

A conversation with Melanie Smith, February 17, 2015

Participants

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Note: These notes were compiled by the Open Philanthropy Project and give an overview of the major points made by Melanie Smith.

Summary

The Open Philanthropy Project spoke with Melanie Smith as part of its investigation into Alzheimer's disease (AD). Conversation topics included the risk factors, genetics, and possible mechanisms of AD, the diagnosis and treatment of AD, and remaining gaps in understanding.

Possible Alzheimer's disease mechanisms

AD and other severe dementias are associated with significant neuronal death. Neurons in the central nervous system have a limited ability to regenerate compared to other cells. It was once thought that the lifetime supply of neurons was set early in life. There is now evidence of brain plasticity and neuron growth well until adulthood, but it's unclear how long this neuronal growth potential lasts. Traumatic brain injury (as seen in boxers and football players) also damages neurons. Because neurons turnover at a much slower rate than other cells in the body, they tend to accrue more damage over time.

Autopsies have shown that brains affected by AD have large ventricles (the four cavities in the brain that produce cerebral spinal fluid) and reduced brain mass (in some areas of the brain over 50% of neurons have died). Parts of the brain related to memory (e.g., the hippocampus) are particularly small in people affected by AD. Amyloid plaques and tau tangles, both abnormal protein structures, are also visible in brains of people affected by AD. The main theories of AD address these two structures.

Tau theory

Neurons are very long cells with branched projections (dendrites). Microtubules (a component of the cellular structure known as the "cytoskeleton") form a path along which neurotransmitters and mitochondria (which generate the cell's energy) are transported to the outer regions of the cell. The protein tau normally helps stabilize these microtubules. Tau's activity is regulated by phosphorylation (i.e. the addition of phosphate groups changes the activity of the protein).

In AD, as well as in other neurodegenerative diseases, tau is often hyperphosphorylated (over-phosphorylated). This is associated with the formation

of abnormal tau aggregates (called tangles) inside the cell. In cells with these aggregates, the tau protein is sequestered away and the microtubules are destabilized and begin to disintegrate. It is not clear whether the hyperphosphorylation is a cause or effect of the tangles. Changes in phosphorylation likely accumulate with age.

The tau theory hypothesizes that neurons die when tau tangles accumulate and neurons can no longer effectively transport neurotransmitters and mitochondria. If tau is overexpressed in a mouse model, the mouse shows hyperphosphorylated tau pathology and neurodegeneration.

Amyloid theory

The amyloid theory hypothesizes that AD is caused by the buildup of amyloid plaques in the brain. Currently, research related to the amyloid hypothesis receives the most funding of all AD related research. Most pharmaceuticals and clinical trials address the amyloid hypothesis.

What are amyloid plaques?

Amyloid is a general term for a three-dimensional shape that a protein can form. A protein has three types of structure:

- A protein's primary structure is its sequence of amino acids.
- This sequence determines the secondary structure, which is a series of interactions that determine the three-dimensional structure.
- A protein's tertiary structure describes the way it interacts with other proteins or protein subunits to form a protein complex.

Normal proteins have unique primary, secondary, and tertiary structures that allow them to carry out their respective functions in the cell. However, many kinds of proteins can form a similar abnormal secondary structure, which facilitates extensive interactions between multiple copies of the same protein and leads to the formation of amyloid fibrils. Amyloid fibrils further accumulate to form plaques outside of the cell.

Amyloid plaques are seen in a variety of neurodegenerative diseases – such as AD Huntington's disease, Parkinson's disease and prion-related diseases like bovine spongiform encephalopathy (mad cow disease). It is unclear if amyloid causes neurotoxicity (neuron death) or if it is a byproduct of neurotoxicity.

Amyloid beta and the amyloid precursor protein

In AD, amyloid plaques are predominantly composed of the protein amyloid beta (A β or A β). A β is a short chain of amino acids that is a product of the amyloid precursor protein (APP) cleavage. A β comes in three forms that are relevant to AD: (A β 40, A β 41, A β 42), which differ by the number of amino acids. A β 42 is the most predisposed to form amyloids. The normal function of A β is not well understood.

