Meeting Report

Trial News: Woes, New Approaches

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HONOLULU: Tomm40 REPORTED TO TRACK WITH BRAIN ATROPHY, COGNITION

Tomm40 has created a stir in Alzheimer's disease (AD) research with the recent proposal that variable-length polymorphisms of this gene, which lives near apolipoprotein E (ApoE) on chromosome 19, can help predict at what age a person may develop late-onset AD (LOAD) [1,2]. This finding came from analysis of several small cohorts in which ages of LOAD onset were determined retrospectively. Now, new work on Tomm40 (aka translocase of the outer mitochondrial membrane 40) confirms the age-of-onset connection in a small prospective study of cognitively normal adults who went on to develop mild cognitive impairment (MCI) or AD. Scientists reported the preliminary findings at the International Conference on Alzheimer's Disease (ICAD) held 10-15 July in Honolulu, Hawaii, along with other data showing that the Tomm40 length variants also correlate with brain atrophy and cognition in asymptomatic middle-aged people. If the results hold up, they could explain why some ApoE3/E3 homozygotes, a supposedly risk-neutral group, have LOAD risk that parallels that of E4 carriers, and may improve stratification of participants in future clinical trials.

Plowing through phylogenetic analyses, researchers led by Allen Roses and Michael Lutz of Duke University in Durham, North Carolina, and Eric Reiman of Banner Alzheimer's Institute in Phoenix, Arizona, came upon a poly-T variant within intron 6 of Tomm40 that greatly improved predictions for when ApoE3 carriers might develop AD. In particular, among autopsy-confirmed ApoE3/4 patients, those with two copies of the long Tomm40 variant (more than 20 poly-T repeats) – aka the "long/longs" – developed AD about eight years earlier than the "short/longs," who had a copy of the short Tomm40 variant (20 or fewer poly-T repeats) along with the long version [3]. In this earlier study, the researchers analyzed several independent cohorts of patients whose LOAD onset ages were documented in medical records.

Because retrospective data can be unreliable, the scientists sought to reproduce those findings in prospective studies of people with known ApoE and Tomm40 status who are being followed with neuropsychological testing for future development of MCI or AD. On an ICAD poster, Richard Caselli of Mayo Clinic, Scottsdale, Arizona, and colleagues including Reiman and Roses, reported preliminary data from 30 participants in the first of several prospective studies in progress for five to 19 years. In short, the results came out as predicted: the "long/long" group developed incident MCI or AD about nine years earlier than the "short/longs" (onset age 73 versus 82). The cohort was too small to correct for ApoE genotype, Caselli noted, but the earlier age of onset in the long/longs did hold for both ApoE3/4 ($n = 10$) and ApoE3/3 ($n = 11$) subgroups.

The Tomm40 length variants also seem to track with other defining measures of AD – namely, brain atrophy and cognition. These preliminary studies involved participants of a longitudinal cohort study called WRAP (Wisconsin Registration for Alzheimer's Prevention) that started in 2001 under the leadership of Mark Sager at the University of Wisconsin in Madison. Participants around a mean age of 54 enter the study asymptomatic and get cognitive testing every few years. Some also receive brain imaging through ancillary studies

led by Sterling Johnson, also at the University of Wisconsin. Forty-six percent of the subjects are ApoE4-positive. Mining the data on 1,400 study participants, the researchers uncovered differences in white matter (measured by diffusion tensor imaging), brain activity (measured by functional magnetic resonance imaging of AD-relevant areas such as hippocampus), and certain measures of learning. Somewhat surprisingly, "the differences were based on whether or not their parents had AD," Sterling said in his ICAD talk. "ApoE was not really giving us all the explanatory power we needed. So we looked for other genetic and lifestyle factors that might predict [the parental history connection]."

