Clinical Effectiveness of Rivastigmine Monotherapy and Combination Therapy in Alzheimer's Patients

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Keywords

Alzheimer's disease; CYP2D6; CYP3A4; Memantine; Pharmacogenetics; Rivastigmine; UGT.

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Received 25 July 2012; revision 31 October 2012; accepted 1 November 2012.

SUMMARY

Background: Rivastigmine is an acetylcholinesterase inhibitor; the genotype data seen alongside the phenotype data explain the mutation or the molecular genetics involved and also help to relate the phenotype of an individual with their genotype. **Aim:** To determine the clinical effectiveness of CYP2D6, CYP3A4, CYP2C9/19, and UGT polymorphism on the steady-state plasma concentrations and therapeutic outcome of rivastigmine monotherapy and combination therapy in patients with Alzheimer's disease. **Result:** In this study, a significant allele frequency was observed for CYP2D6*3 polymorphism in patients under rivastigmine combination therapy ($A>del = 0.50$ [patients] and $A>del = 0.20$ [controls]), UGT2B7 ($T = 0.17$ [patients] and 0.33 [Controls], and UGT1A9*5 $A = 0.58$ [patients] and 0.26 [Controls]). The drug levels and P value of responders/nonresponders were found to be $0.17 \pm 0.08/0.22 \pm 0.16$ and 0.574 for rivastigmine and $0.18 \pm 0.11/0.66 \pm 0.63$ and 0.009 for rivastigmine in combination therapy and $1.40 \pm 0.65/0.59 \pm 0.84$ and 0.05 for memantine in combination therapy. **Conclusion:** Poor metabolizer subjects of UGT2B7 polymorphism in patients under rivastigmine combination therapy have higher drug levels with a poor response to the drug treatments.

doi: 10.1111/cns.12036

Introduction

Alzheimer's disease (AD) is a progressive, degenerative brain disease. The mainstay of current management of patients with AD involves drugs that provide symptomatic therapy. Rivastigmine is an acetylcholinesterase inhibitor (ACHEI); It is the only cholinesterase inhibitor (ChEI) with significant inhibition of butyrylcholinesterase inhibitors (BuChEs) [1]. BuChE is widely distributed in the central nervous system and may play a role in cholinergic function and neurodegeneration [2]. A different treatment for AD that has recently been introduced is Namenda® (memantine, Hcl). This drug is an n-methyl-D-aspartate (NMDA) receptor antagonist that exhibits noncompetitive inhibition of glutamate binding at the receptor site. The development of this drug was based on the observation that excess glutamatergic neurotransmission followed by increased intraneuronal calcium contributes to AD progression [3]. There have been clinical trials that have shown that memantine slows the cognitive, functional, and behavioral decline in patients with moderate to severe AD. Clinicians are well aware that individual dose requirements of drugs for patients can vary widely, even in the absence of obvious confounding factors

such as drug–drug interactions. For many drugs, a significant component of this interindividual difference can be attributed to variability in drug metabolism [4]. Importantly, metabolism cannot be reliably predicted by simple anthropomorphic characteristics such as body mass, body surface area, gender, age, or race.

Hepatic cytochrome P450 isoenzymes (CYP450) are minimally involved in rivastigmine and memantine metabolism. The genotype data seen alongside the phenotype data explain the mutation or the molecular genetics involved and also help to relate the phenotype of an individual with their genotype. This may help in predicting the phenotype from genotype within a population. However, the activity of central nervous system (CNS) drugs can be influenced by genetic alteration that can affect the drug target molecule.

The allele and genotypic frequency of phase I and phase II drug-metabolizing enzymes has been reported in different populations and revealed wide ethnic variation. This is the first study to the best of our knowledge to be performed in India on patients with Alzheimer's disease (AD) investigating the genotypes encoding the drug-metabolizing enzymes of phase I and phase II and the drug levels. Although some studies have seen the impact of CYP

polymorphisms on the drug levels for the patients on donepezil monotherapy [5–7], so far no studies have correlated with relevant CYP and UGT genotypes in patients with rivastigmine monotherapy and combination therapy with drug levels and clinical response.