APP is a protein found in many tissues and concentrated in the synapses of neurons. Its primary function is not known. APP is embedded in the cellular membrane and is cleaved or “snipped” by multi-protein complexes called secretases that include the presenilin proteins, which are mutated in some forms of familial AD.

- If the APP is cut by the enzyme alpha secretase, there is no formation of A β and no consequence for AD.
- If the APP is cut by the enzyme beta secretase, it yields two smaller segments, one of which can be cut again by the enzyme "gamma secretase" to create A β 42.

Evidence for amyloid hypothesis

Compared to other theories, the amyloid hypothesis has the most evidence supporting it. Most genetic risk factors for AD are related to amyloid plaques.

- APP is found on chromosome 21. People with Down syndrome (who have three copies of chromosome 21) have an increase risk of developing AD at a young age. This is evidence of some sort of dosage effect.
- The allele ApoE4, a variant of ApoE gene, is the largest known genetic risk factor for late onset, sporadic AD. ApoE4 is associated with increased amyloid plaques in the brain.
- Mutations in the genes for APP and the presenilin proteins (sub-components of gamma secretase responsible for cutting APP) are linked to early onset, familial forms of AD.
- If A β is overexpressed in a mouse model, the mouse displays memory impairment and associated neurodegeneration.

Possible mechanisms of amyloid toxicity

The actual mechanism by which A β and amyloid plaques cause damage is still unknown. There are several theories:

1. The actual amyloid fibers are problematic to the cell in some way (e.g., by interfering with normal protein function or impeding cell signaling).
2. Oligomers (self-associating proteins that are a precursor to fibers) are toxic to the cell, perhaps by interfering with cell signaling.
3. Another subunit of APP may be neurotoxic. One theory is that one end of the APP (the N-terminus) interacts with death receptors and triggers apoptosis (programmed cell death).

It is unclear whether amyloid plaques are inert or reactive. It is possible that amyloids are sequestering other neurotoxic elements.

It is easy to stain for amyloid and identify it in the brain on autopsy. New imaging technology using positron emission tomography (PET) can detect amyloid plaques in living patients. It remains difficult to visualize the smaller, potentially toxic structures, such as oligomers.

Cholinergic hypothesis

The cholinergic hypothesis proposes that in AD, neurons that signal with the neurotransmitter acetylcholine (ACh) die and that the lack of ACh in the brain causes the symptoms of AD. This is the oldest theory of AD, but because the drugs designed to target ACh signaling have limited efficacy in decreasing symptoms and do not slow disease progression, this theory is no longer widely accepted.

Normally, ACh is cleared from the synaptic cleft (the space between the axon of one neuron and the dendrite of another neuron) by the enzyme acetylcholinesterase (AChE). A drug, Aricept (generic name: donepezil), was designed to inhibit AChE, which would increase ACh signaling. Aricept is still widely used, but it is not very effective.

AD risk factors

Age is the biggest risk factor for AD. Many people over age 80 have multiple forms of dementia, including AD.

It is not clear how the brain ages. Several risk factors are linked with age and the development of AD and other dementias.

Oxidative damage and stress

Cellular metabolic reactions, which create energy in the form of ATP (adenosine triphosphate), also create free oxygen radicals. Free oxygen radicals will attempt to bond to other molecules. When proteins bond with free radicals, they are often damaged, flagged for degradation or form aggregates that disrupt cellular functioning. When too many proteins are damaged, the cell will trigger apoptosis. In the brain, this would mean death or damage to proteins and neurons.

Neuroinflammation

Activated immune cells can cause inflammation in the brain, which also creates free oxygen radicals. Inflammation related to aging and AD is “sterile inflammation,” because there is no foreign material or bacteria that can explain the immune cell activity. The role of immune cells in the brain and the contribution of these cells to AD pathology remain poorly understood, but it is an active area of research.

Type II diabetes and metabolic syndrome are associated with slightly increased levels of neuroinflammation. These conditions are also linked with slightly higher rates of AD.