Puzzling over these findings, which were reported last fall at the Clinical Trials on Alzheimer's Disease meeting in Las Vegas, Nevada, the researchers recalled the recent buzz over Tomm40 and wondered whether Tomm40 length variants might help tease out the differences they had seen related to family history. Johnson focused on E3 homozygotes because of their curious bimodal distribution on AD risk charts. Though E3 has historically been regarded as the risk-neutral ApoE variant, in reality, there is a subgroup of E3 carriers who seem just as prone to AD as people with the high-risk E4 allele. Johnson's team analyzed 120 healthy E3/3 WRAP participants (mean age 57), assessing their Tomm40 status and measuring gray matter volume in the ventral posterior cingulate and precuneus (brain regions affected early in AD) by structural MRI. Comparing participants with two "short" Tomm40 alleles to those with two "long" Tomm40 alleles, the researchers found that the latter had lower gray matter volume in the analyzed brain areas.

Sager and colleagues analyzed more than 700 asymptomatic WRAP participants (mean age 54) with a family history of AD, and similarly compared short/short and long/long subgroups for cognitive differences. Consistent with their greater brain atrophy, the long/long subjects, regardless of ApoE genotype, did worse on several measures of the Auditory Verbal Learning Test.

If confirmed in larger samples, the findings may be "very important to explain why some E3/3s develop AD at earlier ages," said Yadong Huang of Gladstone Institute of Neurological Disease at the University of California, San Francisco. Among E3 homozygotes, about a quarter have the long/long Tomm40 genotype that confers greater AD risk.

The new data may also hint at possible synergistic effects between ApoE and Tomm40 at mitochondria, which help maintain synapses and falter in early AD. Huang and colleagues have shown that proteolytic fragments of ApoE, which form more commonly from E4 than E3, interact with neuronal mitochondria, throwing off membrane potential and contributing to cytoskeletal structures that contain phosphorylated tau [4]. Tomm40 is a mitochondrial membrane protein needed for shuttling proteins into the organelle. "If Tomm40 causes problems, then when the ApoE fragment comes in, that might make it even worse," Huang speculated.

Whether and how the Tomm40 poly-T variants influence mitochondrial function to begin with remain unclear. Because they are intronic, the polymorphs do not affect Tomm40's protein sequence and have yet to demonstrate effects on expression, leaving in question their biological effect in neurons, suggested John Hardy of University College London, UK, in an e-mail to Alzheimer Research Forum (ARF). In his view, it seems more parsimonious that LOAD risk variability derives from ApoE promoter polymorphisms that are known to govern expression of ApoE [5,6] and which are in the same haplotype block as TOMM40. Genetic variability in the ApoE promoter was first shown to be important in risk assessment in AD in 1994 [7]. Hardy noted that this mechanism plays out in another disease, where missense variants in complement factor H are the major risk factor for macular degeneration but in which genetic variability in the promoter is known to both influence gene expression and predisposition to disease [8].

Still, the ICAD data suggest the Tomm40 length variants "clearly have some effect – especially on E3/3s, where there are no confounding effects due to E4," Huang told ARF. Whether those effects involve synergism with ApoE remains to be seen. On the one hand, studies with transgenic mice that express human E4 have shown that E4 alone can drive cognitive decline. That suggests to Huang that E4 messes with learning and memory independent of Tomm40, since mice are unlikely to have the same Tomm40 length variants that have been studied in people. In collaboration with Roses, Huang hopes to do the converse experiment – that is, put the human Tomm40 "long" allele into transgenic mice with or without ApoE4 – to see whether Tomm40 effects require E4.

In the meantime, Roses has submitted an application to the U.S. Food and Drug Administration for a prevention trial in which the Tomm40 genotype would serve as a key criterion for selecting high-risk patients to test an investigational AD drug. The trial, called Opportunity for the Prevention of Alzheimer's

(OPAL) (http://www.opalstudy.org/index.html), would enroll cognitively normal seniors from the ages of 60 to 87 and judge, based on age and Tomm40, whether they are at high or low risk for developing AD in the next five years. Most low-risk participants would go into the placebo arm while the high-risk group is randomized to receive drug or placebo. In this manner, the five-year study would, as Roses hopes, serve a dual purpose: validate Tomm40 as a genetic marker, and test the ability of an investigational AD drug to delay LOAD onset. The trial could start next year, Roses told ARF.

