were seen four times (from June 2009 to March 2013) and were submitted to the MMSE test, the Consortium to Establish a Registry for Alzheimer's Disease battery (CERAD), and the Pfeffer Functional Activities Questionnaire. CERAD memory evaluation was further divided into five components: incidental recall (CERAD-INC), immediate recall 1 and 2 (CERAD-RM1, CERAD-RM2, respectively) and delayed recall after five minutes (CERAD-R). Each aspect was analyzed individually and compared between groups at baseline and after 12 months of treatment with donepezil. All patients had blood samples (10 mL) collected to obtain donepezil plasmatic concentration (DPC) measurements and for *APOE* and *CYP2D6* genotyping.

After 12 months of treatment, good clinical responders were defined as those who scored $\geq -1$ on the MMSE [23], and bad responders scored $< -1$ on the MMSE, both in comparison to baseline.

DPC was determined through a liquid chromatography–electrospray ionization tandem mass spectrometry (HPLC–ESI-MS/MS) method.

The analyses were performed using a Waters system (New Castle, DE, USA), composed of a 1525 binary pump, a 2777 sample manager, a TCM/CHM column oven, and a Quattro LC triple quadrupole mass spectrometer, equipped with an electrospray ion source used in the positive ionization mode. MassLynx v.4.1 software was used for data acquisition and analysis. LC separation was performed on an ACE C18 column (100 mm × 4.6 mm i.d.; 5 μm particle size) from ACT (Aberdeen, Scotland), at 30°C. The mobile phase consisted of 2 mM aqueous ammonium acetate (pH 3.2) adjusted with formic acid and methanol (38:62), at a flow rate of 1 mL/min. The run time was 2.8 min, and the injection volume was 20 μL. Indapamide was used as an internal standard, and the biomarkers were extracted from the plasma samples via a protein precipitation procedure using methanol. The concentration range evaluated in the calibration curve was between 0.5 and 250 ng/mL. The method was developed and validated according to the Food and Drug Administration guidance for bioavailability and bioequivalence studies [24]. The allelic forms investigated were ε2, ε3 and ε4 for APOE and *3, *4, *5, *6, and *10 for CYP2D6. The method used was restriction fragment length polymorphism (RFLP) or allele-specific polymerase chain reaction [6, 25].

The Ethics Committee from the Federal University of Minas Gerais approved the study. The patients and their family caregivers signed the informed consent forms.

*Statistical analysis*

The categorical variables are presented as frequency and percentage values. The normal continuous variables are presented as mean and standard deviation (mean ± sd) values; otherwise as medians, with minimum and maximum values. In the univariate data analysis, Chi-Squared test and Fisher's exact test were used for comparisons between the categorical variables. The Student's t and Mann-Whitney tests were used to compare the means and medians of the continuous variables, respectively, adopting the 0.05 significance level. When we compared the months of DPC, we used Friedman for the groups of good and bad responders.

## RESULTS

Donepezil was prescribed to 55 patients, 42 of whom took 10 mg and completed the 12-month follow-up. The others, who received 5 mg, were excluded from the present analysis. Table 1 shows the demographic and clinical features of the 42 patients.

Table 2 displays the comparison between demographic and clinical variables, such as age, sex, level of schooling, diagnosis, severity of disease, and history of depression. No statistical difference was observed between groups.

Good and bad responders groups were investigated concerning MMSE and CERAD performance after 12 months of treatment with donepezil (Table 3),

