BroadcastMed | Grand Rounds: Identifying and Translating Biomarkers for Neurodegenerative Diseases: Experience with Sphingolipids

SPEAKER 1: So we're going to go ahead and get started. It's a real pleasure to have Dr. Michelle Mielke as our speaker today. She received her bachelor's of science in neuroscience at the University of Pittsburgh and a doctorate in psychiatric/neuroepidemiology from the Johns Hopkins Bloomberg School of Public Health. She was on the faculty at Hopkins prior to joining Mayo in 2011.

Michelle is currently an associate professor in HSR in the division of epidemiology and in the department of neurology. She works as a translational epidemiologist to further understanding of the ideology and epidemiology of neurodegenerative diseases and neuropsychiatric disorders. She's PI of several NIH and foundation-funded clinical and epidemiological-based grants, focusing on Alzheimer's disease and other degenerative conditions. She's published over 100 manuscripts and presented at multiple national and international conferences and consortiums. And she's going to talk today about identifying and translating biomarkers for neurodegenerative diseases. Michelle--

DR. MICHELLE MIELKE: So, thank you very much for the kind introduction. So today I wanted to talk to you a little bit and give some background about some of the work we are doing with Sphingolipids and Alzheimer's disease, particularly with trying to translate some of the animal work to humans and also into new therapeutic targets and biomarkers. And I think translational work in general is difficult. However, at Mayo, with the cross-disciplinary research really fosters a lot more collaboration than at some other institutions.

But I think-- and we all think of our own diseases as being extremely complicated. And I think neurodegenerative diseases are complicated for two additional reasons. One, because we can't just go in and take a biopsy of the brain, like we might some other organs. And even when we try and take a look at neuroimaging of the brain, it's really only a snapshot. We can't quite get at the mechanisms. And two, we really don't have any, I don't want to say good, but our animal models of Alzheimer's disease are lacking.

Mice just don't develop Alzheimer's disease. And we can't get the same type of phenotype and biology that we do in humans. So trying to work with what we can and identify mechanisms in animals and translate them to humans is difficult, to say the least. I think I've heard that we've been able to cure 60-- or with 60 different targets, cure Alzheimer's disease in mice at this point. But so far, we haven't been able to do it in humans.

I think I'm supposed to go through these quick. So what I want to start out with was just some basic information as to why we're interested in looking at sphingolipids in the central nervous system, and discuss a little bit on basic science research linking ceramides to Alzheimer's

disease neuropathology in animals, and then how we're starting to translate that to humans. And if you've been at a Metabololomics Core meeting, you'll probably be and have seen this before. The first couple of slides will be familiar. But hang in there, because we have some really exciting, I think, work at the end, where we're actually starting to really understand some of these mechanisms.

So lipids are extremely important in the central nervous system. They are important for the structure of the neuronal cell membranes. And the type of lipids in the membrane directly affect the solubility and fluidity of the membrane, including what passes in and out of the neurons. Lipids are greatly affected by oxidative stress. And this is especially important within the CNS, because there are low antioxidants in the brain relative to the rest of the body.

Third, a lot of times, when we think of lipids, we think of elevated or high triglycerides, high cholesterol, as being bad. But when we're talking about brain function and, I think, in general diseases, low cholesterol or low lipid are also bad. And so it's really the homeostasis of the lipid metabolism that is important in preventing loss of synaptic plasticity and neurodegeneration. And then lastly, and this is more recent in the past decade or so, we found that lipids aren't just insulation. They're not just a structural component. They actually can act as second messengers and have bioactive properties. And that's one thing that's very intriguing about ceramides and many of the sphingolipids I'll be talking about, where we do think that they're involved in various cellular cascades.

So ceramide is kind of the bedrock of the sphingolipid pathway. And ceramides are a product of sphingomyelin metabolism or can be created through de novo synthesis. There is some sphingolipids that you can pick up through the diet. But quite a bit of it is developed or created within the body. And they're also important because ceramides are precursor to more complex sphingolipids.

