The Science Behind MS

Presented by:

Dr. Esther Melamed, MD, PhD

Alexis Crispino Kline:
Hello and good evening, everyone. [Next slide, please.] I am so excited to welcome everyone and say Happy MS Awareness Month. My name is Alexis Crispino Kline, and I'm the Director of Mission Delivery and Grants Management at MSAA. Dr. Melamed, our speaker for tonight's feature, The Science Behind MS, is absolutely brilliant, and I'm so excited for the information she has in store to share with us.

But before I introduce her and hand the night over, I did want to take just a few moments to highlight some of MSAA's programs and services. [Next slide, please.] So, if you've contacted us before, you've likely met our Helpline specialists, but if you haven't had the opportunity yet, MSAA has a national toll-free helpline that is staffed by individuals with a background in counseling or social work who are familiar with MS.

Helpline specialists can share information about MSAA's programs and services as well as resources from the broader MS support community. For eligible individuals, MSAA also has a durable medical equipment program and a cooling garment program. Both programs provide tangible items that address some of the symptoms of MS, such as cooling vests for those experiencing heat sensitivity or four-prong walkers for mobility support.

MSAA also hosts an MRI Access Fund for eligible individuals who are experiencing barriers to receiving a cranial C-spine MRI, to receive a diagnosis of MS, or to track disease progression. So, if you have questions about these programs or like more information about any of MSAA's programs and services, please don't hesitate to reach out to us. [Next slide, please.]

And without further ado, I am delighted and honored to introduce our esteemed speaker for this evening's program, Dr. Esther Melamed, MD, PhD, founded the Multiple Sclerosis and Neuroimmunology Clinic at Dell Medical Center in Austin and specializes in the care of adults with multiple sclerosis, neuromyelitis optica, transverse myelitis, and a variety of neuroinflammatory conditions. She believes in taking a holistic and individualized approach to patient care and enjoys counseling patients and their families on diagnosis and management of their condition. So, without further ado, I am excited to hand the evening over to Dr. Melamed, and we're going to switch over computer screens. Welcome!

Dr. Esther Melamed:

Great. Thank you so much for the warm introduction, Alexis, and it's my pleasure to speak tonight. I will go ahead and share my screen and we will get started All right. So as Alexis mentioned, I am at Dell Medical School. I'm an Assistant Professor of Neurology and Director of Research for our Post-COVID program. And it's really an honor to be here today to talk to you about the science behind multiple sclerosis and what we have learned over the years.

Many of you have asked yourselves at some point, why me? Why was I the one that got MS? Today, we will explore the reasons behind the why of MS, but before we start, I wanted to share a book by one of my favorite authors, Harold Kushner, that I often recommend to my patients, which explores what to do when getting a chronic condition or when other things happen in life that we do not like. And it's a really wonderful read. I highly recommend this book and I hope that you enjoy it as much as I have and my patients have. [SHOWS BOOK ENTITLED *When Bad Things Happen to Good People*]

So, today we will talk a little bit about the immunology of MS and then we'll explore the different risk factors, including the genetics of multiple sclerosis, sex effects and environmental influences in MS, the role of the gut microbiome diet and alcohol in multiple sclerosis, and the role of viruses.

So, let's go ahead and get started. Multiple sclerosis is a chronic autoimmune neuroinflammatory disease, which is characterized by demyelination and neuroaxonal loss, which results in lesions within the eye, within the brain and within the spinal cord. And as a result, individuals with MS may develop serious neurological disabilities. Here, picture this actress Jamie-Lynn Sigler, who is best known for playing the daughter of the mobster Tony Soprano on the award winning HBO drama The Sopranos. And she revealed in January 2016 that she had been diagnosed with MS and that she had had it since age 20, which was 15 years earlier. And she had revealed this illness to a few trusted colleagues earlier on, but generally kept it as a secret because she was not ready to go public. And this is the case for many patients who do not feel comfortable sharing that they have the disease.

And it actually is the case that today, with so many treatments that are now available with over 20 drugs that are FDA approved for MS, it is really the disease that has become chronic, for which we don't have a cure yet, although we have many, many medications and many exciting research trials into alternative mechanisms of drugs that could reverse neurodegeneration, such as stem cell treatments. And so today many patients can continue to look wonderful and to feel at their best when they're able to be treated appropriately, compared to 15, 20, 25, 30 years ago when we had no medications. And really the diagnosis of MS was more of a sentence of lifelong disease that would lead to neurodegeneration without really any way to stop the disease.

Now, today we will not talk too much about medications during today's talk. I will be happy to discuss any questions after the talk about this topic, and we will focus today on the risks of multiple sclerosis and the disease that ensues as a result of these risk factors. So let's talk a little bit about the symptoms. MS really affects every part of the body. And as the name suggests, there are multiple symptoms and neurological disability can result, which can lead to pain, muscle weakness, depression, cognitive impairment, walking impairment, and really no organ of the body is spared. When the brain is hit, people can develop fatigue, concentration, attention difficulties and depression and anxiety. When the spine is affected, people develop mobility difficulty, pain, bowel and bladder dysfunction and difficulties with tingling and other sensory symptoms.