Patients and Methods

Patient Recruitment

A total of 18 patients (eight men and 10 women) for rivastigmine monotherapy and 18 patients (13 men and five women) for combination therapy with memantine aged ≥ 55 years with mild to moderate AD were recruited from the outpatient department of Neurology, Neuroscience Centre, All India Institute of Medical sciences, New Delhi. The controls ($n = 40$) were collected from the hospital staff and their relatives or from the unrelated attendants of patients for allele and genotypic frequency comparisons.

Standard Protocol Approvals, Registrations, and Patient Consents

The approval of the study for the experiments on human subjects was obtained from our institute ethical committee on human experimentation. Written informed consent was obtained from each patient and controls or from the relatives in case of critically disabled persons with dementia.

Inclusion Criteria for Patients

(1) North Indian subjects; (2) diagnosis of probable AD according to National Institute of Neurological and Communicative Disorders–Stroke and Alzheimer's Disease and Related Disorders Association work group (NINCDS-ADRDA) [8], diagnostic manual and statistics for mental disorders of the American Psychiatric Association (DSM IV), and responder or nonresponder status defined by Mini-Mental State Examination (MMSE) [9] with mild–moderate disease severity; and (3) taking drugs namely rivastigmine (monotherapy and combination therapy with NMDA antagonist, memantine) for at least 4 weeks.

Exclusion Criteria for Patients

(1) Unwilling or unable to fulfill the requirements of the study; (2) had clinically significant and unstable medical illness and had undergone medical/surgical hospitalization within 1 month before the study; (3) known or suspected history of dementia secondary to abuse of psychoactive substances, vision and hearing sufficient for compliance with testing procedures; (4) laboratory values within normal limits or considered to be not clinically relevant by the investigator; (5) comorbidity of primary psychiatric (i.e., schizophrenia, major depression onset before the AD onset) or neurological disorders (i.e., stroke, Parkinson's disease, seizure disorder, or head injury with loss of consciousness within the past year); and (6) known suspected history of alcoholism or drug abuse. Patients suffering from diabetes mellitus, hyperlipoproteinemia, cancer, sickle cell anemia, and liver disease were excluded from the study.

Inclusion Criteria for Controls

(1) The healthy subjects were neither related to one another nor related to patients with AD; (2) We selected all age-, ethnicity-, and sex-matched controls in the range of 55–90 years; and (3) MMSE score was well above the limit for the normal cognitive level and not satisfying DSM IV and NINCDS-ADRDA criteria for diagnosis of AD.

Exclusion Criteria for Controls

(1) Controls taking any form of medication or having had surgery or suffered trauma in the past 30 days were excluded from the study; (2) controls unwilling or unable to fulfill the requirements of the study; and (3) controls with history of bleeding and thrombotic or cardiac disorders were also excluded from the study.

Responder/Nonresponder Assessment Criteria

According to the National Institute for Health and Clinical Excellence (NICE) requirements, a responder was defined as a patient who showed improvement or no deterioration in cognition as evaluated by means of MMSE (NHS, 2009, <http://www.nice.org.uk>).

Blood Collection

In the case of both patients as well as controls, 6 mL of peripheral blood samples was collected in 2% EDTA as well as plain vials. Plasma was isolated within 2 h by centrifugation at 4°C and 7840 *g* for 20 min and stored at -20°C for drug analysis and other tests. Peripheral blood leukocytes were also stored at -20°C for extracting DNA and performing the molecular tests.

Genetic Analysis

Genomic DNA was extracted from 2 mL of blood through phenol–chloroform method. Polymerase chain reaction (PCR)-based restriction fragment length polymorphism assay was performed for phase I drug-metabolizing enzymes with the primers and restriction enzymes as already specified [10,11]. Sequencing was carried out commercially through Xcelaris, Ahmadabad, to confirm the RFLP results.