Ceramides, like many other liquids, do have a structural role. For example, they bind was sphingomyelin and cholesterol in lipid rafts, which determines what proteins are binding to the lipid rafts and what types of cell signaling you might get. But they're also particularly important because they, more than some of the other sphingolipids, such as sphingomyelin, act as second messengers that regulate cellular differentiation, proliferation, and apoptosis.

So, like a lot of things in the body, at low levels they have benefits. They can promote cell division, are important in injury-induced cytokine production. But when you have long-term

elevated ceramides, they activate signaling cascades. They Increase inflammation, promote free-radical generation, sensitize neurons to oxidation. And there's more and more research out now. When I go to sphingolipid meetings, much discussion on ceramides and autophagy, as well as other forms of cell death. And so it looks like there is a lot of important components with this pathway in controlling cell function.

So this is actually a bit of a simplified version of the pathway. And I want to highlight just a couple of lipids here. So as I talked about, ceramide is kind of the bedrock of the pathway and can be formed through a de novo synthesis as well as metabolism from sphingomyelin and some other more complex sphingolipids. Sphingomyelin can be metabolized into ceramide. And I'll be talking about this a little bit more later. Sphingomyelin, unlike ceramide, is primarily a structural lipid. It's a type of phospholipid and is primarily found in the cell membranes. And it's really the interaction between cholesterol, and sphingomyelin, and a couple of other lipids in the membrane that determine what's going to bind to some of the lipid rafts.

Ceramides, as I mentioned, are pro-apoptotic and pro-inflammatory. And in contrast to ceramides, sphingosine-1-phosphate is anti-apoptotic and anti-inflammatory. And so there's a lot of research going on with a variety of diseases, looking at the ratio between ceramides and sphingosine-1-phosphate. So for example, in cancer right now, one therapeutic mechanism is to try and increase ceramides and target it toward the tumor so that it can kill the tumor cells since it's pro-apoptotic and then also inhibit sphingosine-1-phosphate. On the other hand, with some cardiovascular work, they're looking at the benefits of sphingosine-1-phosphate for vascular endothelial function.

So there are a wide variety of diseases that are being looked at with this pathway. And what I've been particularly interested in are all the neurodegenerative and neuropsychiatric conditions that are associated with this pathway. And I'm actually running out of room, so I don't have Alzheimer's disease up here. But you can see frank mutations and sphingomyelinase cause Niemann-Pick's disease, mutations in glucoceramidase, cause Gaucher's disease. Mutations in galactoceramidase cause Krabbe's disease.

So important mutations cause very critical neurodegenerative diseases. But we also know that slight changes are also associated with other diseases, such as Lewy body Parkinson's disease. There's suggestions that galactosylceramide may be associated with ALS, sphingomyelinase with major depression. And interestingly, the first oral medication for MS was fingolimod which is sphingosine-1-phosphate agonist.

So one thing that comes out of what you look at here is, can this actually be a biomarker that's specific for Alzheimer's disease? And I'll show you that we now have evidence that ceramide may be a specific predictor of neurodegeneration in Alzheimer's disease. But it is interesting to think about this. And a lot of times neurodegenerative diseases or neurodegenerative pathology occurs together. So for example, people with Alzheimer's disease often have Lewy body dementia, and they often have depression. And by studying this pathway, this may be some link as to why some of these co-occur together.

So as I mentioned, there is specificity. And basic science studies have shown important links between ceramides and Alzheimer's disease pathology. So the exposure of cultured neurons to amyloid beta, which is the central pathology of Alzheimer's disease, directly increases ceramide levels by activating neutral sphingomyelinase. And inhibiting this increase in ceramide levels can protect the neurons from cell death, apoptosis Amyloid can also indirectly increase ceramides through an oxidative stress-mediated mechanism. And on opposite spectrums, ceramides can modulate base activity to increase amyloid beta levels. So in other words, amyloid can increase ceramides, and ceramides can further increase amyloid beta. Which comes first? We don't know. But there may be a potential positive feedback loop.