Table 1
Demographic and clinical characteristics of 42 patients taking donepezil who followed-up for 12 months after treatment. Descriptive analyses

| Variables | | n (%) |
|---|---|---|
| Diagnosis | AD | 37 (88.12) |
| | AD+CVD | 5 (11.9) |
| Age | ≤70 | 4 (9.5) |
| | 71–80 | 19 (45.2) |
| | ≥81 | 19 (45.2) |
| Sex | Male | 13 (31.0) |
| | Female | 29 (69.0) |
| Schooling | Illiteracy | 6 (14.3) |
| | 1 to 4 years | 29 (69.0) |
| | 5 to 8 years | 4 (9.5) |
| | ≥9 years | 3 (7.1) |
| CDR | Mild | 27 (64.3) |
| | Moderate | 15 (35.7) |
| Past history of depression | Yes | 10 (24.4) |
| | No | 31 (75.6) |
| | Missing | 1 |
| Responder | Good | 22 (52.4) |
| | Bad | 20 (47.6) |
| Donepezil dose - 3 m | 5 mg | 18 (46.2) |
| | 10 mg | 21 (53.8) |
| | Missing | 3 (5.9) |
| Donepezil dose - 6 m | 5 mg | 10 (25.6) |
| | 10 mg | 29 (74.4) |
| | Missing | 3 (7.8) |
| Donepezil dose - 12 m | 10 mg | 42 (100.0) |

which showed no difference. Nevertheless, the comparison of the 12-month performance with the baseline in Table 4 showed an increase in MMSE and Pfeffer scores among good responders, although there was a concomitant decrease in clock drawing performance, CERAD-RM2, and CERAD overall scores. On the other hand, bad responders showed a marked decline in MMSE scores, but a similar impairment in clock drawing ability.

Table 5 shows the distribution of responders according to different allelic forms of *APOE* and *CYP2D6* polymorphisms. Some patients were not genotyped due to lack of sample availability (missing data).

The genetic population was under the Hardy Weinberg equilibrium for all of the polymorphisms. The main genotype of *APOE* was ε3ε3, followed by the presence of ε3ε4/ε4ε4. Only two patients presented the genotypes ε2ε4/ε2ε3. Among the *CYP2D6* polymorphisms, the wild-type genotype (wt/wt) was the most frequent, followed by the *CYP2D6**10 and *CYP2D6**4 alleles. No associations were found between the *APOE* or *CYP2D6* polymorphisms and the pattern of clinical response at 12 months of treatment ($p > 0.05$).

*APOE* polymorphisms were divided between ε3 and ε4 carriers and non-carriers and *CYP2D6* polymorphisms between homozygote wt versions and their homozygote or heterozygote polymorphic version carriers (*3, *4, *5, or T). These groups had their MMSE and CERAD-INC performance compared in Table 6. CYP2D6*4 carriers showed better

Table 2
Association among demographic and clinical characteristics of 42 patients taking donepezil who followed-up for 12 months of treatment

| Categorical Variables | | Responders | | *p*-value |
|---|---|---|---|---|
| | | Good | Bad | (test) |
| Diagnosis | AD | 26 (70.3) | 11 (29.7) | 0.637 |
| | AD+CVD | 3 (60.0) | 2 (40.0) | (Fisher) |
| | Total | 29 | 13 | |
| Age | ≤70 | 2 (50.0) | 2 (50.0) | 0.445 |
| | 71 – 80 | 15 (78.9) | 4 (21.1) | (Fisher) |
| | ≥81 | 12 (63.2) | 7 (36.8) | |
| | Total | 29 | 13 | |
| Sex | Male | 8 (61.5) | 5 (38.5) | 0.495 |
| | Female | 21 (72.4) | 8 (27.6) | (Fisher) |
| | Total | 29 | 13 | |
| Schooling | Illiteracy | 5 (83.3) | 1 (16.7) | 0.929 |
| | 1 to 4 years | 19 (65.5) | 10 (34.5) | (Fisher) |
| | 5 to 8 years | 3 (75.0) | 1 (25.0) | |
| | ≥9 years | 2 (66.7) | 1 (33.3) | |
| | Total | 29 | 13 | |
| Clinical Dementia rating | Mild | 18 (66.7) | 9 (33.3) | 0.739 |
| | Moderate | 11 (73.3) | 4 (26.7) | (Fisher) |
| | Total | 29 | 13 | |
| Depression in the past | No | 20 | 11 | 0.458 |
| | Yes | 8 | 2 | (Fisher) |
| | Total | 28 | 13 | |
| | Missing = 1 | | | |

