Nutrition, Genetics, and Stem Cells in Rare Neuroimmune Disorders

You can view this presentation at: youtu.be/Fc3QCWya1zY

[00:00:00] **Roberta Pesce:** Welcome back. We hope you enjoyed connecting with other members from our community and are ready for our next talk. I am joined by Dr. Michael Levy, Associate Neurologist at Massachusetts General Hospital and Harvard Medical School. You've probably seen him yesterday as well, and today he will be giving a talk about the research that he has been conducting on nutrition, genetics, and stem cells. So, Dr. Levy, over to you.

[00:00:32] **Dr. Michael Levy:** Thank you so much. Just want to make sure everyone can see my slides. Roberta, am I good to go?

[00:00:39] **Roberta Pesce:** Yes. You're good to go. Perfect.

[00:00:42] **Dr. Michael Levy:** Yeah. I only have 20 minutes to go through a lot of research, so I'm going to touch on several different topics, including stem cells and nutrition and genetics, and I'm going to update you on the research that we're doing. And if there are any questions that you would... If there are details you would like more of, I'm always available off-line.

[00:01:04] So, let's start with stem cells for neuroimmune diseases. This is the current state of the field, about how many different types of trials there are going on in spinal cord injury, multiple sclerosis, and neuromyelitis optica worldwide, and you can see how many have US sites. And this is across all different types of stem cell trials, and I'm going to explain that there are two different types that we consider.

[00:01:35] And then, beyond the specific diagnosis that they're targeting and the type of stem cell trial, there's also the route of administration. You can see there are many different ways that you can put stem cells into your body, including direct surgical transplantation or IV or combinations of those.

[00:01:55] And then there are many different types of stem cells. There are types that come from the bone marrow. There are types that come from the peripheral nervous system, from the central nervous system. There are types that come from the part of the brain that can reproduce, the olfactory ensheathing cells. There are fetal-derived stem cells. And then there are induced pluripotent stem cells, which are the newest, hottest thing, for which a Nobel Prize was awarded just a few years ago, and that allows us to create stems

cells from your own body. So, you take those stem cells, you put them in a dish, and then you make them go back in time to pluripotent stem cells.

[00:02:31] So, there are many different types of stem cells to be used, and you can see the various combinations of all the different types of the design, trial designs you can have. There are two basic types of stem cells. There's ones that are designed to become neural, okay, that could replace your damaged nervous system, and then there are types that are really designed more to impact the immune system. And if you have a disease like multiple sclerosis or neuromyelitis optica, you might be thinking, "Well, I really want both," but stem cells are really designed to do one or the other.

[00:03:11] When you take stem cells from the bone marrow, generally speaking, those are designed to become immune cells, because that's where your immune system is made. And so, when you take bone marrow cells and you use them in a stem cell trial, the implication is you're trying to impact the immune system. Then, normally, there are lots of other cells in the bone marrow that become bone cells. That's osteoblasts, adipocytes, which are fat cells, and chondrocytes that form cartilage. But among those, there are also cells that will become immune cells.

[00:03:45] Now, mesenchymal stem cells are generally the ones that become non-immune cells, so those are the ones listed there. And when you take mesenchymal stem cells and you culture them in a dish, you can push them to become more neural, more like cells that are neurons. And so, the hope was, well, maybe if they're in the right environment, like injected into the spinal cord or into the spinal fluid, that maybe they can become neural cells and help regenerate damaged nervous system.

[00:04:17] That was the hope behind the Cleveland Clinic trial that enrolled 24 patients with multiple sclerosis, and the infusion was given IV. And what emerged out of that is that there were no safety issues. You can take a person's own mesenchymal stem cells and inject them back into the blood, and it's not harmful. The hope was that it would somehow either modulate the immune system, or, as I said, try to replace damaged tissue in the nervous system, but that did not emerge as anything that... There was no sign that that's what actually happened.