When the eyes are affected there can be blurry vision or double vision. When speech is affected, there can be difficulty with pronouncing words or difficulty with talking for long periods of time. The gastrointestinal tract can be affected, leading to swallowing difficulty, constipation and bowel incontinence or urgency. And the bladder, likewise, can be affected and people will experience urgency with urination and incontinence. And muscles can be affected, leading to tremors, spasticity and weakness.

There are different types of MS that can result over the years, and the first type is called clinically isolated syndrome, which results in either damage to the optic nerve, leading to optic neuritis, or damage to the spinal cord, leading to transverse myelitis. Now sometimes people can have MS that's already starting many years earlier, that is underway by the time the first symptoms occur.

And when people are caught very, very early on, they might be labeled with a disease process called radiologically isolated syndrome. And over the years MS can either be relapsing-remitting in its nature, where over time, potentially, progress to secondary-progressive multiple sclerosis in some people. In others, MS can start from the very beginning as a primary-progressive course where there are not many relapses, there are not too many MRI new lesions that happen, but there's a very, very progressive course from the very beginning. And we often listen very carefully to the patient's history and clinical science to try to distinguish what type of MS they're presenting with when they come in for the first time.

So a little bit about the statistics of MS. There are 2.5 million people worldwide and there's an increasing incidence of MS at this time. There are about 200 new cases that are diagnosed each week in the United States. And MS is really the leading cause of non-traumatic neurological disability in young adults. The total lifetime costs per person can exceed $4 million, and the average yearly healthcare costs can range from $30,000 to $100,000. So it's a pretty expensive disease that affects quite a few people.

Now, interestingly, when a study was done in 1975, we thought that there were about 300,000 to 400,000 people that were affected with multiple sclerosis in the United States. And that has been the figure that has been cited over the years. However, in 2019 a new study that was performed by Wallin and colleagues from Georgetown University using health insurance data sets, they calculated that the prevalence of MS was actually twice the prevalence that it had been in 1975, which was very surprising. And that number is now known as about a million people in the United States.

So, why do we think that there is this increased number? We're going to come back to this question because it's an interesting one. And one of the questions that arises is do we have more cases of MS because we're better at diagnosing MS? And in terms of the diagnostic criteria, it's called the McDonald Criteria, which many of you may already be familiar with, and it presumes that there must be a separation of lesions in time and in space. In time, meaning that a person must have at least two different instances of neurological symptoms. And by space, we mean that lesions have to be found in different characteristic areas of the brain or spinal cord. Now, for the 2017 update to the McDonald Criteria, MS can actually now be diagnosed at the initial presentation.

If a patient also has oligoclonal bands in the cerebrospinal fluid, and some of you may have had a spinal tap when you were diagnosed with multiple sclerosis, and what we're looking for in the cerebral spinal fluid is the production of antibodies in the central nervous system. In the right context, these antibodies or autoantibodies can be diagnosed as an additive characteristic for

the diagnosis of MS due to suggesting that there's autoimmunity inside the cerebrospinal fluid compartment.

Before we get any further, I'd like to make sure that we get, we all get a good foundation of how the immune system works. So, there are multiple organs in the body that help to orchestrate the immune system, and they include the tonsils, the adenoids that help to filter the pathogens that come into the mouth, the thymus and the bone marrow where immune cells are born and mature. And we will talk more about them in the next couple of slides. Now, different lymph nodes like the axillary and the inguinal nodes and the Peyer's patches, where many of our immune cells live and pathogens are shown to them, is where the immune cells learn about these different pathogens.

There's another very interesting part of the immune system called the appendix. And it is a storehouse of good bacteria. It sort of acts to reboot our digestive system and the appendix, some experts believe, is really a useless remnant of our evolutionary past. But many now recognize it as actually part, and important part, of our gut microbiome, which we'll come back to and talk about a little bit more later on.

So, what happens inside the bone marrow? Inside the bone marrow, there are hematopoietic stem cells which differentiate into myeloid and lymphoid genitor cells. And lymphoid cells are made up of T and B cells, while myeloid cells are made up of monocytes, neutrophils, basophils, eosinophils, platelets, erythrocytes. Many of these cells are monitored when patients are placed on immune therapies and hematopoietic stem cells are the targets of the stem cell transplants that many of you may have heard about.

So, what happens in the thymus and in the bone marrow, the two really important immune organs in our body? Well, within the thymus, T cells are made and they learn to recognize cell from non-cell. They are presented different types of proteins and they learn to be able to either bind to them strongly or not so strongly. And depending on how well they learn, it determines whether these T cells will lead to autoimmunity down the line or not. Similarly, B cells are made in the bone marrow, and they mature here before they go into the bloodstream and to different lymphoid organs. And B cells also learn how to make antibodies and how to mature either into reactive or non-reactive B cells.

