Retromer Sorting

A Pathogenic Pathway in Late-Onset Alzheimer Disease

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During the tail end of the 20th century, a “golden period” in Alzheimer disease (AD) research, many of the pathogenic molecules of the autosomal dominant form of the disease were isolated. These molecular defects, however, do not exist in “sporadic” late-onset AD, the form of the disease that accounts for more than 95% of all cases. Pinpointing the pathogenic molecules of late-onset AD has, therefore, become an urgent goal, both for understanding disease mechanisms and for opening up novel therapeutic avenues. The retromer sorting pathway transports cargo along the endosome–trans-Golgi network, and retromer defects were first implicated in late-onset AD by a study that combined brain imaging with microarray. A range of studies have confirmed that defects in this pathway can play a pathogenic role in the disease. Herein, these findings will be reviewed, the details of the retromer sorting pathway will be discussed, and a biological model that can account for the disease’s regional selectivity will be elaborated.

Arch Neurol. 2008;65(3):323-328

Isolating the primary molecular defects of autosomal-dominant early-onset Alzheimer disease (AD) heralded a new era in AD research and served as the cornerstone on which biological insights into the disease have been made. In particular, expressing these molecules in cells and then in genetically engineered mice resolved many questions about the processing of the amyloid precursor protein (APP) and the neurotoxic effects of its cleaved product, the Aβ peptide.1 Nevertheless, the pathogenic molecules causing the early-onset form of the disease are not defective in sporadic late-onset AD, the dominant form of the disease accounting for most cases. Although a complex disorder—emerging from an interplay of genetic and epigenetic factors—isolating the pathogenic molecules of late-onset disease is acknowledged as the next important step in unraveling its causes and developing effective treatment.

As with all neurodegenerative diseases, a focus on different levels of analysis can provide clues about pathogenic molecules. Historically, a focus on histological abnormalities was the first level that offered early insight into the molecular biological features of AD. When, in 1984, the Aβ peptide was finally identified as the core of amyloid plaques (isolated from menin-govascular tissue, not the brain),2 this led to the identification and cloning of its parent protein, APP.3 The cloning of APP was a required step for elucidating its metabolic pathway, serially cleaved by β-site APP-cleaving enzyme (BACE) and then by the γ-secretase, liberating Aβ and initiating the amyloid cascade.1 A focus on genetic mutations was a second level of investigation, and during the 1990s linkage analyses successfully isolated mutations in APP and the presenilins as pathogenic defects underlying early-onset AD.4 Linkage analysis, however, has proved less successful for pinpointing pathogenic molecules underlying complex disorders, including late-onset AD.

When gene expression techniques like microarray were introduced in the late 1990s,5 they provided an unprecedented...
opportunity to focus on brain tissue itself as a third level of analysis amenable to molecular discovery. In principle, because the expression profile of affected brain cells is a reflection of genetic and epigenetic factors, techniques like microarray are well suited for pinpointing molecules underlying complex disorders. In practice, however, microarray has a number of analytic challenges, such as poor signal-to-noise ratio and high false positivity, hampering its utility and dampening the overall enthusiasm for this approach.

Early failures of microarray, however, have not impugned its technical validity but have simply emphasized the importance of using more sophisticated experimental designs to overcome its analytic challenges. With this in mind, an approach called “imaging-guided microarray,” specifically designed to address the analytic limitations inherent to microarray when applied to disorders of the brain, was recently introduced. A detailed description is provided elsewhere, but in general the approach relies on in vivo imaging to first construct a spatiotemporal model hypothesizing a priori how a pathogenic molecule should behave—anatomically and across age groups. Then, the spatiotemporal model is used as a guide in generating microarray data and in analyzing the gene expression data set. By converting a microarray experiment from one that is typically hypothesis free to one that is hypothesis driven, imaging-guided microarray naturally addresses many analytic challenges. As in any hypothesis-driven study, the results are only as good as the hypothesis and, therefore, as discussed later, any microarray finding needs to be independently confirmed and validated.

THE RETROMER SORTING PATHWAY IMPLICATED BY IMAGING-GUIDED MICROARRAY

Alzheimer disease begins in the hippocampal formation before sweeping over the neocortex, ravaging the mind and causing dementia in its wake. The hippocampal formation itself, however, is a circuit made up of separate but interconnected subregions—the entorhinal cortex, the dentate gyrus, the CA3 and CA1 subfields, and the subiculum. Each hippocampal subregion expresses a unique molecular profile, accounting for why each subregion is differentially vulnerable to mechanisms of disease.