Determination of Rivastigmine and Memantine

The LC-MS/MS method was used for the analysis of rivastigmine and memantine with minor modifications [12,13]. For the quantification of rivastigmine and memantine, an isocratic mobile phase containing 70% acetonitrile with 0.1% formic acid and 30% aqueous 10 mM ammonium acetate containing 0.1% formic acid was used. The mobile phase was filtered with 0.22- μL filter, online degassed, and pumped at the rate of 0.5 mL/min. The samples were loaded into 96-well tray and injected using autosampler kept at 20°C. The total LC run time was 5 min, and 20 μL of sample was subjected for quantification. Using TurboIonSpray (ESI) source, the chromatographic eluents were subjected to ionization

in the positive ion mode at 5500 V. Quantification was performed using multiple reaction monitoring (MRM) mode, based on parent/product ion transition 1 (251.2/206.2) and transition 2 (251.2/86.1). The transitions for homatropine were 276.1/142. The main instrument parameters of the mass spectrometer are as follows: source-dependent parameters were gas 1 (60 psi), gas 2 (40 psi), curtain gas (20 psi), ion spray voltage (5500 V), and temperature (400°C). Compound-dependent parameters for analyte such as declustering potential, entrance potential, collision energy, and cell exit potential, were set at 50, 10, 20, and 4 V for transition 1 and 93, 10, 34, and 15 for transition 2, respectively. Collision-activated dissociation and dwell time were kept at 8 (arbitrary value) and 100 ms. Homatropine was used as an internal standard (IS), and the conditions were followed as mentioned in Nirmal et al. [14].

Standard Preparation

Stock solution of rivastigmine and memantine was prepared in blank plasma. The stock concentration of 1 mg/mL was used for all three drugs. This stock solution was appropriately diluted with blank plasma to reach the required concentrations ranging from low to high. Standard calibration curve was plotted by taking 20 µL of standard from serial dilutions and plasma was extracted using 200 µL of extraction solvent. The above mixture was vortexed for 1 min using a cyclomixer and subjected to centrifugation at 7840 *g* for 10 min. The clear supernatant of 100 µL was transferred to 96-well plates and subjected to quantification using the LC-MS/MS method. Stock solution (1 mg/mL) of internal standard (IS) was prepared by dissolving homatropine in 50% methanol. It was further diluted to reach 500 ng/mL in the extraction solvent containing 70% acetonitrile with 0.1% formic acid.

Sample Preparation

Twenty microliters of plasma samples was extracted using 200 µL of extraction solvent, vortexed for 1 min using a cyclomixer, and subjected to centrifugation at 7840 *g* for 10 min. Extraction solvent consisted of 70% acetonitrile containing 0.1% formic acid and 500 ng/mL homatropine (internal standard). The clear supernatant of 100 µL was transferred to 96-well plates and subjected to quantification using the LC-MS/MS method.

Statistical Analysis

Statistical analysis was carried out using Stata 9.0 (Statacorp, college station, TX, USA). Data were presented as *n* (%) or mean ± SD/median (range) as appropriate. Demographic and clinical characteristics of patients between the drug groups were compared using Fisher's exact test (categorical variable) and one-way ANOVA (continuous variable). The difference in allele and genotypic frequency was compared between patients with AD and controls using chi-square test. The effect of each of the gene polymorphism on patients with AD compared with control was found using logistic regression analysis. The results were reported as OR (95% CI). The difference in median drug levels and MMSE among the 0, 1, and 2 mutant alleles in each of the genotypes was

tested using Kruskal–Wallis test. The *P* < 0.05 was considered significant.

Results

Demographic and clinical characteristics of patients with AD enrolled in our study are shown in Table 1. A Total of 18 rivastigmine and 18 rivastigmine and memantine cases were recruited. Their mean age and standard deviation of each group were 68.4 ± 6.10 and 65.7 ± 7.79, respectively. The change in MMSE score was not found to be significant.

Genotypes

The PCR-RFLP was performed for CYP2D6*2 (2850C>T), CYP2D6*3 (2549A>del), CYP2D6*4 (1846G>A), CYP2D6*10 (100C>T), CYP2D6*17 (1023C>T), CYP3A4*4 (1389A>G), CYP3A4*5 (15820C>G), CYP3A4*18 (293T>C), CYP2C9*2 (430C>T), CYP2C9*3 (1075A>C), CYP2C19*2 (681G>A), CYP2C19*3 (636G>A), UGT2B7(802C>T), UGT1A6 (T181A), UGT1A6 (R184S), and UGT1A9*5 (766G>A) drug-metabolizing enzymes. The allele distribution was found between patients with AD and controls. All these polymorphisms were polymorphic in our study and were seen to be under Hardy–Weinberg equilibrium (cutoff *P* < 0.01). Sequencing was performed to authenticate the RFLP results. Allelic frequency for rivastigmine monotherapy and combination therapy is given in Tables 2 and 3.

