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Editorial

The 7th Leonard Berg Symposium, Part 3

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Alzheimer Research Forum

Is Rare Familial Alzheimer's a Model for the Millions?

One question that pervaded the 7th Leonard Berg Symposium is whether dominantly inherited AD really is the same disease as the common late-onset forms that afflict some 35 million people around the globe, according to a recent report. "Can we generalize from FAD to all AD?" asked Martin Rossor of University College, London.

The question is important in part because the interest drug developers take in testing prevention in families with strong AD genetics hinges on their confidence that success in the few will translate into success in the many. In private, some industry leaders express doubt about that point, and some companies exclude eFAD families from their trials for this reason. That eFAD indeed models late-onset AD (LOAD) to a great extent, if not in all aspects, is a premise of DIAN, and the comparison of DIAN and ADNI data may settle the issue definitively. In the meantime, scientists at the Symposium discussed what clues they have so far. See below for talks taking stock of LOAD/eFAD similarities and differences in the clinic, pathology, biochemistry, genetics, and imaging.

First, a word about nomenclature. Autosomal-dominant AD is the form in which a child of an affected parent faces 50-50 odds of inheriting a pathogenic mutation in either the AβPP or the presenilin 1 or 2 genes. It is variously referred to as "familial" or "FAD," "early-onset" or "EOAD," "early-onset familial" or "eFAD." Strictly speaking, none of the terms is interchangeable. Familial AD often is merely a clustering, not a Mendelian autosomal-dominant inheritance pattern; early-onset AD is often sporadic in the sense that neither affected relatives, much less a gene mutation, is known. The term used in the Alzforum section on this set of aggressive forms of AD – eFAD – does not quite catch all people it means to include, either, because recently, autosomal-dominant pathogenic presenilin mutations were spotted in some rare families who get this form of AD in their seventies, i.e., with a late onset [1, 2]. The WashU researchers sidestepped this confusing nameology by using yet another term: "dominantly inherited Alzheimer disease." DIAD conveniently stands opposite LOAD and echoes DIAN, making DIAN the network for the DIAD crowd.

At the Leonard Berg Symposium, speakers stressed that DIAD/eFAD overall appears to model LOAD quite closely. However, it is not a carbon copy, and for the sake of discussion, the differences got center stage for a session. DIAD is heterogeneous. The way people decline can vary a bit from person to person, said Rossor, whose center follows members of 10 families with AβPP mutations and 34 families with presenilin 1 mutations. Certain clinical differences do track with genetic ones. For example, people who get DIAD because their AβPP gene is duplicated frequently have seizures, brain hemorrhages, and white matter changes. In these extremely rare families, some affected relatives start having seizures when they are teenagers, and depression and then dementia follow within a decade thereafter.

With presenilin, some 175 different mutations are published, but clinicians have no comprehensive description of how their preclinical period unfolds. DIAN is aiming to accomplish that and in the process may match up genotypes to phenotypes. Based on what clinicians observe, it is already clear that some presenilin cases come with added signs that are atypical for AD.

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For example, some patients have a paralyzing weakness of the legs called spastic paraparesis, and stumbling and falls can be among the first signs mutation carriers report. One new Bulgarian family is described in the literature this month [3].

Corresponding to this clinical phenotype, Bill Klunk of the University of Pittsburgh Medical School, Pennsylvania, showed slides of DIAD/eFAD patients with spastic paraparesis who have heavy amyloid deposition in their cerebellum, an area typically spared early on in LOAD. Pathologically, a particular kind of plaque called cotton wool plaque – shaped a bit like a fuzzy ring with a hollow in the middle – appears to match up with this symptom [4,5], but again, there is no definitive link between particular mutations, a particular pathology, and a characteristic clinical phenotype. Myoclonus (a form of muscle twitching) and rigidity also can be part of presenilin-mutant AD, as is Lewy body pathology [6]. Not all mutation carriers in one family will develop these additional symptoms. On the other hand, people with DIAD lack certain deficits that are sometimes seen in sporadic AD. An example Rossor gave was impairment of visual processing and is sometimes referred to as posterior cortical atrophy, or the posterior variant of AD.