So, when T cells and B cells are able to learn what is ourselves and what is foreign during this schooling within the thymus and the bone marrow, they go on into the rest of the body and that person will not develop body immunity. However, when T cells and B cells mistake ourself for a foreign invader due to incomplete schooling in the thymus and the bone marrow, or due to later unlearning the lessons that they may have learned earlier. That is where autoimmunity may be starting. Now, this question has yet to be resolved. And some argue that autoimmunity may actually start within the central nervous system rather than within the peripheral immune system. And that's a very interesting question that much research is currently looking into but now, you know, a little bit about how the peripheral immune system is made up of and how it's functioning.

So, what happens in multiple sclerosis? Well, ultimately, due to the immune system being autoreactive or reactive to the body's self, T cells enter into the central nervous system and attack myelin. However, that's really the tip of the iceberg. While we see on the brain MRI this damage to the white matter, which is made up of axons, neuronal axons, which have myelin, there's actually a lot of grey matter damage that occurs to the cell bodies of the neurons as well, something that we cannot see very well on the typical MRIs, but something that ultimately leads

to a lot of cognitive difficulties and other symptoms of multiple sclerosis that do not necessarily map to the white matter areas in the brain.

Now, there is also another really important component of disease pathogenesis that has to do with dysregulated gut microbiota. And there is a very important gut-brain axis in terms of how the microbes in the immune system within the gut communicates with the brain and spinal cord. And depending on those communications that can also lead to autoimmunity, not only in multiple sclerosis, but in many other autoimmune diseases. And we're going to come back and talk more about the gut microbiome in multiple sclerosis.

But first, we're going to turn to genetics in MS. Genetic contribution to susceptibility of MS is pretty well established. And overall, the contribution of the known MS loci to the genetic risk of illness is thought to be about 20 to 30%. The highest genetic risk is in monozygotic twins, or in a child whose both parents are affected. And so, you can see that these two lines lead us to the highest lifetime risk, which really doesn't cross more than 30%. And the risk falls for dizygotic twins and siblings. And is less than 2 to 5% chance in a child whose one parent has multiple sclerosis and really less than 1% chance for cousins, adopted kids, step siblings, and in the general population. And so, what I want you to remember from this slide is that although there is very strong association with genetics, it's actually not more than 30% and we're going to come back to this point and talk more later on.

In 1972 the first association was reported for major histocompatibility complex or MHC. And in 2019 the International MS Genetic Consortium described 233 genetic loci based on analysis of genetic data of 47,000 MS patients and 68,000 control subjects and established a reference map of the genetic architecture of MS, which now includes about 200 genes on autosomal chromosomes which are the non- X and Y chromosomes, one X chromosomal gene, and 32 disease variants that are outside of the major histocompatibility complex, which was the first reported association. And most of the genes are involved in the peripheral immune system, like with T cells and the B cells that we already talked about. But there are also important genes within this network that have to do with brain function and especially microglial function, which are one of the important immune cells in the brain that have been implicated in the pathogenesis of multiple sclerosis

So, what is this MHC? Major histocompatibility complex, and especially class 2 of the MHC, is found on chromosome 6, and there are important loci here that either lead to increased risk or actually protective risk. So, the biggest association with increased risk of MS is HLA-DRB1*15:01. And having this allele leads to patients presenting at a lower age of onset and increases the progression of multiple sclerosis. On the other hand, HLA-B*44:02 actually is a protective allele and it leads to an association with preserved brain volume over time and reduced burden of lesions in the brain. So, there's a yin-yang between the MHC genes that may both be protective and that may increase the risk.

Now, some of you may have had the experience that you develop other symptoms as a result of having MS that may or may not lead to a diagnosis of another autoimmune disorder. In this pyramid here, shows that there is actually a pretty high genetic correlation between having multiple sclerosis and having other autoimmune diseases. So, in purple here, this tells us the higher genetic correlation, if you look at this key over here, and so you can see that there is a higher association between multiple sclerosis and other autoimmune diseases like type 1 diabetes and Crohn's and lupus and primary biliary cirrhosis. Now interestingly, celiac disease actually has a negative genetic correlation and is not as highly associated with MS. But as I like to tell my patients, autoimmune diseases like to go in groups. And so, if there is one present

there may be another autoimmune disease hiding. And it's important to keep a close eye on symptoms that occur and ask your doctor whether there is an important consideration of another autoimmune disease that ought to be diagnosed and subsequently treated.

So going back to the question, as we said, there was only a low 30% of the risk that is associated with genetics for MS. And given that genetics has not really changed that much between 1975 and 2019, when we've seen this doubling of the risk of MS, the question is what is going on? Why is there such a high increase over not that long of evolutionary time? And if genetics doesn't explain it, what are the other causes that could explain the rise and the continued rise of MS?