Table 3
Cognitive tests administrated in 42 patients who took donepezil (10 mg) after 12 months of treatment: comparison between good and bad responders

| Categorical variables | Responders – n (%) | | |
|---|---|---|---|
| | Good | Bad | *p*-value (test) |
| MMSE 12 m ($n = 42$) | 16.21 ± 4.51 | 13.62 ± 4.64 | 0.105 (T) |
| Clock drawing 12 m | 0 (0–4) | 0 (0–4) | 0.767 (MW) |
| CERAD-INC 12 m | 2 (0–5) | 0 (0–3) | 0.129 (MW) |
| CERAD-RM1 12 m | 3 (0–7) | 2 (0–5) | 0.667 (MW) |
| CERAD-RM2 12 m | 3(0–8) | 3 (0–6) | 0.576 (MW) |
| CERAD-R(5) 12 m | 0 (0–4) | 0 (0–4) | 0.588 (MW) |
| CERAD12 m | 14 (0–19) | 15 (11–18) | 0.710 (MW) |
| Pfeffer 12 m | 26 (11–30) | 28 (16–30) | 0.280 (MW) |

For watch draw and CERAD (cognitive tests), except for MMSE, the number of valid patients are: $n = 35$ (21 good, 14 bad responders, 7 missing; total = 42). For functional tests (Pfeffer). $n = 41$ at baseline (22 good responders, 19 bad responders, 1 missing). $n = 42$ at 12 months (22 good responders, 20 bad responders). For parametric test we write: mean ± sd (standard deviation). For no parametric tests we write: median (minimum, maximum values). MW, Mann-Whitney test; T, Student's *t*-test; CERAD-INC, CERAD incidental memory; –RM(1), ready memory attempt 1; –RM(2), ready memory attempt 2; –R(5), recall after 5 minutes.

Table 4
Cognitive tests administrated in 42 good and bad responders who took donepezil (10 mg): comparison between baseline and after 12 months of treatment

| Categorical Variables | | Timeline | | *p*-value |
|---|---|---|---|---|
| | | Baseline | 12 months | |
| MMSE | Good | 15.00 ± 4.38 | 16.21 ± 4.51 | 0.002* (T) |
| | Bad | 17.46 ± 5.22 | 13.62 ± 4.64 | 0.001* (T) |
| | Overall | 15.76 ± 4.73 | 15.40 ± 4.65 | — |
| Clock drawing | Good | 1 (0–5) | 0 (0–4) | 0.017* (MW) |
| | Bad | 1 (0–5) | 0 (0–4) | 0.014* (MW) |
| CERAD-INC | Good | 1 (0–4) | 2 (0–5) | 0.13 (MW) |
| | Bad | 2 (0–4) | 0 (0–3) | 0.15 (MW) |
| CERAD-RM1 | Good | 3 (0–6) | 3 (0–7) | 0.65 (MW) |
| | Bad | 3 (0–5) | 2 (0–5) | 0.30 (MW) |
| CERAD-RM2 | Good | 3 (0–8) | 3 (0–8) | 0.04* (MW) |
| | Bad | 3 (0–6) | 3 (0–6) | 0.80 (MW) |
| CERAD R(5) | Good | 0 (0–4) | 0 (0–4) | 1.00 |
| | Bad | 0 (0–4) | — | — |
| CERAD Total | Good | 14 (0–19) | 12 (0–18) | 0.03* (MW) |
| | Bad | 14.42 ± 2.39 | 13.91 ± 2.26 | 0.42 (T) |
| Pfeffer | Good | 24 (2–30) | 26 (11–30) | 0.008* (MW) |
| | Bad | 26 (15–30) | 28 (16–30) | 0.09 (MW) |