More recently, there has been more work looking at ceramides in tau, which is the other pathology that's typically found in Alzheimer's disease. And it's been found that ceramides can modulate PP2A activity, leading to tau phosphorylation. So based on this work, one of the potential hypotheses is that, if amyloid can increase ceramides, and ceramides phosphorylate tau, then perhaps if we can block the increase in ceramides, we can prevent neurodegeneration. And this is particularly important for Alzheimer's disease, because it's the neurodegeneration that is associated with the clinical symptoms and the functional deficits, more so than amyloid. So if you only have amyloid, and you don't have neurodegeneration, you're not likely to show the dementia phenotype.

So when I first started looking at sphingolipids, I came about it from an epidemiological perspective. So ideally, if we could identify a blood-based biomarker of cognitive progression or Alzheimer's disease, that would be great, much less expensive than neuroimaging and much less invasive than CSF. And in Europe, it seems to be a little bit easier to get CSF. But in the US, it's still fairly difficult. And so can we identify something in the blood that's predictive of Alzheimer's disease?

So we looked in three or four different studies and basically came up with the same finding, that ceramides are predictive of cognitive decline, regardless of whether you're cognitively normal, MCI, or Alzheimer's disease. So a minor cohort of cognitively normal individuals, high ceramides were associated with risk of memory impairment and Alzheimer's disease. In fact, when we looked at the tertiles, there were very few people in the lowest tertile trial of ceramides that developed Alzheimer's disease over a period of 10 years.

Among a group of mild cognitive impairment patients, again high ceramides were associated with memory decline; hippocampal volume loss; therefore linking it to changes in the brain; and then also white matter integrity, primarily in the posterior cingulate, which is one of the earlier areas affected by Alzheimer's disease. And this is something we now have some preliminary data that I'm not going to show today, but among cognitively normal people, also showing that high levels of ceramide are associated with white matter changes 10 years later.

And then also in a group of Alzheimer's patients, high ceramides were associated with cognitive decline. So after a period of about five years, people that were in the lowest tertile had about a 10 point less decline on the MMSE compared to people in the highest tertile, which is quite significant.

Now in all these studies, when we look cross-sectionally, we tend not to see a difference between normal MCI or AD. Or it's very subtle. And so this indicates that these sphingolipids are not going to be a diagnostic marker. But they tend to always come out as a marker of disease progression or predict disease progression.

So based on these epidemiologic studies, which suggests that the ceramides are a risk factor, can we determine now or start looking to determine whether these are actually biomarkers of disease processes? And so one of our next questions was how do these markers relate to Alzheimer's disease pathology?

And there's only been about a handful of studies that have looked at the relationship between sphingolipids, amyloid, and tau in humans. A couple of brain studies show that increase in acid sphingomyelinase, which again leads to increased ceramides, positively correlated with amyloid and tau in Alzheimer's brains. And another study that showed that ceramide synthases varied. Or upregulation and downregulation varied based on the stage of the Alzheimer's pathology.

There's been just a couple of CSF studies, none of which looked at the correlation with CSF

amyloid beta and tau. And the CSF study suggested that there's elevations in ceramides in individuals that have mild AD, but that once you get to later stages of AD, the levels are essentially similar to cognitively normal individuals. And so this suggests that the levels of ceramides, at least in the CSF, can vary based on the disease severity.

So we wanted to extend this finding. And a few years back I had collaborated with the group at the University of Wisconsin, Madison, with the Wisconsin Registry of Office of patient-- or the Wisconsin Registry of Alzheimer's-- I can never remember these acronyms. Anyway, it's children of parents with confirmed Alzheimer's disease. And they had collected CSF from a variety of individuals that were cognitively normal and age 36 to 69 years old.

