Influence of rs1080985 single nucleotide polymorphism of the CYP2D6 gene on response to treatment with donepezil in patients with Alzheimer’s disease

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Background: Recent data indicate that the rs1080985 single nucleotide polymorphism of the cytochrome P450 (CYP) 2D6 gene may affect the response to treatment with donepezil in patients with Alzheimer’s disease. There is also evidence that the common apolipoprotein E (APOE) polymorphism may affect the response to treatment with donepezil in Alzheimer’s disease. We investigated the association between response to donepezil and the rs1080985 single nucleotide polymorphism, the minor allele (G) of which was previously reported to be associated with a poor response to this drug in patients with Alzheimer’s disease. The common APOE polymorphism was also assessed for its relevance to the outcome of this treatment.

Methods: Analysis of CYP2D6 and APOE polymorphisms was undertaken in 88 naive Caucasian patients with Alzheimer’s disease. All patients received treatment with donepezil for at least 10 months, and the response to treatment was then assessed according to the National Institute for Health and Clinical Excellence criteria.

Results: No significant differences were observed in distribution of the CYP2D6 rs1080985 single nucleotide polymorphism or common APOE polymorphism between responders (68.2%) and nonresponders (31.8%) to treatment with donepezil.

Conclusion: Our results suggest that neither the CYP2D6 nor the APOE polymorphism influences the response to treatment with donepezil in a Polish population with Alzheimer’s disease.

Keywords: Alzheimer’s disease, CYP2D6, APOE, donepezil, pharmacogenetics, single nucleotide polymorphism

Introduction
Donepezil is currently used for symptomatic treatment of mild to moderate Alzheimer’s disease. Differential response to this treatment has been observed. Interindividual genetic variants of genes responsible for drug metabolism (cytochrome P450 [CYP]) or genes associated with the pathogenesis of Alzheimer’s disease (apolipoprotein E [APOE] common polymorphism) may influence the pharmacokinetic and pharmacodynamic properties of donepezil.1

After oral administration, donepezil undergoes significant first-pass metabolism by hepatic microsomal CYP enzymes to several metabolites. The CYP enzymes involved in the metabolism of donepezil are CYP3A4 and CYP2D6.2 The CYP2D6 gene (CYP family 2, subfamily D, polypeptide 6) is located on chromosome 22q13.1–13.2. This locus is highly polymorphic, with a large number of allelic variants identified and
resulting in different degrees of enzymatic activity. The G allele of the single nucleotide polymorphism, rs1080985 (C<sup>−1584</sup>→G), in the promoter region of the CYP2D6 gene, was associated with higher gene expression and greater enzymatic activity in vivo<sup>4,5</sup> and a poor response to treatment with donepezil in Italian patients with Alzheimer’s disease.<sup>6–8</sup>

APOE is a multifunctional protein playing a key role in the metabolism of cholesterol and triglycerides, and in tissue repair and inflammation. The APOE gene is located on chromosome 19q13.2, and has three major isoforms encoded by the ε2, ε3, and ε4 alleles. The ε4 allele is associated with hypercholesterolemia and an increased risk of Alzheimer’s disease, while the ε2 allele is associated with the opposite effect. The relationship between response to donepezil and common APOE polymorphism in patients with Alzheimer’s disease has been investigated in several studies, but the results are controversial.

The aim of our study was to assess the effect of the rs1080985 single nucleotide polymorphism in the promoter region of the CYP2D6 gene on the clinical outcome of treatment with donepezil in a Polish population with Alzheimer’s disease. The influence of the common APOE polymorphism was also assessed for potential relevance to the outcome of treatment with a cholinesterase inhibitor.