For clock draw and CERAD (cognitive tests), except for MMSE, the number of valid patients are: $n = 35$ (21 good, 14 bad responders, 7 missing; total = 42). For functional tests (Pfeffer). $n = 41$ at baseline (22 good responders, 19 bad responders, 1 missing). $n = 42$ at 12 months (22 good responders, 20 bad responders). For parametric test we write: mean ± sd (standard deviation). For no parametric tests we write: median (minimum, maximum values). CERAD-INC, CERAD incidental memory; –RM(1), ready memory attempt 1; –RM(2), ready memory attempt 2; –R(5), recall after 5 minutes; MW, Mann-Whitney test; T, Student's *t*-test. *Significant $p < 0.05$.

performance at CERAD-INC than their wt counterparts, while an opposite trend was observed in*3 carriers, although not statistically significant. There was no difference in MMSE performance (Table 6) or in CERAD-RM1, CERAD-RM2, CERAD-R, or CERAD overall performance (data not shown).

Table 7 presents the donepezil concentration variation through the 12 months of follow-up, which could have influenced the treatment response. In none of the time points analyzed did a significant influence on the pattern of clinical response emerge ($p > 0.05$).

In addition, in Table 7, we compared DPC over the study period using Friedman test. Good responders presented a higher DPC at 12 months than at 6 months ($p = 0.003$) and a higher DPC at 12 months than at 3 months ($p < 0.001$). Among bad responders, there was no need for multiple comparisons because in the Friedman test the result was non-significant ($p = 0.097$).

It is important to note that the DPC increased during the treatment period when the groups were analyzed together, without distinguishing the

Table 5
Associations among ApoE and CYP2D6 polymorphisms and response after 12 months of treatment. Univariate analyses

| Polymorphisms | Alleles | Responders | | *p*-value (test) |
|---|---|---|---|---|
| | | Good | Bad | |
| APOE ε3 | ε3 + | 27 (93.1) | 10 (76.9) | 0.162 (Fisher) |
| | ε3 – | 2 (6.9) | 3 (23.1) | |
| | Total | 29 | 13 | |
| APOE ε4 | ε4 + | 14 (63.6) | 8 (36.4) | 0.514 (Fisher) |
| | ε4 – | 15 (75.0) | 5 (25.0) | |
| | Total | 29 | 13 | |
| CYP2D6*3 | wt/wt | 23 (63.9) | 13 (36.1) | 0.284 (Fisher) |
| | *3/*3/ *3/wt | 4 (100.0) | 0 (0) | |
| | Total | 27 | 13 | |
| CYP2D6*4 | wt/wt | 21 (72.4) | 8 (27.6) | 0.672 (Fisher) |
| | *4/wt | 5 (62.5) | 3 (37.5) | |
| | Total | 26 | 11 | |
| CYP2D6*5 | wt/wt | 10 (55.5) | 8 (44.5) | 0.257 (Fisher) |
| | *5/wt | 3 (100.0) | 0 (0) | |
| | Total | 13 | 8 | |
| CYP2D6*6 | wt/wt | 27 (67.5) | 13 (32.5) | |
| CYP2D6*10 | CC | 13 (61.9) | 8 (38.1) | 0.721 (Fisher) |
| | CT/TT | 11 (73.3) | 4 (26.7) | |
| | Total | 24 | 12 | |

response type (Table 8). Additionally, the same tendency was observed in patients with *APOE*ε4 and *APOE*ε3 carriers and *CYP2D6* wild type. The polymorphism*3 at 12 months was associated with higher plasma levels of donepezil compared with the wild-type counterparts ($p < 0.05$).

## DISCUSSION

We observed that the good response pattern was influenced by the concentration of donepezil but not by *APOE* and *CYP2D6* polymorphisms. Higher concentrations of DPC in good responders at 6 and 12 months of treatment corresponded to a better response.

In the present study, patients with mild and moderate dementia (AD and AD+CVD) who received 10 mg of donepezil were followed for 12 months.

