American Journal of Alzheimer's Disease & Other Dementias®
2015, Vol. 30(2) 139-144

DOI: 10.1177/1533317514539540
aja.sagepub.com
SAGE

Effect of *APOE* and *CHRNA7* Genotypes on the Cognitive Response to Cholinesterase Inhibitor Treatment at Different Stages of Alzheimer's Disease

Ianna Lacerda Sampaio Braga, MD[1], Patricia Natalia Silva, PhD[1], Tatiane Katsue Furuya, MSc[1], Leonardo Caires Santos, MSc[1], Belisa Caldana Pires, BCh[1], Diego Robles Mazzotti, PhD[1], Paulo Henrique Bertolucci, PhD[2], Maysa Seabra Cendoroglo, PhD[3], and Marília Cardoso Smith, PhD[1]

Abstract

The loss of cholinergic transmission is considered to be an important cause of Alzheimer's disease (AD). Treatment with acetyl cholinesterase inhibitors (ChEIs) shows benefits; however, great heterogeneity has been observed in patient responses. We evaluated apolipoprotein E (*APOE*) and α7 nicotinic receptor (*CHRNA7*) single-nucleotide polymorphisms (SNPs) and associated these SNPs with pharmacological responses to ChEIs in a Brazilian population with AD. We studied 177 outpatients using ChEIs, and they were classified as responders and nonresponders according to variation in Mini-Mental State Examination (MMSE) status. The analysis of *APOE* genotypes showed that patients with the ε4 allele had a worse response than those without the ε4 allele. We observed an association between the *CHRNA7* T allele and a better response to treatment with ChEIs in patients with mild AD (MMSE $\geq$ 20). The SNP rs6494223 of *CHRNA7* as well as *APOE*ε4 could be useful for understanding the response to ChEI treatment in patients with AD.

Keywords

pharmacogenomics, Alzheimer's disease, cholinesterase inhibitor, *CHRNA7*, *APOE*

Introduction

Alzheimer's disease (AD) is the most common cause of dementia affecting older people and is associated with the loss of cholinergic neurons in parts of the brain.[1,2] Acetylcholinesterase inhibitors (ChEIs) such as donepezil, galantamine, and rivastigmine prevent the breakdown of acetylcholine (ACh) released into synaptic clefts and thus enhance cholinergic neurotransmission.[3] There is no disease-modifying therapy for AD yet. The target of drug therapy in AD is based on the improvement, stabilization, and retardation of cognitive decline, enhancement of behavioral symptoms, and reduction in caregiver burden.[4]

Most studies have reported interindividual differences in drug response that may be due to variability in drug metabolism related to clinical, behavioral, and genetic factors and mainly hereditary polymorphisms.[5] Apolipoprotein E (*APOE*) is a primary genetic risk factor for AD.[6] Polymorphic variants of the *APOE* gene (19q13.2) are associated with a risk-conferring allele of the gene (encoding *APOE*ε4) or confer protection from AD (*APOE*ε2).[7] A higher frequency of responders has been observed in non-ε4 bearers and males,[8,9] indicating that gender could be a more powerful determinant of short-term outcome to ChEI treatment than *APOE* status. In contrast, other studies do not support the hypothesis that APOE and gender are predictors for the therapeutic response of patients with AD to tacrine or donepezil.[10,11] The absence of at least 1 ε4 allele and a specific gender are not predictive of the response to donepezil treatment in AD.[11] Petersen et al[12] showed that *APOE*ε4 carriers exhibited a better response to donepezil in mild cognitive

[1] Disciplina de Genética, Departamento de Morfologia e Genética, Universidade Federal de São Paulo (UNIFESP), São Paulo-SP, Brazil
[2] Disciplina de Neurologia Clínica, Departamento de Neurologia e Neurocirurgia (UNIFESP), São Paulo-SP, Brazil
[3] Disciplina de Geriatria e Gerontologia, Departamento de Medicina (UNIFESP), São Paulo-SP, Brazil

Corresponding Author:
Marília Cardoso Smith, PhD, Department of Morphology and Genetics, Universidade Federal de São Paulo, 740 Botucatu St, Ed. Leitão da Cunha, Vila Clementino, São Paulo-SP, Brazil.
Email: macsmith.morf@epm.br

impairment (MCI). Similar results were found by Bizzarro et al.[13] However, Rigaud et al[11] did not find any significant difference between *APOE*ε4/4-related responders and nonresponders to donepezil.