**Patients and methods**

Consecutive patients admitted to the Outpatients Memory Clinic, Department of Neurology, University Hospital, Cracow, Poland, were screened for enrollment in the study. Of 361 patients, 245 were excluded because of: refusal to enter into the study (n = 16), lack of a reliable caregiver (n = 32), and concomitant therapy with drugs metabolized extensively by CYP2D6 (anticholinergics, anticonvulsants, antidepressants, β-blockers, opioids, antipsychotic drugs, n = 197). Finally, 116 patients with Alzheimer’s disease, older than 65 years of age at onset of the disease, and without a family history of Alzheimer’s disease were included. Onset of the disease was defined as the age at which memory loss or change in behavior was first noted. The diagnosis of probable Alzheimer’s disease was made according to the National Institute for Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria,<sup>9</sup> with a mild to moderate degree of disease severity. The evaluation included medical, neurologic, and neuropsychologic examinations, interviews with a close informant, laboratory testing, and computed tomography/magnetic resonance imaging. Cognitive status was evaluated by neuropsychologic examination.<sup>10</sup> Patients included in the study were treated with donepezil 5 mg/day for one month, after which the dose was increased to 10 mg a day. Follow-up visits were scheduled at months 6 and 9 of treatment with donepezil 10 mg. The response to donepezil was defined according to the criteria recommended by the National Institute for Health and Clinical Excellence.<sup>11</sup> A responder was defined as a patient who showed improvement or no deterioration in cognition (evaluated by Mini Mental State Examination [MMSE] and the Clock Drawing Test [CDT]), and showed evidence of global improvement on behavioral or functional assessment (instrumental activities of daily living, IADL). All patients with Alzheimer’s disease were Caucasian and of Eastern European descent. All participants gave their informed consent and the study was approved by the local ethics committee.

**DNA analyses**

Real-time polymerase chain reaction was performed using the protocol provided to assess rs1080985 single nucleotide polymorphism of the CYP2D6 gene. To determine the APOE genotype (APOE ε2, APOE ε3, APOE ε4 alleles), we genotyped two single nucleotide polymorphisms (rs429358 and rs7412, National Center for Biotechnology Information) using the TaqMan assay (Applied Biosystems, Foster City, CA, USA).

**Statistical analysis**

Demographic data between the groups were compared by χ² (gender) or t-test (age). The genotype frequencies were compared between responders and nonresponders using the χ² test. For abnormally distributed variables, differences between the groups were tested using the Mann-Whitney U-test. Hardy–Weinberg equilibrium was verified for all tested populations (SAS Genetics version 9.1, SAS Institute Inc, Cary, NC, USA). Adjusted odds ratios (OR) with 95% confidence intervals (CI) were estimated by logistic regression, controlling for APOE carrier status, gender, age, and MMSE at baseline. The level of significance was set at P < 0.05.

We hypothesized that response to the drug would be able to be detected in two thirds of the 116 patients who entered the study, while the remaining one third of patients would show no drug response, according to response rates from previously published studies.<sup>2,6,12,13</sup> We also assumed the presence of an alternative genotype (GG or GC) in 40% of subjects versus the wild-type genotype CC present in 60% of patients. Under these assumptions, the study has a power of 80% to detect an association between alternative
CYP genotypes and response to the drug in patients with Alzheimer’s disease at the level of significance of 0.05 if the OR of association is lower than 0.23 or higher than 3.7.

Calculated post hoc, the power in this study of 88 patients who actually completed follow-up (response to the drug detected in 68% of subjects and alternative genotype present in 39% of subjects) was 7.2% for an OR of 0.8 and 5.45% for an OR of 0.9 (lack of response to the drug detected in 32% of nonresponders and alternative genotypes present in 47% of responders).

Results

Of 116 consecutive patients (mean age 72.72 ± 7.04 years, 78 females [67.24%]), 28 (mean age 73.61 ± 7.24 years, 18 female [64.3%]) were excluded because they did not attend the follow-up visit at 7 or 10 months from initiation of treatment. Analysis of the rs1080985 single nucleotide polymorphism showed that 67 patients were CC homozygotes (57.76%), 38 were CG heterozygotes (33.45%), and 11 were GG homozygotes (9.48%). No differences were found between these observed frequencies and the expected Hardy–Weinberg frequencies for this locus (P = 0.12).

