Genetics of Dementia

Genetic discovery informs our understanding of disease mechanisms and potential drug targets.

An Interview With Rudolph E. Tanzi, PhD

What Should Clinical Neurologists Know About ApoE4?

Many clinicians may know that having 1 copy of the *apolipoprotein E ε4* (*ApoE4*) allele (heterozygous) is the most common risk factor for late-onset Alzheimer disease (AD) with average age 70 at onset. Clinicians may not realize, however, that people who have 2 copies of *ApoE4* (homozygotes) can have early onset under age 60 that appears similar to familial AD. People with 1 copy of *ApoE4* have a 3- to 4-times increased risk of AD; those with 2 copies have a 12- to 14-times higher risk of AD.[1]

Importantly, however, having an *ApoE4* allele does not guarantee AD will develop because the gene is not fully penetrant. There are known *ApoE4* homozygotes over age 80 with no signs of dementia. This suggests there are many other genes involved in susceptibility that exacerbate or mitigate risk of *ApoE4*. People may have many other alleles of dozens of genes that interact with *ApoE4* or cause risk independently of *ApoE4*.

ApoE4 is a common allele present in 20% of the overall population and 50% to 60% of persons with AD. The wildtype allele, *ApoE3*, is present in over 80% of the population, and the *ApoE2* allele, which is protective for AD, is present in only 2% to 3% of the population. Having 1 *ApoE2* allele and 1 *ApoE4* allele reduces risks as much as having no *ApoE4* alleles.[1] Having *ApoE2* and *ApoE3* decreases risk for AD and increases overall life expectancy.[2] Homozygosity for *ApoE2* (2 copies) is rare.

When Would You Recommend Genetic Testing?

We typically do not recommend genetic testing for only *ApoE*, and when testing is done, we recommend genetic counseling for the patient and family members as part of the process. Even for those with a strong family history of early onset AD and cognitive symptoms, the current value of genetic testing is questionable because there is, as yet, no actionable therapy for secondary prevention based on results of genetic testing. There are potential negative consequences. For exam-

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ple, if someone finds out they are a homozygote, they are also finding out there is a 50% chance that their children will have at least 1 *ApoE4* allele and this can affect important life decisions. Thus, the recommendation for genetic counseling. There are commercially available tests, and individuals can do as they wish, but we see no clinical reason for it at this time.

What Are We Learning From Familial AD?

The genes that cause familial AD, including *amyloid precursor protein* (*APP*), which I discovered during my doctoral research,[3] and presenilin 1 and 2 (*PSEN1/2*), which Peter St George Hyslop, Gerard Schellenberg, and I found,[3] have approximately 300 identified mutations. Unlike the *ApoE4* allele, most mutations of *APP* and *PSEN1/2* guarantee AD will develop by age 60 and often by age 50. Onset of AD before age 60 is most commonly caused by mutations in *PSEN1*, and then *APP*, followed by *PSEN2*, although as mentioned, people who are homozygous for *ApoE4* without mutations in *APP* or *PSEN1/2* may also have early onset AD.

With onset under age 60 but no family history, the first thing to look for is censoring (ie, did the parents live long enough to develop AD). If both parents lived to over age 70 and died of other causes or did not develop AD, it is unlikely the person being evaluated has familial AD caused by *APP* or *PSEN1/2*. It is possible they are *ApoE4* homozygotes with each parent contributing 1 *ApoE4* allele. This is the gray zone of onset at age 50 to 60, in which AD could be familial or not. In contrast, onset under age 50 is almost always familial due to fully penetrant *APP* or *PSEN1/2* mutations.

Evaluating the family history for individuals with onset between age 50 and 60 is a key step, after which genetic testing could be considered, with genetic counseling in place because of the reasons previously mentioned. In addition, although there are laws to prevent health insurance companies from denying coverage on the basis of genetic test results, this is not the case for long-term care insurance or life insurance and individuals need to consider the risk of such companies being able to find their genetic results. It is important for clinicians to be able to share these medicolegal risks as patients and families consider whether to pursue genetic testing.

The bulk of those mutations do not increase amyloid β (Aβ), although that misconception is prevalent. Rather,

these mutations increase deposition of the $A\beta_{42}$ isomer, by increasing the ratio of $A\beta_{42}$ to $A\beta_{40}$ (the more common isomer), and this is what causes plaques to form.[4,5] PSEN1/2 are parts of an enzyme called γ secretase, making up the mouth of this Pacman-shaped enzyme that cuts *APP* to make $A\beta$ of different lengths. When mutations in *PSEN1/2* make the area of the cleavage site smaller—closing the "mouth"—*APP* doesn't fit as well and the ratio of $A\beta_{42}$ to $A\beta_{40}$ increases even though overall production of $A\beta$ doesn't increase.[5] Likewise, *APP* mutations can make it more difficult for APP to fit into the mouth. $A\beta_{42}$ is the form of amyloid that aggregrates and forms neurotoxic plaques and induces the development of neurofibrillary tangles (NFTs) made from tau.

