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Q&A

Alzheimer Disease: Advances in Pathogenesis, Diagnosis, and Therapy

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Alzheimer disease (AD)[9], the most frequent cause of dementia in Western societies, affects approximately 5.5 million people in the US and more than 35 million people worldwide. Common symptoms of this disease include memory loss, confusion, irritability, and aggression. Normal bodily functions are progressively lost, with AD becoming fatal within 3 to 9 years after diagnosis.

The major risk factor for AD is advanced age. After the age of 65 years, the incidence of AD doubles every 5 years. As the size of the elderly population increases, particularly the "baby boomers," the prevalence will approach 30 to 60 million cases in the US alone by 2050.

The pathogenesis of AD is not clear, but the major pathogenetic mechanisms that have been suggested include the accumulation of misfolded proteins in the aging brain, which causes oxidative and inflammatory damage, which in turn leads to energy failure and synaptic dysfunction. A very small proportion (<1%) of AD cases are familial with early onset, and such cases have been linked to mutations in genes encoding amyloid precursor protein (APP) and presenilins 1 and 2. There is also a strong genetic association between one variant of apolipoprotein E (apo E) and the risk for developing AD.

Over the last 10 years, there has been increased interest in understanding the pathogenesis of this disease and in the development of new therapies. We interview 4 specialists and examine the latest advances in pathogenesis, diagnosis, and therapy for this debilitating disease.

Can you summarize briefly some new insights into the pathogenesis of AD?

JoAnne McLaurin: We have understood for years that the biggest risk factor for AD is aging. We are now starting to unravel the effects of aging on memory function and link this back to disease progression. New data have arisen that link metabolic disorders, such as diabetes and AD. These insights have led to the proposal that certain lifestyle changes can help to prevent or delay the onset of AD. Notable recent findings are the benefits of vitamin B supplementation and exercise for preventing AD in the healthy elderly population.

David M. Holtzman: There have been many new insights into AD over the last several years. I believe one of the most important realizations is that the pathology that underlies the disease appears to begin many years before any outward signs and symptoms of the disease are present. For example, the buildup of amyloid-β protein (Aβ) may occur as early as 10–15 years before symptom onset. Misfolded τ protein also starts accumulating in the neocortex about 3–5 years before symptom onset. Changes in these biomarkers can be detected in the cerebrospinal fluid (CSF) as well as by neuroimaging techniques, such as amyloid imaging. The good news is

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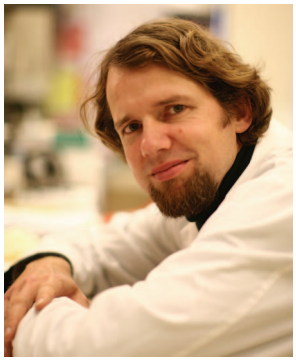
[9] Nonstandard abbreviations: AD, Alzheimer disease; APP, amyloid precursor protein; apo E, apolipoprotein E; Aβ, amyloid-β protein; CSF, cerebrospinal fluid; NSAID, nonsteroidal antiinflammatory drug.

that therapeutic trials can now be developed with the goal of delaying or even preventing the clinical manifestations of disease through the use of these specific biomarkers.



Gerold Schmitt-Ulms: In my view, 3 developments stand out: (i) An increasing number of observations have drawn attention to the possibility that oligomeric assemblies of misfolded proteins may be operative in the cell-to-cell spread of a range of common neurodegenerative diseases, including AD, Parkinson disease, and polyglutamine diseases; (ii) the discovery of novel candidate genes with possible involvement in the pathogenesis of AD has placed a spotlight on synaptic signaling, vesicular protein trafficking, and cellular lipid biology; and (iii) a gradual shift in perception has led to acknowledgment of the humbling complexity of all aspects of AD pathogenesis. Not only are the molecular events that govern the generation of Aβ and τ deposits far more intricate than previously thought, but initial avenues for diagnosis and treatment also have turned out to be too crude.

