The APOE4 conundrum

By Lev Osherovich, Senior Writer

An industry-academic partnership between **Merck & Co. Inc.** and **The J. David Gladstone Institutes** is hoping that modulating APOE4, a variant of apolipoprotein E that is a risk factor for Alzheimer's disease, will help treat the disease. However, controversy about how E4 influences AD has led other pharma to pursue a completely different approach.

Apolipoprotein E (APOE) is a lipid-ferrying protein found in the serum and brain. About 40–50% of AD patients are APOE4 (E4) carriers and develop the disease earlier and progress more rapidly than patients with other APOE variants.¹

E4 has been fingered as the cause of negative results for at least two AD therapeutics in the clinic. Data from a Phase II trial of **Elan Corp. plc** and **Wyeth's** bapineuzumab and a Phase III trial of **GlaxoSmith-Kline plc's** Avandia rosiglitazone suggested that E4 carriers were more resistant to both therapeutics than noncarriers.

Bapineuzumab is a mAb against β -amyloid (A β), the misfolded extracellular protein fragment that is suspected to cause AD. Avandia is a peroxisome proliferator-activated receptor- γ (PPARG; PPAR γ) agonist approved to treat type 2 diabetes.

Targeting E4 is a new tactic that could blunt the impact of AD in a sizable number of patients, said Richard Hargreaves, VP and head of worldwide basic research for neuroscience and ophthalmology at Merck. "E4 does not cause AD, but it increases vulnerability to the disease as well as influencing age of onset," he said. "Distinct therapeutic approaches for these patients could offer a tremendous advantage in combination with traditional therapies" such as blocking A β production or stimulating neuronal survival.

Many theories

The E4 protein has been extensively studied since its discovery as an AD risk factor in the 1990s, but its precise role in the disease remains controversial, said Elan CSO and EVP Dale Schenk. "For all of the years and millions of dollars spent on APOE research, there's not a single thing that's 100% definitive" about how E4 hastens AD, he told *SciBX*.

Elan and Wyeth are stratifying E4 carriers and noncarriers into separate arms of an ongoing Phase III trial of bapineuzumab, with carriers receiving only a low dose because of concerns about vascular side effects seen in carriers given higher doses.

Views on E4's role in AD fall into three camps, with each view leading to distinct ideas about how to tackle the protein and thus treat the disease.

One possibility is that higher AD risk in E4 carriers results from the cardiovascular consequences of altered lipid transport by the mutant lipoprotein. Indeed, E4 carriers also have greater risk of atherosclerosis.² However, AD and cardiovascular disease don't strongly correlate, so researchers have largely moved on to look for effects of E4 in the CNS.

A second hypothesis is that E4 influences the production of neurotoxic A β aggregates. Indeed, a recent imaging study in humans suggests that E4 carriers have higher A β levels.³

Finally, a hypothesis put forth by Gladstone president Robert Mahley and fellow Gladstone investigators Karl Weisgraber and Yadong Huang predicts that E4 may itself be toxic to neurons independently of A β .⁴

Mahley is also professor of pathology and medicine at the **University of California, San Francisco**. He thinks neuronal E4 is misprocessed to produce a toxic intracellular fragment that damages the neuron's metabolic machinery (see **Figure 1, "Possible mechanisms of APOE in AD"**).

"E4 can directly impact nerve survival," said Mahley. Whereas APOE produced in most tissues is secreted into the blood and acts as a lipid carrier, Mahley said neuron-derived APOE has a second function as a stress-induced neuroprotective factor.

According to Mahley, E4—but not other APOE variants—undergoes abnormal proteolytic cleavage that destroys the protein's neuroprotective activity and instead produces a toxic protein fragment. This E4 fragment enters the cytosol, where it interacts with mitochondria to disrupt energy metabolism.

Consequently, Mahley expects that therapeutics aimed directly at A β are unlikely to work in E4 carriers.

"My interpretation of A β -directed trials is that they fail in E4 carriers because their approach is not targeting the right mechanism," he said. "We would have to develop a distinct set of therapies in E4 carriers."

Thus, Mahley told *SciBX*, small molecules that neutralize E4-derived fragments or prevent their formation could be useful for treating patients with the variant.

Merck has put its money on Mahley's theory. Under a four-year deal announced in 2006, the pharma paid \$3.3 million up front in exchange for exclusive development rights to E4-modulating compounds. Gladstone receives annual licensing fees and is eligible for royalties.

Mahley said a major focus of the screening effort is to identify molecules that modify the structure of E4 to make it mimic APOE3 (E3), a benign APOE variant.

"In order to convert E4 into an E3-like structure, compounds will have to bind specifically to E4" and nudge the defective protein into a healthier conformation, said Hargreaves. Fortunately, E4 differs from E3 only by a single amino acid substitution. As a result, the conformational change could be relatively subtle.

Mahley said the small molecule screening project could also identify compounds that block the proteolytic processing of E4 or prevent the toxic fragment from interacting with mitochondria.

The development status of the joint effort is undisclosed.

Mahley's hypothesis is not widely accepted in the AD field, according to Gary Landreth, professor of neuroscience at **Case Western**

Figure 1. Possible mechanisms of APOE in AD. Two studies suggest different theories of how APOE4 (E4) works in Alzheimer's disease (AD), and thus they offer different ways of blocking its action. APOE4 is a variant of apolipoprotein E (APOE) that is linked to a higher risk of AD and poor response to therapies in clinical trials.

