Journal of Alzheimer's Disease 5 (2003) 491–497
IOS Press

Discussion

Live discussion: Amyloid-β degradation: The forgotten half of Alzheimer's disease[1]

Live Chat held 12 September 2002 with Wes Farris and Malcolm Leissring, Brigham and Women's Hospital and Harvard Medical School.
Posted 11 October 2002.
Participants: Chastity Whitaker (*Ohio State University*), *Chris Eckman* (*Mayo Clinic, Jacksonville*), *Claudia Almeida* (*Cornell University*), *Douglas Feinstein* (*University of Illinois, Chicago*), *Craig Atwood* (*Case Western Reserve University*), *Elizabeth Eckman* (*Mayo Clinic, Jacksonville*), *Keith Crutcher* (*University of Cincinnati*), *Lou Hersh* (*University of Kentucky*), *Malcolm Leissring* (*Harvard Medical School*), *Matthew LaVoie* (*Harvard Medical School*), *Nilufer Ertekin-Taner* (*Mayo Clinic, Jacksonville*), *Paul Shapiro* (*Elan Corporation*), *Reisuke Takahashi* (*Cornell University*), *Rina Yamin* (*Boston University School of Medicine*), *Stefan Mansourian* (*Harvard Medical School*), *Steve Estus* (*University of Kentucky*), *Sylvain Lesné* (*University of Caen, France*), *Tony Turner* (*Karolinski Institute, Stockholm, Sweden*), *Wes Farris* (*Harvard Medical School*).

Moderator: Gabrielle Strobel

Malcolm Leissring and Wes Farris: We will start by discussing what genetic evidence there might be for the involvement of decreased amyloid-β (Aβ) degradation as a cause of Alzheimer's disease (AD).

Chris Eckman: Nilufer, this question may be best for you.

Nilufer Ertekin-Taner: The main lines of genetic evidence, in my opinion, come from the mouse/rat knockout/mutant studies showing elevations of brain and plasma Aβ levels in mice that lack the NEP (neprilysin) and PLAU (plasminogen activator-urokinase) genes, and in rats, that have mutations in insulin degrading enzyme (IDE). In addition, our group has shown in three different series that variants in PLAU are associated with risk for late-onset Alzheimer's disease (LOAD), and that some of these variants are associated with elevated plasma Aβ levels in LOAD families. I can continue, but would like to give others the chance to reply.

Gabrielle Strobel: It looks as though two separate Aβ-degrading enzymes – IDE and PLAU – could be under the linkage peak found on chromosome 10. How likely is that?

Malcolm Leissring and Wes Farris: According to Tony Brookes at the Karolinska Institute in Stockholm, there may be at least two, possibly three, with α-T-catenin being the third.

Claudia Almeida: What is PLAU?

Nilufer Ertekin-Taner: PLAU is the gene name for urokinase-type plasminogen activator (uPA). There is also evidence from Rudy Tanzi's (Massachusetts General Hospital) and Brookes' laboratories about significant associations with variants in IDE and AD.

[1] Note: The transcript has been edited for clarity and accuracy.

ISSN 1387-2877/03/$8.00

Chris Eckman: The knockout studies show elevations of Aβ clearly in brain for ECE (endothelin converting enzyme), NEP and IDE knockouts and in plasma for PLAU knockouts. To me, this is considerable.

Malcolm Leissring and Wes Farris: Has anyone looked at plasminogen knockouts?

Gabrielle Strobel: I vaguely recall a talk by Luc Buee (Lille, France) at the World Alzheimer Conference in Stockholm on tPA (tissue plasminogen activator) knockout mice (raised for a different purpose) that had thioflavin-positive Aβ plaques in brain.

Nilufer Ertekin-Taner: This work was presented at Hot Topics.

Sylvain Lesné: I am one of the coworkers of that story.

Gabrielle Strobel: Sylvain, tell us more, please.

Steve Estus: I have heard this and been puzzled. We have preliminary data that tPA knockout/Hsiao mice have less Aβ, if anything.

Malcolm Leissring: Hmm . . . different genetic backgrounds?

Sylvain Lesné: Well, we now have extra data confirming the role of the tPA/plasmin axis in Aβ degradation.

Nilufer Ertekin-Taner: What is the age of your tPA knockouts when they start showing Aβ deposits, Sylvain?

Sylvain Lesné: Between 12 and 14 months old. We have preliminary data about plasmin knockout mice, and we will soon have AβPP-tPA knockout mice at the same age.