In terms of pathology, DIAD can be heterogeneous as well, said Bernardino Ghetti of Indiana University Medical School in Indianapolis. Diffuse plaques, neuritic plaques, and cotton wool plaques are the major types, and vascular deposits can be extensive in some families. Other rare families have abundant pathology in their cerebellum coupled with concomitant ataxic symptoms of poor movement coordination, and yet other presenilin mutations give rise to mixed dementia/parkinsonism reflected by cotton wool plaques in both AD- and PD-vulnerable regions. Some of the most aggressive forms of DIAN, where patients die in their thirties, show large amorphous cotton wool plaques without much neuritic structure. The vasculature in these cases is also severely damaged, with large plaques breaking through vessel walls in many places, Ghetti said. Intriguingly, Ghetti noted that these cotton wool plaques contain little $A\beta_{1-42}$ but a lot of N-terminally truncated forms of $A\beta$, either with or without pyroglutamated residues on positions glu-3 and glu-11 [7].

Biochemically, the heterogeneity of the 175 published presenilin 1 mutations has triggered much debate among molecular biologists about how the mutations cause AD. At this point of the debate, a frequently heard view holds that individual differences among mutations tend to have one feature in common. That is, they disturb the function of the γ-secretase enzyme complex in such a way that the enzyme generates a pathologically altered distribution of $A\beta$ peptide variants, Bart de Strooper of the VIB Institute in Leuven, Belgium, said. In short, the mutant enzyme makes more of $A\beta$ long forms and fewer of its short forms. The Familial Adult Children Study (FACS) aims to capture the dynamics of this process by measuring in real time the production and metabolism of $A\beta$ in the CSF of people with DIAD. In St. Louis, FACS leader Randy Bateman noted that 26 of the planned 36 participants in this study have enrolled as of last August, and that the last nine of those have also completed all DIAN procedures.

Several speakers hinted that the most aggressive presenilin mutations might even express themselves developmentally. Ghetti said that families with myoclonus and seizures have ectopic neurons in the white matter, i.e., neurons that presumably migrated to the wrong place in utero [8]; John Ringman of the University of California, Los Angeles, noted a trend toward less education among PS1 mutation carriers in Mexican American families he studies.

"Overall, I think FAD is actually more heterogeneous than sporadic AD," Rossor summed up. This may be counterintuitive to conventional wisdom, which loosely views LOAD as resulting from many different age-related insults and eFAD as being due to mutation of amyloid-generating genes. On the other hand, a focus on variations and extremely rare cases runs the risk of losing the forest for the trees, Bateman wrote. "In our clinical experience, the majority of DIAD families do not have seizures, myoclonus, or other "atypical" features, all of which are sometimes seen in LOAD, as well," Bateman wrote. "We scientists love to look for differences, but the main point we agree on is that DIAD appears highly similar to LOAD overall."

Perhaps the most significant aspect of DIAD heterogeneity is the age span at which carriers begin to get overtly ill. Presenilin mutations can become apparent from the teenage years to the seventies. Those are the extreme ends, however; most families have a 10- to 15-year spread around a mean age of onset in the forties or fifties. Besides presenting uncertainty for the carrier and practical challenges for future prevention trials, this broad range harbors some scientific promise, too, Alison Goate of Washington University said at the Leonard Berg Symposium: "There are other things besides the presenilin mutation going on that determine age of onset. They could be genetic or environmental." If these things could be understood and exploited ther-

apeutically, even a modest five-year delay in the age of onset (of LOAD in this case) would be a boon to public health.

Some clues on what these factors might be have already come out of research on LOAD. Genetically, the ApoE gene plays the single biggest role, with each E4 risk allele bringing down age at onset by some five years, Goate said. ApoE interacts strongly with head injury in that ApoE4 carriers are more likely to develop dementia after they sustain brain trauma. For example, boxers with dementia pugilistica tend to have E4 alleles [9]. Moreover, genetics research has generated a long list of genes that play smaller but possibly important roles in LOAD. LOAD genetics just got a boost from the discovery of three new genes this past summer, but they have not been checked in DIAD/eFAD yet. How about ApoE in DIAD/eFAD? Even this oldest LOAD risk gene has not been systematically studied in eFAD families; however, it is known that in large Colombian kindreds with the presenilin 1 E280A mutation, carriers who have E4 become symptomatic at a younger age than E3 carriers, and E2 carriers stay well some years longer still. "Even in this early-onset FAD kindred, E4 influences when a carrier will get sick," Goate said.