Normal APOE carries lipids between various cells in the brain. Earlier studies have suggested that E4 particles generated by oligodendrocytes carry fewer lipid molecules than other APOE variants. Work by Bales *et al.* suggests that in E4 carriers, the smaller E4 particles compete with full-sized APOE variants [1a], leading to insufficient lipid delivery and higher levels of β -amyloid (A β) aggregation. That, in turn, leads to neuronal degeneration [2] and, eventually, to AD [3]. Preclinical efforts at **Eli Lilly and Co.** and **Pfizer Inc.** aim to stimulate normal APOE expression in E4 carriers to overcome this defect.

An alternative theory proposed by Mahley *et al.* postulates that E4 originating from within neurons is proteolytically cleaved into fragments that interfere with mitochondrial activity [1b], leading to neurodegeneration and AD. Other APOE variants are not subject to this proteolytic processing and are thus not toxic. Preclinical efforts at **Merck & Co. Inc.** aim to block the cleavage of E4.

Reserve University. He said Mahley's studies involved cell-culture models that do not take into account APOE's normal role as an extracellular lipid transporter and its effect on A β deposition, which is thought to drive AD.

"A lot of other people have tried those experiments and haven't been able to ascertain whether E4 is toxic," said Landreth.

Mahley countered that neuronal intracellular APOE is distinct from the lipid-carrying extracellular form derived from nearby glial cells. He also argued that A β aggregation is likely to be a downstream consequence of E4's harmful effects on neuronal health and is thus not the primary cause of pathology in E4 carriers.

"There has been a very narrow-minded approach to the pathogenesis of AD," said Mahley. "A strong group of very good investigators who convinced themselves that A β was the major factor in AD has hijacked the field."

Amyloid burden of evidence

Indeed, evidence for the mechanism espoused by Landreth—that excessive A β deposition is due to defective lipid transport by E4—has been presented by a team at **Eli Lilly and Co.** in *The Journal of Neuroscience*.⁵

The Lilly group monitored the brains and serum of transgenic mice expressing three human isoforms of APOE and human amyloid- β (A4) precursor protein (APP).

Earlier studies in humans had shown that carriers of the APOE2

(E2) isoform have lower A β levels and AD risk than E3 and E4 carriers. The Lilly team saw similar effects of APOE alleles on A β deposition in mice. In addition, they found that levels of APOE itself varied depending on the allele. E4 mice had less APOE than E3 mice, which in turn had less APOE than E2 mice.

The upshot was that APOE levels correlated inversely with A β levels, with E2 mice having the highest APOE and lowest A β . The study was conducted by a team led by Kelly Bales, now a senior principal research scientist at **Pfizer Inc.**, and Steven Paul, EVP of science and technology at Lilly.

To Bales the findings suggest that APOE normally antagonizes A β deposition and that the E4 version of APOE is less able to do so than other variants.

"The dominant role of APOE is to clear A β generated in the brain," said Bales. "Here, we saw isoform-specific effects of APOE on A β clearance."

Paul's paper "is completely in line with the effect of E2, E3 and E4 in humans," said David Holtzman, professor of neurology at **Washington University in St. Louis School of Medicine**. "I think it's very clear that the main effect of E4 on AD pathogenesis is via A β aggregation in the brain."

The Lilly findings are consistent with a mouse study published last year by a team led by David Riddell, associate director of discovery neuroscience at Wyeth.⁶

Riddell said the two studies collectively show that "low levels of E4

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—Robert Mahley,
The J. David Gladstone Institutes

directly contribute to AD and a range of other neurodegenerative disorders by its inability to promote the clearance of A β levels.”

Paul, Landreth and Holtzman believe that the high levels of A β found in E4 patients result from the low lipid-carrying capacity of E4 compared with other forms of APOE (see **Figure 1, “Possible mechanisms of APOE in AD”**).

Opposite strategies

If the primary problem with E4 is inadequate lipid transport, Bales thinks having more of the protein around may be a solution. “I’m beginning to think that even if you’re an E4 carrier, upregulating APOE expression may have a beneficial effect,” she said.

However, according to Mahley’s theory, raising E4 levels would have negative consequences—increasing the production of toxic intracellular fragments and exacerbating the disease.

“If our theory is right—if you can upregulate the expression of APOE—you might find a viable strategy for treating or preventing AD,” said Paul. “However, if Bob Mahley’s right, you wouldn’t want to upregulate E4. We have a bit of a conundrum on what’s the right theory.”

But Merck’s Hargreaves said the two proposed APOE mechanisms are not mutually exclusive. One possibility is that although E4 inside neurons may promote toxicity, boosting lipid transport by E2 or E3 outside of the neurons could help fight A β deposition. If so, increasing levels of APOE could be especially beneficial in E4 heterozygous patients if the toxic effects of E4 can be cancelled out by higher levels of the other, more functional variant.

One way to raise APOE levels is to agonize the liver X receptor (LXR), a transcription factor that regulates lipid metabolism. Indeed, preclinical studies suggest that LXR agonists can reduce A β levels *in vivo*.⁷

Riddell said the main challenge to agonizing LXR to treat AD is avoiding unwanted peripheral side effects involving lipid metabolism.

Wyeth and partner **Karo Bio AB** discontinued development of LXR-623 in 2007 after patients in a Phase I atherosclerosis trial developed hyperlipidemia.

Wyeth is being acquired by Pfizer.

Osherovich, L. *SciBX* **2**(24); doi:10.1038/scibx.2009.962

Published online June 18, 2009

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