Nilufer Ertekin-Taner: Could measurements at different age points possibly explain differences between Steve's and Sylvain's results?

Chris Eckman: Nilufer, you and Steve Younkin (Mayo Clinic, Jacksonville) have looked at knockouts in this system already. It might be worthwhile to state the data again for everyone.

Craig Atwood: I may have missed this, but are the tPA knockouts on a wildtype or AβPP-transgenic background?

Steve Estus: The tPA knockout mice have a B6 background. Most of the mice in the initial run were F2s, which we ran out to 11 months old to quantify Aβ in collaboration with Steve Younkin.

Craig Atwood: Thanks, Steve. So it is mouse Aβ that is depositing. Quite convincing.

Sylvain Lesné: As I mentioned at the Stockholm meeting, three mice had clearly at least 30-40 Aβ "plaques." Did your mice come from Peter Carmeliet's lab (University of Leuven, Belgium)?

Stefan Mansourian: Have you been able to confirm that these are Aβ plaques by immunocytochemistry? It is sometimes possible to get spurious "plaques" by thioflavin-T staining.

Sylvain Lesné: Absolutely. I performed immunocytochemistry with an Aβ42-specific antiserum.

Stefan Mansourian: Were you able to repeat this with any other Aβ42 antiserum, or with Aβ40 antiserum?

Wes Farris: Any thoughts on why this would be the only mouse model with deposition of endogenous rodent Aβ?

Nilufer Ertekin-Taner: In Steve Younkin's laboratory, measurements of plasma and brain Aβ were done in PLAU knockout mice. We found significant elevations in plasma Aβ in knockout mice compared to wildtype. This increase was more enhanced when the mice were aged. We did not see significant elevations in brain Aβ levels.

Sylvain Lesné: We did not check whether tPA knockouts were eliciting enhanced plasma Aβ levels.

Craig Atwood: Did you try mouse versus human Aβ-specific antibodies?

Sylvain Lesné: This is currently under investigation by us. The deposits are also immunoreactive to an Aβ1-10 directed antiserum, so we are also looking this way.

Wes Farris: Any thoughts on why this mouse model would show deposits of endogenous Aβ, unlike the presenilin model? It has been generally believed that rodent Aβ, with its three amino acid differences from human Aβ, doesn't aggregate *in vivo*.

Gabrielle Strobel: Nilufer, that is interesting. Malcolm and Wes, how about plasma Aβ, and also about insulin levels in IDE knockout mice?

Wes Farris: The elevation of both plasma Aβ and insulin are currently being analyzed in the IDE knockout mice. Preliminary evidence suggests that plasma insulin is increased about 2.8-fold in our IDE knockout mice.

Gabrielle Strobel: Malcolm and Wes, you wrote about rats with IDE mutations. Do they have AD-like behavior or learning deficits? Or pathology? Or, assuming there are more Aβ protofibrils around, LTP (long-term potentiation) impairment, as in the (Walsh et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. Nature 2002 Apr 4;416(6880):535-9) experiments?

Wes Farris: We have not looked for any of the phenotypes you mentioned yet – just Aβ levels. Because the Aβ is the endogenous rodent peptide, protofibrils probably won't form.

Craig Atwood: Wes, would high levels of insulin compete for IDE with Aβ aggregation?

Wes Farris: Craig, I do not think that Aβ aggregation is induced by IDE, but high insulin levels can act as a competitive inhibitor of IDE *in vitro*, leading to decreased Aβ degradation.

Malcolm Leissring: IDE does not induce Aβ aggregation. That original finding was later found to be flawed.

Wes Farris: Lou, have you looked at increases in any other IDE substrates in your IDE knockout mice?

Malcolm Leissring: So we have one group (Lesné's) that has found deposits and increased Aβ in tPA knockouts, but two others that have found no differences (Takaomi Saido, The Institute of Physical and Chemical Research, Japan) – see his comment to the background text of this discussion, or the opposite (Estus).

Sylvain Lesné: I spoke with Professor Saido last July, and we were surprised by the divergent results.

Craig Atwood: What about degradation of Aβ by IDE?

Douglas Feinstein: Is it known how IDE function is normally regulated, e.g., by phosphorylations?

Lou Hersh: There is no evidence I am aware of for any regulation of IDE.