On the basic science front, other studies presented at ICAD added to the emerging connection between insulin and regulation of both amyloid-β (Aβ) and tau. Ewan McNay, University at Albany, State University of New York, in collaboration with Suzanne Craft, University of Washington, Seattle, reported that rat models of type 1 and type 2 diabetes show impaired production, accumulation, and clearance of Aβ. In other studies with wild-type rats, McNay and colleagues reported that small Aβ oligomers known as ADDLs (amyloid-derived diffusible ligands) may mediate these effects. Hippocampal injection with synthetic ADDLs into wild-type rats caused problems on a spatial memory task and with glucose transporter translocation and insulin signaling. Evidence for a potential role for Aβ oligomers also appeared on a poster by Fernanda De Felice, Federal University of Rio de Janeiro, Brazil, and colleagues. In cultures of rat hippocampal neurons, the researchers found that – similar to what occurs in type 2 diabetes – ADDLs trigger abnormal phosphorylation of insulin receptor substrate-1 and that c-Jun N-terminal kinase might be responsible. Cheng-Xin Gong and colleagues at the New York State Institute for Basic Research on Staten Island found depressed insulin-PI3K-AKT signaling in the brains of patients with AD and type 2 diabetes. In quantitative Western analyses of postmortem brain tissue, these researchers correlated the downregulation of insulin-PI3K-AKT signaling components with calpain-1 overactivation and abnormal tau phosphorylation. – Esther Landhuis.

ANTI-Aβ OLIGOMER HEADED FOR PHASE 3 CLINICAL TRIAL

Top-line data announced this week from a Phase II clinical trial of the Aβ oligomer blocking drug scyllo-inositol (aka AZD-103, or ELND005) have given Elan Corporation PLC (http://www.elan.com) and part-ner Transition Therapeutics Inc. (http://www.transition therapeutics.com/) reason to take the compound into Phase III. Overall, the drug did not significantly improve cognition and function in participants with mild to moderate AD. It therefore missed its formal co-primary outcome. Even so, the companies' press release states that AZD-103 had an effect on clinical endpoints in an exploratory analysis. This suggested to the company scientists that a subgroup of patients may have improved on the medication. The compound achieved pre-determined target levels in the cerebrospinal fluid (CSF) and had effects on CSF Aβ, noted Transitions CEO Tony Cruz in a conference call to investors August 10. "Any changes in the biomarkers may be suggestive that the drug is affecting the underlying pathology," Cruz said.

"I tend to be encouraged whenever an AD drug development project moves forward (despite the disappointing track record in pivotal trials)," said Paul Aisen, University of California, San Diego, who was not involved in the trial. "But, of course, we need to examine the specific findings of the Phase II study."

One of several isomers of the cyclic sugar alcohol inositol, scyllo-inositol prevents formation of Aβ oligomers, which are believed to be the most toxic forms of the peptide. The inositol isomer appears to block phospholipids, such as phosphatidylinositol, from promoting Aβ oligomerization. Scyllo-inositol prevents accumulation of Aβ in the brains of mouse models and protects them from cognitive decline.

For this Phase II trial, a total of 351 participants with mild to moderate AD were given 250 mg, 1,000 mg, or 2,000 mg of the drug twice daily. The two top doses were subsequently dropped over safety concerns. The 250 mg dose appeared to have a favorable safety profile so far. According to Cruz, clinical significance was not expected in the trial because it was not sufficiently powered to detect such changes.

Nevertheless, given the safety profile of the 250 mg dose and the effects on CSF and on clinical outcomes in exploratory analysis, the companies, after consultation with "15–20 advisors and key opinion leaders in the field," said Cruz, have decided to move forward into Phase III. Alzforum readers may remember that the γ-secretase modulator flurizan also showed promising signs in Phase II, only to fail in a subsequent Phase III trial. In that case, indications were that the drug failed to find its intended target, as it never reached levels in the CSF that came close to its effective concentration. Alzhemed, an Aβ aggregation inhibitor, also showed promising Phase II data, apparently reducing CSF Aβ

in mild to moderate AD patients, but it, too, failed in Phase III. The field will be hoping for something better from scyllo-inositol. – Tom Fagan.

