Pharmacogenomics and the Treatment of Sporadic Alzheimer's Disease: A Decade of Progress

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Abstract: Several lines of evidence indicate that apolipoprotein E (apoE) plays a central role in the brain's response to injury and neurodegeneration in the adult. The coordinated expression of apoE and several of its accessory proteins appears to regulate the transport and internalization of cholesterol and phospholipids during development and normal brain reinnervation in the adult. The discovery, a few years ago, that a genetic variant in the apoE gene called apoE4 strongly links to both sporadic and familial late onset Alzheimer's disease (AD) has raised the possibility that a dysfunction of the lipid transport system in the brain could be central to AD pathophysiology. Pathophysiological evidence obtained in autopsy-confirmed sporadic AD cases clearly indicate that the presence of apoE4 allele in humans directly compromises cholinergic function in the adult brain and indirectly modulate the efficacy of medications designed to enhance the cholinergic activity in diseased brain. The apoE4 allele was found to significantly increase the risk of progression to dementia for persons exhibiting amnestic mild cognitive impairment (aMCI), a transitional state between the cognitive changes associated with normal aging and early AD. Furthermore, two accessory enzymes involved in cholinergic neurotransmission called butyrylcholinesterase and paraoxonase-1 were shown i) to display polymorphic variants that increase the risk of developing AD and ii) to modulate drug responsiveness in AD subjects exposed to cholinomimetic agents. This article reviews the most critical findings in this field and reassess the potent clinical value of pharmacogenomics of neurodegenerative diseases and dementia.

INTRODUCTION

Pharmacogenomics, the study of the interplay between drug efficacy and/or toxicity versus genetics, is not characterized by the nature of the chemical entity nor by the biochemistry of the response but, by the fact that a response may lack uniformity and that this lack of uniformity has a genetic basis [Kalow 1995]. Traditionally, pharmacogenetics focused on the interaction between genetic polymorphisms in drug metabolizing enzymes (such as the P450 system), drug transport enzymes, drug response and toxicity. However, more recently, the science of pharmacogenomics has begun to focus on genetic polymorphisms in the drug target genes underlying the disease process, onset, progression and the response to therapy [Farlow *et al.* 1998; Poirier *et al.* 1995; Poirier 1999; Nebert *et al.* 1999; McCarthy and Hilfiker 2000].

This review article will focus on the pharmacogenomic role of three specific yet prevalent genetic risk factors associated with the common form of Alzheimer's disease (AD) called apolipoprotein E (apoE), butyrylcholinesterase (BuChE) and Paraoxonase 1 (PON).

GENETIC RISK FACTORS IN SPORADIC ALZHEIMER'S DISEASE

AD is a progressive neurodegenerative disease that has a strong genetic basis [Bertram and Tanzi 2004]. The disease is characterized by the progressive loss of memory, functional decline, behavioural symptoms and finally death. It is estimated that by the 2030, there will be some 10 million AD patients in North America alone. The treatment of this disease represents a large financial burden on the health care system that has been estimated to be in the 100 billion dollar range in North America. In 1993, it was shown that the frequency of a particular polymorphism in the apoE gene, called apoE4 variant, was increased in both sporadic and some forms of familial AD [Poirier *et al.* 1993; Rebeck *et al.* 1993; Strittmatter *et al.* 1993]. The initial observations were rapidly confirmed and it is now an established fact that the apoE4 allele represents the most important and common genetic risk factor ever identified for the development of sporadic AD around the world.

ApoE has been extensively studied in non-nervous tissues as one of several proteins that regulate lipid transport and metabolism. ApoE facilitates cholesterol, phospholipids and vitamin E transport between different cell types and different organs. It binds to large lipid-protein particles called lipoproteins. This binding increases the ability of large lipid complexes to transport cholesterol and phospholipids in the blood and the parenchyma of the brain. The mature form of apoE found in human plasma and in the brain is a single glycosylated 37-kDa polypeptide containing 299 amino acids. It was shown to coordinate the mobilization and redistribution of cholesterol in repair, growth and maintenance of myelin and neuronal membranes during development or after neuronal cell injury or neurodegenerative conditions [Beffert *et al.* 1998]. In the brain, apoE coordinates the redistribution of cholesterol and phospholipids during membrane remodelling associated with synaptic plasticity and dendritic remodelling (Fig. (**1**)) [Poirier 1994]. Deletion of the apoE gene in apoE knockout mice causes an age-dependent reduction of synaptic contacts in the cortex and a marked impairment of synaptic plasticity (and reinnervation), a significant loss of cholinergic activity in the hippocampal area but most impor-

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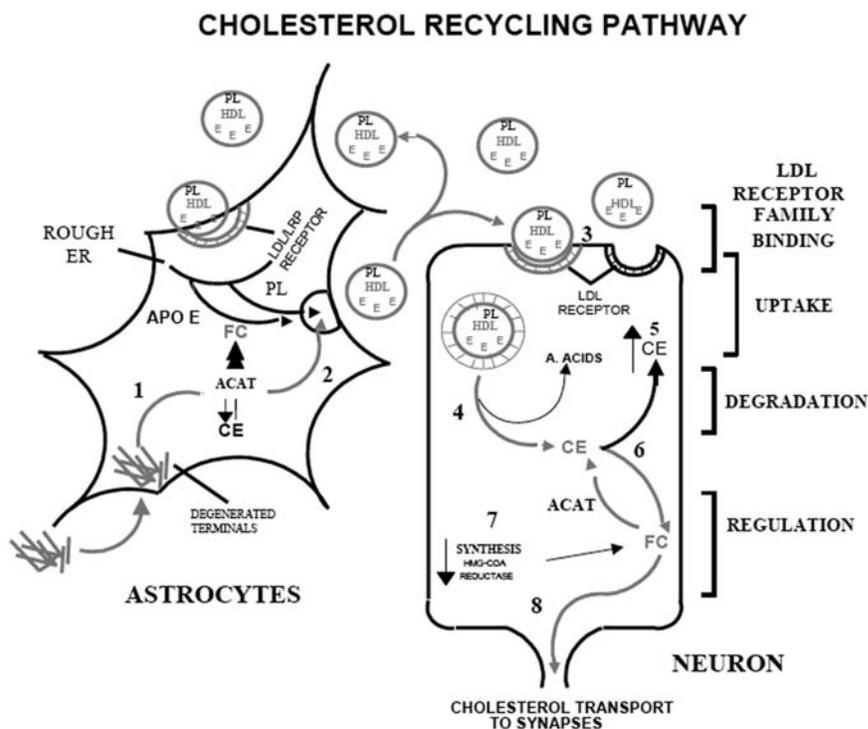E-mail: judes.poirier@mcgill.ca