Biochemical analyses of the brains of the patients with AD have revealed deficits in neuronal nicotinic acetylcholine receptors (nAChRs) and reduction in the activities of Ach, acetylcholinesterase (AChE), and choline acetyltransferase (ChAT). More recently, a study demonstrated that the T allele of rs6494223, a single-nucleotide polymorphism of the nicotinic Ach receptor α 7 (*CHRNA7*) gene, showed a reduction of 50% in the probability of progressing to AD within 5 years.[14] To the best of our knowledge, there are no studies concerning this polymorphism and cholinergic inhibitor drug response. The aim of this study was to evaluate the influence of rs6494223 *CHRNA7* on the response to ChEI therapy in patients with AD during 2 years of follow-up.

Materials and Methods

Participants

This investigation was a retrospective cohort study. This study was approved by the local ethics committee on human experimentation from the Universidade Federal de São Paulo (UNIFESP). All participants were informed of the study protocol. Written informed consent for research according to the Helsinki's Declaration was obtained from each patient or from relatives or a legal guardian in the case of seriously disabled patients with dementia.

From July 2009 to March 2010, we recruited outpatients of the Department of Neurology and Neurosurgery (Behavior Neurology Section) from UNIFESP. This is a neurological reference center in Brazil, designed to diagnose patients with dementia and supply appropriate medications according to government guidelines. Approximately 81.8% of patients with AD were of European origin, 5.3% were of African origin, and 12.9% were of Asian origin. All of the patients had treatment duration of 3.71 $\pm$ 1.72 years and presented with 2.77 $\pm$ 1.27 comorbidities.

Patients underwent a standard clinical evaluation at the beginning of outpatient care, including a structured interview, physical examination, routine biochemical screening, and central nervous system imaging by a specialized physician with experience in assessment scales and diagnosing AD. Cognitive status was evaluated by means of the Mini-Mental State Examination (MMSE).[15,16] Differential diagnosis among AD, mixed dementia, and vascular dementia was based on the Hachinski Ischemia Score.[17] Diagnosis of MCI was made according to Petersen's criteria for amnestic MCI.[18] Functional status was assessed using the Activities of Daily Living index[19] and the Instrumental Activities of Daily Living scales.[20] Once the patient was diagnosed with AD, ChEIs were prescribed, and every 6 months a new cognitive assessment with MMSE score was performed.

Inclusion criteria were (1) age $\geq$60 years; (2) diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association Work Group criteria; (3) ChEI drug prescription for at least 6 months; and (4) written informed consent for research. Patients were excluded from the study when they were unwilling or unable to fulfill the requirements of the study or had a suspected history of dementia secondary to abuse of a psychoactive substance, cases of non-AD dementia (normal pressure hydrocephalus, subdural hematoma, Parkinson's disease [PD], frontotemporal dementia, primary progressive aphasia, vascular dementia [Hachinski $\geq$7]), history of psychiatric symptoms of depression, and an established diagnosis of MCI.[18]

Drug dosage administration followed international recommendations as well as the Brazilian Academy of Neurology recommendations. Daily doses from 6 to 12 mg of rivastigmine, from 5 to 10 mg of donepezil, and from 8 to 24 mg of galantamine were used. The patients were naive to ChEI therapy.

All demographic and clinical characteristics were collected by a structured interview, clinical evaluation, and review of records from the neurologic unit. The MMSE analyses were carried out in categories, defined by the first MMSE at the reference center, to determine the relationship between genotype and response to treatment according to disease severity. Mild AD was defined as an MMSE $\geq$20 and moderate to severe AD as an MMSE <20.

According to the National Institute for Health and Clinical Excellence (NICE) requirements,[21] a responder was defined as a patient who showed improvement or no deterioration in cognition comparing MMSE scores at the baseline with MMSE scores after 6, 12, and 24 months of treatment. The data records were collected during 2 years, and every 6 months, the patients were classified as responders or nonresponders.