[00:04:53] Now, there are many other trials going on worldwide, some that are being injected into the spinal fluid, for example, as I mentioned. And again, there's always... We're not really sure what we're doing. I'll just start with that, that we're really at the point where we're exploring which type of cell to use, which approach to use, whether we should be modulating the immune system or the nervous system, and which disease would be most amenable. A lot of these questions are still unanswered. The hope with regenerative stem cells is that you're going to replace damaged tissue.

[00:05:27] Now, you can get regenerative stem cells from many different places. You can get them from embryonic sources. The idea with embryonic stem cells is they can become anything. You could also get them from adult tissue. As I said, we now have the ability to take cells from your skin, for example, put them in a dish, convince them that they're actually embryonic stem cells, and then turn those into neural cells that can then regenerate damaged tissue.

[00:05:54] And the type of cell we want to create to transplant are progenitor cells. These are the ones that will shortly become a mature cell. But a progenitor cell, you can see, I showed them here as very small and transplantable. You can't just take a mature neuron, or a mature nerve cell, or a mature supportive cell, or a mature myelin-producing cell and expect that you can just fit it into a syringe and inject it into the brain and it would survive. These cells are very fragile.

[00:06:25] But these progenitor cells are very small and mobile, and they're responsive to their environment. That's why we inject progenitor cells. And then, once the progenitor cells reach their target tissue, then they would mature into one of the functioning cells, like neurons or supportive cells called astrocytes. So, these progenitor cells are being developed for different trials. And as proof of concept, you can inject them into a mouse or a rat, for example, that doesn't make its own myelin, and show that you can produce all of this beautiful myelin. And that's what's shown here in yellow and in orange, is myelin that has never been seen in this rat, which was born genetically without any myelin.

[00:07:14] So, these cells can really produce myelin, and the hope is one day we'll be able to inject these cells into people who have a demyelinating spinal cord or optic nerve. Our focus in the lab recently has been on optic nerve. And I think I have these as videos, so I'm just going to ... For those of you who are a little queasy, you may not want to look...

[00:07:39] Video: "Can be discarded, and should not be replaced after injection."

[00:07:42] **Dr. Michael Levy:** What this is, is an optic nerve of a rat, which we can isolate out fairly easily. This is about a 10-minute procedure. And then once we have the optic nerve, then... I'm going to show you. We can then isolate it and use a needle to...

[00:08:04] Video: "Needle. Lower the tip of the..."

[00:08:07] **Dr. Michael Levy:** We can use this needle to inject... In this case, we're injecting a dye, but normally we would be injecting stem cells into this optic nerve. I apologize if it's a little fuzzy, but that's where it is. And then this is what the rat looks like afterwards.

[00:08:23] Video: "Anesthesia. Do not leave an animal..."

[00:08:26] **Dr. Michael Levy:** And just want to show you what the optic nerve looks like afterwards. When you take it out, you can see exactly where you've injected... It's coming. Be patient. Here it is. Here's the optic nerve. This is where the eye would be. This is where we injected, and you can see the blue dye. And this is just 30 minutes later. It hasn't diffused very far. But what we found is when we inject stem cells here, they can diffuse all along the optic nerve. And what we're doing now is trying to demonstrate in, after optic neuritis, that these stem cells can survive, respond to their environment, and hopefully produce myelin.

[00:09:09] This is an example of what those stem cells look like as they're traveling down the optic nerve. And we could also do this in spinal cord. Here's a setup of, in our lab, of how we do that, injections into the spinal cord, and proof of where those stem cells end up. This is a cut of the spinal cord showing that we can deliver stem cells safely to this rat. So, the next step with this rat, of course, is to generate a transverse myelitis model, and show that those stem cells can help in the healing process. So, that's only 10 minutes, I know, a quick run-through of where we are with stem cells, and transverse myelitis, and optic neuritis.