And here are all the other possible factors that may be contributing. They may include being female over male. So, there's a big sex difference in MS that we know of. It may be, have to do with living at a greater distance from the equator, may have to do with having a low vitamin D level, being a smoker, being obese, having dysbiosis within the gut microbiome, which means that there are different bacteria, viruses or fungi compared to healthy controls. Having leaky gut syndrome as a result of that, and the type of diet people consume.

So, let's now turn our attention and talk about sex effects and environmental influences in MS. There is an important sex difference, and the question is why? So why are females affected at three times more commonly than males with MS? And what is different between males and females that may be contributing to this epidemiologically observed trend? Now we know that onset of puberty correlates with an increased onset of disease in women, but not men. About 17% higher relapse rate is observed in women compared to men, and men tend to have more disability when they're affected and progress to secondary progressive MS much faster. And men also experience more atrophy in the brain and more cognitive decline. So why is that?

Well, there are two big factors that are different between men and women. One of them is hormones. And studies have shown that testosterone may be protective in males with MS and may decrease brain atrophy, and lower testosterone would lead to worsening of brain atrophy and worsening of symptoms. And that's something that we test in men to see what the level of testosterone may be.

Now, for women, physiological levels of estradiol and progesterone have had unclear effects on disease onset and relapses but have been shown to improve symptoms. Now, we also know that 50% of postmenopausal women report worsening of MS symptoms. The treatment with estriol has been shown to be protective in a mouse model of multiple sclerosis, also called experimental autoimmune encephalomyelitis, or EAE. And the role of oral contraceptives and hormonal therapy, post menopause, have been somewhat controversial. But overall, we also know that during pregnancy MS tends to get better, and post-pregnancy MS tends to get worse. And so there is a lot of suggestions of hormones may be influencing the course of multiple sclerosis in men and women.

Now the other difference between men and women is the fact that women have an X and an X chromosome, two X chromosomes, and males have an X and Y chromosome. And as a result, that also leads to differences between men and women. So, for example, it depends on how many X chromosomes we have because it determines the level of gene expression from the X genes as some of the X genes actually escape a process called X inactivation, which is supposed to equate the number of X genes between males and females. But what happens, actually, is that there are a number of genes that are, actually many of them are immune genes

or brain related genes that end up being expressed, being found, at higher levels in females compared to males. And that's one difference between men and women.

It also makes a difference on whether we get the X chromosome from mom or dad. And this process is called imprinting, and there are different marks that are placed on the X chromosome depending on whether it came from mom or dad. And that may also be one of the differences that may relate to disease pathogenesis. The Y chromosome does not have very many genes. Many of them are involved in Spermatogenesis, but there are other non-gonadal genes on the Y chromosome and those could be having effects on the immune system that may also lead to differences in how men and women present with MS. And then we already mentioned that X escapees, genes that are expressed at a higher level in females compared to males, will make male and female X X and X Y cells different.

Well, those are some of the differences between males and females and the sex difference in MS. But what about where people live? It turns out that living farther from the equator, in colder climates, actually increases the risk of MS. And it is currently unknown whether this is due to UV exposure or low vitamin D level or types of infections that are found farther from the equator, genetics or perhaps an artifact. But the only thing to say is that there is a very, very strong trend for this and it has been shown in study after study that depending on where people live and living farther from the equator increases the risk of developing MS. And when people move from living closer to the equator to living farther from the equator, their risk increases after they have moved.

Now, very interestingly, in a couple of countries like Italy and Scandinavia, there's actually this reverse gradient. So, the gradient doesn't fit for the general idea. But what tends to explain the reverse gradient in Italy is that there is a genetic frequency of HLA-DRB alleles at the point where people live that are closer to the equator compared to farther from the equator, where there is this reverse gradient. So, genetics explains it in this case. In Scandinavia, the reverse gradient is actually explained by the fact that there is a higher intake of vitamin D because the people may be located closer to coastal regions where there may be higher consumption of vitamin D rich fatty fish. And another really interesting example is in Japan where the cases have gone up over the years. There are over 20,000 cases now of demyelinating diseases like MS and neuromyelitis optica compared to a thousand of cases in the 1980s. And the explanation there is that there has been westernization of the diet in Japan, and it may not have to do with the actual equator but may just have to do with the fact that people are eating a different diet and that may be more pro-inflammatory. But even within the United States we can see this very, very sharp line with the northern states having 110 to 240 cases per 100,000, people in southern states having 57 to 78 cases per 100,000 people. So it's a really interesting effect with latitude. And so the presence, just to summarize, the presence of HLA-DRB1 may have an influence. The gradient may be due to low UVB exposure versus low vitamin D levels. And then another interesting aspect is that the frequency of oligoclonal bands in multiple sclerosis also increases with latitudes. Another representation that this may actually be a real effect.