Analysis of the APOE polymorphism showed that nine patients were ε2/ε3 heterozygotes (7.76%), 54 were ε3/ε3 homozygotes (46.55%), one was an ε2/ε4 heterozygote (0.86%), 43 were ε3/ε4 heterozygotes (37.07%), and nine were ε4/ε4 homozygotes (7.76%). No differences were found between these observed frequencies and the expected Hardy–Weinberg frequencies for this locus (P = 0.57). The patients lost to follow-up did not differ significantly in age at onset of the disease, sex, MMSE, CDT, and IADL score, or CYP2D6 polymorphism when compared with the rest of the group (data not shown).

During follow-up, three patients complained of nausea and vomiting when the donepezil dose was increased to 10 mg; however, when the dose was reduced and after 2 weeks it was again increased up to 10 mg, these patients were still eligible for assessment after 6 and 9 months. Of the 88 patients who were followed up, 60 were identified as responders. There was no significant difference with regard to age, sex, or scores on the MMSE, CDT, and IADL between responders and nonresponders. The characteristics of the patients at baseline are summarized in Table 1.

The CYP2D6 and APOE polymorphisms were in Hardy–Weinberg equilibrium (P = 0.12, and P = 0.57, respectively). No differences were found between the rs1080985 genotype distribution in the two groups (P = 0.79; Fisher’s Exact test, Table 1). Logistic regression analysis adjusted for age, sex, MMSE score at baseline, and APOE polymorphism showed no association between CG heterozygotes (P = 0.70, OR 0.80; 95% CI 0.30–2.28) or GG homozygotes (P = 0.90, OR 0.90; 95% CI 0.15–5.40) and response to treatment.

Table 1 Demographic and clinical characteristics of patients with Alzheimer’s disease who completed the follow-up visit

| Characteristics | Responders (n = 60) | Nonresponders (n = 28) | P-value |
|-----------------|---------------------|------------------------|--------|
| Age at onset of AD (years) | 72.23 ± 7.40 | 72.86 ± 6.10 | 0.27 |
| Age at baseline (years) | 74.36 ± 7.44 | 75.14 ± 5.8 | 0.16 |
| Women, n (%) | 39 (65) | 21 (75) | 0.35 |
| IADL score | 12 (9–12) | 14 (11.5–17.5) | 0.06 |
| MMSE score | 23 (14–29) | 22 (17–25) | 0.20 |
| CDT score | 8 (5–10) | 5.5 (3–9) | 0.06 |
| APOE polymorphism | | | |
| 2/3, n (%) | 4 (6.66) | 3 (10.71) | OR 0.83, 95% CI 0.33–2.01, P = 0.66 |
| 3/3, n (%) | 29 (48.34) | 11 (39.28) | OR 0.69, 95% CI 0.28–1.72, P = 0.43 |
| 2/4, n (%) | 1 (7.67) | 1 (3.57) | OR 0.73, 95% CI 0.28–1.89, P = 0.52 |
| 3/4, n (%) | 21 (35) | 13 (4.6) | OR 0.63, 95% CI 0.13–3.10, P = 0.42 |
| 4/4, n (%) | 5 (8.34) | 2 (7.14%) | (dominant G allele) |
| rs1080985 SNP | | | |
| GG, n (%) | 6 (10%) | 2 (7.14%) | OR 1.44, 95% CI 0.26–7.65, P = 0.67 |
| CG, n (%) | 22 (36.7%) | 9 (32.14%) | OR 0.61, 95% CI 0.23–1.57, P = 0.51 |
| CC, n (%) | 32 (53.3%) | 17 (60.7%) | OR 0.76, 95% CI 0.38–1.60, P = 0.50 |

Notes: *Mean ± standard deviation; †mean, interquartile range; ‡genotype with ε4 allele versus other genotype; §genotype with ε4 or ε2 allele or versus ε3ε3 genotype; ¶genotype with ε4 allele versus ε3ε3 genotype; ‖genotype with ε2 allele versus ε3ε3 genotype; single nucleotide polymorphism.