Rémi Quirion: Recent data from genome-wide association studies suggest possible (albeit minor) roles of genes such as *CLU*[10] (clusterin) and others in the pathogenesis of AD. Other studies are investigating the significance of intracellular vs extracellular amyloid components in the etiology of AD. In contrast to a few years ago, interest has now shifted to the role of intracellular amyloid components (such as APP) in AD and their association with other cytoplasmic proteins and organelles, although some still argue that the amyloid hypothesis has been overemphasized. Others aim to investigate the role of caspases, cell death, excitotoxicity, and other pathways to identify a potential primary role for microglia and neuroinflammatory processes in AD. In the end, it is likely that these various phenomena are interrelated, with a more prominent role for one pathway over another in a given subgroup of patients. AD should not be considered a single entity; it is probably far more complex than the simple amyloid cascade hypothesis.

It appears that the incidence of AD is increasing. Is this true, and if "yes," can you speculate as to why this may be happening?

JoAnne McLaurin: One of the biggest contributors to the incidence of AD is the population demographics in North America. Our aging population continues to increase dramatically as the baby boomers reach 60, and it is speculated that in 2015, for the first time in Canada, people over the age of 60 will outnumber those under the age of 15. Furthermore, the healthcare system has improved the longevity of life in general, a fact also contributing to the increase in the number of people living with AD.

David M. Holtzman: I am not aware that the incidence of AD is increasing. It is true that as our population ages the prevalence of AD is increasing. Thus, as heart disease, stroke, and cancer have better treatments, AD is going to become an even bigger and bigger public health problem.

Gerold Schmitt-Ulms: While undoubtedly AD has been around for a very long time, the perception of an increasing prevalence is not just a reflection of a heightened awareness. Aside from changes to demographical patterns (age remains the most important risk factor for this disease), epidemiological studies have proposed links between obesity or diabetes (risk factors themselves experiencing a surge in recent times) and late-onset AD. Furthermore, with more diseases being preventable or treatable, neurodegenerative disorders may increasingly replace other causes of death. It is difficult to put exact numbers to this trend due to the challenges associated with the acquisition and interpretation of retrospective epidemiological data for AD, a dilemma exacerbated by the fact that the method of AD diagnosis itself has been a moving target.

Rémi Quirion: Well, this is not really true. Yes, there are more reported cases, but this is because the average age of the population is increasing worldwide, and age is still the main contributor in AD. The increase in AD is not due to the appearance of any new virus or a pathogen.

[10] Human genes: *CLU*, clusterin; *APOE*, apolipoprotein E; *PICALM*, phosphatidylinositol-binding clathrin assembly protein; *APP*, amyloid beta (A4) precursor protein; *PSEN1*, presenilin 1; *PSEN2*, presenilin 2; *BIN1*, bridging integrator 1; *CR1*, complement component (3b/4b) receptor 1 (Knops blood group).

It is generally believed that AD runs in families. Can you outline briefly the genetic association of this disease? Are there any new clues?

JoAnne McLaurin: Geneticists predict that they have identified the mutations/duplications in all the genes associated with early-onset AD, but the identification of risk factors that are linked to late-onset AD is the present challenge. The greatest risk factor associated with AD is the apo E4 allele, which increases a person's risk of developing AD by 50%. However, as the cause of the majority of cases of AD is still unknown, additional risk factors within families need to be identified. Genetic-association studies have identified at least 42 other candidate genes, but these have not been confirmed in large pedigrees to understand the link to familial inheritance.

David M. Holtzman: Late-onset AD (age of onset >60 years) accounts for more than 99% of AD cases. There is no question that late-onset AD runs in families. One of the biggest reasons for this is *APOE* (apolipoprotein E) genotype. There are 3 flavors of apo E: E2, E3, and E4. One copy of the E4 allele is associated with a 3-fold increased risk for AD, and 2 copies of E4 are associated with a 12-fold increased risk. E2 is associated with a decreased risk for AD. Much of the familial association of AD is accounted for by *APOE* genotype, but not all. There are other genetic changes that also contribute to the genetics of AD, though it is unlikely that any one of these in isolation contributes more than apo E. Some recently identified genes that also contribute to AD risk include *CLU* and *PICALM* (phosphatidylinositol-binding clathrin assembly protein), among others.