Correlation of Drug Levels and Genotypes

The patients were classified as responders and nonresponders according to the MMSE scores. Nonresponders were having

Table 1 Demographic and clinical characteristics

Demographic characteristics	Rivastigmine	Rivastigmine and memantine
Age (years)		
<i>n</i>	18	18
Mean ± SD	68.4 ± 6.10	65.7 ± 7.79
Male/female	8/10	13/5
MMSE baseline (4 weeks)		
<i>n</i>	18	18
Mean ± SD	18.3 ± 6.5	18.8 ± 5.02
MMSE (16 weeks)		
<i>n</i>	17	16
Mean ± SD	17.8 ± 6.64	18.2 ± 6.34
Weight (Kg)		
<i>n</i>	18	18
Mean ± SD	78.8 ± 6.40	72.4 ± 5.79
Systolic pressure		
<i>n</i>	18	18
Mean ± SD	128 ± 13.2	126 ± 10.5
Diastolic pressure		
<i>n</i>	18	18
Mean ± SD	83.6 ± 9.24	80.3 ± 2.58

MMSE, Mini-Mental State Examination.

Table 2 Allele frequency of phase I drug-metabolizing enzyme on rivastigmine monotherapy

Gene	Allele frequency		<i>P</i> value	OR (95%CI)
	Patients (n = 18)	Controls (n = 40)		
CYP2D6*2	C = 0.56	C = 0.65	0.53	0.76 (0.31, 1.92)
2850C>T	T = 0.39	T = 0.35		
CYP2D6*3	A = 0.75	A = 0.80	0.54	0.75 (0.27, 2.18)
2549A>del	Adel = 0.25	Adel = 0.20		
CYP2D6*4	G = 0.61	G = 0.58	0.71	1.16 (0.48, 2.83)
1846G>A	A = 0.39	A = 0.43		
CYP2D6*10	C = 0.72	C = 0.63	0.30	1.56 (0.61, 4.13)
100C>T	T = 0.28	T = 0.38		
CYP2D6*17	C = 0.47	C = 0.61	0.15	0.56 (0.23, 1.35)
1023C>T	T = 0.53	T = 0.39		
CYP3A4*4	A = 0.56	A = 0.59	0.74	0.87 (0.36, 2.10)
1389A>G	G = 0.44	G = 0.41		
CYP3A4*5	C = 0.61	C = 0.66	0.59	0.80 (0.33, 1.98)
15820C>G	G = 0.39	G = 0.34		
CYP3A4*18	T = 0.69	T = 0.74	0.63	0.80 (0.31, 2.15)
293T>C	C = 0.31	C = 0.26		
CYP2C9*2	C = 0.58	C = 0.64	0.57	0.79 (0.33, 1.93)
430C>T	T = 0.42	T = 0.36		
CYP2C9*3	A = 0.72	A = 0.66	0.27	1.65 (0.61, 4.79)
1075A>C	C = 0.22	C = 0.34		
CYP2C19*2	G = 0.67	G = 0.71	0.61	0.80 (0.32, 2.08)
681G>A	A = 0.33	A = 0.29		
CYP2C19*3	G = 0.61	G = 0.60	0.99	1.04 (0.43, 2.56)
636G>A	A = 0.39	A = 0.40		

higher C/D median plasma drug levels than responders, and hence, we wanted to confirm that whether it was due to the combined effect of the gene or due to a single gene variation. Thus, the patients were classified according to the genotypes based on monotherapy and combination therapy of rivastigmine and correlated with the drug levels. Clinical outcome of the drugs was demonstrated by means of MMSE score (MMSE [4 weeks] – MMSE [16 weeks]). A higher negative score indicates better clinical response to the drug. Patients were divided into three groups by the number of mutant alleles. Each polymorphism has three groups wherein subjects having 0, 1, and 2 mutant alleles are homozygous extensive metabolizer (EM), heterozygous EM, and poor metabolizer (PM), respectively (Tables 4 and 5). These classifications were made according to the Varsaldi et al. [5].