Familial AD can be heartbreaking because patients know what to expect. In a family I work with, there is a mother of 2 children in her early 20s who realizes that by the time her children are the same age, she may already not remember them. To have a drug that would reverse that by addressing the mechanism affected by these mutations would be a godsend for that 2% 3% of people with familial AD, especially if patients can take it early before $A\beta$ deposition and tangle formation begin.

CLINICAL GEMS

Consider genetic testing for known AD-associated genes *ApoE*, *APP*, and *PSEN1/2* with AD onset before age 60 only after a detailed family history and genetic counseling.

We have been working for a long time on an allosteric γ secretase modulator that keeps the PSEN cleavage site available—keeping the mouth open, so to speak. This potential drug will start phase 1 clinical trials, hopefully this year, and if it is safe and works as we think it will be, PSEN will make more $A\beta_{38}$ and less of the amyloidogenic $A\beta_{42}$. By addressing the mechanism affected by mutations, this potential pharmacotherapy could address many of the various alleles responsible for familial AD.[6]

What Can We Learn from AD in Down Syndrome?

Down syndrome is a constellation of syndromes due to a large number of genes that are multiplied due to 3 copies of chromosome 21 that causes Down syndrome. What is notable is that individuals with partial trisomy 21, who do not have an additional *APP* gene, do not develop AD pathology or symptoms. This further confirms that $A\beta_{42}$ deposition into plaques is the cause of AD.[7]

Isn't There Still Debate About Whether $A\beta$ or Tau Causes AD?

The causative role of $A\beta$ was initially questioned because in mice transfected with familial AD gene mutations, they develop $A\beta$ plaques and eventually neuroinflammation, but not NFTs.

What is clear now is that the mice don't develop NFTs because they do not express the correct ratio of isoforms of tau that make AD-type tangles. In culture, when a 3-dimensional matrix of neurons and glia derived from human stem cells—developed in my lab in 2014—is used, cells transfected with mutated *APP* and *PSEN1/2* make $A\beta$ plaques and NFTs follow with subsequent neurodegeneration. $A\beta_{42}$ deposition directly leads to formation of NFTs composed of helical tau aggregates.[8]

The second part of debate, which I think is good for clinicians to know, is related to all the amyloid-directed drug trials that have failed to show clinical improvements in cognition.[9] Even the recently approved aducanumab, a monoclonal antibody directed against $A\beta$ plaque, was approved by the FDA "based on the surrogate endpoint of reduction of $A\beta$ plaque in the brain,[10]" not because it improved AD symptoms.

This doesn't mean that $A\beta_{42}$ isn't the cause of AD. Brain imaging shows that $A\beta$ plaques begin forming 2 to 3 decades before AD symptoms occur, and we have yet to address amyloid pathology at this stage when amyloid starts driving tangle formation.[11] In fact, by the time symptoms of mild cognitive impairment start to appear, amyloid plaques are starting to decrease. Chronic traumatic encephalopathy (CTE) supports this as well—the precipitating event of head trauma occurs decades before NFTs and cognitive symptoms. Targeting amyloid at the symptomatic stage is akin to giving statins to someone who has already developed congestive heart failure.

This is why many people are advocating for clinical trials that use biomarkers not only to identify participants but also to measure whether a drug is having an effect. If we can get a safe and cost-effective drug that reduces biomarkers in people with high-risk biomarker levels approved, and then longitudinally follow people using it for 10 or even 20 years, that is when we will know if a drug positively affects cognitive symptoms.[9]

What Should Clinicians Know About AD Genomics?

What is striking about GWAS findings is that most of the genes identified in GWAS play a role in immunity and neuroinflammation or cholesterol metabolism.[12,13] The immune and neuroinflammatory process is directly related to the neuronal death and neurodegeneration of AD. In the first GWAS for AD in 2008, we found CD33,[12] which we later identified as an innate immune effector of neuroinflammation.[13] CD33 controls the microglial activation state, turning microglia from housekeepers that clear amyloid while you sleep into killers that destroy neurons as if there were a local infection. You can think of it as evolutionarily, the microglia didn't get the memo that humans now live to age 80 and neurons die for reasons other than infections.[13] The second innate immunity molecule found in GWAS in 2011, triggering receptor expressed on myeloid cells-2 (TREM2), turns CD33 off to switch microglia back to that nondestructive housekeeping

state.[13] Cholesterol metabolism directly affects Aβ production and regulates neural plasticity because it is the rate limiting factor in generating new axons, neurites, and synapses.