Abbreviations: AD, Alzheimer’s disease; CDT, Clock Drawing Test; CI, confidence interval; IADL, instrumental activities of daily living; SNP, single nucleotide polymorphism; OR, odds ratio; MMSE, Mini Mental State Examination; APOE, apolipoprotein E.
Discussion

The variable therapeutic response to donepezil observed in patients with Alzheimer’s disease may be caused by differences in the efficacy of cholinesterase inhibitors resulting from individual genetic variation in genes that influence the pharmacokinetic and pharmacodynamic properties of this drug. Identification of specific genetic polymorphisms associated with a good response to treatment with donepezil would allow an individually designed approach to therapy for patients with Alzheimer’s disease.

To date, over 20 studies have investigated the relationship between responsiveness to cholinergic therapy and common APOE polymorphism. In general, their results can be divided into two groups, ie, those showing no effect of APOE status on response to treatment and those showing that patients with the ε4 allele had a better response to treatment than others.13–18 Only two studies showed a better response to treatment in patients with Alzheimer’s disease not carrying the ε4 allele of the APOE gene.19,20 The studies showing response to treatment had a longer observational period (12–36 months) than those not finding such an association (3–12 months).13,17,18

Two studies that analyzed the interaction between APOE status and rs1080985 did not find a direct interaction between APOE and CYP2D6 polymorphisms.7,8 In one of these studies, a marginal significance for frequency of the APOE ε4 and rs1080985 G allele was found, but an independent effect of APOE ε4 on response to donepezil was not found.8 In our study, APOE ε4 was not significantly associated with the therapeutic response to donepezil. None of the three studies supported the hypothesis of a direct interaction between APOE and CYP2D6 polymorphisms on response to treatment with donepezil.

Factors influencing the response to donepezil were evaluated in a few studies and yielded inconsistent results. Darreh-Shori et al showed better therapeutic results after 2 years of treatment, in patients who had an increased donepezil concentration in blood and cerebrospinal fluid.21 However, a study by Yuan-Han et al showed that patients treated with donepezil who did not respond to the drug after 6 months had a higher plasma concentration of donepezil. To explain these inconsistent results, a therapeutic window in the treatment of Alzheimer’s disease by donepezil was postulated; an increased concentration of the drug may not improve cognitive and global function, but may induce clinical tolerance that may lead to a poor therapeutic response.13,22

The plasma concentration of donepezil is dependent on CYP2D6 polymorphism. A number of allelic variants causing either absent, decreased, or increased enzymatic activity have been described,3 allowing for categorization of the populations into three groups, ie, poor metabolizers, extensive metabolizers, and ultrarapid metabolizers. The efficacy of donepezil after 3 months of treatment in 42 Italian patients with Alzheimer’s disease23 and in another Italian population of 57 patients with Alzheimer’s disease after 6 months4 showed that poor metabolizers are better responders. However, a study by Chianella et al24 evaluating 92 patients after one year of treatment with donepezil reported a general tendency towards a higher frequency of faster metabolizers in responders.

The phenotypic outcome of rs1080858 (C–1584G) in the promoter region of the CYP2D6 gene remains controversial.5,5 The influence of rs1080985 on response to treatment with donepezil was assessed in three Italian studies.5–8 The frequency of the G allele was higher in nonresponders than in responders, and the presence of the G allele was associated with a poor response to treatment with donepezil; however, the effect size of the modulation of donepezil response was small to moderate. We were not able to replicate these results in our study. We did not find an association between the rs1080985 single nucleotide polymorphism of the CYP2D6 gene and response to treatment in a Polish population. The G allele was found more often in responders than in nonresponders, but the difference was not significant.

Although data regarding the CYP2D6 polymorphism are inconclusive, they do not rule out the role of rs1080985 as a marker of response to therapy. It was suggested that another genetic variant of CYP2D6 may be in linkage disequilibrium with rs1080985 and influence the phenotypic effect of this polymorphism.23 Further, other as yet unknown factors beyond the concentration of donepezil and CYP2D6 polymorphism influencing poor therapeutic responses need to be clarified.

Genetic replication studies are important, especially in different ethnic groups, to confirm the first observation usually in small populations. So far, the results of published studies encourage continued evaluation of the role of rs1080985 polymorphism in response to treatment with donepezil in different populations.

Disclosure

The authors report no conflicts of interest in this work.
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