CHOLESTEROL RECYCLING PATHWAY

Fig. (1). Apolipoprotein E-dependent lipid recycling pathway in the injured or damaged CNS. Degenerating neurons and terminals are initially internalized and degraded by astrocytes and/or microglia. The non-esterified cholesterol released during breakdown of terminals is used as free cholesterol (FC) for the assembly of an extracellular apoE/cholesterol/lipoprotein complex, or converted into cholesterol esters (CE) for storage purposes. The newly formed apoE/cholesterol/lipoprotein complexes are then directed toward a) the blood brain barrier and transferred into the blood stream presumably through the ependymal cells surrounding the ventricles and/or b) to specific brain cells requiring lipids. ApoE complexes then binds to the different family members of the LDL receptor family but it is through the LDL receptor pathway that the complex is internalized and the cholesterol released for dendritic proliferation and/or synaptogenesis. As a consequence of the internalization process, cholesterol synthesis in neurons (*via* the HMG-CoA reductase pathway) becomes progressively repressed. E: ApoE; PL: phospholipids; A. Acids: Amino acids; CE: Cholesterol Ester; FC: Free Cholesterol. ACAT: Cholesterol acetyltransferase. ER: Endoplasmic Reticulum.

tantly, an irreversible loss of cognitive performance as early as 3 months after birth; highlighting the crucial role played by apoE in response to brain damage. memory, neuronal cell losses and cholinergic activity [Fisher *et al.* 1998; Krzywkowski *et al.* 1999; Masliah *et al.* 1995; Poirier 1994].

In contrast to rodents, human apoE is encoded by a four-exon gene (3.6 Kb) on the long arm of chromosome 19 and three major isoforms of apoE (E4, E3 and E2) differing by a single unit of net charge, which can be easily detected by iso-electrofocusing (Fig. (**2**)). These isoforms are expressed from multiple alleles at a single apoE genetic locus, giving rise to three common homozygous phenotypes (E4/4, E3/3 and E2/2) and three common heterozygous phenotypes (E4/3, E4/2 and E3/2) [Utermann *et al.* 1980]. Although these different isoforms have been quite well characterized in periphery, we know very little about the effects of these variants on brain physiology, especially in the aging central nervous system.

The strong association between the apoE4 allele and AD led us to propose that a selective dysfunction of the lipid transport system controlled by apoE during synaptic remodelling in the CNS could be central to the pathophysiological

process that characterizes apoE4 AD subjects [Arendt *et al.* 1997; Poirier 1994]. Alternatively, defects in accessory proteins involved in the cholesterol homeostasis (such as the apoE/cholesterol receptors or cholesterol synthesizing enzymes) have been invoked to explain some of the pathophysiological features of non-E4 AD subjects. The so-called "lipid metabolism disturbance theory" predicted an association between poor drug response and AD pathology driven by the apoE4 genotype. Introduction of the human apoE3 and apoE4 isoform by mean of genetic recombination in apoE-deficient mice was shown to restore the brain response to damage, synaptic integrity and the inherent ability of the brain to scavenge toxic peptides such as the beta amyloid [Blain *et al.* 2006; Holtzman *et al.* 1999].

Polymorphisms in more than two hundred genes have been associated to AD [Bertram *et al.* 2007]. Unfortunately, only a small number of studies have been thoroughly replicated worldwide and a handful of genetic variants were found to affect drug responsiveness in sporadic AD. In addition to apoE, we will also discuss BuChE and PON who exhibit common polymorphic variants which have been associated to both i) risk of developing AD and ii) drug responsiveness in AD and other related dementias.

Apolipoprotein E Polymorphism in Human

	E4	E3	E2
ALLELE FREQUENCY ON CHROMOSOME 19 IN EASTERN CANADA POPULATION:	0.15	0.770	0.08
IN EASTERN CANADA ALZHEIMER CASES:	0.38*	0.59*	0.03

PROTEIN CODED BY EACH ALLELE (APO E IS 299 AMINOACIDS LONG)	APO E4	APO E3	APO E2
- SITE 112	ARG	CYS	CYS
- SITE 158	ARG	ARG	CYS

CANADIAN POPULATION PHENOTYPES		RELATIVE CHARGE	%	ISOELECTRIC PROFILE
HOMOZYGOTES	E4/4	+2	3.9	
	E3/3	+1	61.8	
	E2/2	0	2.0	
HETEROZYGOTES	E4/3		20.6	
	E4/2		9.8	
	E3/2		2.0	

Fig. (2). Apolipoprotein E polymorphisms, population phenotypes distribution and biochemical properties.

APOE4 AND CHOLINERGIC DYSFUNCTION IN ALZHEIMER'S DISEASE

The biological basis for the pharmacogenomic response in sporadic AD stems from the post-mortem analyses of autopsy-confirmed control and age-matched AD subjects in which, key cholinergic markers were examined in relation to their apoE4 allele burden. A marked reduction in choline acetyltransferase (ChAT) activity, the rate limiting step in acetylcholine synthesis, in the hippocampus and temporal cortex of AD cases was reported to be inversely proportional to the apoE4 allele copy number [Allen *et al*. 1997b; Soininen *et al*. 1995b] (i.e. as apoE4 allele copy number increased, ChAT activity decreased). Similar gene dose response associations were reported with other cholinergic pre-synaptic markers such as acetylcholine esterase activity (AChE: [Arendt *et al*. 1997; Poirier *et al*. 1995; Soininen *et al*. 1995c]), nerve growth factor receptor (NGF receptor: [Arendt, *et al*. 1997]) and nicotinic receptor density . Table **1** summarizes the reported findings so far. Recently, Cohen *et al* described a marked effect of the apoE4 allele on *in vivo* distribution volume of M2 receptor sites in the human brain using positron emission tomography [18F]FP-TZTP tracing techniques [Cohen *et al*. 2003; Cohen *et al*. 2006].

These changes are consistent with the fact that nicotinic and M2 receptors, like ChAT, have a preferred pre-synaptic location in the hippocampal formation. In contrast, pirenzepine sensitive "M1" receptor sites remain relatively unaltered in the hippocampus of AD subjects with different apoE genotypes [Poirier *et al*. 1995].

Altogether, these studies indicate that the cholinergic pre-synaptic structures are preferentially damaged in apoE4 allele carriers (ChAT, Nicotinic receptor, NGF receptor, AchE

and M2 receptor) whereas post-synaptic markers are clearly spared in the brain of autopsied AD subjects, irrespective of the apoE genotype. These results also indicate that the AD patients who have the most to gain in term of cholinergic benefit (or enhancement) belong to the apoE4 carrier group.

The apoE4 allele-cholinergic dysfunction association weakens in oldest old and, as it was shown for the E4 genetic risk level, it becomes non significant in subjects aged 80 years and older [Reid *et al*. 2001; Svensson *et al*. 1997]. In contrast, strong pharmacogenomic responses have been reported in mild cognitively impaired subjects (pre-AD prodrome) receiving AChE-specific cholinomimetic medications [Gold *et al*. 2004; Petersen *et al*. 2005].