Gerold Schmitt-Ulms: AD is a heterogeneous disorder with both sporadic and familial forms. The genetic component is most conspicuous in a relatively small percentage (<1%) of families that carry disease-causing germline mutations in 1 of 3 genes—*APP* [amyloid beta (A4) precursor protein], *PSEN1* (presenilin 1), or *PSEN2* (presenilin 2)—that will invariably lead to AD, often at a relatively young age. A second group of individuals may harbor a genetic risk factor in their genome that has less than absolute disease penetrance but increases their chance to develop late-onset AD in their lifetime. Carriers of the apo E4 allele, for example, are significantly overrepresented among AD subjects due to a well-documented ability of this allele to lower the age of disease onset. A flurry of recent genome-wide association studies has added many candidate AD-risk genes [*PICALM*; *BIN1*, bridging integrator 1; *CLU*; *CR1*, complement component (3b/4b) receptor 1 (Knops blood group); and others]. It may emerge that some of these confer only a minor increase in AD risk by themselves but contribute to a considerable cumulative risk in a given individual through complex interactions with other genes or environmental factors.

Rémi Quirion: There is no doubt that genes play a key role in AD. However, truly familial cases of AD are rather rare (at most 10% of all cases) and are due to mutations in the *APP*, *PSEN1*, or *PSEN2* gene. In addition, the apo E4 allele plays a role in the incidence of both familial and sporadic AD, but as a risk rather than an etiological factor. Genome-wide association studies have revealed novel genes possibly involved in AD, such as *CLU* (apo J), but their penetrance is usually low. It is not clear if these genes play major roles in the etiology of AD or if they are preferentially associated with disease progression or minor phenotypes observed in AD.

We need early diagnostic modalities for AD, which, in turn, could lead to early treatments with better outcomes. What are the latest diagnostic advances for this disease?

JoAnne McLaurin: A large amount of effort is being targeted to identify more-specific and earlier biomarkers for AD, both in CSF and plasma. Imaging agents, for example positron emission tomography and MRI, are being refined and tested to diagnose and to follow disease progression or resolution. These efforts are designed for earlier identification of patients at risk for AD such that preventive strategies or novel drug therapies can be initiated before the onset of significant cognitive impairment.

David M. Holtzman: It is clear that measurement of Aβ42, τ, and p-τ (phosphorylated τ) concentrations, especially when examined as a ratio of τ to Aβ42, can identify the presence of AD pathology in living people who already have dementia, as well as in people who are cognitively normal. Importantly, these measures can identify cognitively normal people who are at high risk of converting to becoming demented over a several-year period. In addition, amyloid imaging is very useful for assessing the amount of fibrillar Aβ that has built up in the brain. It can also identify people who are cognitively normal but are likely to progress over the next several years to dementia. In addition to these markers, structural and functional MRI may be very useful for determining how much neurodegeneration may be present and for monitoring therapies.

Gerold Schmitt-Ulms: To this day, early AD diagnosis is primarily based on tests that interrogate cognition and behavior. Modalities that increasingly find application and offer considerable promise are structural and functional neuroimaging (MRI, single-photon

emission computed tomography, and related methodologies) and molecular profiling–based diagnostics from body fluids. It is apparent that AD does not present as a unitary disorder but rather as a group of syndromes that not only progress at different rates in different individuals but also may be triggered by a spectrum of related but nonetheless distinct molecular etiologies. Thus, the ability to detect the disease early will likely require lockstep advances in the characterization of AD subtypes and their differential diagnosis.

Rémi Quirion: The earliest and most predictive biomarker for AD is the CSF ratio of Aβ to τ, especially when coupled with amyloid imaging in brain structures. Hopefully, both markers will turn out to be diagnostically sensitive enough and to respond to future therapeutic approaches, as "real-world" biomarkers should.

Are there any good treatments/preventive strategies for AD now? What is in the pipeline?

JoAnne McLaurin: There are presently over 200 clinical trials under way evaluating AD therapeutics within North America. To date, clinical trials have been somewhat successful in terms of developing symptomatic treatments, while clinical trials for disease-modifying therapies have failed to show effects on cognitive performance. The field is shifting toward addressing clinical impact at early stages of disease, where the benefit to the patient is greater. The pipeline for new compounds/treatment strategies is deeper today than it has been in the last 20 years.