Still, the first 4 genes, *ApoE*, *APP*, *PSEN1*, and *PSEN2* plus the first 40 genes identified with GWAS account for only half of the genetic risks for AD, leaving the other half unknown. This is where we enter the "dark matter" of the genome—the rare variants. These are not found with standard GWAS chips because of the nature of that technique, which requires use of common single nucleotide polymorphisms (SNPs) that are approximately 1000 to 2000 base pairs apart. Anytime association of disease with a common SNP is seen on GWAS, there are dozens, if not hundreds, of other SNPs traveling with it, due to "linkage disequilibrium." This is why functional testing is necessary, and even then, the SNPs don't cover the rare variants that occur in the population with less than 1% frequency but account for 77% of the variance in an individual's genome. To address this, we have recently carried out whole genome sequencing on thousands of people with AD and found 13 rare variants associated with AD, all of which were associated with neuronal function and synaptic integrity and function.[14]

Approximately 100 AD-associated gene variants have been identified, about half of which are protective and half of which are risk factors, the majority of which have no identified function yet. The question then, is how to assess an individual's risk, and we think it will most likely be with use of whole-sequencing genome studies or imputed GWAS data to develop a polygenic risk score. A good polygenic risk score does not depend on individual genes and gene functions, but rather is based on patterns of inheritance of individual segments of the genome. This is a big priority with multiple labs racing to develop an effective polygenic risk score, but we're not quite there yet.

CLINICAL GEMS

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Can Genetics Inform Development of Treatments?

There is a very interesting axiom across multiple diseases that was a true revelation for me. Gene mutations that cause early onset forms of disease inform us of what needs to be treated earliest and, preferably, presymptomatically. For example, with the role of cholesterol in heart disease, Brown and Goldstein found the early onset hyperlipidemia and heart disease in a person's 50s was caused by a mutation in the low-density lipoprotein (LDL) receptor.[15] This discovery led to the realization that cholesterol must be treated earlier to reduce risk for heart disease.

Applying this to AD, the genes we know so far are *APP*, *PSEN1*, *PSEN2*, and *ApoE* homozygosity, which are all involved in accelerated amyloid deposition.[1-6] Regardless of the trigger—whether it be concussions for CTE, frontal lobe dementia genes, or mutations in *APP*, *PSEN1*, or *PSEN2* for AD—that causes plaques and NFTs, these pathologies need to be targeted early, preferably before symptoms occur. The late-onset genes are involved in neuroinflammation and synaptic plasticity, which point to resilience and neuroprotection having an important role.[11-14] The importance of protection from neuroinflammation is supported by data from people with large amyloid plaque and NFT loads at autopsy who had no clinical symptoms of dementia, because what is consistently found in these so-called *mismatch brains* is a complete absence of evidence for neuroinflammation. To help symptoms in people with later-stage AD, we believe that neuroinflammation and synaptic resilience are the best targets, as elucidated by the late-onset AD genes found by GWAS.

Other forms of dementia (eg, Lewy body disease, frontotemporal dementia, or corticobasal degeneration) have an entirely different set of associated genes and genetic risk factors, some of which overlap with other neurodegenerative diseases (eg, amyotrophic lateral sclerosis or Parkinson disease). In these dementias, Aβ plaques do not form, but Lewy bodies, NFTs, or other types of tau aggregates occur. This suggests that the tau or α-synuclein pathology and subsequent neuroinflammation and neurodegeneration are like brush fires spreading through the brain. The match that lights that fire (eg, a concussion, α-synuclein proteinopathy, or *C9orf72* mutations) may be different in each type of dementia, and the proteinopathies may have different molecular structures and distributions, but the end neurodegenerative processes are the same.

Can You Envision an Eventual Gene Therapy for AD?

As discussed, to treat people who have symptoms, it is important to target the later processes of neuroinflammation. The challenge with gene therapy targeting neuroinflammation that is driven by microglia is that delivery of gene therapy uses viral vectors. Microglia function to combat viruses making this delivery unfeasible. We just published a proof-of-concept study in which we downregulated CD33 in mice by developing a way to have astrocytes express the viral vector and gene therapy in the form which is then released in exosomes that we called vexosomes because they carry the viral vector. Microglia also function to clear the space between synapses by phagocytosing exosomes and so also took up the vexosomes. The antiCD33 microRNA thus delivered to microglia reduced CD33 expression by approximately 30%, which also reduced amyloid plaque as well as neuroinflammation and neurodegeneration.[15]

Doing that in humans will be more complex, of courses, but for gene therapy the primary target seems to be the neuroinflammatory genes. The exception may be in turning

down *APP* in people with Down syndrome, who have an extra copy of *APP* anyway, and this could be less complicated because only neurons would have to be targeted.

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Gene therapy for dementia should get microglia to go back to housekeeping— keep calm and phagocytose on.

Ultimately though, gene therapy will need to downregulate the neuroinflammatory activity of microglia so that they don't act as if there is an infection and kill brain cells. Gene therapy for dementia should be directed at getting microglia to go back to housekeeping, keep calm, and phagocytose on, so that's what we're hoping to do with gene therapy and with small molecules. ■

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Disclosures
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