APOE4 AND CHOLINOMIMETIC DRUGS IN ALZHEIMER'S DISEASE

A 1995 retrospective pilot analysis of the clinical data of the so-called 30-week pivotal tacrine clinical trial (a dual acetylcholinesterase / butyrylcholinesterase inhibitor) led to the breakthrough discovery of potential link between the presence of the apoE4 allele and the clinical outcome in AD subjects with mild-to-moderate AD treated with cholinomimetics [Poirier *et al*. 1995]. This analysis revealed that a majority of subjects not carrying the E4 allele (nearly 50% of all subjects) displayed marked improvement on the Caregiver-rated Clinical Impression of Change (CGIC) scale but more modest genotype-dependent response on the Alzheimer Disease Assessment Scale-cognitive portion (ADAScog). However both the apoE4 and non-E4 subjects treated at the highest dose of Tacrine (160 mg/day) showed significant improvement of symptoms when compared to the placebo group (Fig. (**3**)) [Poirier 1999]. Closer examination of the tacrine dataset revealed that most of the pharmacogenomic

Table 1.   Apolipoprotein E4 and Cholinergic Integrity in Alzheimer's Disease

Cholinergic Markers	Effect of E4 Allele	References	Country
Pre-synaptic Markers			
ChAT Activity	↓↓	a,b,c,d,i	Can, Fin, UK(2), Ger
AchE Activity			
• Post-Mortem	↓	d,e,	Ger, Fin
• CSF	↓	f	Swe
M2 Receptor Sites			
• Post-Mortem	↓↓	a	Can
• *In Vivo*	↓	g	USA
Nicotinic Receptor Sites	↓↓	a,d	Can, Ger
NGF Receptor Sites	↓↓	d	Ger
NBM Cholinergic Neuron Atrophy	↓↓	h	Ned
Post-Synaptic Markers			
M1 Receptor Sites	-------	a	Can
Non-Replication with Older Patients			
ChAT and Nicotinic Sites	-------	Nordberg *et al*., 1996, Corey-Bloom *et al*., 2000/2001	Swe USA

Abbreviations: ChAT: choline acetylcholine transferase, AchE: acetylcholine esterase, M2: muscarinic type receptor, NGF: nerve growth receptor, NBM: nucleus basalis of Meynert.

Countries : Can: Canada, Swe: Sweden, Fin: Finland, Ger: Germany, Ned: Nederland, UK: United Kingdom, USA: United States.

References: a: [Poirier *et al*. 1995]; b: [Soininen *et al*. 1995a]; c: [Allen *et al*. 1997a]; d: [Arendt *et al*. 1997]; e: [Soininen *et al*. 1995c]; f: [Rreh-Shori *et al*. 2006]; g: [Cohen *et al*. 2006]; h: [Salehi and Swaab 1999]; i: [Lai *et al*. 2006].

effects stem mainly from the placebo group where the apoE4 allele was shown to markedly modulate the rate of decline, consistent with previous and subsequent publications on the natural progression of AD [Dal *et al*. 1996; Frisoni *et al*. 1995; Stern *et al*. 1997]. There are also reports of opposite findings in population-based studies [Craft *et al*. 1998; Murphy, Jr. *et al*. 1997; Slooter *et al*. 1999]; suggesting profile differences in naïve subjects as opposed to cohorts of memory clinic subjects. It was also shown that the action of apoE4 on disease progression varies significantly with the stages of the disease [Farlow *et al*. 2004; Gold *et al*. 2004; Jonker *et al*. 1998; Petersen *et al*. 2005] as well as for the actual loss of cholinergic activity from early to more severe stages of the disease [Davis *et al*. 1999; DeKosky and Scheff 1990; Gilmor *et al*. 1999].

This initial series of analyses gave a first indication of a possible pharmacogenomic interaction between apoE gene polymorphisms and existing pharmacological treatments for sporadic AD. This observation spawned many follow up studies which confirmed that an individual's apoE allele carrier status may have to be considered by the physician in choosing the most appropriate cholinergic treatment for mild to moderate AD cases. But more importantly, the nature of the cholinergic agent (mono *vs* dual cholinesterase inhibitors) used directly impact on the extent of the pharmacogenomic effect on efficacy parameters.

The analysis of the influence of apoE genotype on tacrine drug response in mild-to-moderate AD cases was further extended in our follow-up analysis of the 30-week trial dataset using intent-to-treat analyses (ITT) and the subsequent open label follow-up study [Farlow *et al*. 1996b]. It was independently confirmed in an analysis by MacGowan *et al*. (1998) in which they reported that apoE genotype affected response to tacrine in the longer term (12 months) as opposed to short term (3 months).

Using quantitative EEG response to tacrine administration, Riekkinen and his colleagues reported similar apoE genotype preference in AD subjects treated with dual Bu-ChE/AChE dual inhibitor tacrine [Riekkinen, Jr. *et al*. 1997]. A similar genotype relationship was also reported with the response to tropicamide, a selective cholinergic antagonist, in healthy cognitively normal elderly subjects carrying different apoE genotypes. The authors observed that the cholinergic antagonist drug [Higuchi *et al*. 1997] caused a marked dilatation in pupil area only in apoE4 carriers; consistent with a neuronal hypersensitivity caused the preferential damage of the cholinergic neurons.

Tacrine, like the more recent and safer rivastigmine, inhibits both BuChE and AchE activities (Table **2**). In contrast, galantamine, metrifonate and donepezil, three mono-specific acetylcholine esterase inhibitors that exhibit little inhibitory
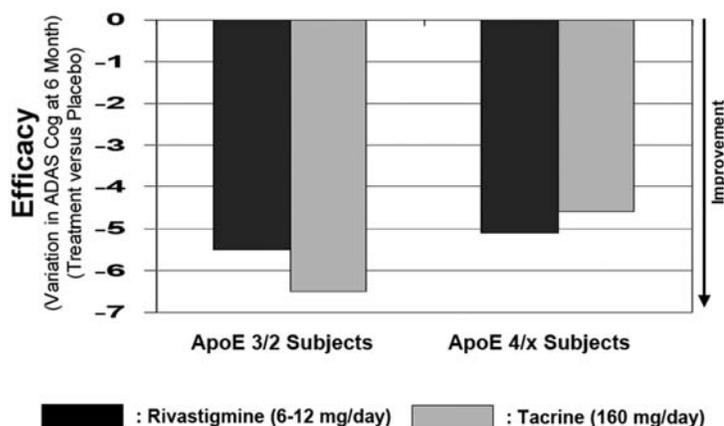
Fig. (3). Efficacy of dual AchE/BuChe Inhibitors in mild to moderate AD cases as a function of ApoE genotype. Effect of apoE4 allele dose on Tacrine and rivastigmine drug responsiveness in mild-to-moderate AD subjects. Graph illustrates the observed results in subjects who completed the drug trials and who received the highest recommended doses of medication. Genotypic determination was performed as described [Poirier *et al.* 1993]. Graph represents overall drug efficacy in presence/absence of apoE4 allele (i.e. group variation in Alzheimer Disease Assessment Scale–Cognition in inhibitor-treated versus placebo cohorts). Efficacy of dual inhibitor drugs is not significantly affected by the presence of the E4 allele in these observed cases (OC) analysis.

effect toward BuChE, were found to display different pharmacogenomic profiles in subjects with AD as well as with aMCI.