Malcolm Leissring: Doug, there are some reports that calcium and ATP may modulate IDE, but the evidence is weak.

Craig Atwood: So, if there are high levels of insulin competing for IDE, this would not prevent Aβ degradation? Or vice versa?

Wes Farris: One hypothesis that we have is that hyperinsulinemia may cause increased levels of Aβ *in vivo*, through insulin's competition with IDE's degradation of Aβ.

Lou Hersh: The problem with insulin and IDE is, where is the insulin and where is the "functional" IDE?

Douglas Feinstein: Is IDE neuronal?

Malcolm Leissring: Functional IDE has been shown to be on the cell surface in numerous studies.

Lou Hersh: Douglas, I do not think IDE has been looked at really carefully in the brain.

Stefan Mansourian: Douglas, yes, it has been found in cultured neurons and PC12 cells (Vekrellis et al. Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. J Neurosci. 2000 Mar 1;20(5):1657-65) and by immunocytochemistry in human and mouse neurons.

Lou Hersh: Stefan, I am not convinced cultured cells reflect the *in vivo* situation, particularly PC12 cells.

Stefan Mansourian: But we have seen IDE in primary neurons by immunocytochemistry.

Douglas Feinstein: IDE localization should be done in mice/rats – possibly developmentally, possibly in the mutant AβPP transgenics.

Malcolm Leissring: Several zinc-metalloproteases that lack signal peptides are found on cell membranes. Examples are IDE, NRDc, neurolysin and thimet oligopeptidase, and perhaps several others . . . .

If there is a problem for IDE, there is likely a problem for all these zinc metalloproteases.

Lou Hersh: Malcolm, I seem to recall that the levels of secreted IDE are very low.

Sylvain Lesné: We see a two- to threefold increase of Aβ accumulation in primary cultured neurons from tPA-/- mice (compared to wildtype). The effect was reversed by adding tPA in tPA-deficient neurons.

Steven Estus: Sylvain, that is amazing. Do you have to add exogenous plasminogen to see this effect?

Sylvain Lesné: No, we did not add exogenous plasminogen into the culture media of our cultured tPA-/- neurons. We also confirmed the requirement of plasmin to let this occur by blocking the effect with α2AP (α2-antiplasmin).

Steven Estus: Sylvain, perhaps you and I are the only ones on this particular thread, but have you had a chance to look at cellular Aβ in this paradigm?

Sylvain Lesné: This is a point I want now to look at.

Steven Estus: Sylvain, our data are not necessarily contradictory, in that we are looking at the tPA/Hsiao mice. Have you looked at AβPP-transgenic animals?

Sylvain Lesné: We use the Parkinson's disease-AβPP mice crossed with our tPA-/- or plasmin-/- mice.

Steven Estus: Sylvain, I misunderstood. Has all of your work been in the Parkinson's disease-AβPPs?

Sylvain Lesné: Not at all; we used single transgenic animals and we are now using bigenic mice to test whether the lack of tPA would potentiate/exacerbate Aβ deposition. Professor Estus, did you observe any changes in Aβ loads in your bigenic mice (tPA-Tg2576) as compared to same-age Tg2576 mice?

Steven Estus: We observed decreased Aβ burden as quantified by ELISA.

Claudia Almeida: Where is Aβ accumulating? extracellularly?

Gabrielle Strobel: Claudia, primarily extracellularly. Intracellular Aβ seems to be detected in neurons. Gunnar Gouras (Weill Medical College of Cornell University) had a presentation in Stockholm about age-related increases of intracellular Aβ42 in AD-vulnerable neurons. What does this mean for our topic today? Anyone?

Douglas Feinstein: Gabrielle, how do they know they are AD-vulnerable if they are still there? Perhaps those are the healthier neurons.

Gabrielle Strobel: Douglas, they were neurons from typically affected brain areas, I believe.

Chris Eckman: I agree with Lou. I think it is telling that several different genes linked to Aβ degradation all elevate Aβ in the knockout animals. These include ECE (1 and 2), NEP, IDE and PLAU. Is this not the ultimate test? Honestly, I think that there are likely to be regional as well as subcellular/extracellular differences regarding which enzyme is involved, but the knockouts are quite telling.

Wes Farris: Lou, do you believe that IDE is on the cell membrane? Where do you beleive it sees Aβ?

Lou Hersh: Wes, intracellularly. That is where it appears to see insulin.