Metal ion homeostasis changes

In the past, it was thought that aluminum and other metals played a role in AD and other dementias. Recent studies have not supported this hypothesis.

AD genetics

Early-onset familial AD

Early-onset familial AD is a type of AD that is inherited in an autosomal dominant pattern (i.e. if one parent carries the gene, all children will develop the disease). This accounts for about 1% of AD cases. Symptoms appear before the age of 65 (and as young as 30-40 in some cases).

This form of AD is better understood than the others. Early-onset familial AD is the result of single mutations in the genes for APP, presenilin 1 or presenilin 2 proteins. Presenilin 1 and presenilin 2 form the subunit of gamma secretase that cuts APP. All three mutations increase levels of A β 42.

Late-onset AD

The genetics of late-onset AD (which appears after age 65) are less understood than those of early-onset familial AD. The largest known genetic risk factor is the ApoE4 allele.

There are three major forms of the ApoE gene:

- **ApoE2** – Associated with lower risk of AD
- **ApoE3** – The most common form
- **ApoE4** – Associated with elevated risk of AD

About 20% of the general population carries at least one ApoE4 allele. 40-60% of those diagnosed with AD have at least one ApoE4 allele. Genome wide association studies have identified other genetic risk factors, but they have a much smaller effect than the ApoE4 allele.

Diagnosing dementia

Clinicians usually diagnose dementia based on:

- Patient interview
- Family interview
- Patient history

Typically, patients (or their family members) report they can longer do basic tasks (e.g., making a recipe, finding their way home, paying the bills, etc.).

Some standard tests can be used to help diagnose dementia and distinguish dementia from "mild cognitive impairment," which is a less severe than dementia and does not interfere with activities of daily living. These tests include:

1. **Montreal cognitive assessment (MoCA)** – Used to test for mild cognitive impairment, which is a precursor to various dementias. Patients are asked to complete tasks such as naming animals or drawing a clock showing a certain time.
2. **Mini-mental state examination (MMSE)** – Used for identifying severe dementia. Tasks are more language-based than the MoCA (e.g., patients are asked to spell "world" backwards and count down from 100 by sevens).

Before a diagnosis of dementia is formalized, clinicians must exclude other possibilities that are more treatable than dementia, such as:

- Thyroid problems
- Vitamin B₁₂ deficiency
- Syphilis
- Electrolyte disturbances (such as low sodium)
- Lead poisoning
- Mercury poisoning

MRI (magnetic resonance imaging) and CT (computerized tomography) scans can also be used to rule out strokes, tumors, or other problems, like too much cerebrospinal fluid (CSF) in the brain. Imaging can also identify brain mass changes that are indicative of AD.

Differentiating between different kinds of dementia

A patient's behavior is important in distinguishing between different kinds of dementia. Dementia with Lewy bodies, a form of dementia closely associated with Parkinson's disease, is associated with personality changes and other neuropsychiatric issues. AD is characterized by a decrease in the ability to form new memories.

Often, AD is a diagnosis of exclusion, i.e. what remains after eliminating other possibilities.

A definitive diagnosis can only be made with an autopsy. During the autopsy, parts of the brain are weighed and the amounts of tau tangles and amyloid plaques are measured.

Biomarkers and imaging

Biomarkers and imaging are beginning to be used in clinical diagnoses, but they are more often used in clinical trials.

It is possible to sample CSF via a lumbar puncture (also known as a "spinal tap") to assess levels of tau and A β . Increases in tau and decreases in soluble A β in the CSF are indicative of AD. Clinical use of the lumbar puncture is limited because it is an unpleasant, invasive procedure.

FDG (fludeoxyglucose 18F) PET (positron emission tomography) scans measure cerebral glucose metabolism and blood flow. Normally, this scan is used to look for cancer but it can also be used to approximately measure synaptic function. AD is associated with decreased metabolism in particular parts of the brain.

It is also possible to use a PET scan to identify amyloid deposits directly using molecules that bind amyloid and can be detected via PET. Results from these PET scans match the distribution of amyloid plaques found in autopsies of patients with AD.