During the past few years, variants of functional imaging have been used to investigate the hippocampus as a circuit—ie, simultaneously investigating multiple subregions—establishing a spatiotemporal profile of AD-related dysfunction. Agreeing with some, although not all, postmortem indicators of disease, the spatial pattern of dysfunction suggests that, early on, AD targets the entorhinal cortex with relative sparing of the dentate gyrus. In contrast to the spatial pattern, the temporal pattern of dysfunction uncovered by the imaging studies was unexpected and could not have been inferred from postmortem indicators alone. Specifically, entorhinal dysfunction detected in early AD was age invariant.

This spatiotemporal profile was used to construct a model predicting how a pathogenic molecule related to AD should behave. Guided by the model, the entorhinal cortex and the dentate gyrus from postmortem brain specimens with and without AD were harvested, purposefully covering a broad age span, and microarray analysis was performed on each tissue sample. The final analysis revealed that, among a handful of hits, the expression level of vacuolar protein sorting 35 (VPS35) best conformed to the full spatiotemporal model of late-onset AD.

Vacuolar protein sorting 35 turns out to be the core component of the retromer sorting pathway. First described in yeast, the retromer sorting pathway consists of a multimeric retromer complex, comprising VPS35, VPS26, VPS29, VPS5, and VPS17. This complex acts as a “coat” that binds and transports the transmembrane receptor VPS10 from the endosome back to the trans-Golgi network (Figure 1). Except for VPS17, mammalian homologues of the retromer complex have been identified and are expressed in the brain and among other tissue types. Previous studies have shown that a primary reduction in any retromer element will lead to secondary degradation of other elements of the complex, causing general retromer dysfunction. Indeed, it was found that VPS35 and VPS26 proteins were differentially reduced in AD. To test whether this finding was potentially pathogenic, small interfering RNA was used to systematically decrease retromer elements in cell culture, showing that this reduction led to increased concentrations of Aβ, while overexpressing retromer elements decreased Aβ levels.

THE NEURONAL RETROMER AND ITS RELATION TO APP PROCESSING

Why would retromer dysfunction cause an increase in Aβ levels? Identifying the type 1 transmembrane receptor sorted by the neuronal retromer might offer clues. In contrast to nonneuronal mammalian cells, the receptor of the neuronal retromer had not been elucidated, although retromer-related molecules are highly expressed in the brain. In an attempt to identify candidate receptors of the neuronal retromer, an analytic approach was applied to the microarray data set; this approach has been used in prior gene expression studies to search for potentially interacting molecules. Underlying this approach is the assumption that molecules that interact with each other are more likely to have expression levels that cross correlate. Because VPS35 serves as the key retromer element that directly binds the retromer receptor, the microarray data set was searched for correlations between the expression levels of VPS35 and type 1 transmembrane molecules, a search that identified, among other molecules, sorLA as a candidate receptor of the neuronal retromer.

sorLA is a complex molecule with multiple domains, including a VPS10 domain and low-density lipoprotein receptor domains. It is this complexity that accounts for its numerous names (eg, sorLA, sorl1, and LR11) and for why this molecule has been grouped together with different families of proteins. Vacuolar protein sorting 10 is the receptor of the yeast retromer, the species in which the retromer was first described, and so it was sorLA’s VPS10 domain that seemed most intriguing. Mammals express a family of 5 VPS10-containing proteins that, together with sorLA, include sortilin, sorCS1, sorCS2, and sorCS3. Because all members of the family are type 1
transmembrane receptors and are highly expressed in the brain, it was proposed that, in contrast to nonneuronal mammalian cells,10 the VPS10 family of proteins might function as receptors of the neuronal retromer9 (Figure 1). More important, work by Scherzer et al17 had previously shown that sorLA is down-regulated in late-onset AD (in their article, they focused on the low-density lipoprotein receptor domain of the molecule, using the name LR11). Shortly thereafter, a collaborative series of studies by the laboratories of Andersen et al18 reported on the cell’s biological properties of sorLA, focusing more on its possible role in sorting APP. Since then, all of these groups have extended their work, suggesting that sorLA might interact with APP or with BACE. Put into the context of the retromer, the neuronal retromer might be involved, directly or indirectly, via sorLA or other VPS10-containing proteins, in sorting APP and/or BACE along the endosome–trans-Golgi network trafficking pathway.9