There are many controversies surrounding the relationship between *APOE*ε4 and a specific pattern of clinical response to ChEI. Cacabelos [26], in one pharmacogenetic study, alleged that patients who are carriers of *APOE*ε4 show worse cognitive response than do non-carriers. Conversely, Barnes et al. [27] and Bizzarro et al. [28] found that the response in *APOE*ε4 carriers taking donepezil was better than in non-carriers. In another study, Rigaud et al. [29] did not obtain the same results as these authors. In this study, no difference was observed in the clinical response, regardless of the presence of *APOE*ε4 (Table 5). Our findings are different from those obtained by other investigators [30, 31], but are in line with the results reported by Rigaud et al. [29], Klimkowicz-Mrowiec et al. [32], and Lu et al. [33]. Lu and colleagues [33] also analyzed the influence of the ε3 allele in clinical response and found an increase in the rate of good responders among non-carriers of ε3 taking donepezil, a result that was not reproduced in the present study. Again, in contrast to our results, the same study found differences in the response between *1/*1, *1/*10, and *10/*10, the latter exhibiting the best response to donepezil measured by MMSE. Although no difference between different *APOE*

Table 6
Cognitive performance after 12 months of treatment (MMSE and CERAD-INC) comparison between APOE and CYP2D6 allelic presentations

| Polymorphisms | | MMSE | | | CERAD-INC | | |
|---|---|---|---|---|---|---|---|
| | | N | Score | *p*-value | N | Score | *p*-value |
| APOE ε3 | ε3 + | 37 | 16 (7–25) | 0.34 | 36 | 2 (0–5) | 0.45 |
| | ε3 – | 5 | 14 (12–21) | (T) | 5 | 0 (0–3) | (MW) |
| APOE ε4 | ε4 + | 22 | 14.95 ± 4.36 | 0.52 | 22 | 1.50 (0–5) | 0.87 |
| | ε4 – | 20 | 15.90 ± 5.02 | (T) | 19 | 1.00 (0–4) | (MW) |
| CYP2D6*3 | wt/wt | 36 | 15.50 (7–23) | 0.06 | 36 | 2.00 (0–5) | 0.10 |
| | wt/*3 or *3/*3 | 4 | 11.00 (11–25) | (MW) | 4 | 0.50 (0–2) | (MW) |
| CYP2D6*4 | wt/wt | 29 | 15.31 ± 4.65 | 0.92 | 28 | 1.00 (0–5) | 0.02* |
| | wt/*4 | 8 | 15.50 ± 4.69 | (T) | 8 | 2.00 (0–4) | (MW) |
| CYP2D6*5 | wt/wt | 18 | 14.28 ± 4.08 | 0.88 | 17 | 2.00 (0–5) | 0.34 |
| | wt/*5 | 3 | 4.77 ± 3.79 | (T) | 3 | 1.00 (0–2) | (MW) |
| CYP2D6*10 | CC | 21 | 14.95 ± 5.18 | 0.49 | 19 | 0.00 (0–4) | 0.12 |
| | CT/TT | 15 | 16.07 ± 4.15 | (T) | 15 | 2.00 (0–5) | (MW) |

CYP2D6 is not shown because we found only one group (wt/wt). CERAD-INC = CERAD incidental memory; MMSE, Mini-Mental State of Examination; MW, Mann-Whitney; T, Student's *t*-test. *Significant $p < 0.05$.

Table 7
Association among donepezil serum concentration after 3, 6, and 12 months of treatment onset and response