Fig. (**3**) contrasts the ADAS-Cog clinical measures obtained in mild-to-moderate sporadic AD receiving the highest recommended dose of a) tacrine (160 mg/day) and b) rivastigmine (6 -12 mg/day) for a period of 6 months as a function of apoE genotype profile [Farlow *et al.* 1996b; Lane and Farlow 2005; Poirier 1999]. The results suggest that in the context of cognitive performance, the dual esterase inhibitors efficacy (treatment versus placebo) are minimally affected by the patient's apoE genotype when compared to placebo [Blesa *et al.* 2006a]. However, it has been suggested that patients with the apoE4 allele may show greater rates of disease progression in the early stages of the disease [Dal Forno *et al.* 2002]. Imaging studies have found that ApoE4 may accelerate the progression of hippocampal atrophy in prodromal and in early AD [Bigler *et al.* 2000; de Leon *et al.* 2001], but once an individual is advanced in age or in the progression of the disease, any influence of APOE ε4 on the

rate of progression is lost [Bigler *et al.* 2000]. Actually, the rate of cerebral atrophy in AD subjects with a mean age of 70 years may be slower in association with ApoE4 relative to other genotypes, whereas in older patients, with a mean age of 80 years [Bigler *et al.* 2000], progression was no different by genotype. This is supported by clinical data that demonstrate that during the prodromal phase of AD, carriers of an ApoE4 allele progress faster than non-carriers [Farlow *et al.* 2004; Gold *et al.* 2004; Jonker *et al.* 1998; Petersen *et al.* 2005]. Fig. (**4**) summarizes the situation observed in the placebo arms of two double-blind clinical trials performed at a ten year interval. In mild disease, progression may be comparable or faster, and in more advanced stages, progression was shown to be consistently slower in ApoE4 carriers relative to non-carriers.

However, the story is somewhat different in patients treated with mono-specific AChE inhibitors that exhibit little or no effect on BuChE activity such as metrifonate, galantamine and donepezil. Farlow and coworkers (1999) examined the influence of apoE genotype on the short-term metri-

Table 2.   Cholinesterase Inhibitor Selectivity* in the Mammalian Brain

Compound	IC50(nM) AChE	IC50(nM) BuChE	Ratio BuChE/AChE
Monospecific Inhibitors			
Donepezyl	5.7	7139	1252
Galantamine	0.35	19	53
Dual Inhibitors			
Rivastigmine	48000	54000	1.1
Tacrine	190	47	0.25

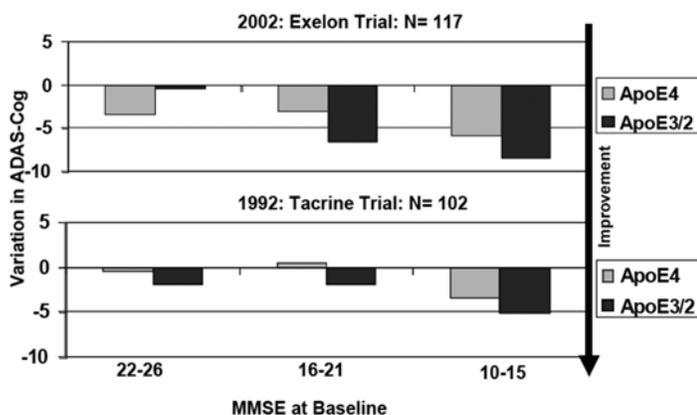* : Adapted from [Giacobini E. 2000].

Fig. (4). ApoE4 and rate of decline in mild-to-moderate AD as a function of disease severity. Effect of apoE genotype on rate of decline on the ADAS-Cog scale in placebo and dual-inhibitors treated AD cases over a 6 month period. Only observed cases (OC) who completed the study were analysed. In the moderate stages, the rate of decline of both placebo and treated subjects is more pronounced in the apoE4-negative subjects as compared to the apoE4 allele carriers. Adapted from [Farlow *et al.* 1996a and Farlow *et al.*, 2004].

fonate treatment (6 months) in mild-to-moderate AD patients. In contrast to tacrine and rivastigmine, metrifonate is much more selective toward the AChE than for the BuChE, although it is still catagorized as a weak dual inhibitor. In this particular study, data pooled from four double-blind placebo controlled clinical trials were analyzed retrospectively for the possible interaction between apoE genotype and cognitive response to metrifonate after 26 weeks for the entire group (n = 959). A significant trend that did not reach significance suggested possible interaction between apoE genotype and treatment effect [Farlow *et al.* 1999a]. One of the major limitations of the above mentioned study is that only a single time point, 6 month, was considered for analysis. A subsequent study on metrifonate that used blood samples from a 3-year open labelled clinical trial in mild-to-moderate AD revealed progressive pharmacogenomic effect of the apoE4 allele emerging after 24 months of treatment [Poirier 1999]. Fig. (**5**) illustrates the results obtained at 6 and 24 months in metrifonate-treated subjects with different apoE genotypes. After 26 weeks of treatment, there was no significant difference in the MMSE score of the two groups (E4 versus non-E4): consistent with the previous [Farlow *et al.* 1999a] meta-analysis. However, the longer-term assessment of the patients' responses indicates a progressive dissociation of the apoE4 and non-E4 groups over time, particularly noticeable after 120 weeks into the trial. After 240 weeks of treatment, one can observe a clear departure of the E4 carriers subjects who show a much faster rate of decline. The absence of a parallel placebo group (for obvious ethical reason) prevents us from determining the efficacy of metrifonate in a stratified population of subjects [Poirier 1999].

As we examine cholinesterase inhibitors which are orders of magnitude more selective to AChE isoform, a slightly different picture emerge: a stronger than expected effect of the apoE4 allele on efficacy parameters. McGowan and colleague first reported the effect of galantamine on cognitive performance in 84 mild to moderate AD patients over the course of 6 months of treatment and found that the best responders did belong to the apoE4 homozygotes subgroup [MacGowan *et al.* 1998]. More recently, Wilcock and col-

laborators and Raskind and collaborators examined galantamine efficacy in two large international multicentre placebo-controlled randomised clinical trials. They report that while subjects with and without apoE4 allele exhibited a significant improvement on the ADAS-Cog scale, sub-analyses clearly identified that apoE4/4 group as the responder cohort with an average 6.6 points improvement on the ADAS-Cog scale [Wilcock *et al.* 2000]. Similar findings were reported more recently by an independent group using patients from eastern Europe [Babic *et al.* 2004].
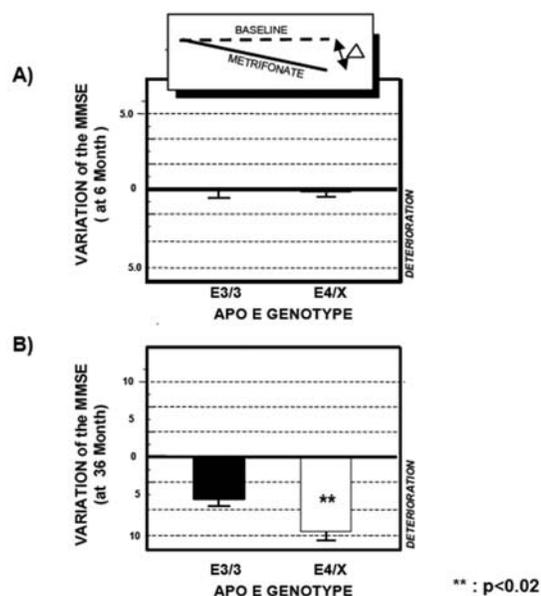