Genetic Analysis

Whole blood was collected in tubes containing 0.1% EDTA and genomic DNA was obtained from peripheral lymphocytes. DNA extraction was performed according to Lahiri and Numberger.[22]

CHRNA7 *gene—polymorphism rs6494223.* Genotypic analysis of rs6494223 was conducted by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) methods. Reactions were performed in a total volume of 25 μL containing 1× PCR buffer: 75 mmol/L of Tris HCl, pH 8.8, 20 mmol/L $(NH4)_2SO4$, 1 mmol/L $MgCl_2$, 0.4 mmol/L deoxynucleotide triphosphate, 1U *Taq* DNA polymerase (Fermentas, Canada), and 0.4 μmol/L of each primer. A 235-bp fragment was amplified from genomic DNA using the forward 5′-TCCCTGGACAGCATAGGAAC-3′ and reverse 5′-GGGGGAAATCAAGTGGTTCT-3′ oligonucleotides. The PCR conditions involved an initial denaturation step of 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 30 seconds, and a final extension

step of 72°C for 7 minutes. Restriction fragments were analyzed on a 1% agarose gel stained with ethidium bromide. Of the amplification products, 4 μL were digested with 12U *Nla*III (10 U/μL) restriction enzyme (New England Biolabs, Ipswich, Massachusetts) for 16 hours at 37°C, subjected to electrophoresis on a 3% agarose gel stained with ethidium bromide and analyzed in ultraviolet light. After digestion, 3 different fragment patterns were observed: a 235-bp fragment (CC genotype), 143- and 92-bp fragments (TT genotype), and 235-, 143-, and 92-bp fragments (CT genotype).

Apolipoprotein E. The detection of APOEε2, ε3, and ε4 alleles was performed using TaqMan Genotyping Assays: C_3084793_20 (rs429358) and C_904973_10 (rs7412; Applied Biosystems, Foster City, California). For each reaction, 0.31 μL of 40× assay, 6.25 μL of 2× TaqMan genotyping mix (Applied Biosystems, Foster City, California), 5.44 μL of DNAse-free H_2O, and 2 μL of DNA (100 ng) were used. The PCR amplification was cycled at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, primer hybridization, and amplification at 60°C for 1 minute.

Statistical Analyses

Allele and genotype frequencies were calculated for the polymorphism, and the chi-square test was used to investigate deviation from Hardy–Weinberg Equilibrium (HWE). The Mann-Whitney *U* test or Student's *t* test was performed to verify polymorphism association with clinical and demographic variables. Logistic regression analysis was performed to verify polymorphism association with nonresponders using sex, age, *APOE* genotyping, and baseline MMSE as covariates in the model. Odds ratio (OR) and 95% confidence interval (95% CI) were also calculated. Individual polymorphism data analyses were performed using SPSS 18.0. A *P* value smaller than the type 1 error rate of 0.05 was considered significant.

Results

Among the 205 recruited patients with AD, 28 did not have MMSE score data recorded during the course of 6 months. Of the remaining 177 patients with AD enrolled in the study, rivastigmine was used by 75 patients (42.4%), galantamine by 31 (17.5%) patients, and donepezil by 71 (40.1%) patients. Of those patients, 113 (63.8%) were classified as responders and 64 patients (36.20%) were classified as nonresponders during the 6-month ChEI treatment. After 2 years of follow-up, 147 patients remained in the study and 30 patients were missed. The reasons for dropout were not recorded.

Demographic and clinical characteristics of the patients according to MMSE and response to ChEIs over the course of 6 months are summarized in Table 1. The main observed comorbidities were hypertension in 48.3% of patients, diabetes in 25.0%, dyslipidemia in 23.8%, and cardiovascular disease in 16.9%. The genotype characteristics and allele frequencies of patients according to treatment response at 6 months of evaluation are described in Table 2.