David M. Holtzman: Many pharmaceutical companies and other groups have developed a variety of treatments that have potential as therapeutic or prevention strategies. There are many promising agents that attack the Aβ peptide. These include anti-Aβ antibodies, γ-secretase inhibitors, γ-secretase modulators, and β-secretase inhibitors, among others. While these treatments are very promising, assuming one can overcome potential side effects, the big issue is at what point in the disease course would these treatments be effective. It seems likely, given what we know about the time course of AD, that they would have the best chance to be effective if used as in prevention. Use of these promising agents, however, has not been attempted in the "preclinical" or "presymptomatic" stage of AD. This appears to be a very important area for future study.

Gerold Schmitt-Ulms: Currently, approved AD drugs (for example, acetylcholinesterase inhibitors and *N*-methyl-D-aspartate antagonists) do not halt the disease but may temporarily improve cognitive ability and provide symptom relief. A range of studies suggest that living a socially, mentally, and physically active life may still constitute the most potent remedy we have against AD. Despite a rather grim picture painted by a recent series of disappointing AD clinical trials, there are some positive developments. The sophistication of AD clinical trials with the potential to interfere mechanistically with AD is increasing, and the intervals between them are shortening. And whereas the predominant focus in past years has been squarely centered on the Aβ paradigm, alternative concepts aimed at targeting downstream events are increasingly gaining traction.

Rémi Quirion: Well not really, unfortunately. Normalizing high blood pressure has been suggested as a preventive strategy, but recent epidemiological data are not clear-cut. Physical and mental exercises are also likely to have protective effects, but, again, more data are required to confirm this hypothesis. Controlling low-level brain inflammation has similarly been suggested to be effective, but results of these clinical studies have been difficult to fully confirm and extend. More studies are under way in that regard with apo E subgroups and cohorts at early stages of the disease.

Some advocate that over-the-counter antiinflammatories such as Ginkgo biloba, lecithin, curcumin, and other chemicals maybe useful. Do you believe that such treatments are beneficial?

JoAnne McLaurin: Clinical trials sponsored by the National Institute on Aging and the National Center for Complementary and Alternative Medicine have failed to show improvement or stabilization of memory in elderly patients with mild cognitive impairment or in mild to moderate AD patients. The large size of the clinical trials suggests that treatment with these natural products may be too late when memory has already started to decline. The beneficial effects of these compounds in transgenic mouse models have primarily been as a preventive strategy, and this finding may account for the lack of translation to date.

David M. Holtzman: While it is possible that the agents mentioned above may be useful, there is no solid evidence to date that support that notion.

Gerold Schmitt-Ulms: There are retrospective epidemiological data on arthritis patients that indicate that long-term administration of nonsteroidal antiinflammatory drugs (NSAIDs) may cause a pronounced reduction in AD risk. This observation is, however, contrasted by AD clinical trial data that repeatedly failed to detect significant benefits following extended administration of NSAIDs (or other natural antiinflammatories). It has been proposed that these conflicting con-

clusions may reflect, at least in part, differences in presymptomatic vs symptomatic cohorts underlying these data. Indeed, the suspicion is increasingly taking hold in the field that drug regimens may need to commence before the prionlike spread of misfolded Aβ and τ aggregates has hijacked an affected individual's brain. More work is needed not only to settle this question but also to delineate more fully the mechanism of action of NSAIDs and the capacity of natural compounds with antiinflammatory efficacy to mimic synthetic NSAIDs in this regard.

Rémi Quirion: Taken one by one and on their own, I doubt that a really significant effect will be seen with these medications. But, these could prove to be beneficial if taken as a mixture and for decades. Well-controlled studies are required to confirm my assumptions.

What is your prediction about new clinical-breakthrough technologies for this disease? Would whole-genome sequencing, advanced imaging, immunology, etc., help to understand the pathogenesis of this disease and facilitate the development of new treatments over the next 10–15 years?

JoAnne McLaurin: Advanced imaging modalities have the potential to make a large impact on the early identification of people at risk or with disease before clinical onset. It is estimated that AD initiation occurs up to 10 years before symptom onset, and therefore noninvasive imaging modalities that have the potential to aid in preclinical early diagnosis may prevent cognitive decline once therapeutics have been identified.