Fig. (5). Variation in MMSE scores in mild to moderate AD subjects treated with Metrifonate. Bars represent variations in MMSE scores at 6 and 36 months compared to baseline values at time zero. Paired T-tests were used to contrast changes over time. **: p <0.02. Apolipoprotein E phenotype was determined by the method of Poirier *et al.*, 1993. MMSE: Mini Mental State Exam score. Adapted from [Schappert, Sevigny, and Poirier 360].
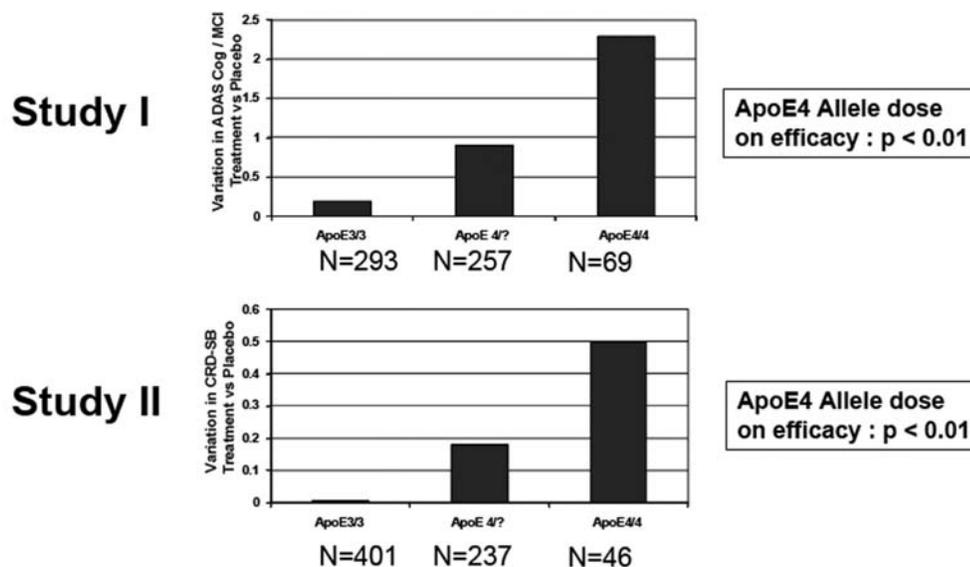
Fig. (6). Impact of ApoE genotype on galantamine efficacy in mild cognitive impairment.

Mean changes from placebo in ADAS-Cog/MCI score (Study 1) and CDR-SB score (Study 2) in galantamine treated subjects as a function of apoE genotype. The allele dose response analysis reveals a significant association between ADAS-Cog-MCI and CDR-SB scores improvement and apoE4 allele copy number. Adapted from Gold *et al.* 2004.

At the highest dose (32 mg/day) of galantamine, Raskind and collaborators report a strong apoE4-dependent improvement on the ADAS-Cog scale when compared to the non-apoE4 group; a pharmacogenomic effect that progressively disappear at lower doses in mild-to-moderate AD cases [Raskind *et al.* 2000]. Consistent with the observation, lead investigators at Johnson and Johnson presented detailed evidence from a two year long, 1600 patients, clinical trial in MCI which revealed a strong apoE4-dependent response in galantamine treated subjects when compared to placebo [Gold *et al.* 2004]. Fig. (**6**) summarizes the results of the first of two parallel studies where ADAS-Cog variations were contrasted with apoE4 allele dose in subjects exposed to either galantamine or placebo for a period of two years [Gold *et al.* 2004]. The efficacy profile of the drug was found to be tightly associated with apoE4 allele dose; the subjects carrying the apoE4/4 genotype exhibiting outstanding response when compared to other genotypes.

Finally, we must now examine the controversial literature pertaining to the most specific and most widely used AChE-specific inhibitor of all, donepezil. The first report suggesting a possible pharmacogenomic effect of the apoE4 allele on donepezil efficacy was published by Lucotte's team in France [Oddoze *et al.* 2000] using an open labelled uncontrolled trial in mild to moderate AD subjects. This observation initially came as a surprise as it suggested that selective AChE inhibitors like donepezil were more affected by apoE4 allelle than the dual inhibitors such as tacrine and rivastigmine. This pilot observation was subsequently confirmed by a small randomized cross over study using donepezil in mild-to-moderate AD [Greenberg *et al.* 2000]. Although the focus of the study was on global clinical improvement, a clear apoE4-dependent effect was documented in the ADAS-Cog scale. Several investigators followed up with open trial designs with no placebo arm and, either succeeded or failed to replicate the original observation with donepezil [Bizzarro *et al.* 2005; Borroni *et al.* 2002; Rigaud *et al.* 2002].

Only two sufficiently large randomized placebo-controlled studies examined this issue the proper statistical power; the first one, performed Windblad and co-workers in North European countries, failed to detect any apoE4 contribution to donepezil efficacy (n=198 subjects) in mild-to-moderate AD [Winblad *et al.* 2001]. In contrast, Lendon and coworkers in England reported a significant contribution of the apoE genotype on donepezil efficacy at the level of cognition and activities of daily living in mild to moderate AD cases (N = 785) [Lendon *et al.* 2002].

While the bulk of the findings suggest a better donepezil response in apoE4 allele carriers with sporadic AD, only recently has the observation been extended to MCI populations [Petersen *et al.* 2005]. In a three year prospective study of the rate of conversion of MCI to sporadic AD, the authors reported that donepezil was not particularly effective at altering the rate of conversion of MCI during the three year period, except for the first 12 months of the study. However, when subjects were stratified by apoE genotype, results clearly show a significant effect of donepezil on the rate of conversion in apoE4 allele carriers during the entire 3 years period; subjects E4-negative not benefiting from the medication (Fig. (**7**)).

Fig. (**7A**) illustrate the effect of apoE4 allele on the conversion of MCI case to diagnosed AD as opposed to apoE4 negative case whereas Fig. (**7B**) illustrates the marked and significant impact of donepesil on the conversion rate from MCI in apoE4 allele carriers only. Subjects without an apoE4 allele failed to respond to donepezil.
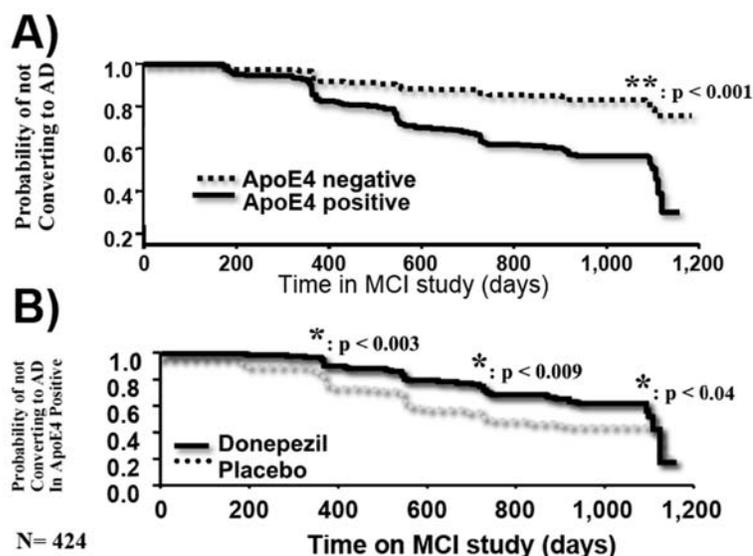
Fig. (7). Kaplan–Meier estimates of the rate of Progression from mild cognitive impairment to Alzheimer's Disease (AD). Panel **A** shows the effect of apoE4 carrier status on the rate of progression to AD whereas panel **B**) illustrates the long term beneficial effect of donepesil treatment on the rate of progression in the apoE4 allele carriers population only. Adapted from [Petersen *et al*. 2005].