Analysis of *CHRNA7* rs6494223 showed that 30.9% of patients (n = 51) were C/C wild type, 52.1% C/T heterozygotes (n = 86), and 17.0% T/T homozygotes (n = 28). No differences were found between the observed frequencies and the expected HWE frequencies ($P = .47$). The analysis of the *APOE* polymorphisms showed that 2.4% (n = 4) of patients had the *APOE* ε2/3 genotype, 1.2% (n = 2) had the ε2/4 genotype, 43.6% (n = 72) had the ε3/3 genotype, 45.5% (n = 75) had the ε3/4 genotype, and 7.3% (n = 12) had the ε4/4 genotypes. No differences were found between the observed frequencies and the expected HWE frequencies for this locus ($P = .70$-$.90$). Logistic regression analysis of *APOE*, adjusted for gender, age, and baseline MMSE, showed that patients with the ε4 allele over 6 months of treatment had the worst response compared to those without the ε4 allele ($P = .023$, OR = 0.454, 95% CI 0.230-0.896). Logistic regression analysis, adjusted for sex, age, presence of the *APOE* ε4 allele, and baseline MMSE, revealed that the T allele of *CHRNA7* SNP rs6494223 ($P = .049$, OR = 1.894, 95% CI 1.003-3.578) was significantly associated with a better response to ChEI treatment in patients with mild AD at the first 6 months. After 2 years of follow-up treatment, the *CHRNA7* T allele and the presence of *APOE*ε4 did not show differences between responders and nonresponders regardless of the severity of the disease.

Discussion

In this study, we observed that allele T of the polymorphism *CHRNA7* is significantly related to a better response to treatment with ChEIs in patients with MMSE ≥20 after 6 months of follow-up. After 24 months of treatment, we did not observe a significant association between the *CHRNA7* T allele and response to ChEIs.

The ChEIs are medications designed to improve cognition and behavior. Previous studies have shown a mild improvement in cognition with an average increase in MMSE of 1.5 to 2 scores at 6 to 12 months.[23] Moreover, its effects on behavior may be related to better cognitive processing and also to reduced apathy and improvement in social interaction.[4]

The response of patients to ChEI treatment is not homogeneous. Clinical studies have suggested that the higher the cholinergic deficits, the better the expected response to treatment with ChEIs.[24] Furthermore, neuropsychiatric features may influence the response to treatment. For example, patients with hallucinations and attention deficits have higher cholinergic deficits and also better responses to ChEIs.[25]

The clinical criteria that distinguish responders from nonresponders are controversial in the literature. The adoption of the NICE criteria has allowed for a more powerful comparison between responders and nonresponders. Our study showed a response rate of 45%. A similar study of 976 older Italians with AD showed a response rate of 43%,[3] while in a study of the American population, the response rate was 34% in patients using donepezil.[26]

Table 1. Selected Demographic and Clinical Characteristics of Patients According to MMSE Score at 6 Months of Evaluation.

	MMSE < 20							MMSE > 20						
	Nonresponders			Responders				Nonresponders			Responders			
Quantitative variables	N	Mean	SD	N	Mean	SD	*P*	N	Mean	SD	N	Mean	SD	*P*
Age, years	25	82.960	7.191	60	80.267	6.994	.112	39	79.795	6.856	53	79.887	5.999	.946
Education, years	24	3.292	2.053	60	3.600	2.763	.623	39	4.205	2.597	53	5.623	4.161	.064
Concomitant diseases	24	2.375	1.439	58	2.707	1.214	.290	38	2.789	1.189	52	2.692	1.292	.716
Concomitant therapies, number of drugs	23	3.913	3.014	57	4.614	2.343	.269	37	4.324	2.286	51	4.353	2.726	.959
Years of AD	25	6.240	2.818	59	5.593	2.614	.314	39	6.026	2.323	53	5.226	2.407	.114
MMSE score at baseline	25	15.400	2.828	60	15.167	3.179	.751	39	23.051	2.695	53	23.000	2.139	.919
MMSE scores after 6 months of treatment	25	12.880	2.774	60	17.950	4.127	<.001	39	20.051	2.883	53	24.226	2.318	<.001
MMSE scores after 12 months of treatment	20	13.250	4.315	56	17.036	4.584	.002	38	20.763	3.132	50	23.860	3.169	<.001
IADL	21	14.095	3.897	55	15.309	5.480	.357	34	17.912	5.276	48	18.896	5.058	.396
ADL	22	4.636	1.364	57	4.491	1.754	.698	32	5.094	1.445	49	5.347	1.071	.368

Qualitative variables	Nonresponders	Responders	*P**	Nonresponders	Responders	*P**
Sex, n (%)	F—21 (84.0%) M—4 (16.0%)	F—50 (83.3%) M—10 (16.7%)	.940	F—28 (71.8%) M—11 (28.3%)	F—31 (58.5%) M—22 (41.5%)	.189
BPSD presence, n (%)	No 2 (9.1%) Yes 20 (90.9%)	No 7 (13.7%) Yes 44 (86.3%)	.580	No 13 (36.1%) Yes 23 (63.9%)	No 11 (21.2%) Yes 41 (78.8%)	.121

Abbreviations: n, number of individuals; BPSD, behavioral and psychological symptoms of dementia; MMSE, Mini-Mental State Examination; IADL, Instrumental Activities of Daily Living; ADL, Activities of Daily Living; SD, standard deviation; AD, Alzheimer's disease; *P*, *P* value obtained by Student's *t* test; *P**, *P* value obtained by chi-square test; F, female; M, male.