So, we mentioned that the latitudinal effect may have to do with vitamin D because of sunshine, which is one of the primary sources of vitamin D. There are many other sources like getting vitamin D from dairy, from eggs, from fish and cereals, mushrooms, or vitamin D supplements. And when we get vitamin D to our body, it goes through several steps before it gets activated. So, it goes through the liver and the kidney. And when people do not have a gastrointestinal tract that's functioning optimally, vitamin D may not be absorbed as well. So, vitamin D is, overall most people believe that vitamin D has a very important role in multiple sclerosis. And

this has been shown in a number of different studies where people who are diagnosed with multiple sclerosis for the first time tend to have very low vitamin D levels. In patients with established multiple sclerosis, vitamin D levels that are higher, above levels of 70 nanomoles per liter, have been found to be associated with a decreased risk of attacks, and lower concentrations increase the likelihood of both relapses and early chronic progression.

And there's also genetic effects of vitamin D. In genome wide association studies have identified genetic abnormalities in genes, encoding specific enzymes in vitamin D, synthesis and conversion, and in patients that have been treated with different types of medications like natalizumab, for example, supplementing vitamin D has been shown to reduce the relapse rate when compared to frequency of prior to vitamin D supplementation.

I should mention that there have been a few studies over the years that have not found beneficial effect of vitamin D, but many of those studies were underpowered or looked at a certain number of studies, not including all of the trials that have been done. And so, although there is still a little bit of a question of whether all of the studies are consistent, overall, the general understanding is that vitamin D is helpful and it is rarely toxic unless levels go above 150 nanomoles per liter, and therefore it's a very, very important risk modulator in multiple sclerosis and is easy to take and easy to supplement and easy to monitor. So, by all means, this is one of the factors in MS that is in our control and should be modulated.

Now a factor that has been linked very closely to MS, and that's a negative factor, is smoking. And smoking, smokers with MS have an increased relapse rate during treatment, faster conversion to secondary progressive MS, and faster disease progression. Smokers among MS patients have a higher percentage of inflammatory cells, called TH17 cells, and lower number of regulatory T cells called CD25 cells. Firsthand smoking increases the risk of MS by about 50%, which is really, really high. And there's also an increased risk by secondhand smoke. And each year of smoking after diagnosis actually accelerates time to conversion to clinically defined secondary progressive MS by 4.7%. And so, this is one of the modifiable risk factors as well, that's a negative risk factor, that is very, very closely linked to inflammation in MS.

And, with that, we're going to transition and talk a bit more about the gut microbiome diet and alcohol. So environmental factors are really thought to be essential for development of MS in genetically susceptible individuals, given that, as we said, again, genetics only accounts for about 30% of the risk. And there are 10 trillion human cells and 100 trillion microbial cells, 20,000 human genes compared to 2-20 million microbial genes. And as a consequence of coevolution the microbiota have become a key integrated component of our biological systems. And thus, the gut microbiota play an important part in maintaining our proper physiological processes.

Now, two studies have demonstrated that transferring the gut microbiota from MS patients to mice can lead to a transfer of MS like the disease experimental autoimmune encephalomyelitis. And in one study, gut microbiota were transferred from MS patients to two mice, and then the mice developed MS. In another very intriguing study, the microbiota were transferred either from an affected or an unaffected twin. And the results demonstrated that the mice got sick when they received the gut microbiome transfer from the affected twin, but not from the unaffected twin.

And so, the role of the gut microbiome has been really solidified with these emerging studies. And subsequent studies have also shown that the gut microbiome in progressive MS and in relapsing-remitting MS are somewhat different. In this picture, you can see that the blue dots

are different from the yellow dots, and the blue dots are healthy control, and the yellow dots are relapsing-remitting MS patients. Here in red are patients with progressive MS compared to healthy controls. And these are all significant. They are less than .05 which is a measure of scientific significance. Whereas when patients were compared to having relapsing-remitting MS versus progressive MS, they were actually not too different, because the value is greater than .05.

However, the disease status, so, whether if patients had very progressive disease, regardless of whether they had relapsing-remitting or secondary-progressive disease, if they had a high EDSS score, which is our measure of disease status, there was a difference in the microbiota composition in those patients. And so here you can see that there are purple and pink differences between the microbiota in relapsing-remitting MS patients and then in secondary-progressive MS patients as well. And disease status was really the number one factor which determined the greatest effect for microbiota. And that was followed by body mass index, race, and sex. And interestingly, in this study, the authors found that a bacteria called akkermansia, which had been previously linked to higher MS activity, was actually linked to lower disability in this patient cohort, which actually underlines the importance of sampling microbiota at different times and at different stages by male and female sex, because there's not really a "one type fits all" signature of gut microbiota and therefore not a "one type, one glove fits all" in terms of probiotics and prebiotics that could be taken by different types of patients.