In terms of environmental factors, little is known in DIAD/eFAD. Research of the same Colombian pedigrees suggested, surprisingly, that people with a higher level of education were diagnosed at younger ages than less educated carriers. This was unexpected because education is thought to be protective by affording some brain reserve. In this study, the result might simply mean that more educated people were being picked up as having a problem earlier because they were performing more demanding jobs [10]. Unlike LOAD, which tends to show up in retirement, eFAD typically gets noticed first at work.

Brain imaging has advanced immensely, and numerous lines of evidence, from ADNI and elsewhere, are gradually bringing into focus a view of preclinical AD for both LOAD and DIAD/eFAD. Imaging has produced its share of puzzling moments, however, where it appeared to highlight the heterogeneity of eFAD. For example, in 2007, when excitement over amyloid imaging began to spread worldwide among AD research groups, Klunk, who co-developed PIB with his friend and colleague Chet Mathis, published the first PIB images of presymptomatic carriers of the C410Y and the A426P presenilin mutations. Their PIB retention began building up with an extraordinarily intense signal in the striatum, an area hit hard in PD and HD, but usually spared in AD [11]. The typical AD regions lit up, too, but much more weakly. This finding seemed at odds with the clinical and pathological picture of AD, and it prompted questions about whether eFAD truly models LOAD. "This unusual distribution was so surprising, I first accused Chet of accidentally giving me a dopaminergic agent, not PIB," Klunk recalled. But PIB it was. Since then, the London researchers have observed a similar striatum-first PIB pattern in members of some of the families they follow, Rossor said.

In the meantime, Klunk's group has obtained repeat scans from those and other DIAD/eFAD research volunteers. He offered this update: Over the course of four years, the PIB pattern spread out from the striatum to a typical AD pattern with increasing binding in cortical regions, whereas the original striatal binding diminished over this period of time. The pattern of PIB retention varied somewhat by mutation. Overall, Klunk said, it looks like in eFAD, amyloid deposition starts in the striatum as early as 10 years before symptoms, peaks before or around the time symptoms appear, and then spontaneously decreases. Neocortical amyloid appears later but then stays. In LOAD, amyloid deposition begins in the neocortex (in the precuneus and anterior cingulate subareas), later spreads to include the striatum, and does not decrease.

"We also see the striatal pattern of eFAD in Down's, the only other Aβ overproduction syndrome," Klunk said. "We think it is related to that but cannot explain it." In the discussion, scientists noted that PIB may not see cotton wool plaques and diffuse plaques. It also does not bind non-fibrillar forms of Aβ, all of which may develop with a slightly different time course and distribution in eFAD versus LOAD.

Imaging Preclinical AD – Can You See It Coming in the Brain?

At the 7th Leonard Berg Symposium, brain imaging researchers of various stripes – some MRI, some FDG PET, some amyloid imaging aficionados – took stock of what brain imaging tells the field about the preclinical period of AD. From their vantage point, a picture is slowly coming into focus whereby a number of different imaging techniques together predict that a person is on the way to developing AD symptoms. For some speakers, the evidence is strong enough to start thinking seriously about how to use images (in conjunction with CSF and cognition) to test prevention of AD in people

who are at high risk, either by virtue of having inherited the ApoE4 risk allele, or a dominant mutation.

Nick Fox of King's College, London, UK, reminded the audience that of the imaging modalities currently used, MRI is one that can yield perhaps the most exquisite view of the brain with a resolution of 1 mm. This can be had with a 10-minute scan session that is robust and practical for the imaging technician and easy to tolerate for the subject, certainly for presymptomatic people and even for many patients. This method is particularly suited for tracking change, for example, shrinking of brain areas, over time, and to measure progression. "We are all interested in drugs that slow or stop progression," Fox said.