Table 2. Genotype and Allele Frequencies of *CHRNA7* (rs6494223) and Presence of the *APOE*ε4 allele According to Treatment Response at 6 Months of Evaluation.

		MMSE < 20				MMSE ≥ 20			
		NR	*R*	Total		NR	R	Total	
CHRNA7		n (%)	n (%)	n (%)	*P*	n (%)	n (%)	n (%)	*P*
Alleles	C	26 (56.5)	69 (59.5)	95 (58.6)	.73	48 (28)	48 (28)	96 (55)	.03
	T	20 (43.5)	47 (40.5)	67 (41.4)		26 (15)	52 (30)	78 (45)	
	Total, n (%)	46 (100)	116 (100)	162 (100)		74 (42)	100 (58)	174 (100)	

		MMSE < 20				MMSE ≥ 20			
		NR	R	Total		NR	R	Total	
APOE		n (%)	n (%)	n (%)	*P*	n (%)	n (%)	n (%)	*P*
Presence of ε4 allele	ε4 noncarriers	14 (29.2)	54 (47.4)	68 (42.0)	.03	34 (43.6)	56 (58.3)	90 (51.7)	.05
	ε4 carriers	34 (70.8)	60 (52.6)	94 (58.0)		44 (56.4)	40 (41.7)	84 (48.3)	
	Total, n (%)	48 (100)	114 (100)	162 (100)		78 (100)	96 (100)	174 (100)	

Abbreviations: *CHRNA7*, α7 nicotinic receptor; MMSE, Mini-Mental State Examination; NR, nonresponders; R, responders; n, number of individuals, *P*, significance value obtained by chi-square test.

A few studies have shown that clinical and genetic variables might influence the response to treatment with ChEIs. Gender has been identified as an important modulator of response to ChEI treatment, with men presenting with a better response than that of women in long-term evaluation when considering cognitive outcome.[27] Moreover, the male gender has been previously described as a predictor of better response during short-term evaluation.[8] Our study showed no significant difference in treatment response when comparing gender.

Apolipoprotein E ε4 is the most studied genetic variable in regard to its effect on clinical outcome and response to treatment with ChEIs. It is well known that patients with genotype ε4/4 have a greater degree of brain atrophy, atherosclerosis, cognitive impairment, and psychotic symptoms.[28] Additionally, this group of patients has a higher degree of cholinergic deficits.[25] The presence of *APOE ε4* was correlated with the worst response to treatment, compared with non-ε4 alleles, in our sample, in agreement with other studies in the literature.[27,29]

The nAChRs are ionic channels that allow the neurotransmission of ACh. Biochemical analysis of brain tissues of patients with AD revealed a reduction in nicotinic receptors, ACh, lower cholinergic synthesis, and also inactivation of the enzymes ChAT and ACh.[30-32] In addition, the clearance of amyloid β and neuroprotection are also related to these receptors. Previous studies have shown that nAChR agonists improve cognition in rats and in patients with schizophrenia. Moreover, it seems that nAChR agonists could function similar to antipsychotic agents, improving memory in rats and monkeys and also acting as neuroprotective factors.[33] The *CHRNA7* polymorphisms are known to be related to schizophrenia and AD.[34,35] In particular, the T allele of the polymorphism rs6494223 is related to a reduced progression of mild cognitive deficits to AD and reduced symptoms of delirium.[14,36-38] The presence of the T allele may be a sign of higher cholinergic deficits, delirium symptoms, and better response to ChEIs. This hypothesis has already been observed in patients with Lewy body dementia and dementia related to PD.[39,40] To our knowledge, this is the first study that detected a better response to ChEIs in patients with AD bearing the polymorphism rs6494223.