So we looked at and measured amyloid beta and tau. And we did find very nice correlations between ceramides and amyloid beta, as well as sphingomyelins and tau. And our strongest correlations were between sphingomyelins and CSF total tau. Now here I'm looking at total tau. I'll show you sphingomyelin C18 next. But sphingomyelin C18 is really the most abundant species in CSF. So it's pretty much driving, essentially, this total now finding.

So next we wanted to see if we could replicate this. And so we looked in the Mayo Clinic study of aging and about 88 cognitively normal individuals, measured ceramides and sphingomyelins, and then also looked at amyloid and total tau. And again, we saw a very consistent finding among these individuals that are 70 years and older, where increasing CSF sphingomyelin C18 or totals sphingomyelins were associated with increasing tau. And what was really nice to see is that when we look and compare to the [INAUDIBLE], the association is very similar.

What's nice about this is that with working with Alzheimer's disease, it's very difficult to have reliable measures of amyloid and tau. So we have two completely different labs that measured tau here and also two completely different labs and methods that measured sphingomyelins and ceramide. And we're still getting the same thing. So this suggests that there is a replication of our original findings.

So looking at correlations between the sphingolipids and pathology is nice to show that there's some relation to the disease. But it doesn't really tell us whether and how the sphingolipids might be related to disease progression and how it influences and affects the pathology. And so we really, in humans, need to start looking at the relationship between amyloid neurodegeneration and cognitive symptoms. And new research is now allowing us to do that.

So 10 years ago, we weren't able to do amyloid imaging in the brain. The only thing we could really do was look at hippocampal volume and white matter hyperintensities. And this is helpful, but it's not something that's specific to the disease. So with the onset of being able to image amyloid in about 2004 to 2006, now we're able to actually determine which individuals have amyloid. And this is particularly important because among cognitively normal individuals age 70 and older, 30% of people will have amyloid. You get to age 80 and older, you have about 44% of people that will have significant amyloid in their brains.

Now, not all of them will go on to develop dementia. But a good portion will. And so we need to try and separate those individuals that have amyloid from those that don't. And by having amyloid imaging through PIB-PET available, that gives us the opportunity now to see in vivo pathology.

And based on that work, about four years ago, there was a new preclinical Alzheimer's staging in relation to Alzheimer's disease. And this was really unprecedented because prior to this, we didn't really have a staging based on biomarkers for Alzheimer's disease. And essentially, the staging indicated that amyloid comes first, followed by neurodegeneration, and then clinical symptoms. And so among cognitively normal individuals, you can see that some of these people already have amyloid imaging. Many of them have neurodegeneration. And then they will go on to develop MCI and dementia.

So this group of cognitively normal individuals is heterogeneous. And when we look at biomarkers, we can't just look and compare cognitively normal individuals to MCI. We need to look and compare those cognitively normal individuals with amyloid versus those without amyloid.

But this cascade was essentially unprecedented, that now we have a potential biomarker model of Alzheimer's disease in humans. Now granted, there is some back and forth that in some people neurodegeneration occurs first, and we need more individualized medicine approach. But at least we have a starting point now.

So after this came out-- and Cliff Jack and many of the researchers at Mayo Clinic Study of Aging were instrumental in determining these preclinical models. They sought within the Mayo Clinic Study of Aging to identify and let's put this to practice. Let's see what this looks like in the general population. And they found that roughly 16% of individuals age 70 and older had only amyloid. About 15% had amyloid and neurodegeneration. And about 23% of individuals had

neurodegeneration but not amyloid.

And so this gave us a starting point to really start looking at the relationship between changes in these markers then and cognitive decline. But again, it also highlights the need that we can't just look at the clinical phenotypes. We have to take the biomarker and pathology into account.