| Donepezil serum concentration | Responder – n(%) | | *p*-value (test) |
|---|---|---|---|
| | Good | Bad | |
| DPC 3m ($n = 36$) | 29.70 | 31.93 | 0.888 (MW) |
| (26 good, 10 bad responders) | (10.90–87.00) | (8.00–107.90) | |
| DPC 6m ($n = 37$) | $47.71 \pm 17.01$ | $46.67 \pm 24.58$ | 0.884 (T) |
| (27 good, 10 bad responders) | | | |
| DPC 12m ($n = 37$) | $66.14 \pm 31.78$ | $50.47 \pm 22.99$ | 0.149 (T) |
| (26 good, 11 bad responders) | | | |
| *p*-value (test) | <0.001* (Friedman Test) | $p = 0.097$ (Friedman test) | |
| Good responders | Bad responders | | |
| DPC 3 months 29.70 (10.90–87.00) | 31.93 (8.00–107.90) | | |
| DPC 6 months 49.44 (6.0–73.9) | 44.79 (16.8–91.8) | | |
| DPC 12 months 59.77 (2.4–127.6) | 47.88 (0.2–84.5) | | |
| $P < 0.001$ – Friedman test | $p = 0.097$ – Friedman test | | |

The concentrations below the table are slightly different of the concentrations inside the table, because outside numbers represent the DPC comparison inside of each group (between good and between bad responders) and in this case we use the Friedman test and median. DPC, donepezil plasmatic concentration.

and *CYP2D6* alleles was observed regarding clinical response classification (good/bad), the analysis of CERAD-INC score showed a better performance among *4 carriers in comparison to their wt counterparts.

Cacabelos [26], in the study mentioned above, concluded that 15% of the AD population may exhibit abnormal metabolism of ChEI, with 50% of those being UM and the remaining 50% being PM. According to this author, 75% of the response to ChEI could be explained by pharmacogenetic and pharmacogenomic factors.

The DPC at 12 months was higher than those were at 3 and 6 months (Tables 7 and 8). Rogers and co-workers [9] suggested a plasma concentration of 50–75 ng/mL for optimal response. In our study, we could not explain the elevation of plasma donepezil concentration across the study in a way that was supported by the current medical literature. One possible explanation might be the residual dose observed in the good responder group at 6 and 12 months compared with the bad responder group, which could explain the better response to treatment in those patients (Table 7).

It is important to note that *APOE*4 and *CYP2D*6 wt individuals showed higher levels of donepezil at 12 months than at 3 and 6 months, which suggests that these alleles are important for maintaining higher DPC over the course of treatment, even at the same donepezil dose (Table 8).

The lack of influence of *CYP2D6* polymorphisms could be explained by the fact that this enzyme is not exclusively responsible for the metabolism of ChEI. *CYP3A4* also has the same function and could fill the role of *CYP2D6*.

Few studies compare therapeutic response among groups of responders in correlation to differences in donepezil concentration between these groups, and there are even fewer cohorts with more than two evaluations of patients. We were not able to find a meta-analysis about this topic, although we found other original studies analyzing the correlation between differences in donepezil concentration and clinical response. Concerning the CYP2D6 polymorphism, Varsaldi et al. [6] analyzed clinical outcomes of 42 Italian AD patients treated with donepezil and their correlation with genetic polymorphisms and with the drug concentrations. They found that 10 heterozygous EM had a better clinic outcome when compared to the 30 homozygous EM and with the two UM. However, there were no differences concerning donepezil serum concentration among the three groups. In 2014, Sonali et al. [34] studied 37 North Indian AD patients using donepezil in monotherapy with similar purpose. In their study, there was no significant difference in donepezil serum concentration among all CYP2D6 alleles analyzed (*2, 3, 4, 10 e 17) or in the clinical outcome. Interestingly, Lu et al. [35] compared the stereoselective metabolism of donepezil and the clinical response of 77 Han Chinese AD patients. This group found that a higher concentration of the enantiomer S-donepezil was associated with a better response to the treatment and, when the racemic mixture was considered for analysis, no association was found. They also analyzed CYP2D6*1 and *10 polymorphism

and found that the *1/*1 and *10/*10 was associated with a better response to treatment and there were significant difference among *1/*1, *1/*10, and *10/*10 phenotypes regarding S-donepezil concentration. This recent study suggests a possible explanation for the conflicting results regarding the association of CYP2D6 polymorphism, donepezil serum concentration, and clinical outcome, which might be the contamination of the previous analysis with R-donepezil.