Some prior imaging studies on presymptomatic eFAD mutation carriers have been done, all very small [12–15]. Early data from Fox's group suggest that, after diagnosis, people with eFAD lose volume rapidly. Fox showed images of one person's hippocampus shrinking dramatically within only two years of diagnosis. Many years prior to diagnosis atrophy rates are slow, but five to two years prior, they noticeably pick up to as much as 3 percent per year in particularly vulnerable areas. Rates come in plural form because they differ by brain area; for example, the entorhinal cortex shrinks particularly fast just before diagnosis. "Serial imaging gives us fantastic insight into structural change prior to diagnosis. The excitement of DIAN is that we can pull more people together to expand on these findings with better power," Fox said.

Technically, the large range of normal variation in brain size between one person and the next makes it important to focus on rates of change in given areas, not absolute measures, and also to focus on the area that changes between two well-registered images (aka the boundary shift interval), without including the volume of the entire brain, Fox noted.

MRI is one of the most established ways of brain imaging. In the past five years, it got increasingly prominent company from amyloid PET, which visualizes in fiery color and in living people pathology that previously could only be seen after a patient had died. Research groups around the globe quickly adopted the amyloid label PIB or similar compounds for study, and as people began to drill deeper, the story became more complicated. Questions arose about what it means that a large fraction of cognitively healthy elderly have amyloid in their brains, about exactly which forms of amyloid PIB binds to, and how being PIB positive related to other liabilities ascribed to the preclinical stage of AD. At the Leonard Berg Symposium, Keith Johnson of Massachusetts General and Brigham and Women's Hospitals in Boston addressed these questions with the data available to date.

Multiple studies show that, by and large, everyone with AD has high PIB retention in their brains, Johnson said. There are exceptions relating to the maturity of plaques and perhaps "strains" of Aβ [16]. "These are interesting and important, but they are exceptions," Johnson stressed. At the early symptomatic MCI stage, people either have a control pattern or an AD-like pattern, not an in-between pattern. The so-called PIB-positive (PIB+) controls, that is, people who are clinically normal but have amyloid in their brains, tend to have it in a stereotyped AD-like distribution with a weaker intense signal. Around the time of diagnosis, PIB binding tends to become saturated and stay relatively stable through the course of clinical disease.

One burning question on the minds of people who study preclinical detection in different populations and with different methods is how the data might all fit together. Johnson offered this hypothesis (see Fig. 1):

By that scenario, the long road to disease would begin with Aβ pathology. Age and genetics (i.e., ApoE genotype) influence a person's propensity to deposit amyloid, and both CSF Aβ and PIB PET can detect it. This would lead to synaptic dysfunction, which impairs cognition directly, and also indirectly over time as the neurons bearing malfunctioning synapses slowly die. It is widely accepted that brain and cognitive reserve influence how long a person can withstand the damaging effects of the encroaching pathology. Bill Klunk, of the University of Pittsburgh Medical School in Pennsylvania, noted that vascular disease is another important environmental factor that acts at this level. The PIB+ clinically normal people currently draw intense interest among researchers. Are they the ones who are on this path to AD? Imaging methods from functional MRI to FDG glucose PET and volumetric MRI exist to visualize and quantify these stages. The standard clinical tests used routinely in diagnosis pick up the latest stage on this flow chart, but increasingly, new findings are converging around more sensitive cognitive measures to pick up earlier, subtler changes.

One way of probing whether this suggested signature of AD holds true is to test whether amyloid deposition in clinically and cognitively normal people relates to the other components in this diagram. "We do this by looking at other measures of AD in PIB+ controls," Johnson said. Here are four examples of doing so that Johnson presented in his talk:

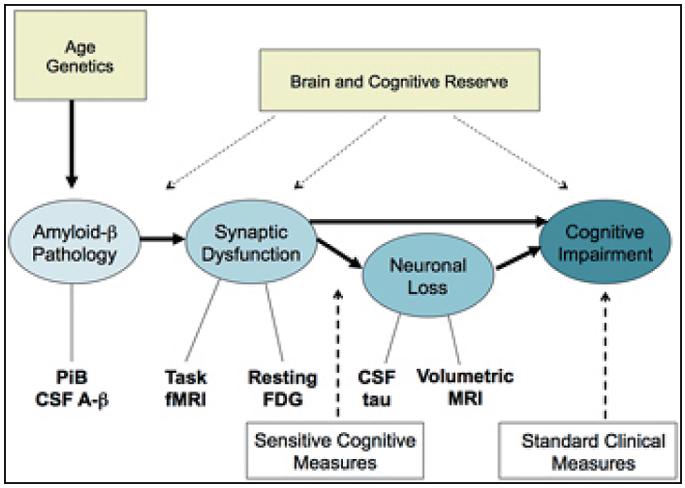

Fig. 1.