So this whole biomarker modeling completely opened up a whole new world. Originally with Alzheimer's disease, we would look at normal MCI and AD. And now we can look at this and we have another layer of complexity. So now we can look at some of the pathology. And that, therefore, allows us to go one step further and look at some of the mechanisms that are related to some of these pathologies.

So when we think of mechanistic studies-- or in the past, it was primarily done on transgenic animal models. But these are not fully translatable. And so we have the option to start looking at this within humans as well.

So what I've been interested in and working on lately is to try and understand what the relationship is between plasma and CSF sphingolipids and amyloid and neurodegenerative pathology across the spectrum of Alzheimer's disease severity. And these are-- I apologize for these. These are a little bit difficult to see. We've been having some problems getting SAS graphs into PowerPoint and making them legible. But there's two things that I want to point out with this. Now among 589 individuals within the Mayo Clinic Study of Aging, we've assayed much of the sphingolipid pathway and are looking at it in relation to cognition, hippocampal volume, FDG hypometabolism, and PIB-PET ratio.

And there's a couple of things that can highlight from this. One, so on the left-hand side, we have baseline memory, change in memory, baseline hippocampal volume change, baseline FDG with change, and then baseline amyloid and change. And what you see by looking at the cross-sectional or baseline associations again is that there are differences in the outcome by normal and MCI. But there aren't' really differences in any of the sphingolipids, whether somebody is normal or MCI, again indicating that this isn't going to be a diagnostic biomarker. And it's only when we start looking at changes in some of the outcomes that we do start to see some differences in-- that we do start to see that ceramides are associated with some of these outcomes.

So this is a lot of numbers. I'm going to be focusing on the ceramides going forward. But I just want to also highlight some of the other work that we're doing as well, with looking at some of

the sphingomyelins, phosphatidylcholines. Gangliocytes are interesting because they've been associated with seeding amyloid. And so that's something we'll be looking at down the road.

But when we first started looking at ceramides, generally when we include everybody-- so all 589 people-- we find that ceramides tend to be predictive of hippocampal volume loss, cognition-- so global memory-- global z-scores as well as memory z-scores. And there's not much association with amyloid except for this particular marker.

And so getting back to what I talked about with the transgenic mouse models, where they showed that amyloid can increase ceramides, and ceramides can increase tau and neurodegeneration, we wanted to see if we could replicate that in this human sample. And we were very excited-- and this is just within the past couple of weeks-- that we have found that association. So when we looked cross-sectionally at the relationship between ceramides and hippocampal volume or FDG or cognition, we didn't see that the association varied by amyloid. But when we looked longitudinally, we do see that the association between ceramides and the neurodegenerative markers depends on whether you have significant amyloid in your brains.

So in this column, we have the specific baseline ceramide. And here is the change in outcome. This column indicates whether ceramide is predicting the change in these outcomes, whether amyloid predicts the change in the outcome, and this is the interaction. So this indicates that elevated ceramides among those individuals that have elevated amyloid at baseline, only those individuals have declines in FDG-PET hypometabolism and declines in hippocampal volume. So this is a direct translation of what we were also finding in humans. And it also suggests that there is specificity of this pathway to Alzheimer's disease.

So in other work, we're now trying to also expand this towards other mechanisms as well. And obviously, that brings in more complexity. And I showed you a three-way interaction last time, and so we're getting even more complicated. But as we're trying to understand some of these underlying pathologies, you can't just look at one at a time. We have to find ways of looking at multiple pathways.

So we've started to look at the relationship between sphingolipids and inflammation and Alzheimer's disease pathology. And inflammation is particularly important because we know, at least based on cellular studies, TNF-alpha and IL-6 can activate or enhancement sphingomyelinase activities, therefore, leading to higher increases in ceramides. Now most of this work had been done, in translation to humans, had been done on patients, so either

looking in diabetics or patients with heart disease.

