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Cholesterol metabolism ADds up

By Lev Osherovich, Senior Writer

Reports by independent teams at **Dartmouth College** and **Albert Einstein College of Medicine of Yeshiva University** suggest that blocking production and transport of cholesteryl esters, the extracellular form of cholesterol, could be useful for treating Alzheimer's disease.[1,2] The findings reveal two cholesteryl ester metabolism enzymes, ACAT1 and CETP, as potential AD targets and could represent a repurposing opportunity for a quartet of pharma companies that have studied the enzymes in the cardiovascular space.

Cholesteryl esters consist of interlinked molecules of cholesterol and are the primary form of cholesterol in the plasma membrane and outside the cell. Cholesteryl esters are made in the endoplasmic reticulum by sterol O-acyltransferase 1 (SOAT1; ACAT1).

Cholesteryl ester transfer protein (CETP) controls the flow of cholesterol between two types of lipoprotein particles, which are protein-lipid complexes that move cholesterol between cells. CETP shuttles cholesteryl esters from high-density lipoprotein (HDL) particles to low-density lipoprotein (LDL) particles.

Hints of a role for cholesterol transport in AD arose in the 1990s when human genetic studies identified variants of apolipoprotein E (APOE), the protein component of lipoprotein particles, as key genetic risk factors.

Together, cholesterol acyltransferase and CETP act to increase cholesterol levels in cardiovascular disease–linked LDL particles and for this reason have been considered good targets for dyslipidemia drugs.

Although the precise role of lipoprotein particles in AD pathogenesis is under debate,[3] the new findings "point to the intracellular esterification process" as a targetable space upstream of APOE, said Samuel Gandy, professor of neurology and psychiatry at **Mount Sinai School of Medicine**.

ACAT1 in mouse

The Dartmouth group focused on ACAT1's role in AD and built on earlier findings from **Massachusetts General Hospital** (MGH) showing that a discontinued cholesterol acyltransferase inhibitor, **Pfizer Inc.**'s CP-113,818, reduced AD pathology in mice.[4]

Team leader Ta-Yuan Chang, professor of biochemistry at Dartmouth, said it has been difficult to study how cholesterol acyltransferase inhibitors improve AD pathology because there are several homologs expressed in different parts of the body. Thus, broad-spectrum inhibitors like CP-113,818 may have multiple effects inside and outside the brain.

To get around this problem, Chang's team compared knockouts of the two major murine cholesterol acyltransferases, ACAT1 and ACAT2 (SOAT2). In mouse brain lysates, ACAT1 knockouts showed less enzyme activity than both wild-type mice and ACAT2 knockouts, suggesting that ACAT1 was the most relevant target in AD.

The team then made ACAT1 knockout mice that overexpressed human amyloid-β precursor protein (APP), which is converted into toxic β-amyloid (Aβ) peptide fragments that form the characteristic amyloid plaques of AD. ACAT1 knockouts had lower Aβ than wild-type animals that overexpressed APP.

Chang's results were published in the *Proceedings of the National Academy of Sciences*.

Chang's study "puts a lot of attention on ACAT1 as a potential AD target," said Dora Kovacs, associate professor of neurology at MGH and **Harvard Medical School** and the senior author of the earlier CP-113,818 study. This may convince big pharma to reexamine CETP inhibitors as potential AD drugs, she said.

The challenge now, said Kovacs, is to understand exactly why low ACAT1 activity improves AD pathology. Inhibiting ACAT1 sets off a series of changes in lipid metabolism, any one of which could potentially influence the course of the disease. Possible mechanisms include changes in APP processing caused by altered membrane lipids or direct effects on Aβ, but Kovacs said "there is no single mechanism that is convincing at the moment."

Chang thinks the effect of ACAT1 deletion is mediated by 24(*S*)-hydroxycholesterol (24SOH), a metabolic intermediate of cholesterol that builds up when ACAT1 is missing. Cultured murine hippocampal slices treated with 24SOH had lower levels of APP than mock-treated hippocampal slices, in line with earlier evidence showing that altering the lipid composition of neurons reduces Aβ production.

"Chang shows that 24(*S*)-hydroxycholesterol has a protective effect," said Gandy. "This is a convincing experiment."

Jin Ye, assistant professor of molecular genetics at **The University of Texas Southwestern Medical Center at Dallas**, was more cautious. Chang's paper "is fascinating, but they don't have a mechanism," he said.

Ye noted that the effect of ACAT1 deletion on overall cholesteryl ester levels was relatively modest, suggesting the protein's role in AD may be independent of its enzymatic activity on cholesterol.

"The critical thing is to see whether the effect of ACAT1 deletion has anything to do with cholesteryl esters," he said.

Ye recommended examining whether point mutations that inactivate ACAT1's enzymatic activity have the same effect as wholesale deletion of ACAT1.

Kovacs suggested further genetic and pharmacological experiments to confirm that increased 24SOH is indeed protective. "If their model is right, blocking 24(*S*)-hydroxycholesterol synthesis in Chang's animal model should make things worse," she said.

Chang has filed for a patent on targeting ACAT1 to treat AD, adding that Dartmouth holds three earlier patents on ACAT1 uses and assays. The patents are available for licensing.

Healthy humans

The Albert Einstein researchers approached the cholesteryl ester pathway from a different angle—human genetics. A team led by Richard Lipton, professor of neurology and epidemiology, found that a loss-of-function variant of the *CETP* gene delayed the onset of AD in a small cohort of human subjects.

Earlier studies showed that carriers of the *CETP* variant had higher levels of protective HDL particles than people with normal *CETP*.[5] In another previous study,[6] "we saw there was a connection between the CETP mutation and longevity in Ashkenazi Jewish centenarians," said Lipton. "We also found that carriers had better mental status."