Rivastigmine Concentrations in Monotherapy and Combination Therapy

The concentration of the plasma ranged from 0.06 to 0.66 ng/mL for rivastigmine (12 mg/day), 0.05 to 2.60 ng/mL for rivastigmine in combination therapy, and 0.05–1.44 ng/mL for memantine in rivastigmine and memantine combination therapy among patients with Alzheimer's.

The drug levels and *P* value of responders/nonresponders were found to be $0.17 \pm 0.08/0.22 \pm 0.16$ and 0.574 for rivastigmine, $0.18 \pm 0.11/0.66 \pm 0.63$ and 0.009 for rivastigmine in combina-

Table 3 Allele frequency of phase I drug-metabolizing enzyme on rivastigmine and memantine combination therapy

Gene	Allele frequency		<i>P</i> value	OR (95%CI)
	Patients (n = 18)	Controls (n = 40)		
CYP2D6*2	C = 0.75	C = 0.65	0.28	1.61 (0.62, 4.44)
2850C>T	T = 0.25	T = 0.35		
CYP2D6*3	A = 0.50	A = 0.80	0.001*	0.25 (0.09, 0.63)
2549A>del	Adel = 0.50	Adel = 0.20		
CYP2D6*4	G = 0.69	G = 0.58	0.22	1.67 (0.68, 4.31)
1846G>A	A = 0.31	A = 0.43		
CYP2D6*10	C = 0.61	C = 0.63	0.88	0.94 (0.39, 2.31)
100C>T	T = 0.39	T = 0.38		
CYP2D6*17	C = 0.56	C = 0.61	0.56	0.79 (0.33, 1.90)
1023C>T	T = 0.44	T = 0.39		
CYP3A4*4	A = 0.47	A = 0.59	0.24	0.62 (0.26, 1.49)
1389A>G	G = 0.53	G = 0.41		
CYP3A4*5	C = 0.67	C = 0.66	0.96	1.01 (0.41, 2.59)
15820C>G	G = 0.33	G = 0.34		
CYP3A4*18	T = 0.78	T = 0.74	0.64	1.24 (0.45, 3.66)
293T>C	C = 0.22	C = 0.26		
UGT2B7	C = 0.83	C = 0.68	0.05*	2.9 (0.41, 2.59)
802C>T	T = 0.17	T = 0.33		
UGT1A6	A = 0.67	A = 0.68	0.78	0.88 (0.35, 2.28)
(T181A)	G = 0.33	G = 0.30		
541A>G				
UGT1A6	A = 0.56	A = 0.60	0.65	0.83 (0.35, 2.00)
(R184S)	C = 0.44	C = 0.40		
552A>C				
UGT1A9*5	G = 0.42	G = 0.74	0.0009*	0.25 (0.10, 0.63)
766G >A	A = 0.58	A = 0.26		
CYP2C9*2	C = 0.72	C = 0.64	0.37	1.47 (0.58, 3.92)
430C>T	T = 0.28	T = 0.36		
CYP2C9*3	A = 0.67	A = 0.66	0.96	1.01 (0.41, 2.59)
1075A>C	C = 0.33	C = 0.34		
CYP2C19*2	G = 0.69	G = 0.71	0.84	0.91 (0.36, 2.41)
681G>A	A = 0.31	A = 0.29		
CYP2C19*3	G = 0.56	G = 0.60	0.65	0.83 (0.35, 2.00)
636G>A	A = 0.44	A = 0.40		

**P* < 0.05 was considered significant.

tion therapy, and $1.40 \pm 0.65/0.59 \pm 0.84$ and 0.05 for memantine in combination therapy. Overall, the drug levels were found to be higher for nonresponders on rivastigmine monotherapy and combination therapy (Figures 1–3). There was no difference in clinical response between subjects on monotherapy and combination therapy.