David M. Holtzman: It is clear that in regard to clinical breakthroughs in AD, CSF biomarkers and amyloid imaging have great potential as antecedent biomarkers for AD. Further, neuroimaging techniques have revealed important insights into disease mechanism as well as provided other important diagnostic and prognostic markers for disease. It is possible that whole-genome sequencing and understanding immunological aspects of AD may lead to further insights. A big diagnostic advance would be the ability to image τ pathology.

Gerold Schmitt-Ulms: The challenges posed by a looming AD pandemic are formidable and will require a concerted effort involving many disciplines beyond those listed above. Thus, 3 advances are critically needed: (i) to address the limitations of existing animal models that mimic facets of the disease but are inadequate for assessing the efficacy of compounds for the treatment of AD in humans; (ii) to build bioinformatics platforms suitable for depositing and extracting useful information from a jungle of cumulatively collected data that are beyond anyone's ability to evaluate and integrate; and (iii) to generate reliable biomarkers for identifying at-risk, but asymptomatic, individuals.

Rémi Quirion: In my view, the key is to refine current biomarkers and find additional new ones that will allow us to diagnose very early and to precisely subclassify AD patients. Treatment approaches could then be targeted to the most appropriate subgroup of patients, similar to how treatments for hypertension can target the kidneys with a diuretic, the vessels with a vasodilator, and the heart with β-blockers, or a mix of those, depending upon each patient.

Hence, novel highly reliable and robust biomarkers are needed, hopefully plasma based for ease and safety of sampling. I am optimistic that plasma biomarkers will be developed over the next 5 to 6 years. Coupled with imaging and genomics, this could allow for detailed characterization of each patient for a more personalized treatment approach.

As for new treatments, we must be optimistic as well as very open-minded about alternative hypotheses and approaches. The dogma-related strategy has certainly not served us well. It is likely that an arsenal of drugs targeting different processes will be required to be truly effective. We should learn from lessons from the HIV-AIDS world, tritherapy being the key to effectiveness.

It was suggested that antibodies might remove amyloid from the brain and restore or stop progression. Any updates on this and a possible "vaccine"?

JoAnne McLaurin: Every large pharmaceutical company has both active and passive vaccine programs at various stages of preclinical and clinical development, the most advanced residing in late phase III human clinical trials. The hurdles for development of an active/passive vaccine are high with side effects (such as vasculitis, encephalopathies, and increased cerebral amyloid angiopathy) or more-practical concerns (such as a high burden for delivery by frequent visits to the clinic and costs) being some of the concerns around this technology. We will need to wait to see the outcome of the present trials to determine whether removal of amyloid is sufficient to arrest cognitive decline.

David M. Holtzman: Evidence from both animals and now humans suggests that antibodies to Aβ have the potential to block further Aβ accumulation as well as to decrease the amount of Aβ pathology present in the brain. Phase III trials of 2 different anti-Aβ antibodies in humans with AD are under way. While these anti-

bodies have promise, they are being tested in patients who already have mild to moderate dementia due to AD. It is possible they will have a clinical effect. However, the biology of AD suggests that the best chance for their success would be to try to utilize such agents in individuals before the development of signs and symptoms of AD.

Gerold Schmitt-Ulms: The last chapter on this approach has not been written; rather, the field is currently trying to incorporate hard-learned lessons into improved AD immunotherapy designs, several of which are currently being tested. What is now understood is that pilot data generated in mice may translate poorly when predicting efficacy in humans and that careful consideration has to be given to every aspect of the study design (including the mechanism of antibody administration, antibody epitope, etc.). Long-term assessments of the efficacy of previous studies have led to conflicting data, some of which suggest that reduced functional decline was achieved in antibody responders.

Rémi Quirion: Indeed, this is a most exciting approach, and it may work for some patients in the end. But we probably jumped too quickly and ran clinical trials that were not optimal. As we find out more about the mechanisms possibly involved in AD, it should be possible to perform clinical trials under better-controlled conditions. In my mind, this approach is not a magic bullet that will solve it all. Rather, it may work only on a subgroup of patients for whom extracellular amyloids play a greater role. That is not necessarily true for the majority of AD cases.

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