TOMMorrow's AD marker

By Lev Osherovich, Senior Writer

Since its discovery as an Alzheimer's disease risk factor in the early 1990s, mutations in APOE have been implicated in up to 50% of AD cases. Now, the researcher who first detected APOE's role in AD is touting a new theory: he points to APOE's immediate chromosomal neighbor, the gene for a mitochondrial protein called TOMM40, as a key contributor to the neurodegenerative disease.

Allen Roses, director of the **Deane Drug Discovery Institute at Duke University**, presented his TOMM40 (translocase of outer mitochondrial membrane 40 homolog) findings at the International Conference on Alzheimer's Disease (ICAD) in Vienna in July. Although the work has not yet been published, Roses is already heading a company, **Zinfandel Pharmaceuticals Inc.**, which plans to commercialize the discovery.

Roses formerly was SVP of genetics research and pharmacogenomics at **GlaxoSmithKline plc**. During an earlier stint at Duke, he led a team that identified the E4 variant of APOE as the most powerful risk factor for the most common, sporadic form of AD. Apolipoprotein E (APOE) is a cholesterol-ferrying protein involved in lipid metabolism in the cardiovascular system and the brain. Several genetic variants of APOE occur in humans, each with different biochemical properties and effects on AD.

E4 carriers have an earlier age of onset for AD and a more aggressive course of disease compared with carriers of other APOE alleles. E4 carriers also have proven more resistant to several investigational therapies than noncarriers, leading companies to stratify such patients into separate arms in ongoing trials.[1]

Roses' earlier discovery of E4's link to AD "is one of the most important and reproducible genetic findings for any complex genetic disorder," said Steven Paul, president of **Lilly Research Laboratories**, the research arm of **Eli Lilly and Co.**, and SVP of science and technology at Eli Lilly.

However, some patients without the E4 allele develop disease earlier and respond more poorly to therapy than others. Geneticists turned to complex methods of genomic analysis to identify other AD susceptibility loci but thus far have not found others with effects as powerful as APOE.

Roses now thinks he has found one and says his data suggest that *TOMM40* is a major susceptibility gene that accelerates disease independently of E4.

Indeed, the findings may help explain why some carriers of a common, benign form of APOE called E3 develop AD earlier than others.

Because the primary causes of AD and the mechanism of APOE's role in the disease are hotly debated, academic and industry researchers have met Roses' claims about TOMM40 with caution. The real test of whether TOMM40 status can predict rapid disease progression will be a prophylactic AD trial using TOMM40 as a diagnostic marker—a trial for which Zinfandel is already recruiting patients.

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>
> ***—Allen Roses, Zinfandel Pharmaceuticals Inc.***

Genetic detectives

Roses told *SciBX* that studies of *TOMM40* began during his tenure at GSK, where he was in charge of hunting down AD risk genes. Genome-wide association studies by GSK and others hinted that the region around *APOE* contributed more to AD risk than could be accounted for by E4 alone, leading him to suspect that nearby genes were hidden players in pathogenesis.

In 2005, Roses' team at GSK sequenced the chromosomal region encompassing *TOMM40* and *APOE* in patients and controls. They used phylogenetic analysis to arrange individual sequences into related groups that reflected the evolutionary history of the chromosomal region.

"We came up with a very interesting region of about 10,000 base pairs" with two distinct types of sequence, said Roses. All E4 carriers, as well as a fraction of E3 carriers, had one variant of the region, whereas most E3 carriers had the other variant.

However, it was not clear which specific mutations within the variable region were most closely associated with AD, so GSK dropped the project. Shortly thereafter, Roses left the company to head drug discovery at Duke. Between jobs, he personally financed the sequencing of additional AD cases and controls and narrowed down the most statistically powerful effect on AD risk to a region within *TOMM40*.

Roses' team at Duke then discovered a sequence length variant in a noncoding region of *TOMM40* that strongly correlated with disease. The team found that E4 carriers always had the longer, high-risk form of *TOMM40*. In E3 carriers, the presence of the long variant of *TOMM40* increased AD risk to a level comparable with that for E4 carriers (*see* **Figure 1, "*TOMM40* in Alzheimer's disease"**).

Overall, carriers of the long form of the variant had nearly 50% higher risk of developing disease than short-form carriers.

Roses then examined the relationship between the *TOMM40* length polymorphism and age of onset in E3 homozygotes, in which the effect of *TOMM40* could be studied independently of E4.

"When the long form of *TOMM40* is present in patients with E3, it appears to cause an average age of onset similar to that in E4 carriers, at about age 70," said Roses. In contrast, E3 patients who had only the short form of *TOMM40* had an average age of onset of 78 (p=0.03).