Discussion

Metabolism of most drugs influences their pharmacological and toxicological effects. Many of the interindividual and interethnic differences in effects of drugs are now attributed to the genetic differences in their metabolism. In this study, we have found CYP2D6*3, UGT2B7, and UGT1A9*5 to be allelic significant among patients with AD. However, this data is distinct from south

Table 4 CYP2D6, CYP3A4, CYP2C9 and 19 frequency, C/D rivastigmine and MMSE change for monotherapy

Mutant allele	n = 18	Frequency (%)	C/D donepezil Median (range)	<i>P</i> value	MMSE change Median (range)	<i>P</i> value
CYP2D6 (*2, *3, *4, *10, *17)						
Homozygous EM	1	5.6	0.05	0.14	2	0.73
Heterozygous EM	4	22.2	0.23 (0.19–0.27)		1 (–5 to 5)	
PM	13	72.2	0.16 (0.05–0.66)		–1 (–4 to 5)	
CYP3A4 (*4, *5,*18)						
Homozygous EM	3	16.7	0.19 (0.15–0.31)	0.90	4 (0–6)	0.15
Heterozygous EM	3	16.7	0.18 (0.05–0.66)		0 (0–2)	
PM	12	44.4	0.17 (0.05–0.35)		–2 (–5 to 5)	
CYP2C9 (*2, *3)						
Homozygous EM	4	22.2	0.18 (0.13–0.21)	0.94	–0.5 (–4 to 4)	0.90
Heterozygous EM	9	50.0	0.15 (0.05–0.66)		0 (–5 to 5)	
PM	5	27.8	0.18 (0.08–0.37)		0 (–2 to 6)	
CYP2C19 (*2, *3)						
Homozygous EM	3	16.7	0.21 (0.16–0.66)	0.49	–2 (–4 to 0)	0.05
Heterozygous EM	8	44.4	0.20 (0.05–0.35)		–1 (–5 to 5)	
PM	7	38.9	0.17 (0.35–0.01)		2 (0 to 6)	

EM, extensive metabolizer; MMSE, Mini-Mental State Examination; PM, poor metabolizer.

Table 5 CYP2D6, CYP3A4, UGT, CYP2C9/19 frequency, C/D rivastigmine in combination therapy and MMSE change

Mutant allele	n = 18	Frequency (%)	C/D rivastigmine Median (range)	P value	MMSE change Median (range)	P value
CYP2D6 (*2, *3, *4, *10, *17)						
Homozygous EM	1	5.6	0.09	0.30	−6	0.09
Heterozygous EM	6	33.3	0.46 (0.20–2.6)		0 (−4 to 2)	
PM	11	61.1	0.35 (0.05–0.36)		0 (−4 to 6)	
CYP3A4 (*4, *5,*18)						
Homozygous EM	1	5.6	0.29	0.51	0	0.13
Heterozygous EM	6	33.3	0.29 (0.09–2.6)		−1.5 (−6 to −2)	
PM	11	50.0	0.42 (0.05–1.05)		0 (−2 to 6)	
UGT1A6 (T181A & R184S)						
Homozygous EM	2	11.1	0.14 (0.05–0.23)	0.18	−2.5 (−3 to 2)	0.10
Heterozygous EM	9	50.0	0.42 (0.08–2.6)		0 (−6 to 3)	
PM	7	38.9	0.34 (0.80–0.16)		0 (−2 to 6)	
UGT2B7						
Homozygous EM	14	77.8	0.32 (0.05–1.05)	0.04*	0 (−6 to 6)	0.52
Heterozygous EM	2	11.1	1.7 (0.86–2.6)		1 (0–2)	
PM	2	11.1	0.58 (0.49–0.67)		2 (0–4)	
UGT1A9*5						
Homozygous EM	5	27.8	0.53 (0.31–0.86)	0.28	0 (−2 to 4)	0.89
Heterozygous EM	5	27.8	0.35 (0.23–1.05)		0 (−3 to 1)	
PM	8	44.4	0.27 (0.05–2.6)		1 (−6 to 6)	
CYP2C9 (*2, *3)						
Homozygous EM	4	22.2	0.58 (0.31–0.86)	0.15	0 (0–4)	0.88
Heterozygous EM	8	44.4	0.35 (0.23–1.05)		0 (−6 to 6)	
PM	6	33.3	0.27 (0.05–2.6)		0 (−4 to 4)	
CYP2C19 (*2, *3)						
Homozygous EM	3	16.7	0.20 (0.09–0.42)	0.33	−4 (−6 to 3)	0.30
Heterozygous EM	8	44.4	0.39 (0.05–2.60)		−0 (−3 to 6)	
PM	7	38.9	0.35 (0.31–0.86)		0 (−2 to 4)	

EM, extensive metabolizer; MMSE, Mini-Mental State Examination; PM, poor metabolizer. * $P < 0.05$ was considered significant.