Our sample was well distributed and the groups were well matched. We studied patients from an patient with AD reference center, which is one feature of our population. The results of this study should be analyzed in the context of some limitations. Further studies in a larger sample of patients might hypothetically increase the detection of effects of polymorphisms and gender on psychotic and delirium symptoms. In addition, a detailed evaluation of ethnic influences with specific biomarkers, follow-up on the behavioral and psychological symptoms of dementia, and applications of scale to measure caregiver stress should be considered.

In conclusion, this study showed that the presence of the T allele polymorphism of *CHRNA7* was a predictor of better response in the first 6 months of ChEI treatment in patients with mild AD. The presence of the *APOE*ε4 allele was a predictor of the worst response to ChEI therapy in the patient population studied here. The genetic heterogeneity of the population with AD could be one of the causes of different responses to current disease treatments.

Acknowledgments

This research was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq).

Authors' Note

Ianna Lacerda Sampaio Braga and Patricia Natalia Silva contributed equally to the article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet*. 2011;377(9770):1019-1031.
2. Cummings JL. Biomarkers in Alzheimer's disease drug development. *Alzheimers Dement*. 2011;7(3):e13-e44.
3. Santoro A, Siviero P, Minicuci N, et al. Effects of donepezil, galantamine and rivastigmine in 938 Italian patients with Alzheimer's disease: a prospective, observational study. *CNS Drugs*. 2010;24(2):163-176.
4. Birks J. Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev*. 2006;(1):CD005593.
5. Pilotto A, Franceschi M, D'Onofrio G, et al. Effect of a CYP2D6 polymorphism on the efficacy of donepezil in patients with Alzheimer disease. *Neurology*. 2009;73(10):761-767.
6. van Es MA, van den Berg LH. Alzheimer's disease beyond APOE. *Nat Genet*. 2009;41(10):1047-1048.
7. Cacabelos R. Pharmacogenomics and therapeutic prospects in dementia. *Eur Arch Psychiatry Clin Neurosci*. 2008;258(suppl 1):28-47.
8. MacGowan SH, Wilcock GK, Scott M. Effect of gender and apolipoprotein E genotype on response to anticholinesterase therapy in Alzheimer's disease. *Int J Geriatr Psychiatry*. 1998;13(9):625-630.
9. Richard F, Helbecque N, Neuman E, Guez D, Levy R, Amouyel P. APOE genotyping and response to drug treatment in Alzheimer's disease. *Lancet*. 1997;349(9051):539.
10. Rigaud AS, Traykov L, Caputo L, et al. The apolipoprotein E epsilon4 allele and the response to tacrine therapy in Alzheimer's disease. *Eur J Neurol*. 2000;7(3):255-258.
11. Rigaud AS, Traykov L, Latour F, Couderc R, Moulin F, Forette F. Presence or absence of at least one epsilon 4 allele and gender are not predictive for the response to donepezil treatment in Alzheimer's disease. *Pharmacogenetics*. 2002;12(5):415-420.
12. Petersen RC, Thomas RG, Grundman M, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med*. 2005;352(23):2379-2388.
13. Bizzarro A, Marra C, Acciarri A, et al. Apolipoprotein E epsilon4 allele differentiates the clinical response to donepezil in Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2005;20(4):254-261.
14. Barabash A, Marcos A, Ancin I, et al. APOE, ACT and CHRNA7 genes in the conversion from amnestic mild cognitive impairment to Alzheimer's disease. *Neurobiol Aging*. 2009;30(8):1254-1264.
15. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12(3):189-198.
16. Bertolucci PH, Brucki SM, Campacci SR, Juliano Y. [The Mini-Mental State Examination in a general population: impact of educational status]. *Arq Neuropsiquiatr*. 1994;52(1):1-7.
17. Hachinski VC, Iliff LD, Zilhka E, et al. Cerebral blood flow in dementia. *Arch Neurol*. 1975;32(9):632-637.