Malcolm Leissring: I think the case for secreted IDE is more difficult, but the case for cell-surface associated IDE is very clear. It is on the surface of neurons.

Wes Farris: Lou, do you think IDE degrades Aβ in membranous vesicles or in the cytosol?

Claudia Almeida: Lou, what is the data that IDE sees insulin intracellularly?

Malcolm Leissring: IDE antibodies. IDE antibodies were injected and reported to affect insulin degradation.

Douglas Feinstein: Lou and Wes, so IDE trafficking could play a role in Aβ removal?

Wes Farris: Doug, IDE clearly plays a role in Aβ degradation in knockout mouse brain, if that is what you mean by trafficking. If this is not what you mean, please clarify.

Douglas Feinstein: Wes, trafficking, as in movement from intracellular organelles to the membrane surface. I would think membrane-IDE might be more efficacious to degrade extracellular Aβ.

Wes Farris: Douglas, I agree with you about membranous IDE being more relevant, but I would say the jury is still out over exactly where IDE degrades Aβ. Our lab has shown IDE on the surface of neurons, but we as yet do not have a good mechanism as to how it gets there (no transmembrane domain and a questionable signaling peptide).

Douglas Feinstein: Wes thanks. I was just looking at a study by Dennis Selkoe (Harvard Medical School) concluding that IDE mostly works extracellularly (Vekrellis et al. Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. J Neurosci. 2000 Mar 1;20(5):1657-65).

Gabrielle Strobel: How about glial cells? Do we know what their contribution is to the secretion of Aβ-degrading enzymes?

Wes Farris: Our lab has shown that microglial cell lines degrade Aβ, mainly via IDE, but I do not know of any specific studies of astroglial Aβ degradation, but they clearly could play an important role.

Chris Eckman: Very interesting question, Gabrielle. The honest answer is, we do not have any idea collectively.

Malcolm Leissring: IDE is expressed in and secreted from mouse microglial cells. It seems they need to be primed first with LPS. Intriguingly, activation of purinergic receptors on microglia causes the release of leaderless cytosolic proteins like IL-1β (and IDE) through a mechanism called microvesicle shedding. Perhaps this is one mechanism by which IDE is secreted.

Lou Hersh: Doug, if I remember, the amount of cell surface IDE is vanishingly small.

Stefan Mansourian: Chris, are ECE-1 and 2 located primarily in neurons or glia?

Nilufer Ertekin-Taner: UPAR (UPA receptor) is found on microglia and its expression was shown to be increased upon treatment with Aβ.

Chris Eckman: ECE-1 and 2 are ubiquitous, with ECE-2 mostly in neurons, but certainly they could play a role there as well as in glia.

Steven Estus: Regarding expression of the plasmin members, plasmin/tPA/PLAU are expressed in neurons; tPA, PAI-1, PAI-2 are also expressed in microglia.

Malcolm Leissring: Chris, does ECE-1 suffer the same problem of not having a signal peptide?

Lou Hersh: In terms of IDE trafficking, we know that some is targeted to the peroxisomes through a C-terminal signal.

Malcolm Leissring: Lou, yes, you make a good point. IDE is known to be trafficked across membranes – definitely into peroxisomes – so why not into other compartments or onto the cell surface?

Douglas Feinstein: Malcolm, hence, increasing IDE levels should be "protective"?

Malcolm Leissring: Yes, that is the theory, at least.

Chris Eckman: ECE is clearly a transmembrane protein and ECE-catalyzed Aβ degradation is optimal at a slightly acidic pH, indicating intracellular to me.

Malcolm Leissring: Chris, which ECE?

Lou Hersh: Malcolm, I do not think the peroxisomal targeting sequence gets proteins to the cell surface.

Malcolm Leissring: IDE also gets to mitochondria.

Lou Hersh: Malcolm, where is the evidence for that?

Malcolm Leissring: Lou, I have not published it, yet, but we have found that the initial 41 amino acids of IDE encode a mitochondrial targeting sequence; EGFP fusions containing this sequence are targeted to mitochondria very efficiently.

Gabrielle Strobel: Are any of those proteases good drug targets, given how promiscuous they seem to be and that we would want to increase their function?

Chris Eckman: Very good question, Gabrielle. The real answer is, we do not know. Clearly, overexpression of some of these has been tried and the mice seem okay. Remember, though, that all of these can certainly also do other things. The real tests still need to be done. Can overexpression of any enzyme (physiological or not) result in decreases in Aβ that are therapeutically useful? I know several of us have this target in our

sights, and I am certain we will be hearing more in the future.