Taken together, it has been proposed that retromer dysfunction would increase the resident time of APP and its cleaving enzymes in the same organelle, accelerating APP processing and accounting for the Aβ elevation observed in retromer-deficient states.9,10

CONFIRMING THE PATHOGENICITY OF RETROMER SORTING

Although a priori modeling and sophisticated statistics can increase the odds that a given microarray finding is relevant to a disease process, microarray findings by themselves do not inform about pathogenicity. As previously described, because tissue samples are harvested years after the disease has begun, it is impossible to know whether the retromer defects observed in AD brain specimens are truly pathogenic or whether the finding simply reflects a secondary response to a sick and dying cell. As with all microarray findings, 3 types of studies can be used to potentially confirm the pathogenicity of retromer sorting in AD.6 First, cell culture studies can test whether manipulating retromer-related molecules affects Aβ production. Second, genetically engineered mice studies can test whether retromer deficiency affects Aβ production in the brain and causes hippocampal dysfunction. Third, genetic studies can test whether polymorphisms in retromer-related molecules increase the risk for late-onset disease.

As mentioned, the first confirmatory studies were reported in a previous retromer study,7 and showed that manipulating retromer-related molecules in cell culture had a commensurate effect on Aβ levels. The second confirmatory studies using genetically engineered mice are under way. A colony of retromer-deficient mice has been recently bred, and the process of establishing their behavioral, electrophysiological, and biochemical phenotypes is under way. These mice have partial reductions in VPS26 and VPS35, thereby modeling the molecular defects found in AD brain specimens. Although still a work in progress, the preliminary results are encouraging.20
Mice can also be used to establish the normal anatomical expression pattern of retromer-related molecules in the brain. With this question in mind, I recently explored the Allen Brain Atlas, which has made available the expression maps of most of the murine genome. Unexpectedly, VPS35 and VPS26 are expressed with highest levels in the pyramidal cells of the hippocampus, more so than in other regions of the brain (Figure 2). This contrasts with the diffuse expression pattern observed for APP, BACE, and sorLA. Interestingly, although presenilin 1 is expressed in many regions of the brain, compared with APP or BACE, presenilin 1 does show some degree of differential expression in the hippocampal formation (Figure 2A). Further-
more, examining the microarray data set of human hip-
campal tissue shows that presenilin 1 has higher ex-
pression levels in the entorhinal cortex compared with
the dentate gyrus ($P < .04$) (Figure 2B). Of course only
suggestive, the fact that retromer-related molecules track
the anatomical pattern of AD provides indirect, but in-
triguing, support for a role in the disease.

Recently, a genetic study was reported by Rogaeva et
al.21 Investigating multiple cohorts with late-onset AD,
they genotyped VPS35, VPS26, and the family of VPS10-
containing molecules. Remarkably, genetic variants in
sorLA were associated with late-onset AD. The research-
ers interpret their results in the context of the retromer
sorting pathway and, indeed, provide direct evidence that
VPS35 binds sorLA and that knocking down VPS26 in
cell culture increases Aβ production.

OVERLAPPING FUNCTIONS OF RETROMER
AND PRESENILIN SUGGEST UNIFYING
MECHANISMS OF DISEASE PATHOGENESIS?

“Localizing the lesion” is a basic tenet in all neurology.
Not only does pinpointing a targeted neuronal popula-
tion promise to improve diagnostic precision, but more
important, this anatomical information provides clues into
a disease’s primary pathophysiological features. As dis-
cussed, in contrast to APP, BACE, retromer, and presen-
ilin are differentially expressed in the pyramidal neu-
rons of the hippocampal formation (Figure 2). Thus,
identifying cellular mechanisms in which retromer and
presenilin play a shared role might expand our under-
standing of the disease process.

Besides Aβ production, to date, there are 2 addi-
tional cellular mechanisms in which retromer and pre-
senilin appear to play an active role. First, like the retro-
mer, a growing number of studies have established that
the presenilins play a general role in sorting type I trans-
membrane proteins, and that disease-causing mutations
in presenilin cause protein mis-sorting (as reported by
Small and Gandy19). Second, and perhaps more interest-
ing, retromer23 and presenilin24 play critical roles in the
Wnt signaling pathway.