So we looked to see whether inflammation was affecting sphingomyelins and ceramides within just cognitively normal individuals in the general population. And we did find that higher levels of both IL-6, TNF-alpha, as well as interferon gamma, which I didn't include here, was associated with a lower sphingomyelin to ceramide ratio, indicating higher ceramide levels. So this is directly replicating some of the cellular and basic science studies.

And so we've started to look at this a little bit within the Mayo Clinic Study of Aging. And so on this left-hand side are individuals that are amyloid negative or have low levels of amyloid in their brains. And on the right side are individuals that are amyloid positive. On our y-axis, we're looking at change in hippocampal volume based on ceramides and TNF-alpha levels.

And we are seeing differences, or interactions between ceramide and inflammation in predicting hippocampal volume, depending on whether somebody has elevated amyloid or not. Obviously, these are a very small sample sizes. And so we're hoping to replicate this in the larger MCSA and just submitted a yesterday, where we will have a couple of thousand visits of individuals with amyloid PET, PIB-PET, FDG-PET, and at least three followups on many individuals. So we can really start looking at this closer and also have the power to start understanding more of these mechanisms.

So where do we go from here? And we've shown that it looks like these sphingolipids can be biomarkers. How can we better translate them to the clinic? And there are some companies right now that are looking at several enzymes and targets within this pathway for Alzheimer's disease as well as other neurodegenerative conditions. So one such pathway right now is looking at sphingomyelinase. And so it was recently shown by Yvette Sheline that citalopram, an SSRI, which reduces or deactivates sphingomyelinase, was associated with reduced amyloid beta. And so this suggests that, perhaps by treating depression or with the use of SSRIs, we can reduce some amyloid levels.

The second big area is based on sphingosine-1-phosphate levels. So as I mentioned, the first oral medication for MS, fingolimod or Gilenya, was a sphingosine-1-phosphate agonist. And sphingosine-1-phosphate has five receptors, and this medication hit four out of the five receptors. So it's been found to be beneficial for MS, but there happens to be a lot of side effects. There has been some suggestion that fingolimod can be beneficial, at least in transgenic mouse models, in reducing neurodegeneration and amyloid as well. But again, with

the side effects, it's not something that would be given long-term to individuals for Alzheimer's disease.

So AbbVie and a couple other companies right now are looking at a specific sphingosine-1-phosphate receptor 5 agonist. And they're finding that these don't-- sphingosine-1-phosphate receptor 5 is primarily found in the brain. And they're finding that they don't have all the side effects that they do with fingolimod, and that there still looks to be benefits on amyloid plaques, and I hear also on symptoms of MS. Although, AbbVie is tending to focus more on AD.

So there are pathways down the road suggesting that we may have therapeutic opportunities. And along with the therapeutic opportunities, as we continue to look at the ceramides, sphingomyelin, other levels within this pathway, we can potentially develop a companion diagnostic determining who has elevated levels and who should be given the medications as they come about. And that's no small task in and of itself, because there aren't clinically useful assays right now for many of these sphingolipids.

So I think, in conclusion, there's consistent evidence for a role of ceramide metabolism in Alzheimer's disease. It initially started with animal models, and now we're seeing some similar, and I think exciting, evidence in humans as well. Importantly, plasma ceramides may predict which persons with elevated amyloid will progress. And this is particularly relevant right now for many of the preventive clinical trials, where they are enrolling individuals that have amyloid but are still cognitively normal and administering an amyloid vaccine. Because we know that not everybody with elevated amyloid will progress to Alzheimer's disease, so if we can identify on an individualized medicine approach who will and who will not, those are the people that we can therefore target therapies to.

And just again, to highlight that, we certainly need a collaborative translational effort, starting to work with basic scientists now to look at and understand some of these pathways as well as some of the new therapeutic animals and transgenic mice models. And then going forward and further, trying to replicate what we're finding in humans to determine whether they are biomarkers, and then also to develop clinically useful assays as well.

So I've had the opportunity to work with many amazing people and across various institutions. I think I'm supposed to show this. And thank you.

[APPLAUSE]