Doubting TOMM

Roses' findings have not yet undergone peer review, and as a result researchers polled by *SciBX* had mixed opinions on whether the findings would stand up to scrutiny.

Dale Schenk, EVP and CSO of **Elan Corp. plc**, said Roses' hypothesis could explain variation in AD risk independently of E4, "which is why it's so interesting and provocative." However, Schenk cautioned that the TOMM40 discovery must be independently validated before gaining acceptance in the AD community.

"Almost any genetic finding needs to be replicated before other geneticists buy into it," Schenk told *SciBX*.

Last month, **Johnson & Johnson** said it will form a new company

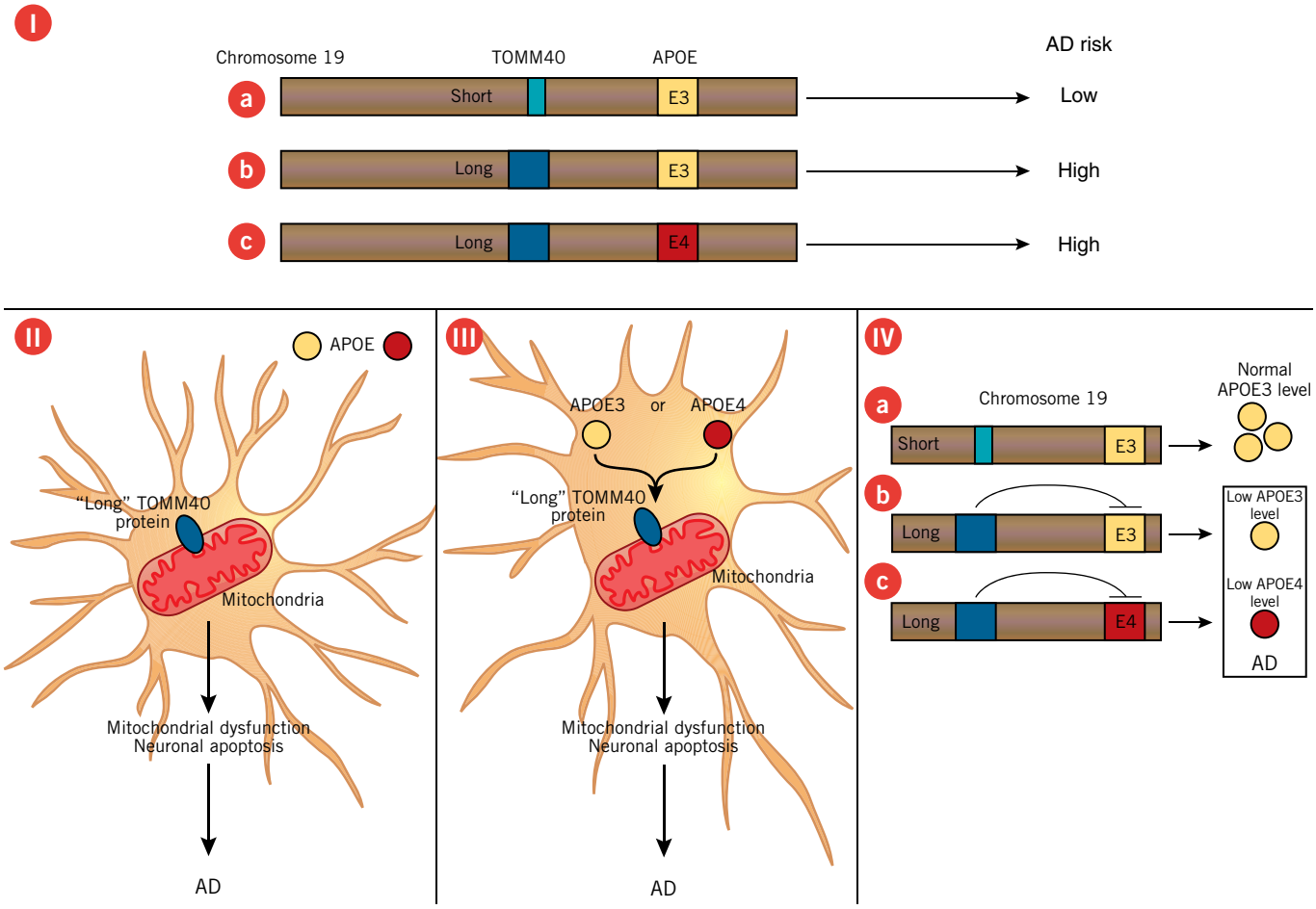

Figure 1. *TOMM40* in Alzheimer's disease. At the International Conference on Alzheimer's Disease in Vienna last month, Allen Roses presented genetic evidence that sequence length variation within the *TOMM40* (*translocase of outer mitochondrial membrane 40 homolog*) gene affects AD risk. The *TOMM40* gene, which encodes a mitochondrial protein, lies on chromosome 19 adjacent to the gene encoding apolipoprotein E (APOE), a well-known AD risk factor. DNA sequencing of 340 individuals revealed two forms of a noncoding region within the *TOMM40* gene.