Which of these 3 overlapping functions—Aβ produc-
tion, transmembrane protein sorting, and Wnt signaling—
might account for the differential expression pattern of
presenilin and retromer in the entorhinal cortex? Aβ pro-
duction is an unlikely candidate because within unaf-
eced specimens, Aβ levels are not higher in the ento-
rhinal cortex compared with other brain regions.25 The
second function, protein sorting, is a better candidate.
Sorting type I transmembrane proteins is important for
synaptogenesis, because these proteins are the domi-
nant players in the synaptogenic process.26 As the main
gateway into the hippocampus, the entorhinal cortex re-
ceives constant input from the whole neocortical mantle,
and integrating this information requires highly active
dendritic remodeling and extremely high metabolic ac-

tivity.27

The overlap of retromer and presenilin in the func-
tion of the Wnt signaling pathway is perhaps the best can-
didate for why they are differentially expressed in the en-
torhinal cortex. Morphologically, the entorhinal cortex

exhibits 2 distinct features. First, at the single-cell level,
entorhinal cortex neurons exhibit a very complex dend-
ritic organization. Indeed, it is for this reason that many
entorhinal cortex neurons are called “stellate” cells, in
contrast to morphologically simpler “pyramidal” or “gran-
ule” cells found in other hippocampal subregions.28 Sec-
ond, an even more unique morphological feature is that
stellate neurons are organized as clusters of “islands” ex-
tending throughout the tangential axis of the entorhinal
cortex.29 The Wnt signaling pathway turns out to play an
important role in establishing complex cellular30 and an-
tomical morphology,31 which can account for why ret-
romer and presenilin are differentially needed in the en-
torhinal cortex.

Thus, entorhinal cortex neurons might differentially
express retromer and presenilin not because these neu-
rons require high Aβ levels in their normal states but
rather to support their unique metabolic and morpho-
logical characteristics. However, once presenilin and ret-
romer are rendered dysfunctional, by genetic or epige-
etic factors, these neurons are expected to differentially
overproduce Aβ because presenilin and retromer can also
affect APP processing. Future studies are required to test
this proposed hypothesis, but if confirmed it would open
up novel therapeutic avenues for treating this devastat-

Accepted for Publication: February 28, 2007.

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Financial Disclosure: None reported.

Funding/Support: This study was supported in part by fed-
geral grant AG025161 from the National Institutes of
Health; the McKnight Neuroscience of Brain Disorders
Award; and the James S. McDonnell Foundation.

REFERENCES

1. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer’s disease: progress
2. Glennner GG, Wong CW. Alzheimer’s disease and Down’s syndrome: sharing of a
unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun.
1984;122(3):1131-1135.
distribution, and genetic linkage near the Alzheimer locus. Science. 1987;235
(4791):880-884.
4. Price DL, Tanzi RE, Borchelt DR, Sisodia SS. Alzheimer’s disease: genetic stud-
5. Lockhart DJ, Dong H, Byrne MC, et al. Expression monitoring by hybridization
to high-density oligonucleotide arrays. Nat Biotechnol. 1996;14(13):1675-
1680.
6. Lewandowski NM, Small SA. Brain microarray: finding needles in molecular
7. Pierce A, Small S. Combining brain imaging with microarray: isolating mol-
29(5):1145-1152.
68-75.


In reply

The case reported by Leussink and colleagues shows striking parallels with our previously described case of a delayed allergic reaction to natalizumab. We fully agree that we must anticipate additional cases of such delayed, type III systemic reactions to natalizumab in the future. The two published cases should help to raise awareness of this type of complication.

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Financial Disclosure: Drs Gold and Hohlfeld have received grant support and consultancy fees from Schering, Teva, Serono, and Biogen Idec.

Incorrect Wording. In the article titled "Retromer Sorting: A Pathogenic Pathway in Late-Onset Alzheimer Disease," by Small, published in the March issue of the Archives (2008;65[3]:323-328), an incorrect word was used when discussing an observation by Small and Gandy. On page 327, left-hand column, "Overlapping Functions of Retromer and Presenilin Suggest Unifying Mechanisms of Disease Pathogenesis" section, second paragraph, second sentence, the sentence should have read as follows: "First, like the retromer, a growing number of studies have established that the presenilins play a general role in sorting type 1 transmembrane proteins, and that disease-causing mutations in presenilin cause protein missorting (as reviewed by Small and Gandy)."