Treatment for AD

The U.S. Food and Drug Administration has approved around five drugs for the treatment of dementia. None of them slow disease progression and most have a modest affect on symptoms.

Currently, there is a chicken-and-egg problem with AD treatment and diagnostics. Because of brain plasticity, a large amount of neuronal death occurs before cognitive impairment appears. However, there is little incentive to improve screening or diagnostic measures because there aren't any effective treatments available. If diagnostics improved, it would be more compelling to develop better drugs and if there were better drugs, it would be more compelling to develop improved diagnostics. Currently, researchers are focusing more on developing drugs that would halt the progression of AD than on improving diagnostics.

Clinical trials

There have been many clinical trials targeting AD – almost all of which have failed. Many compounds that have worked well in animal models have failed in human trials.

Most clinical trials have tested drugs on patients with moderate or severe dementia. Researchers are beginning to use longitudinal trials to test the effects of drugs used on mild dementia.

Specific drugs

- **Monoclonal antibodies**
 - **Crenezumab** – a humanized monoclonal antibody that targets all forms of A β
 - It destroyed A β in mouse models, but didn't work well in a Phase II trial with humans. There was no difference in clinical endpoints and no improvement seen in amyloid PET imaging, volumetric MRI, or CSF tau samples. There may have been a slight reduction in cognitive decline.
 - It is possible that Crenezumab would work better when used earlier to treat mild dementia.
 - **Bapineuzamab** – a humanized monoclonal antibody that reduces tau levels in the CSF.
 - Bapineuzamab did not have an affect on A β . Despite the reduction in tau, Bapineuzamab did not lead to clinical improvement.
 - **Aducanumab** – a fully human antibody produced by Biogen that targets A β . A small, early stage trial indicated that it reduced A β in the brain. Biogen is beginning a phase III trial.
 - Another antibody was found to increase soluble A β (which implies less A β in amyloid plaques), but it did not lead to clinical improvements.

- **AChE inhibitors** – e.g., Aricept (generic name: donepezil)
 - These are based on the cholinergic hypothesis. Aricept is not very effective, but continues to be prescribed, possibly because it allows doctors and patients to do something to ‘treat’ AD.
- **NMDA (N-methyl-D-aspartate) receptor antagonists** – e.g., Namenda (generic name: memantine)
 - **These** compounds inhibit NMDA receptors, which play an important role in learning and memory.
 - High levels of glutamate (a neurotransmitter) signaling through NMDA receptors can cause overstimulation and neurotoxicity. NMDA receptor antagonists decrease this signaling and stop any possible overstimulation. These compounds were originally designed as anti-influenza drugs.

Other targeted therapies, such as compounds designed to inhibit beta secretase or presenilins directly, have not worked well. Some therapeutic interventions (including vaccines) have attempted to target oligomers (the amyloid precursors), but these have not been shown to be effective therapies.

There are multiple pathways involved in AD pathology, and we may need to target more than one pathway in order to see a clinical response. In Melanie's view, it's possible that a combination of drugs will be needed to treat AD.

AD research

Key unanswered (or incompletely answered) questions include:

- 1) What causes neurons to die?
- 2) Do sporadic and familial (autosomal dominant) AD share the same underlying pathophysiology?
- 3) How can we make it easier to translate findings in the (in vitro or in mice) into the clinic?
- 4) How does ApoE4 contribute to AD pathology?
- 5) What are potential therapeutic targets besides tau and A β ?
- 6) How can we differentiate AD from other dementias in the elderly?
- 7) Can we better understand risk factors for sporadic AD to determine who may benefit from interventions?
- 8) How can we generate better human neuronal cultures? Are induced pluripotent stem (IPS) cells a way to do this?
- 9) Are there AD subtypes that might benefit from different therapies?
- 10) What is the pathophysiology of disease progression? How do biomarkers change over time? How are different areas of the brain affected (PET, MRI)?
- 11) Are there lifestyle modifications that influence disease progression? (Exercise is promising).

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