These results, combined with the other clinical trials with various AChE inhibitors, strongly support the notion that the cholinergic system which is particularly affected by the presence of the E4 allele in AD becomes a target of choice for cognitive enhancing drugs designed to block acetylcholine degradation. The exact molecular cascade responsible for the apoE4 effect in human is unclear, however, we know from apoE knock-out and apoE4 knock-in experiments in rodents that apoE plays a central in the delivery of key phospholipids precursor used in the production of acetylcholine in the cholinergic system. The selective damage of the presynaptic cholinergic compartment in human apoE4 allele carriers is certainly consistent with a hypersensitivity of the post-synaptic cholinergic sites and the better than expected response observed in apoE4 carriers [Poirier 1999].

On the other hand, several reasons can be invoked to explain the diverging apoE4 dependent clinical responses that exist between dual inhibitors (rivastigmine, tacrine) versus mono-specific AChE inhibitors (metrifonate, donepezil, galantamine): population biases, sampling error or more simply, the biochemistry and pharmacology of the different inhibitors and the fact that BuChE is, by itself, a genetic risk factor, for common AD [Lehmann *et al*. 1997]. This issue will be further discussed below. It is well known that highly selective acetylcholinesterase inhibitor like donepezil exhibits virtually no butyrylcholinesterase activity. Tacrine and rivastigmine, on the other hand, have multiple effects on butyryl-cholinesterase activity, nicotinic receptor binding and even glutamatergic modulation. It is thus quite possible that target selectivity of each compound is affected differently by the presence of apoE4 allele in those subjects.

EXPERIMENTAL DRUGS IN DEVELOPMENT AND THEIR RELATIONSHIP TO THE APOE4 ALLELE

In recent years, the implementation of phase III clinical trials in the field of dementia has led to a careful analyses of the apoE (and other) gene(s) contribution to drug response. Unfortunately, analyses of the clinical trials in MCI, mild, moderate and severe AD and other dementia were done in a retrospective manner to allow the future commercialization of the product under development to proceed without stratification. While this approach has led to some controversy as to the full public disclosure and publication of both positive and negative pharmacogenomic results [Hedgecoe 2006; Sinha 2006; Wiebusch *et al*. 1999] by the pharmaceutical industry, a few companies have chosen to publish or present some of their clinical trial results at international meeting.

Here are a few examples. The pharmacogenetic profile of Xanomeline (Eli Lilly) was assessed in a phase II drug trial in mild-to-moderate. This compound, which is a M1 specific cholinergic agonist, apparently bypasses the pre-synaptic cholinergic terminals and directly stimulates the post-synaptic receptor sites in the brain. Since the M1 sites are particularly insensitive to the apoE4 allele in post-mortem brains of AD subjects [Poirier *et al*. 1995], it was postulated that the pharmacogenetic profile of Xanomeline would less dependent on the apoE4 allele than existing acetylcholine esterase inhibitors. Patients exposed to a 75-mg dose of Xanomeline were monitored over a period of 6 months using the ADAS-Cog as primary outcome variable. Fig. (**8**) illustrates the observed apoE4 allele dependent dose response observed Xanomeline administration; the near complete absence of response of the apoE4/4 AD subjects suggests that some pre-synaptic cholinergic components must be present for xanomeline to exert its post-synaptic effect. A second follow-up Xanomeline drug trial (phase IIb) was implemented by Eli Lilly in 1997 and the data analysis of more than 180 mild-to-moderate Alzheimer's disease patients revealed a clear apoE4-dependent pharmacogenomic profile which was virtually identical to the one presented in Fig. (**8A**) [Alstiel *et al*. 1998]; with apoE4/4 showing no improvement after 6 months of treatment. The development of this agent for the
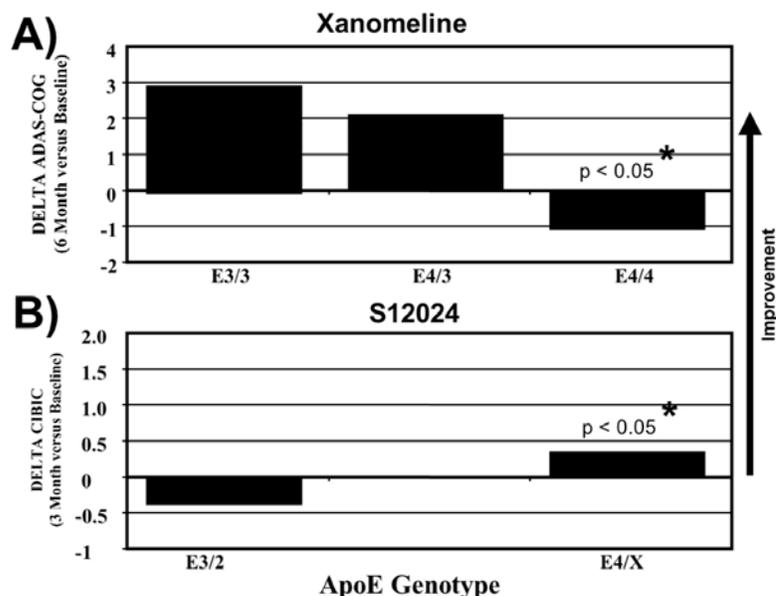
Fig. (8). Effect of xanomeline (**A**) and S12024 (**B**) on disease progression in mild to moderate AD cases as stratified by apoE genotype. Xanomeline, a M1 cholinergic receptor agonist developed by Eli Lilly, was administered during 6 months whereas S12024, a noradrenergic/vasopressinergic agent from Servier, was tested for only 3 months. Adapted from [Richard *et al.* 1997] and [Altstiel *et al.* 1998]. Results are expressed as mean differences between CIBIC or ADAS-Cog scores in treated versus placebo subjects.

treatment of AD was subsequently abandoned due to side effect issues and the observe pharmacogenomic effect.

ApoE was also shown to affect non-cholinergic drugs as well. Richard and collaborators examined the influence of apoE genotype on drug responsiveness of the experimental vasopressinergic/noradrenergic Servier drug called S12024 in mild-to-moderate AD [Richard *et al.* 1997]. The authors showed that while there was no significant benefit of the drug in the AD group as a whole, the stratification of the AD patients into E4 and non-E4 carriers clearly showed that the E4 carriers were significantly responding to the medication drug whereas the non-E4 carriers were not (Fig. **8B**).