To prospectively test whether the loss-of-function *CETP* allele affected AD progression, Lipton's team genotyped a cohort of 523 healthy elderly people of various ethnicities and tracked them over the course of 15 years, testing annually for AD onset.

The team found that carriers of the *CETP* allele developed AD more slowly than noncarriers. Overall, homozygous carriers of the allele had about one-third the risk of developing AD compared with homozygotes for an equally common but more functional *CETP* variant (p=0.04).

Heterozygous carriers of the *CETP* allele had about half the risk compared with homozygous noncarriers.

The powerful effect of the *CETP* variant on AD risk observed by Lipton runs counter to results from larger genomewide association (GWA) studies.

"It matches nicely with the ACAT1 story, so it's neat if it's true. But the genetic data are not compelling," said Rudolph Tanzi, professor of neurology at MGH. "Five previous studies show that there's nothing at this locus to say that this is a protective gene."

According to Tanzi, a meta-analysis of the five earlier studies showed that the *CETP* allele modestly increases the risk of AD, thus contradicting Lipton's conclusions.

Lipton acknowledged that his team's sample size was small compared with the standard GWA approach. However, he countered that his study was prospective and was designed to tease out genetic factors that might be overlooked by conventional GWA studies.

"Typically when you do a GWA study, you look for genes associated with increased, not decreased, risk," said Lipton. "You look for genetic variants that are overrepresented in AD cases, but I don't think much work has been done looking at protective genes."

Lipton said the protective effect of CETP may have been overlooked because of a statistical anomaly called prevalence-incidence bias, in which protective mutations that also affect longevity distort the results of GWA studies.

"You're less likely to develop AD if you carry the protective CETP allele," he said. "But it's also associated with longer survival after diagnosis, so carriers of the gene are overrepresented" among patients.

In retrospective approaches like GWA studies, the protective effect of the *CETP* gene and its overrepresentation in patients "cancel each other out," Lipton added.

He noted that the loss-of-function CETP allele "appears to protect against many types of vascular disease. One hypothesis is that CETP exerts its effect purely in the vascular system, or it could perhaps be like the mechanism in the ACAT1 paper, where there's less accumulation of amyloid in the brain" as a result of lower cholesteryl ester levels or higher 24SOH levels.

UT Southwestern's Ye noted that mice do not have a *CETP* gene, which will frustrate preclinical testing of CETP inhibitors as potential AD therapeutics. He recommended overexpressing human *CETP* in transgenic knock-in AD mice to see whether this would exacerbate the neurodegenerative disease.

Meanwhile, to determine whether the protective effect of lower CETP activity depends on cardiovascular benefits, Lipton hopes to follow up with a *post hoc* analysis of AD incidence in patients enrolled in CETP inhibitor trials.

He has filed a patent on targeting CETP to prevent AD. This patent is available for licensing from Albert Einstein.

Tox talk

For industry, the biggest challenges in targeting ACAT1 and CETP are concerns about the safety and inadequate brain permeability of earlier compounds.

"Cholesterol acyltransferase inhibitors are traditionally difficult to develop because they have drastic side effects," said Kovacs.

In 2003, Pfizer dropped its most advanced cholesterol acyltransferase inhibitor, avasimibe (CI-1011), after the compound failed Phase III testing in dyslipidemia.

Daiichi Sankyo Co. Ltd. also was developing a cholesterol acyltransferase inhibitor, pactimibe (CS-505), but discontinued the compound in 2005 after a failed Phase III trial in coronary atherosclerosis.

Pactimibe missed the primary endpoint and showed unfavorable effects in the secondary endpoints.[7] Pfizer has not disclosed data from its avasimibe trial, which has frustrated some academics.

"The main stigma around these compounds is the unknown experience of Pfizer," said Gandy. "If they're never going to release their data, it's up to academics to figure out if there were any potential toxicity issues."

CETP is the target of anacetrapib (MK-0859) from **Merck & Co. Inc.** and dalcetrapib (JTT-705; R1658; RG1658) from **Japan Tobacco Inc.** and **Roche**. Both are in Phase III trials for dyslipidemia and atherosclerosis.

Another CETP inhibitor, Pfizer's torcetrapib, was discontinued after a Phase III trial showed a higher risk of death in dyslipidemia patients taking the compound.[8]

Chang noted that in Pfizer's failed trials of avasimibe and torcetrapib, the ACAT1 and CETP inhibitors were given in combination with statin therapy. He suggested this could have produced unfavorable cross-interactions in peripheral organs such as the liver.

Ye is concerned about the potentially deleterious consequences of inhibiting CETP. Even though blocking CETP would redistribute cholesteryl esters from LDL to HDL in the periphery, the effect of CETP inhibitors on brain cholesterol is harder to predict.

"The main stigma around these compounds is the unknown experience of Pfizer. If they're never going to release their data, it's up to academics to figure out if there were any potential toxicity issues."

—Samuel Gandy, Mount Sinai School of Medicine

"We need CETP for a reason, but we don't quite know why yet," said Ye.

If the brain-specific role of cholesteryl ester metabolism in AD can be shored up, it could renew medicinal chemistry efforts for these targets, said Chang.

"We need to develop new, more specific and safe ACAT1 inhibitors that can overcome the blood brain barrier," he said.

Synthesizing such inhibitors is exactly the plan for Kovacs. MGH has filed a patent on Kovacs' method of subcutaneous delivery of ACAT1 inhibitors to treat AD.

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COMPANIES AND INSTITUTIONS MENTIONED

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