Finally, in relation to the additional cognitive tests, no differences were found between the good and bad responders in the CERAD list of words and clock drawing (Table 4).

Some limitations in the present study may be considered, such as the small sample size and the fact

Table 8
Verification of the plasma concentration of donepezil in all patients based on APOE and CYP2D6 polymorphisms. Univariate analyses

| Concentration of donepezil (ng/mL) | | 3 months | 6 months | 12 months | *p*-values |
|---|---|---|---|---|---|
| All donepezil patients | | 29.70 (8.0–107.9) | 49.44 (6.0–91.8) | 56.95 (0.2–127.6) | 3–6 m: 0.003* 3–12 m: 0.003* 6–12 m: <0.001* |
| APOE ε3 | ε3 + | 29.71 (10.9–107.9) | 49.44 (6.0–91.8) | 56.95 (0.2–127.6) | 3–6 m: 0.006* 3–12 m: 0.001* 6–12 m: 0.005* |
| | ε3 – | 29.27 (8.0–50.5) | 46.83 (20.9–72.8) | 60.04 (38.0–106.8) | 3–6 m: 0.180 3–12 m: 0.180 6–12 m: 0.655 |
| | *p*-value (test) | 0.679 (MW) | 0.946 (MW) | 0.845 (MW) | |
| APOE ε4 | ε4 + | 33.53 (8.0–107.9) | 52.51 ± 13.75 | 57.51 (36.4–127.6) | 3–6 m: 0.006* 3–12 m: 0.003* 6–12 m: 0.056 |
| | ε4 – | 28.13 (10.9–88.7) | 46.22 ± 20.43 | 55.48 (0.2–100.3) | 3–6 m: 0.107 3–12 m: 0.053 6–12 m: 0.069 |
| | *p*-value (test) | 0.869 (MW) | 0.291 (T) | 0.289 (MW) | |
| CYP2D6*3 | wt/wt | 30.17 (8–107.9) | 46.45 (6–91.8) | 51.69 (0.2–106.8) | 3–6 m: 0.021* 3–12 m: 0.005* 6–12 m: 0.013* |
| | wt/*3 & 3/*3 | 29.12 ± 9.18 | 61.90 ± 10.94 | 123.75 (48.9–127.6) | 3–6 m: 0.109 3–12 m: 0.068 6–12 m: 0.285 |
| | *p*-value (test) | 0.454 (MW) | 0.182 (T) | 0.018* (MW) | |
| CYP2D6*4 | wt/wt | 29.57 (8.0 – 107.9) | 48.61 ± 17.12 | 63.79 ± 31.76 | 3–6 m: 0.004* 3–12 m: 0.000* 6–12 m: 0.002* |
| | wt/*4 | 50.5 (29.8 – 88.7) | 53.94 ± 25.39 | 54.65 ± 30.24 | 3–6 m: 0.465 3–12 m: 0.715 6–12 m: 0.686 |
| | *p*-value (test) | 0.112 (MW) | 0.560 (T) | 0.499 (T) | |
| CYP2D6*5 | wt/wt | 33.06 (8.6 – 107.9) | 52.08 ± 16.48 | 54.42 (0.2–127.6) | 3–6 m: 0.028* 3–12 m: 0.048* 6–12 m: 0.075 |
| | wt/*5 | 30.5 (10.9 – 43.2) | 36.92 ± 4.66 | 46.28 (40.70–87.90) | 3–6 m: 0.285 3–12 m: 0.109 6–12 m: 0.109 |
| | *p*-value (test) | 0.529 (MW) | 0.144 (T) | 0.434 (MW) | |
| CYP2D6*6 | wt/wt | 38.97 ± 23.34 | 47.77 ± 19.02 | 61.52 ± 30.44 | 3–6 m: 0.006* 3–12 m: 0.001* 6–12 m: 0.004* |

(*Continued*)