Citicoline (cytidine 5'-diphosphocholine), an endogenous intermediate in the synthesis of membrane lipids and acetylcholine, has been used in the treatment of neurodegenerative disorders like AD. Alvarez and coworkers (1999) published findings of a double-blind placebo controlled study in mild to moderate AD patients who received citicoline for 12 weeks. As compared to placebo, citicoline improved cognitive function only in those patients carrying at least one apoE4 allele.

PHARMACOGENOMIC CONSIDERATIONS FOR DISEASE-MODIFYING DRUGS

Drugs acting on amyloid metabolism may modify the clinical course of AD. Such drugs under testing include tramiprosate (Alzhemed®, Neurochem Pharma), R-flurbiprofen (Flurizan®, Myriad), and a number of monoclonal antibodies (Elan, Pfizer, Roche). Another etiological hypothesis is also being investigated using rosiglitazone (Avandia®, GSK): in a Phase IIB clinical trial of rosiglitazone in mild to moderate AD, Roses and colleagues designed the clinical trial as a 24-week monotherapy trial with the primary endpoint being improvement of the ADAS-cog scores [Risner *et al.* 2006].

Rosiglitazone, a peroxisome proliferator-activated receptor gamma (PPARδ) agonist and an approved anti-diabetic pharmacotherapy, is an insulin sensitizing agent, a mild anti-inflammatory drug [Feinstein *et al.* 2002] and a potent apoE inducer medication [Yue *et al.* 2004]. The authors were surprised with the initial analyses in the ITT population of 511 patients to determine that no significant clinical effect was observed at any time points. Using ApoE4 carrier status, the patient population was subsequently segregated to examine therapeutic response. AD patients without an ApoE4 allele responded to all three doses of rosiglitazone while those patients who carried an ApoE4 allele did not respond at the lowest dose, but had greater responses at the two higher doses. This crucial observation led to the design of the follow-up prospective phase 3 clinical trial in which AD patients are *a priori* stratified on the basis of their respective apoE genotype. Results are expected in 2008.

The plan of analysis for efficacy in clinical trials using potentially disease-modifying drugs do includes pharmacogenomic analyses, looking for patterns of clinical response based on genotype. If it is found that indeed only certain groups of patients respond to an individual drug or a class of drugs, a very significant change will take place in acceptance for reimbursement by third party payers and in the guidelines for clinical use of the disease-modifying drugs, relative to the way symptomatic drugs for AD have been handled up to now (vide supra).

On the negative side only persons with a pre-defined genomic profile will get the drug/drug class prescription and reimbursement, possibly with no alternative disease-modifying treatments. It is possible that future clinical trials will pre-screen for genomic profile and enroll only patients who fit a profile of fast decliners (for instance apoE4 in amnestic MCI) or fit a need for alternative treatments to ones already

approved. The genetic profile of those enrolled in the study will automatically be known to caregivers and other family members, with concern to first degree relatives (for instance if only carrier of apoE4 are enrolled, each first degree family member has a 50% chance of being a carrier).

On the positive side disease-modifying drugs will allow for the best drug/drug class use for individual patients, facilitating compliance and reimbursement. Clinical trials with enrichment for specific genotype will be shorter, with a smaller number of subjects, and allow for 'proof of principle' in phase II prior to a much larger number of subjects in phase III.

PARAOXONASE 1 AND BUTYRYLCHOLINESTER-ASE GENETIC VARIANTS

Paraoxonase-1 (PON-1) is an arylesterase with multiple biological activities. Interestingly, it is also a potent endogenous, biologically active, choline esterase inhibitor [Costa *et al.* 2005; Kondo and Yamamoto 1998]. PON-1 derives its name from the ability to hydrolyze paraoxon, an active metabolite of parathion characterized by toxic cholinesterase properties. By hydrolyzing paraoxon, PON-1 provides protection against exogenous organophosphate poisoning. In addition to that, PON-1 is also largely responsible for the antioxidant activity of high-density lipoproteins (HDL), the sole lipoprotein complex found in the brain to carry apoE. Serum PON-1 levels and activity in humans display up to 40-fold inter-individual variability and are genetically determined by a common polymorphism of the PON-1 gene. The PON-1 gene is a member of a multigene family, also including PON-2 and PON-3, located on chromosome 7q21.3-22.1. The molecular basis for the PON-1 gene polymorphism is a Gln→Arg substitution at residue 192, that results in three possible genotypes: QQ, QR, and RR. The Q allozyme, that has Gln at residue 192, has low paraoxon-hydrolyzing activity, while the R allozyme, that has Arg at residue 192, shows high activity.

Recently, Pola and collaborators [Pola *et al.* 2005] evaluated whether the 192 Q/R polymorphism of the PON-1 gene might influence responsiveness to a 9 month treatment with cholinesterase inhibitors in subjects affected by AD. Results indicate that subjects carrying the R allele of the PON-1 genotype are more likely to respond to cholinesterases treatment, compared to QQ homozygous individuals. The biological significance of this finding might be based on the fact that the mutations responsible for the Q/R polymorphism of the PON-1 gene result into the synthesis of PON-1 proteins with different hydrolyzing activities, as the R allele is associated with a higher activity-enzyme and the Q allele with a lower activity-enzyme.

In humans, BuChE (EC3.1.1.8) occurs in plasma and in most tissues including certain regions of the brain [Bartels *et al.* 1992]. Despite today's extensive knowledge about allelic BuChE variants and their pharmacogenetic impact, its physiological function still remains uncertain. The 574-amino-acid glycoprotein is coded by a single-copy gene on chromosome 3q26.1–26.2. Several dysfunctional BuChE mutations have been characterised, both at the phenotypic and genetic level [Primo-Parmo *et al.* 1996]. Since 1965, several studies have

reported the co-localisation of the enzyme with senile plaques and neurofibrillary tangles, the hallmarks of the pathology of Alzheimer's disease and the severe loss of cholinergic neurons in AD brains has been found to be accompanied by higher than normal levels of BuChE [Gomez-Ramos and Moran 1997; Perry *et al.* 1978]. Recently, it has been suggested that higher BuChE levels may play a role in the maturation of senile plaques [Gomez-Ramos and Moran 1997]. The K-variant has been identified as by far the most frequent functional mutation of BuChE [Bartels *et al.* 1992]. The K-polymorphism is found in various ethnic populations with homozygote frequencies between 1% and 4%. Interestingly, a concentration dependent anti-A-beta aggregating role has been suggested for normal form of BuChE [Diamant *et al.* 2006]. This involves an interaction between a 40 amino acid residue segment in the C-terminal of BuChE with the soluble form of Aβ (i.e. it is distal from the BuChE enzymatic site). This is consistent with the fact that genetic BuChE-K variant, which has been associated with a higher risk of developing AD, is also characterized by higher amyloid plaque density relative to BuChE wild-type cases [Ghebremedhin *et al.* 2002; Lehmann *et al.* 2000]. The BuChE -K polymorphism has been reported to occur with higher frequency in late-onset AD patients, especially in carriers of the apoE4 allele [Lehmann *et al.* 1997; Wiebusch *et al.* 1999]. For all these reason, BuChE was deemed to act as a potential pharmacogenomic modulator of cholinomimetic therapy in AD and other related disorders.

