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 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 stage 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-
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; Krzywowski 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.
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, pirenzipine 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
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 BuChE/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
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 allele 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 e4 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50(nM) AChE</th>
<th>IC50(nM) BuChE</th>
<th>Ratio BuChE/AChE</th>
</tr>
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<tbody>
<tr>
<td>Monospecific Inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donepezyl</td>
<td>5.7</td>
<td>7139</td>
<td>1252</td>
</tr>
<tr>
<td>Galantamine</td>
<td>0.35</td>
<td>19</td>
<td>53</td>
</tr>
<tr>
<td>Dual Inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rivastigmine</td>
<td>48000</td>
<td>54000</td>
<td>1.1</td>
</tr>
<tr>
<td>Tacrine</td>
<td>190</td>
<td>47</td>
<td>0.25</td>
</tr>
</tbody>
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* : Adapted from [Giacobini E. 2000].
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 categorized 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 isofrom, 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 collaborators 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. (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].

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].
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 allele 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 medicate (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 donepezil on the conversion rate from MCI in apoE4 allele carriers only. Subjects without an apoE4 allele failed to respond to donepezil.
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 role in the delivery of key phospholipid precursors 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 postsynaptic 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 butyrylcholinesterase 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. (8A) 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 presynaptic 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
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 trami-prostate (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 (PPARγ) 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 present...
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 BUTYRYLCHOLINESTERASE 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 cholinesterase 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 5q26.1–26.2. 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 Ab (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; Wiesbusch 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].
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 GlaxoSmithKline pharmaceuticals is attempting these days with its first prospective, pharmacogenomics, double-blind placebo controlled phase 3 clinical trial in mild-to-moderate AD subjects treated with rosiglitazone [Risner et al. 2006].

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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
Allen, S. J.; MacGowan, S. H.; Tyler, S.; Wilcock, G. K.; Robertson, A. G.; CGIC = Caregiver-rated Clinical Impression of
74
REFERENCES
CNS = Central Nervous System
ITT = Intent-To-Treat
NGF = Nerve Growth Factor
PON = Paraoxonase