(**I**) The most common form contains a short sequence repeat and correlates with an average age of AD onset of 78 years in carriers of the AD-neutral *APOE* allele *E3* [**a**], whereas an alternative, longer repeat sequence correlates with average age of AD onset of 70 years in *E3* carriers (n=105, $p<0.03$) [**b**]. The long form is also present in nearly all carriers of *APOE* allele *E4* [**c**], which was previously shown by Roses to correlate with early AD onset.

Three possible mechanisms could explain the contribution of TOMM40 to AD.

(**II**) APOE functions outside of the neuron, physically separated from an abnormal form of TOMM40. Abnormal TOMM40 could lead to mitochondrial dysfunction, apoptosis and eventually AD in an APOE-independent manner.

(**III**) Alternatively, intracellular APOE protein and TOMM40 protein could interact at the mitochondrial surface to disrupt mitochondrial metabolism, leading to apoptosis and AD.

(**IV**) Finally, length variation in the *TOMM40* gene could influence the expression of the adjacent *APOE* gene. The short form of *TOMM40* could lead to strong transcription of *APOE* and thus normal E3 protein levels that protect against disease [**a**]. However, the long form of *TOMM40* could dampen expression of *E3* [**b**] or *E4* [**c**], leading to inadequate APOE protein levels and subsequently to AD.

to acquire the rights of Elan to an AD immunotherapy program that is partnered with **Wyeth**. The program includes four products targeting β-amyloid (Aβ).

Rudolph Tanzi, professor of neurology at **Massachusetts General Hospital**, doubts that *TOMM40* is a significant player in AD. He noted that many other sequence variants besides the *TOMM40* length variant are tightly linked with the *APOE* locus and that Roses' methods don't conclusively prove that the length variant causes accelerated disease onset.

Instead, Tanzi suggested the apparent association between AD and *TOMM40* variants could be a statistical fluke due to small sample size or the powerful effect of E4 and a counteracting effect by E2, another *APOE* variant that protects against AD.

"I honestly think that it's nothing more than a reflection of its chromosomal association with *APOE*," said Tanzi.

Roses countered that both he and GSK had collectively sequenced a sufficient number of patients to dispel statistical doubts. He said the conclusions hold up even when the effect of E2 is excluded.

Alison Goate, professor of genetics at **Washington University in St. Louis**, told *SciBX* she thinks Roses' data corroborate earlier genomewide association studies, but she was cautious about his interpretation.

"People have previously found polymorphisms in *TOMM40*, but it wasn't possible to disentangle those variants from the correlation with E4," she said.

Goate did note that Roses' deep-sequencing strategy is "the next step forward," giving the clearest picture yet of variation near the *APOE* locus and thus teasing out genetic effects that are hard to find using conventional genomewide association analysis.

"When you do deep sequencing, you find all the variants in the genomic region that show association with disease," she said. "If you have two groups of patients that differ in their age of onset, you can ask what other sequence variants correspond with the disease."

Chang-En Yu, associate professor of gerontology and geriatric medicine at the **University of Washington School of Medicine**, leaned more into Roses' camp. "Though people think E4 is the main genetic cause of AD, we cannot completely explain AD risk" using E4 alone, he said.

In 2007, Yu and colleagues reported that *TOMM40* polymorphisms were tightly linked to higher AD risk in Caucasians.[2] Earlier this year, a study by Japanese researchers showed similar results in Japanese AD patients.[3]

"Nobody can jump to a solid conclusion at this point, but I think that *TOMM40* is a very good candidate gene," said Yu.

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Mechanism questions

Even if the genetic association between *TOMM40* and AD is validated, how the gene influences AD remains opaque. Several models are possible, each with different implications about targeting *TOMM40* with therapeutics.

Because TOMM40 helps transport other proteins into the mitochondrial interior, defects in TOMM40 could compromise mitochondrial function, weakening neuronal health independently of APOE's role in AD (*see* **Figure 1.II**).