18. Petersen RC, Doody R, Kurz A, et al. Current concepts in mild cognitive impairment. *Arch Neurol*. 2001;58(12):1985-1992.
19. Katz S, Akpom CA. A measure of primary sociobiological functions. *Int J Health Serv*. 1976;6(3):493-508.
20. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist*. 1969;9(3):179-186.
21. NHS - National Institute for Health and Clinical Excellence. *Alzheimer's Disease - Donepezil, Galantamine, Rivastigmine and Memantine* 2011; www.nice.org.uk/guidance/TA217. Accessed June 23, 2011.
22. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res*. 1991;19(19):5444.
23. Martinelli-Boneschi F, Giacalone G, Magnani G, et al. Pharmacogenomics in Alzheimer's disease: a genome-wide association study of response to cholinesterase inhibitors. *Neurobiol Aging*. *2013*;34(6):1711. e1717-e1713.
24. Frankfort SV, Appels BA, de Boer A, et al. Identification of responders and reactive domains to rivastigmine in Alzheimer's disease. *Pharmacoepidemiol Drug Saf*. 2007;16(5): 545-551.
25. Lemstra AW, Kuiper RB, Schmand B, van Gool WA. Identification of responders to rivastigmine: a prospective cohort study. *Dement Geriatr Cogn Disord*. 2008;25(1):60-66.
26. Burns A, Yeates A, Akintade L, et al. Defining treatment response to donepezil in Alzheimer's disease: responder analysis of patient-level data from randomized, placebo-controlled studies. *Drugs Aging*. 2008;25(8):707-714.
27. Wattmo C, Wallin AK, Londos E, Minthon L. Predictors of long-term cognitive outcome in Alzheimer's disease. *Alzheimers Res Ther*. 2011;3(4):23.
28. Cacabelos R, Martinez-Bouza R. Genomics and pharmacogenomics of dementia. *CNS Neurosci Ther*. 2011;17(5): 566-576.
29. Cacabelos R, Martinez R, Fernandez-Novoa L, et al. Genomics of Dementia: APOE- and CYP2D6-related pharmacogenetics. *Int J Alzheimers Dis*. 2012;2012:518901.
30. Benzi G, Moretti A. Is there a rationale for the use of acetylcholinesterase inhibitors in the therapy of Alzheimer's disease? *Eur J Pharmacol*. 1998;346(1):1-13.
31. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J Neurol Neurosurg Psychiatry*. 1999;66(2):137-147.
32. Takada-Takatori Y, Kume T, Izumi Y, et al. Roles of nicotinic receptors in acetylcholinesterase inhibitor-induced neuroprotection and nicotinic receptor up-regulation. *Biol Pharm Bull*. 2009;32(3): 318-324.
33. Dziewczapolski G, Glogowski CM, Masliah E, Heinemann SF. Deletion of the alpha 7 nicotinic acetylcholine receptor gene improves cognitive deficits and synaptic pathology in a mouse model of Alzheimer's disease. *J Neurosci*. 2009;29(27):8805-8815.
34. Ancin I, Barabash A, Vazquez-Alvarez B, et al. Evidence for association of the non-duplicated region of CHRNA7 gene with bipolar disorder but not with Schizophrenia. *Psychiatr Genet*. 2010;20(6):289-297.
35. Joo EJ, Lee KY, Kim HS, Kim SH, Ahn YM, Kim YS. Genetic Association Study of the Alpha 7 Nicotinic Receptor (CHRNA7) with the development of Schizophrenia and Bipolar disorder in Korean Population. *Psychiatry Investig*. 2010;7(3):196-201.
36. Carson R, Craig D, Hart D, et al. Genetic variation in the alpha 7 nicotinic acetylcholine receptor is associated with delusional symptoms in Alzheimer's disease. *Neuromolecular Med*. 2008; 10(4):377-384.
37. Carson R, Craig D, McGuinness B, et al. Alpha7 nicotinic acetylcholine receptor gene and reduced risk of Alzheimer's disease. *J Med Genet*. 2008;45(4):244-248.
38. Sabbagh MN, Shah F, Reid RT, et al. Pathologic and nicotinic receptor binding differences between mild cognitive impairment, Alzheimer disease, and normal aging. *Arch Neurol*. 2006;63(12): 1771-1776.
39. Kurtz AL, Kaufer DI. Dementia in Parkinson's disease. *Curr Treat Options Neurol*. 2011;13(3):242-254.
40. Court JA, Ballard CG, Piggott MA, et al. Visual hallucinations are associated with lower alpha bungarotoxin binding in dementia with Lewy bodies. *Pharmacol Biochem Behav*. 2001;70(4):571-579.