Moderate-to-severe AD, Parkinson's disease dementia and dementia with Lewy body patients with the BuChE-K allele, which encodes for lower expression of the enzyme, decline less rapidly than those with wild-type BuChE [Holmes *et al.* 2005; O'Brien *et al.* 2003]. These preliminary observations prompted the analysis of the BuChE genetic variants in several clinical trials involving dual AChE/BuChE inhibitor rivastigmine. BuChE wild-type carriers younger than 75 years showed differential efficacy to cholinesterase inhibitor therapy. Patients receiving rivastigmine displayed significantly greater treatment responses over 2 years than patients receiving donepezil. In contrast, BuChE-K variant carriers experienced similar long-term treatment effects with both agents, although adverse events were more frequent in rivastigmine-treated patients (Fig. (**9**)). In most dementia variants (Lewy Body, Parkinson's Disease, Moderate-to Severe AD cases), BuChE-K carriers display rate of deterioration markedly slower in more advanced dementia than those case expressing the wild type variant of the BuChE gene [Bullock and Lane 2007]. Additional clinical studies are now required to determine the overall impact of the presence of the K variants on the efficacy (treatment versus placebo) of drugs commonly use to treat sporadic AD.

BuChE activity is relatively high in thalamic nuclei that project to frontal cortical structures involved in attention, executive function, and behavior. However, the largest pool of BuChE in the brain is found in the glia, particularly those in deeper cortical and subcortical structures [Wright *et al.* 1993] . BuChE enzymatic activity may relieve the acetylcholine blockade of pro-inflammatory responses in parenchymal regions distal from synaptic sites and the inhibition of BuChE may have the opposite effect [Bullock and Lane 2007].
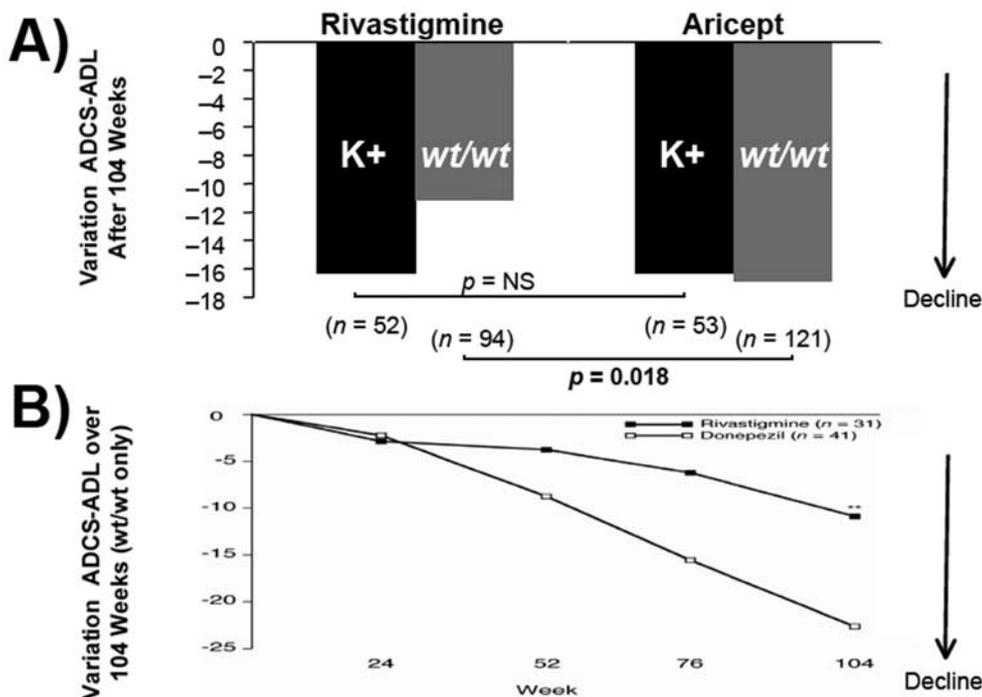
Fig. (9). Changes from baseline on activity of daily living (ADCS-ADLs) in Intent to Treat-Last Observation Carried Forward after 2 years of treatment with rivastigmine or donepezil in patients younger than 75 years as stratified by butyrylcholinesterase genotypes. Panel **A**) contrast the results obtained as a function of genotype at the 104 week time point. Panel **B** illustrated the time course changes observed for both drug in the wild type cohort only. K: Kallow variant, wt: wild type variant. Adapted from Blesa *et al.* 2006 and Bullock *et al.* 2007.

Loss of white matter causes a progressive disconnection of widely distributed neural networks that result in cognitive decline, particularly in the processing speed that underlies mechanisms of attention and working memory. Thus, deterioration of executive function, attention and functioning – which may more related to frontosubcortical pathology – would be expected to be more marked in those with BuChE wt/wt as the wild-type is more highly expressed in the periphery and may also be in the brain. This appears to be the case [Bullock and Lane 2007], and this genotype also means greater functional improvement in moderate AD treated with a BuChE and AChE inhibitor relative to a selective AChE inhibitor, particularly in younger patients [Blesa *et al.* 2006b; Bullock *et al.* 2005].

CONCLUSION

It appears that not only is the apoE4 allele an established risk factor for the development of AD; it also has a significant influence on the response of AD patients to treatment with both cholinergic and non-cholinergic drugs. Similar but more preliminary conclusion can be drawn from the BuChE and PON-1 retrospective pharmacogenomic studies. All these results are intriguing from a mechanistic viewpoint and suggest that the underlying biochemistry and pathology of E4 versus non-E4 carriers, BuChE-K versus wild type or PON-1 Q versus P alleles AD patients are different within the same disease group. Further investigation is needed to determine the biochemical cascade responsible for the pharmacogenetic effect of these different alleles. The synergistic effect of the apoE4 and BuChe-K allele on risk level in AD [Lehmann *et al.* 1997; Wiebusch *et al.* 1999] could also

similarly impact treatment efficacy. This could lead to the development of a new class of AD drugs that are specifically designed to modify the pharmacogenetic impact of apoE4, for example, such that those patients would respond to a drug therapy that would ordinarily work only in non-E4 carriers. This is precisely what GlaxoSmith Kline pharmaceutical is attempting these days with it's first prospective, pharmacogenomics, double-blind placebo controlled phase 3 clinical trial in mild-to-moderate AD subjects treated with rosiglitazone [Risner *et al.* 2006].

ABBREVIATIONS

ApoE	=	Apolipoprotein E
AchE	=	Acetylcholine esterase
AD	=	Alzheimer's disease
ADAScog	=	Alzheimer Disease Assessment Scale-cognitive portion
aMCI	=	amnestic mild cognitively impaired
BuChE	=	Butyrylcholinesterase

ChaT   =   Choline acetyltransferase

CGIC   =   Caregiver-rated Clinical Impression of Change

ITT   =   Intent-To-Treat

NGF   =   Nerve Growth Factor

CNS   =   Central Nervous System

PON   =   Paraoxonase

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