Cleaving schizophrenia

By Lev Osherovich, Senior Writer

A study published in the *Proceedings of the National Academy of Sciences* points to γ -secretase, a major Alzheimer's disease target, as a new player in schizophrenia.¹ Although AD work has focused on γ -secretase antagonists, agonists of the brain enzyme could have applications in schizophrenia if questions about timing and specificity can be answered.

Historically, the proteins that influence schizophrenia have been hard to identify, and, as a result, marketed therapies are only a stop-gap for psychosis. The mouse and cell-culture studies reported in *PNAS* and conducted by a team led by Bart De Strooper, professor of human genetics at the **University of Leuven**, provide new insights into the molecular mechanisms underlying schizophrenia.

γ -secretase is a protease complex that resides in the endosomal compartment underneath the plasma membrane of neurons. Initially discovered as a genetic risk factor for AD, it processes a number of proteins involved in brain development and synaptic activity. The *PNAS* study underscores the importance of γ -secretase in neurological processes beyond its role in producing AD-associated β -amyloid (A β) peptides.

γ -secretase consists of four subunits, and its active site is located within the main subunit (presenilin). The other three subunits—nicastrin, presenilin enhancer 2 homolog (PEN2) and anterior pharynx defective 1 homolog (APH1)—play an essential role in stabilizing the complex and recruiting at least three-dozen different substrates. Digestion of target proteins by γ -secretase liberates activated intra- and extracellular fragments with diverse roles in transcription and intercellular signaling.

Although A β is the most well-known substrate for γ -secretase, other proteins cut up by the enzyme play critical roles in brain development and activity. It is not known how γ -secretase picks its targets.²

De Strooper's study addressed the function of Aph1. Earlier studies showed that a variant of APH1 called APH1A allows γ -secretase to recognize and cut the signaling protein Notch. De Strooper's team examined the function of another APH1 variant called APH1B, linking it to the processing of a schizophrenia-associated protein called neuregulin 1 (NRG1).

Tim Dejaegere, a graduate student and lead author of the study, said the work "connects neuregulin signaling, one of the most important strands of schizophrenia research, to γ -secretase."

Although humans only have the *APH1B* gene, rodents have two identical homologs—*Aph1B* and *Aph1C*. Thus, to study *Aph1B*'s function, it was necessary to knock out both variants in mice. De Strooper's team did that and then characterized the effects of these mutations on brain physiology and behavior.

Although the knockouts developed normally, they responded poorly in an assay of prepulse inhibition (PPI) compared with the response of wild-type controls. PPI, a measure of how well the nervous system adapts to loud noises, is markedly worse in schizophrenic humans and mouse models of schizophrenia than it is in healthy controls.

De Strooper's team found that the PPI defect of *Aph1B* and *Aph1C* double knockouts could be corrected by the antipsychotic drugs haloperidol and clozapine.

Moreover, the knockouts displayed excessive agitation compared with wild-type controls when injected with amphetamine. As antipsychotics antagonize dopamine signaling and amphetamine promotes it, the team reasoned that the knockouts had higher dopamine activity, a hallmark of schizophrenia.

Indeed, the knockouts had higher levels of dopamine metabolites in the ventral striatum, a part of the brain involved in reward and decision-making, than wild-type mice. The consequence of this increase in dopamine activity was a defect in working memory. Although *Aph1B* and *Aph1C* double knockouts could learn the location of a hidden underwater platform as quickly as wild-type mice, the mutant mice later forgot the location and needed more time than wild-type mice to find it again.

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—Steven Wagner,
TorreyPines Therapeutics Inc.

High Nrg1

To determine how disrupting *Aph1B* and *Aph1C* could promote schizophrenia, De Strooper's team looked at the processing of two brain proteins, Nrg1 and v-erb-a erythroblastic leukemia viral oncogene homolog 4 (ErbB4). Both proteins are substrates for cleavage by γ -secretase and had previously been linked to schizophrenia in human and mouse studies.³

The team found that a transmembrane fragment of Nrg1 accumulated in brain homogenates from *Aph1B* and *Aph1C* double knockouts but not from those of wild-type controls. By contrast, ErbB4 processing was unaffected. The team obtained similar results with NRG1-expressing cultured cells treated with small molecule γ -secretase inhibitors.

Finally, the team found that a mutant form of human NRG1 linked to schizophrenia suffered from the same defect in processing caused by *APH1B* and *APH1C* disruption. In cell culture, NRG1 bearing the schizophrenia-associated V321L mutation accumulated as a fragment of the same size as the one caused by γ -secretase inhibition.

The study thus established that some forms of schizophrenia arise because γ -secretase cannot properly cleave NRG1. The results also shed light on how NRG1 normally functions to prevent schizophrenia.

Ordinarily, NRG1 cleavage by γ -secretase produces both intra- and extracellular fragments. The intracellular fragment, which is a transcription factor, triggers a backward signal into the nucleus of the cell, presumably altering gene expression. The extracellular fragment, however, goes on to interact with receptors on the surface of neighboring neurons. Until now, it was not known whether NRG1-related schizophrenia is due to a problem with the intracellular or extracellular portions of the protein.

Knocking out *APH1B* and *APH1C* “specifically disrupts backward signaling but not the production of secreted forms” of NRG1, DeJaegere told *SciBX*. Without proper cleavage by γ -secretase, “the transmembrane fragment of NRG1 stays stuck in the membrane, and the intracellular fragment is not being produced,” he said.

David Talmage, associate professor of pharmacology at the **State University of New York at Stony Brook**, told *SciBX* that De Strooper’s study “begins to uncover the normal functions and substrates” of γ -secretase.