Now, this is another interesting aspect, showing that there's a regional distribution of the gut microbiome within the world, and there are different predominant bacteria that are found in Western countries compared to Eastern countries and across the equator. There's also a notable sex difference in the gut microbiome in this study in type 1 diabetes, they were shown that the mice that received microbiome from males was protective compared to the mice that received microbiome from females. And even the male microbiome transfer was protective in the female mice from type 1 diabetes. And this was another very interesting study showing that in mice that were fed chow versus high fat diet, there was an important sex difference in those mice. And this sex difference was missed when mice of different genetics were looked at all together. And so, this study really speaks to the fact that when we study the microbiome, it really does need to be done in people by region, by culture, because as we see, there is a regional distribution of the gut microbiome and there are also important sex differences.

The other interesting aspect of the gut microbiome is that it can profoundly shape innate and adaptive immune systems, both locally within the gut and systemically. And in the central nervous system, the gut microbiota have been demonstrated to regulate the permeability of the blood brain barrier, which is the entrance to the brain and spinal cord from the blood, has been shown to activate microglia, the native immune cells within the brain and spine, to limit astrocyte pathogenicity, another type of immune cell within the brain and spine, and the gut microbiota can also influence myelinating genes, which is very, very interesting.

And so there is this gut CNS axix that has been coined that involves effects by the gut microbiota on the adrenal glands, the HPA axis, which is involved in stress mitigation on the immune system, on the gut, on the brain, on the endocrine pathway and is just such an important area to study and to understand better because really what starts in the gut really changes the rest of the body because so much of the immune system is found in the gut. Some say that it's up to 80% of the immune system.

And among the known environmental factors in MS that can modulate the gut microbiome is our diet. So, one of the studies that has looked at different types of diets, so here they looked at low

polysaccharide diet versus high polysaccharide diet, and polysaccharides are found in plants, fungi and algae, and they can include pectin and inulin. And what this study showed was that a diet that is higher in polysaccharides can actually fix leaky gut syndrome, which is due to inflammation within the gut. And it works via these short chain fatty acids which can go from the gut inside the blood and inside the brain and spinal cord. And so there have been a number of diets that have been developed in MS, and they include paleo diets, like Wahls Protocol, low fat diets like Swank and McDougall, Mediterranean diet, which has been well known for a long time as a cardioprotective diet, intermittent fasting diet, gluten free diet and best bet diet, which for those of you who may not be familiar with this diet, it's a diet that's based on eating a lot of fish and vegetables, but also supplementing with vitamin D, calcium, magnesium, omega 3, B12, and vitamin C.

And so, what does a healthy diet in MS look like? Well, it looks like, although there is not one diet that one should ascribe to, what is common among the different diets for MS is a lot of fruit, a lot of vegetables, and a lot of fish, and a lot of healthy grains. And for some people, dairy may be okay. For others it may not be okay, but there's really not one type of diet that fits all. It's really just about getting a lot of healthy foods that are cardioprotective. My favorite is the Mediterranean Diet.

So briefly, what is the role of alcohol in multiple sclerosis? We often get a question from patients on, okay, now that I'm diagnosed with MS, should I stop drinking alcohol or should I drink more alcohol? And it actually turns out that alcohol consumption is as high as 40% in MS patients. Men are more likely to abuse alcohol at higher levels, and alcohol at higher levels can magnify motor and cognitive symptoms in MS. However, it turns out that alcohol at lower levels may actually be anti-inflammatory, and there's a lot of epidemiological data to suggest that there is a dose dependent U-shaped relationship between MS severity and alcohol dose, meaning that at lower levels alcohol is protective and at higher levels it is detrimental.

In this study that was done in my lab, we showed that mice that were exposed to low alcohol levels at the time before they showed any symptoms of MS-like disease, they already had a difference in their microbiota. And these blue dots were animals that already started to have a more protective microbiota as a result of drinking low level of alcohol. And it actually turns out that across autoimmune diseases, this phenomenon has been described, and with high levels of alcohol, there can be increased in-gut leakiness and an increase in immune cells like T cells and immunoglobulins and a lower number of B cells, a higher number of cytokines that can be very inflammatory to blood vessels and can be inflammatory to the brain, to the heart and to the liver, and lead to overall systemic inflammation with high doses of alcohol.

However, with low doses, low to moderate doses of alcohol, there is actually an increase in protective bacteria like akkermansia, leading to protective short chain fatty acids, like acetate and protective cardiovascular factors like polyunsaturated fatty acids and an increase in the good cholesterol, the HDL, which all lead to contribute to an inhibition of autoimmunity, or an improvement in autoimmunity, in different autoimmune diseases like MS, thyroid disease, rheumatoid arthritis and diabetes. And so, at this point, it looks like low to moderate dose alcohol could actually be protective, but high dose alcohol is definitely detrimental.