Nilufer Ertekin-Taner: Gabrielle, your point about potential drug targets is interesting. If we could detect the people who develop AD because they have "defective" forms of these enzymes, then one could think about compensating for their defect, as opposed to overexpressing them. Ultimately, genetic tests that could develop as a result of the current research could help us identify such individuals, who would be candidates for treatment.

Douglas Feinstein: Nilufer, the possibility is that the proteases are not defective per se, but their subcellular localization, modifications, etc., are, so genetics would not necessarily work.

Nilufer Ertekin-Taner: Douglas, what I meant by defective is not simply underexpression, but includes all of the potential problems with folding, trafficking, etc., to which you are referring. If we can find the genetic mutations leading to these problems, then we have a test for identifying individuals at risk.

Douglas Feinstein: Nilufer, I absolutely agree.

Gabrielle Strobel: Are there drugs to induce the overexpression of proteases?

Malcolm Leissring: We are working on it. On the therapeutic side, what does everyone think of the reports that NEP is upregulated by injection of Aβ?

Chris Eckman: The data look decent, but as with everything, a confirmation would be good.

Lou Hersh: I agree with Chris. This is surprising and needs to be replicated. We have not seen NEP upregulated in Aβ mice.

Chris Eckman: Many people have tried Aβ injections over the years to create AD models. The reality is that it never worked, presumably because it was cleared, by what I do not know. Remember, there is a wealth of other ways to remove peptides in addition to direct catabolism.

Malcolm Leissring: Good point, Chris.

Wes Farris: Nilufer, what is your latest thinking about the chromosome 10q linkage?

Nilufer Ertekin-Taner: Wes, evidence from multiple laboratories indicates the existence of a locus (loci) on chromosome 10 that is linked with risk for AD. Our evidence suggests that this locus/loci act(s) through Aβ. Multiple genes reside under the peaks, and we need to sort them out by both genetic and functional studies. I do think it possible that multiple genes exist on chromosome 10q that yield these signals.

Gabrielle Strobel: Is this cluster of three possible LOAD risk factor genes, then, why this peak is so large? Are other genetic risk factors for LOAD more scattered throughout the genome and therefore harder to find?

Nilufer Ertekin-Taner: Gabrielle, multiple factors could cause the linkage peak to be large or narrow (study size, recombination information, heterogeneity). The existence of multiple genes in a region will not necessarily lead to narrower peaks – quite the contrary.

Wes Farris: Nilufer, what are your best candidate genes (that are public) to date?

Nilufer Ertekin-Taner: Wes, we presented our data on two genes at the World Alzheimer Conference, PLAU and VR22. Variations in the former are associated with LOAD in our case-control series and Aβ in the families, but do not account for the linkage signal. Variations in the latter account for a substantial proportion of our linkage signal, however.

Douglas Feinstein: This may be a repeat question, but has anyone examined if inflammatory stimuli decrease IDE or other putative degrading enzymes?

Wes Farris: Douglas, LPS (lipopolysaccharide) increases secretion of IDE, but I do not think that inflammatory stimuli decrease IDE. I not aware that this has been studied though.

Malcolm Leissring: Doug, to my knowledge, I have not seen much work looking at the IDE/inflammation connection, but the possibility that cytokines and IDE might be secreted by similar mechanisms from microglia is interesting.

Douglas Feinstein: I was thinking along the lines of a cyclic phenomenon in which low amounts of Aβ–> inflammatory–> decrease IDE, etc.

Paul Shapiro: Are there any decent commercially available antibodies for IDE?

Wes Farris: Paul, not yet, but I heard that Richard Roth may make his monoclonal commercially available. Nilufer, unfortunately I missed the international AD conference this year. What is VR22? I heard you may have found some allelic association to IDE in some families – is this correct?

Nilufer Ertekin-Taner: Wes, VR22 is a novel α-T-catenin. We do not see an effect for the IDE polymorphisms we tested in the families.

Gabrielle Strobel: Wes, check out the news coverage of Nilufer's excellent talk on the Alzheimer Research Forum website; it has a short summary (http://www.alzforum.org/new/detail.asp?id=624).

Chris Eckman: I think it is great, finally, to see people really interested in Aβ removal. I honestly believe that this will result in the identification of additional individuals at risk, and may someday result in new therapeutics.