The study, he said, shows that “there are other isoforms of the γ -secretase complex with different specificities for substrates” beyond A β and Notch.

According to Talmage, De Strooper’s results are in accord with recent findings that another AD-linked protease, β -secretase (BACE1), also plays a role in NRG1 processing.⁴ BACE1 acts prior to γ -secretase in cleaving A β and NRG1, and *Bace1* knockout mice have PPI defects and other schizophrenia-like phenotypes similar to those found in De Strooper’s *Aph1B* and *Aph1C* double knockouts.⁵

Go gamma

Whether targeting BACE1 and γ -secretase will have any effect on schizophrenia depends on whether the neurological effects of altering NRG1 activity occur only during development or are due to ongoing effects in mature neurons, according to Talmage.

If problems with NRG1 processing lead to abnormal neural connections, not much can be done. But if restoring NRG1 cleavage can help adult neurons function better, γ -secretase could be a target for next-generation antipsychotics.

Talmage suspects the latter may be true.

“We have a good sense that γ -secretase contributes to neuronal plasticity in adults, so I’m guessing that there’s a continuing requirement” for NRG1 activity, he told *SciBX*.

Todd Golde, professor of neurosciences at the **Mayo Clinic**, agreed. “The real challenge is determining whether this is a developmental or a signaling phenotype,” he said.

DeJaegere told *SciBX* that experiments are under way to work out the temporal requirements for γ -secretase activity in NRG1 processing.

Uninhibited

If γ -secretase is indeed a good target for schizophrenia, a rich pharmacopeia already exists to tweak its activity. However, most therapeutics that target γ -secretase are antagonists being developed for AD. The ideal schizophrenia compound would be a selective γ -secretase agonist.

At least seven companies are developing γ -secretase antagonists in hopes of decreasing the production of neurotoxic A β fragments.

The most advanced inhibitor is **Eli Lilly and Co.**’s LY450139, which is in Phase III testing to treat AD. Last week, the company announced a \$300 million deal with **TPG-Axon Capital** and **Quintiles Transnational Corp.**’s NovaQuest unit to fund the pharma company’s ongoing Phase III IDENTITY (Interrupting Alzheimer’s Dementia by Evaluating Treatment of Amyloid Pathology) trial of the compound.

Other companies with γ -secretase programs include **Wyeth**, **Merck & Co. Inc.** and **Eisai Co. Ltd.**

First-generation γ -secretase inhibitors not only block A β production, but they also shut down signaling by Notch, which plays critical roles in the cardiovascular, immune and nervous systems.

Concerns about Notch-related side effects have prompted some companies to develop compounds that selectively prevent A β cleavage without affecting Notch.

“There’s been a large investigation of A β , but we’re just beginning to understand the other substrates” of γ -secretase, said Guilio Pasinetti, professor of neuroscience at **Mount Sinai School of Medicine**.

Because these compounds function by altering the enzyme’s preference for its substrates, some of them could potentially agonize NRG1 cleavage.

According to Pasinetti, the challenge will be to sort out which, if any, compounds selectively affect *APH1B* variants of γ -secretase without undesired side effects on other substrates such as A β or Notch.

Pasinetti is an investigator on a Phase IIb AD trial of NIC5-15, a selective γ -secretase inhibitor from **Humanetics Corp.**

Modulators needed

Golde told *SciBX* that De Strooper’s study bolsters the case for compounds that modulate γ -secretase activity rather than inhibit it outright. Whereas inhibitors “prevent production of all forms of A β ,” modulators change the enzyme’s substrate specificity or cleavage site. Certain modulators could in principle agonize *APH1B* γ -secretase isoforms without affecting other γ -secretase complexes.

Adele Rowley, director of pharmacology at **Cellzome Inc.**, seconded this view. The findings raise concerns “for γ -secretase inhibitors but not for γ -secretase modulators,” she said.

The biotech company recently extended a partnership with **Johnson & Johnson** to develop γ -secretase modulators through 2010. Rowley declined to discuss the status of Cellzome’s program.

At the same time, γ -secretase compounds in testing for AD also should be examined for undesired effects on NRG1 processing.

Rowley noted it is still unknown whether the “inhibition of NRG1 cleavage in the adult human would be deleterious.” Thus, she said, “it will be important to understand how different γ -secretase complexes are modulated or inhibited by existing compounds.”

Rowley told *SciBX* that “modestly substrate-selective γ -secretase inhibitors” could be effective against AD without necessarily perturbing NRG1 processing.

Golde said it would be straightforward to use cellular and animal

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assays to sort out the selectivity of existing γ -secretase compounds in inhibiting A β production but not NRG1 cleavage.

In fact, **TorreyPines Therapeutics Inc.** uses a cellular assay to identify γ -secretase modulators to treat AD.

Steven Wagner, the company's CSO, told *SciBX* that De Strooper's study has "obvious implications with respect to synaptic plasticity and cognition in schizophrenia and AD."

According to Wagner, TorreyPines has identified "over 1,200 compounds which favor the right γ -secretase profile" needed to selectively prevent toxic A β fragment production.

Although the company has focused on AD, Wagner sees an opportunity to use the company's γ -secretase modulator library for schizophrenia and other cognitive indications.

"Perhaps by modulating γ -secretase cleavage of other substrates, we could have some hope of possibly using these compounds for other neuropsychiatric disorders," he said.

Pasinetti agreed, noting that it seems likely that "you can generate steric modulators of NRG1 cleavage" from scaffolds designed to modify A β cleavage sites.

Dejaegere told *SciBX* that the University of Leuven has filed patents for the use of *Aph1B* and *Aph1C* double knockout mice as a disease

model and that the technology is available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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