LILLY HALTS IDENTITY TRIALS AS PATIENTS WORSEN ON SECRETASE INHIBITOR

Eli Lilly and Company (http://www.lilly.com) announced yesterday that it has halted its Semagacestat γ-secretase inhibitor program. Preliminary results from two ongoing Phase III trials [the IDENTITY and IDENTITY-2 (http://www.clinicaltrials.gov/ct2/show/ NCT00762411?term=Alzheimer%27s&id=IDENTITY &rank=1) trials] revealed that the drug not only failed to slow cognitive decline in people with mild to moderate AD, but that it actually made them worse (see company press release, http://newsroom.lilly.com/releasedetail. cfm?ReleaseID=499794). Cognition as measured by the ADAS-cog and Activities of Daily Living Scale declined faster in volunteers in the treatment arms compared to those on placebo. "Obviously, we are clearly disappointed about the results for patients, their caregivers, and everyone else," said Eric Siemers, Medical Director, Alzheimer's Disease Team at Lilly, in an interview with ARF.

"The billion-dollar questions on everyone's mind are whether this is a body blow to the amyloid theory and what this means for all the planned prevention trials using similar drugs," suggested Murali Doraiswamy, Duke University Medical Center, Durham, North Carolina. Siemers said that there are numerous explanations for the outcome, among them being the possibility that lowering $A\beta$ in the brain makes cognition worse. That would be a difficult pill to swallow for supporters of the amyloid hypothesis, and would raise questions about pursuing other γ-secretase inhibitors and BACE inhibitors as therapies for AD. "I would be surprised if that was the explanation, but at this point we really do not know," said Siemers.

Other possible explanations for the accelerated deterioration envisage roles for C-terminal fragments of $A\beta$PP in cognition. Blocking γ-secretase would lead to an increase in the β-secretase product C99, and a decrease in the $A\beta$PP intracellular domain (AICD), which is cleaved from C99 by γ-secretase. "Perhaps the increase in C99 may have some deleterious effect, or maybe decreasing AICD has an adverse effect," suggested Siemers. Either scenario would raise questions

about pursuing any type of γ-secretase inhibitor, trials of which are currently ongoing.

Alternatively, the disappointing trial outcome may have nothing to do with $A\beta$PP or $A\beta$. "The general concern about γ-secretase inhibitors has been mechanism-based toxicity," according one of the trial site investigators, who wished to remain anonymous. "The factor(s) that led to faster clinical decline need to be understood in order to guide progress with secretase inhibition or modulation, or, more broadly, anti-amyloid treatment in the future."

Toxicity concerns stem from γ-secretase's penchant for processing a multitude of substrates. In particular, researchers in the field have tried to develop modulators that, while lowering production of the more toxic $A\beta_{42}$, do not block cleavage of Notch, a crucial signaling molecule that regulates the fate of a wide variety of cell types. Semagacestat is not "Notch sparing," and even though the doses used in the IDENTITY trials (140 mg per day) were conservatively chosen to reduce $A\beta$ production by about 25% daily, Notch signaling may have suffered. "This needs close investigation, especially since the increase in skin cancer in the treated patients also suggests Notch signaling inhibition," suggested Bart De Strooper, KU Leuven, Belgium. "The implication is that we should explore now, even more, Notch- (and other substrate-) sparing modulation of γ-secretase activity (e.g., blocking selectively Aph1B-γ-secretase)."

Siemers suggested that one positive thing that came from the trials was a justification for the rationale of using biomarker analysis to determine adequate dosing. In Phase II trials, the company used CSF analysis to determine whether the drug got into the brain and had an effect. "The fact that people were worse shows that we did get [the drug] into the brain and have an effect," said Siemers. "Obviously it is not the effect that we wanted, but in a sense it tells you that this biomarker strategy does help you make some conclusions."

Siemers said that Lilly will eventually release more data from the trials, which together enrolled more than 2,600 patients worldwide. Dosing has stopped, but the company plans to follow patients for at least another six months. Lilly has also been going through a major restructuring that has led to some downsizing.

Tom Fagan.

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