So, I'm going to turn to a question that has been in the news recently, and that is the role of viruses in MS. There are a number of viruses in the herpes family that have come to attention over the years. One of them is human herpesvirus 6, HHV-6, which has been found in oligodendrocytes. These are the cells that make myelin, and it is found in a number of MS patients and has been implicated as a pathogenic virus. Now, another one that's really common

in the general population and in the MS population is cytomegalovirus. And there has been somewhat inconsistent association of MS with CMB infections. And interestingly, EBV or Epstein-Barr virus has come to the forefront as one of the leading herpes viruses that could be accountable for MS. And in a recent study that was done at Harvard, showed that the EBV infection increased the risk of developing MS by 32 times. And we're going to come back and talk a little bit more about that study.

So, what happens with EBV infection? Well, as a result of the EBV infection, which starts in the... which is transmitted through saliva, EBV gets inside the tonsils, and it then makes a home within B cells. And in the B cells it can actually stay dormant for a really long time, for a lifetime. And at some point, either stress or sleep deprivation or other infections can lead to its reactivation. And as a result, it can actually start to wreak havoc in the body, which we'll talk about in the next slide.

And there is a mimicry in action that can happen as a result of EBV. And first, I wanted to talk about what happens in nature. There is mimicry where a moth or a butterfly can look like an owl in order to protect themselves. And oftentimes in the animal world, some animals may use mimicry in order to hide from predators, while predators can mimic their prey in order to get to them. Like, for example, some spiders that look like ants in order to be able to prey on the ants. So, what happens with EBV is that there are proteins that make up the EBV virus that actually may look like some myelin proteins. And so, as a result, T and B cells that naturally make cytokines and make antibodies to attack EBV and to kill B cells that harbor EBV, may as a result of seeing EBV proteins that look like myelin actually then start to attack myelin because they think that myelin looks like the virus, and that is called molecular mimicry.

And that was explored in these two studies that came out in January 2022, one from Harvard in Science and one from Stanford in Nature. And the question is whether EBV is the cause of MS based on these studies. So briefly, the study that was done by the lead author, Alberto Ascherio, and his group looked at 800 veterans with MS and 1500 matched veteran controls, and they were able to collect blood samples on these veterans over time, because they were in the military and they all gave samples of blood over time, which were saved by the military for research purposes. And what they were able to see was that over time, patients who did not have EBV at the beginning actually converted to having positive EBV over time. And it was 33 out of 35 EBV negative MS patients that converted and 90 out of 107 controls that converted to EBV over time, which, if you do the calculations, turns out to be that EBV is associated with developing MS 32 times higher than in the general population.

And they also looked at CMV and did not find a difference. Those patients did not have an increased risk of developing MS compared to patients who were positive for EBV, and actually turned out that only one out of 800 patients did not test positive for EBV. Everyone else did. And they similarly showed that there was an association between neurofilament light chains increasing in patients with MS and not increasing in patients who didn't have MS. And neurofilament light chains are a measure of inflammation over time. And this happened in patients who were EBV positive.

Now, in a study by Lawrence Steinman and William Robinson as lead authors, they showed that there was molecular mimicry but there was a similarity between an EBV protein called EBNA1 and a glial protein called GlialCAM. And they showed that anti-EBNA and anti-GlialCAM antibodies were common in MS patients and that they looked pretty similar. There was a big part of these two proteins that overlap in terms of sequence and the EBNA1 immunization,

when they immunized mice with a viral protein, led to worse development of disease, here in red, for the mice that developed EBV compared to the mice that got a placebo.

And so, this study suggested, these studies suggested that EBV is really important in MS. But the question is, is EBV infection sufficient to cause MS? And it turns out that nearly everyone is infected with EBV, but only a small fraction develop MS, and other factors are important, like genetic susceptibility, vitamin D levels, being male versus female, as we have talked. And so, Robinson and Steinman concluded that infection with EBV is likely the initial pathogenic step, but additional fuses must be ignited for the full pathophysiology.

Now, what are the implications of EBV infection in MS? Well, now that we know that this is really important, and of note, it's not something new; we knew that EBV was important for a long time, but it's just these studies have underlined the importance of EBV with a higher number of patients. And so now that we realize that EBV is probably really, really important in MS, can we screen for EBV in family members of patients with MS who are genetically predisposed? Could we perhaps develop a vaccine against EBV? Could we treat patients with antivirals against EBV? And could we potentially prioritize B cell therapy in MS patients who are positive for EBV over other medications earlier on in their treatment to try to kill the B cells that have the EBV in them. And interestingly, medications like ocrelizumab and rituximab have been thought to be beneficial in MS because they target the EBV infection.

So, with that, in summary, EBV may be the initial trigger for MS, but there are also multiple other risk factors that contribute to MS pathophysiology that include genetics, sex differences, latitude, vitamin D levels, smoking, gut microbiome dysbiosis, and diet. And so, I will end by pointing you to a quote which is credited to Hippocrates: "Let food be thy medicine, and medicine be thy food". And with that, I will stop sharing. I wanted to thank you for your attention, and I'll be happy to take any questions.