Roses favors the idea that TOMM40 interacts with an intracellular, neuronal form of APOE that has been implicated in AD by researchers at the **Gladstone Institute of Neurological Disease**. According to this theory, defective TOMM40 is harmful in its own right but could also enhance the toxic effects of neuronal APOE, leading to mitochondrial dysfunction, apoptosis and neuron loss (*see* **Figure 1.III**).

Although the length polymorphism occurs in a noncoding region of TOMM40, Roses thinks the polymorphism could affect *TOMM40* mRNA or protein levels, which in turn could affect mitochondrial activity and TOMM40's interactions with APOE.

"We and a couple of other labs have shown that E3 and E4 bind to the outer mitochondrial membrane by differentially binding to TOMM40," said Roses. Thus, he believes that TOMM40 protein could be a direct player in mitochondrial dysfunction in AD.

An alternative explanation may be that variations in *TOMM40* influence the expression of the nearby *APOE* gene through a knock-on effect (*see* **Figure 1.IV**).

The possibility that *TOMM40* polymorphisms affect *APOE* gene expression is "a much more reasonable argument," said Schenk. "It's been known for some time that at the transcriptional and translational level, there are differences in *APOE* expression" caused by nearby DNA.

Indeed, Yu's team recently reported that *TOMM40* polymorphisms correlated with different levels of *APOE* mRNA and protein in postmortem samples from AD patients.[4]

Goate said her team has also seen *TOMM40* mutations affecting levels of Aβ, the aggregation-prone protein fragment most closely associated with AD.[5]

"I'm agnostic about the mechanism" of TOMM40's role in AD, said Goate. "But I certainly believe that there's something more to it than APOE."

Roses' team at Duke is testing the three possibilities by examining mitochondrial activity and *APOE* expression in mice bearing *TOMM40* transgenes either together or separately from *APOE* transgenes.

The mitochondrial link

If TOMM40 protein is indeed a direct player in AD, mitochondria may be key therapeutic targets.

"Roses' data imply that a mitochondrial protein may have relevance to AD," said **Medivation Inc.** CMO Lynn Seely. The company's Dimebon latrepirdine is an oral small molecule believed to enhance mitochondrial function. The compound is in a Phase III AD trial and is partnered with **Pfizer Inc.**

Roses believes that the best way to counteract *TOMM40* and *APOE* dysfunction is to increase mitochondrial numbers. Peroxisome proliferation–activated receptor-γ (PPARG; PPARγ) agonists could serve this purpose because they also stimulate mitochondrial proliferation.

An earlier effort to test whether PPARγ agonists could do this was run by GSK. There, Roses oversaw Phase II and III trials of the PPARγ agonist Avandia rosiglitazone to treat moderate-to-advanced AD. Earlier this year, GSK reported that an extended-release formulation of

Avandia failed a Phase III trial, and the company halted further development of the drug in AD.

Roses attributed the clinical setback to a patient population that was too far advanced in the disease process to benefit from higher numbers of mitochondria. "As a treatment where the cells are already dead or dying, it won't be as effective," he noted.

Zinfandel, where Roses is president and CEO, is collaborating with clinics in Russia to identify a large number of middle-aged *TOMM40* long-form carriers and noncarriers who have not yet developed AD. Roses plans to treat these individuals with low doses of a to-be-determined PPARγ agonist over several years. If his theory is correct, *TOMM40* long-form carriers will develop AD more quickly than noncarriers, but disease progression in both groups could be slowed by the PPARγ agonist.

The study should thus test two hypotheses: the role of long-form *TOMM40* in accelerated AD progression and the ability of PPARγ agonists to ameliorate AD.

Roses said that running such a trial in Russia is attractive because of the country's relative genetic homogeneity and good medical records. He also noted the regulatory challenge of doing a prophylactic trial using an unvalidated biomarker and a therapeutic agent with a history of adverse cardiovascular events at higher doses.

"Getting the FDA to do something that hasn't been done before is very difficult," he said.

Roses has filed patents for the use of *TOMM40* repeat polymorphisms to predict AD risk and on new methods of identifying genetic diseases used in his studies. The diagnostic technology is being licensed to **Shiraz Pharmaceuticals Inc.**, another company headed by Roses.

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COMPANIES AND INSTITUTIONS MENTIONED

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Elan Corp. plc (NYSE:ELN), Dublin, Ireland
Eli Lilly and Co. (NYSE:LLY), Indianapolis, Ind.
Gladstone Institute of Neurological Disease, San Francisco, Calif.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
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