Table 8
(*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| CYP2D6*10 | CC | 30.03 (8.0 – 107.9) | 46.25 ± 19.54 | 60.28 ± 30.37 | 3–6 m: 0.100<br>3–12 m: 0.003*<br>6–12 m: 0.008* |
| | CT &TT | 35.41 (18.0 – 88.7) | 47.97 ± 20.89 | 60.06 ± 30.48 | 3–6 m: 0.086<br>3–12 m: 0.314<br>6–12 m: 0.767 |
| | *p*-value (test) | 0.725 (MW) | 0.821 (T) | 0.984 (T) | |

Legend: ε3ε3 ($n = 24$); ε4ε4 and ε3/ε4 ($n = 25$). **APOE: 10 mg donepezil:** 10 mg ε3ε3 ($n = 17$) ε3ε4 and ε4ε4 ($n = 18$). 10 mg ε3ε3 ($n = 17$) ε3ε4 and ε4ε4 ($n = 18$). 10 mg ε3ε3 ($n = 16$) ε3ε4 and ε4ε4 ($n = 19$); **CYP 2D6 *3:10 mg donepezil:** wt/wt ($n = 30$) wt/*3 and *3/*3 ($n = 4$). wt/wt ($n = 32$) wt/*3 and *3/*3 ($n = 3$). wt/wt ($n = 32$) wt/*3 and *3/*3 ($n = 4$); **CYP 2D6 *4:10 mg donepezil:** wt/wt ($n = 27$) wt/*4 and *4/*4 ($n = 4$). wt/wt ($n = 27$) wt/*4 and *4/*4 ($n = 5$). wt/wt ($n = 27$) wt/*4 and *4/*4 ($n = 7$); **CYP 2D6 *5:10 mg donepezil:** wt/wt ($n = 14$) wt/*5 and *5/*5 ($n = 3$). wt/wt ($n = 14$) wt/*5 and *5/*5 ($n = 3$). wt/wt ($n = 16$) wt/*5 and *5/*5 ($n = 3$); **CYP 2D6 *6:10 mg donepezil:** wt/wt ($n = 34$) – 3 months. wt/wt ($n = 35$) – 6 months. wt/wt ($n = 36$) – 12 months; **CYP 2D6 *10:10 mg donepezil:** CC ($n = 20$) CT/TT ($n = 10$). CC ($n = 20$) CT/TT ($n = 11$). CC ($n = 20$) CT/TT ($n = 12$). The *p* values between columns represent the comparison between the wild type (wt/wt) versus wt/polymorphism, and the *p* values between lines represent the comparison among the months (3, 6, and 12 months). MW, Mann-Whitney; T, Student's *t*-test. *Significant $p < 0.05$.

that five patients (10%) taking donepezil abandoned the treatment because of side effects, although this rate was not above the rates found in patients from our sample who took other ChEIs [22].

The study population is very old (19 out of 42 patients were aged 82+ years) and with low educational level (35 out of 42 patients had less than 4 years of education). Given the high age and the low schooling of our sample, some caution shall be taken with regard to the generalization of our results. However, our sample is representative of patients who regularly come to our outpatient clinic and is probably representative of the population of dementia patients seen in most developing countries, especially in Latin America. Hence, given the naturalistic design of our study, it reflects our reality.

In addition, since we had a small sample, we divided our patients in two groups (slow decliners and fast decliners), without emphasizing the group who scored > 1 in the MMSE at 12 months in comparison to baseline. Moreover, this is not the main outcome of our study, but it is a tool which was already been employed by other researchers to classify patients in clinical trials and in other longitudinal studies [17–19].

In summary, we investigated the association of *APOE* and *CYP2D6* polymorphisms, as well as the dose and plasma concentration of donepezil, with the clinical response in mild to moderate dementia patients presenting comorbidities. This was a 12-month study in a naturalistic or "real-life" setting. Studies in other populations or from different countries are necessary to assess specific genetic backgrounds. However, it seems that pharmacogenetics and pharmacogenomics are important but are not the only reason for the clinical and cognitive response to treatment in AD.

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