– Cortical thickness: In MGH studies of cognitively normal PIB+ people, increasing amyloid is associated with a thinning cortex in the precuneus, anterior and posterior cingulate, and some temporoparietal and medial frontal cortical areas. These areas have come to represent a network that represents functional connection. "These areas talk to each other," Johnson said.
– Hippocampal volume: In the MGH/BWH studies, this measure does not seem to relate to amyloid deposition in the cognitively normal volunteers but does by mildly impaired stages of CDR 0.5 and 1. "This suggests to us that the cortex thins first and the hippocampus a bit later," Johnson said.
– Brain connectivity: A recent study reported that the more highly linked an area is – in other words, if it is a hub of intense connectivity – the more amyloid it tends to have [17]. This month, the researchers reported an amyloid-related disruption of intrinsic connectivity among asymptomatic elderly [18]. "This suggests a disconnection where one area is unable to talk with other, previously connected areas anymore," Johnson said. In July, the same researchers reported that using fMRI, they found that brain amyloid in non-demented people mapped to their inability to deactivate the default network when focusing on a task [19].
– Cognitive function: It is becoming increasingly clear that amyloid deposition is tied to cognitive impairment. IQ and education are important here because people high on those measures are able to tolerate amyloid pathology longer. People with low IQ/education scores show a much stronger link between amyloid in their precuneus (a particularly early area) and how they perform on neuropsychological tests [20]. And preliminary analyses of longitudinal data showed a detectable decline in a delayed word recall test. People declined along with increasing precuneus PIB. These are subtle effects on difficult tests, Johnson noted; the first difference the MGH/BWH studies see in those tests is that people with PIB in precuneus lose the practice effect, i.e., they do not improve anymore with repeated testing. "We really do not understand in biological terms what cognitive reserve is and how it preserves function," Johnson noted.

The Society for Neuroscience conference, held 17-21 October 2009 in Chicago, featured even newer data along these lines. To quote but one example, Deepti Putcha, working with Reisa Sperling at Brigham and Women's Hospital in Boston, reported that cognitively normal older people who performed poorly on a challenging memory test (RVALT) had hyperactivation of their hippocampus along with cortical thinning in AD-vulnerable areas of the medial temporal lobe. These data are fresh out of the scanner, and concomitant amyloid and connectivity imaging data on this new aging cohort still needs to be analyzed, Putcha said.

These and other emerging clues on how multiple preclinical markers link up together in the same people will hopefully bring into focus a predictive signature over the course of the next five years, as longitudinal studies under way at several institutions progress. But even now, the field has advanced far enough to start planning prevention research, argued Eric Reiman of

the Banner Alzheimer's Institute in Phoenix, Arizona. Evaluating promising presymptomatic treatments in the general population takes too long and is too costly, but doing so in high-risk groups such as ApoE4 carriers and presymptomatic carriers of DIAD/eFAD mutations has come entirely within reach, Reiman said. Adding to the other speakers' presentations a brief summary of his group's research, Reiman noted that he has followed ApoE-genotyped volunteers – that is, people at three defined levels of risk – with brain imaging for well over a decade. People with the ApoE4 allele have decreased glucose utilization and subtle metabolic changes already as young adults, predating even the earliest biochemical marker to date, i.e., CSF Aβ. These FDG PET abnormalities do not progress continuously from early adulthood, but they flag early on where the AD changes will develop later, Reiman said [21–24].

The wealth of imaging and biomarker data the field has accumulated to date provides a sufficient foundation for proof-of-concept trials of biomarker outcomes, not clinical outcomes, with several hundred subjects, Reiman said. He spoke forcefully for starting an era of prevention research. This will not be a quick achievement, but it is time to begin gathering drug/biomarker data that can open a new regulatory path to approval. Such trials would also show research volunteers who have allowed scientists to poke, question, and scan them for decades that the scientists, in turn, will use all that data toward something tangible that truly matters to the volunteers and their families.

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