India where a genotype study of 447 residents from four south Indian states revealed a polymorphic distribution that differed somewhat from that seen in Caucasians, those of African descent,

and Far Eastern Asians [15]. The high-functioning alleles, *1 and *2, were most prevalent in these states, while the intermediate *10 allele was present in 10%, whereas it was found to be 5% in

Figure 1 Drug levels of rivastigmine for nonresponders and responders for patients on rivastigmine monotherapy.

Figure 2 Drug levels of rivastigmine for nonresponders and responders for patients on rivastigmine and memantine combination therapy.

Africans and up to 70% in Far Eastern Asians. The PM alleles, *3, *4, and *5, were present at low frequencies, which leads to the suggestion that PM rates in the south Indian states are below 5%. PM frequency in North India has been reported to occur at a frequency of 3% [16] in contrast to south India. Rivastigmine monotherapy was having better clinical response for PM, even though the drug level was comparatively lower than EM subjects. The interindividual variation in the drug response could be due to interindividual differences in the expression of CYP3A4 and CYP3A5 and the drug transporter P-glycoprotein [17]. In a recent study, CYP3A4 and CYP3A5 polymorphisms are unlikely to influence donepezil metabolism and/or clinical outcome [6]. However, genetic variants identified in the CYP3A4 and CYP3A5 genes have only a limited impact on the CYP3A-mediated drug metabolism [18]. Therefore, the identification of the genotype for the ABCB1

Figure 3 Drug levels of memantine for nonresponders and responders for patients on rivastigmine and memantine combination therapy.

gene may provide further clues for the individualization of immunosuppressive drug therapy.

The impact of SNPs UGT2B7, UGT1A6, and UGT1A9*5 and clinical response to the drugs for AD have not been studied till date. According to the genotype classification, we found PM subjects of UGT2B7 polymorphism in patients under rivastigmine combination therapy to have higher drug levels with a poor response to the drug treatments. This indicates that these subjects classified as poor metabolizers having higher drug levels might be above the therapeutic range to give a poor response to the drug. However, the possibility of subclinical comorbid condition among these subjects cannot be ruled out. An important limitation of the study is the small sample size due to the strict inclusion and exclusion criteria. It is also notable that the earlier studies covered were of Caucasian origin, but the exact reason for the poor response is still unclear; however, we can suggest UGT polymorphism may play a role in steady-state plasma concentration and clinical outcome of rivastigmine combination therapy, but this needs to be confirmed by larger and extensive studies.

CYP2C9 is a major cytochrome P450 enzyme that is involved in the metabolic clearance of a wide variety of therapeutic agents, including nonsteroidal antiinflammatories, oral anticoagulants, and oral hypoglycemics. Disruption of CYP2C9 activity by metabolic inhibition or pharmacogenetic variability underlies many of the adverse drug reactions that are associated with the enzyme. CYP2C9 polymorphisms vary dramatically between different ethnic populations. An early genotyping study by Goldstein's group demonstrated that the CYP2C9*2 and CYP2C9*3 variants that are common in Caucasians were represented in African-Americans but at much lower allele frequencies (1–2%) [19]. CYP2C9 gene presents genetic polymorphism, with significant effects on the disposition of several important drugs as amitriptyline, nortriptyline, diazepam, imipramine, propranolol, and selective serotonin reuptake inhibitors. Besides the presence of the completely functional allele CYP2C9*1, the individual variability of CYP2C9 activity is associated with the presence of the null alleles CYP2C9*2 and CYP2C9*3, which has been described as responsible for about

87% of all PMs in Caucasian populations and 100% in Asians [19,20]. The marked interethnic difference in the distribution of CYP2C9 alleles and genotypes makes it an important component for the study of interindividual differences in drug metabolism. In this study, we could not find any association between CYP2C9 and CYP2C19. Thus, associational studies investigating the relationship between genetic polymorphisms in metabolic enzymes and neurotransmitter receptors and Alzheimer's treatment outcome provide a step toward the individualization of Alzheimer's treatment by enabling the selection of the most beneficial drug in terms of its efficacy and low side effect profile.

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Acknowledgments

We acknowledge the financial support from the Department of Science and Technology (F.No.SR/SO/HS-0004/2009).

Conflict of Interest

The authors declare no conflict of interest.