Alexis Crispino Kline:
Dr. Melamed, that was so wonderful. Thank you so, so much for that. We've got a few questions that came in during the program. I'd love to take a few moments and propose those to you, if that's okay.

Dr. Esther Melamed:
Absolutely.

Alexis Crispino Kline:
So, we had some questions come in about if you think that taking probiotic supplements will help with MS, and the same about the benefits of intermittent fasting.

Dr. Esther Melamed:
Yes. So, based on our understanding of the gut microbiome's importance in multiple sclerosis, it certainly seems to be the case that taking probiotics and supplements would be helpful. However, what is not very well understood is what are the beneficial probiotics and what are the less beneficial probiotics because the bacteria can be either good guys are bad guys at different times of the disease. And so, it may not be the best to take the same type of probiotic at different points of pre diagnosis or post diagnosis, relapse versus remission. And what I generally recommend to my patients is to eat a very balanced diet that is rich in prebiotics, such as fruits and vegetables, as well as eat a lot of fermented foods that have active cultures, like yogurts, if people are not lactose intolerant. And that is probably the best way of getting natural

pre and probiotics until we can figure out exactly which ones and at what doses to take at specific points in time of the disease process.

Alexis Crispino Kline:
And so, I guess just a follow-up question to that, because I know that you had mentioned it a few times, how might someone know that they have a leaky gut or who would they talk to about finding that out?

Dr. Esther Melamed:
So, leaky gut can manifest in different gastrointestinal symptoms. So, people may have bloating or diarrhea or just a lot of inflammation in the body and there is not necessarily a diagnostic test, like a blood test or an MRI test that can be done, but it can be a sign of inflammation and when people change their diet, the leakiness of the gut can improve over time and the gut can heal. And so, listening to the body, listening to the symptoms that the body has in terms of the gastrointestinal... in terms of the GI, can be really helpful in terms of that diagnosis outside of specific tests that could be run.

Alexis Crispino Kline:
Thank you so much. That's really helpful. And that might be something that someone might talk to their, they could talk to maybe their primary care provider or their neurologist, their MS provider about that.

Dr. Esther Melamed:
Yeah. One of the things that we can do in animals is we can actually give animals a dye, and see, if they take it orally, whether we can detect it in the blood. And when animals develop inflammation due to an MS-like disease, there will be a lot more of this dye in the blood compared to inside the gut. Naturally, we would not administer dyes to patients just for diagnosis. But sometimes that's actually accomplished when people have diagnostic procedures done for other reasons.

Alexis Crispino Kline:
Okay. Thank you so much. And so, we had another interesting question come out. It was how do you treat someone that has multiple autoimmune diseases and are they on multiple auto immune treatments?

Dr. Esther Melamed:
Yeah. So that's a really great question because there can be a number of autoimmune diseases that one has, and the first step is to diagnose the different autoimmune diseases. And then we try to kill two birds with one stone. We try to, because we have so many medications with different mechanisms of action, we can try to pair a medicine that might be useful in two different autoimmune diseases. And if that's not possible, then certainly there can be disease specific medications that are added. For example, if a patient has lupus and MS, there may be an addition of lupus specific medications in addition to potentially a B cell therapy that would target both MS and lupus.

Alexis Crispino Kline:
Thank you so much. And so I'll ask one more question, if that's okay and it will be kind of a broad one, but if you have any guidance or feedback or suggestions that you like to share with some of the patients that you see or just the folks you care about and that you want to extend something that you think is really great, you have in your back pocket?

Dr. Esther Melamed:
Probably the general advice is to try to do all the things that keep our heart healthy, which includes exercise and eating a healthy diet and getting regular sleep and trying to have less stress to the degree that's possible. And very importantly, supplementing vitamin D. We know that all of these factors work not only for cardiovascular health, but also for the immune system. And it's really, really important to make sure that cardiovascularly people stay fit in order to improve their outcomes in an autoimmune disease like multiple sclerosis. So that would be the general advice to make sure that you're going to your internal medicine doctor in addition to your neurologist and you're doing appropriate yearly health screenings for cardiovascular health.

Alexis Crispino Kline:
Well, thank you so, so much. Dr. Melamed, that was really just a wonderful program. And just so everybody knows, too, this program tonight will be available on-demand on the MSAA MSi website in the upcoming weeks. I know I'll be going back to read the transcripts and maybe put it slow and really absorb all the great information that you shared with us tonight.

If anybody has any questions or wants to reach out to us at MSAA, please don't hesitate to give us a call, chat with us or email. And we really hope everybody has a really wonderful night. And thank you all so, so much for being here with us. And have a great night, y'all.

Dr. Esther Melamed:
Thanks for joining and thanks for the opportunity to speak tonight.