[00:09:50] I want to get to this new data we have from nutrition, where we surveyed people with NMO using this diet history questionnaire and a survey of their clinical data. And what emerged out of this, what we're trying to figure out is, is there anything related to the patient's nutrition that impacts their disease course. And hypothesis number one is that a higher body mass index, so the more you weigh for your height, the more that correlates with poor outcomes. That tends to be true in almost all demyelinating diseases. And in our study, it was true as well, that a higher body mass index correlated... I'm sorry, a normal body mass index correlated to the best overall general health.

[00:10:41] What about high fiber diet and vitamin D? We thought that that would improve quality of life, and that tended to only be true in aquaporin-4-positive people, and we're not exactly sure why. It's possible that aquaporin-4 people have more transverse myelitis, and therefore have more gut-related issues from the transverse myelitis, and where a high fiber diet may be more important in preventing constipation. We're not exactly sure. But it turns out in our other patient populations, it didn't seem to be as important as in the aquaporin-4 people.

[00:11:17] One interesting thing that emerged out of this nutrition survey is that the more dairy you take in, the more pain you have. Now, I know that, for me, I'm lactose intolerant. If I eat uncooked lactose, I get bloated, and I wondered if that could be playing a role here. It's something that we did not control for. We didn't ask people if they were lactose intolerant, and we probably should have. But then the thinking is, "Well, if you know you're lactose intolerant, then why are you consuming lactose?" We're all guilty of it. But it is something that emerged out of this nutrition study.

[00:11:50] Now, a lot of this data was really designed to give us ideas for what we're going to do next. And probably the next thing we're going to do with collaborators is what's called elimination studies. This is where you eliminate certain things from your diet, and then you survey people how they do before and after the elimination. And here are the types of things we could eliminate. And just going in, I don't know what's going to help. We thought that a high-gluten diet, for example, was associated with bad outcomes, and that did not turn out to be the case.

[00:12:20] It might be that people already know if they're gluten sensitive, they already know to avoid gluten, and so they're not eating gluten, but maybe there are some people who still don't know and who may not be gluten-sensitive, per se, but may still have a better outcome if they avoid it altogether. This is really unknown, and we're not sure how to even advise patients, at this point, about their nutrition.

[00:12:47] And then the last topic in the area of research is genetics. If you attended last year's SRNA, you may have heard of our initial finding of a mutation in the gene associated with familial transverse myelitis. So, this is the type of transverse myelitis that occurs one time, not the recurring type, like multiple sclerosis or neuromyelitis optica. And we had access to two families. One family had two sisters that were affected. That's these dark circles. And then one family had just this one affected person. And we sequenced their genome and found a mutation in one gene called VPS37A. And then their family members had a carrier, each a boy and girl, in this family that were unaffected, as well as healthy people. And this family had, I think, one more healthy person left off this pedigree. I apologize for that. But since we did this initial study, we started looking for other people with transverse myelitis who have mutations in the same gene, and we found several more, not with the exact same mutation, but with other mutations in this same gene.

[00:14:05] So, we're really keen on trying to understand what VPS37A does and how a mutation can cause transverse myelitis. First, if you just look at where this gene is expressed, it's actually in almost every cell in the body. This is the gut. These are brain tumor cells, so even brain tumor cells express this protein. It's almost vital for life. And we discovered, as I'll show you, that when you knock it out in mice, it's not compatible with life, and so these mice are not even born. So, VPS37A is critical for life, but if you have a mutation in it, we're not quite sure exactly why that causes transverse myelitis. Now, one interesting thing about this particular mutation, called 234, that's the amino acid position where the mutation was found in our first three patients, is that it encodes for a protein called, an amino acid called leucine, and then the mutation forces it to produce isoleucine. If you look throughout the mammal kingdom, you'll find that... animal kingdom... you'll find that leucine is remarkably conserved. Almost every animal that we looked at that has VPS37A has a leucine at that 234 position, and so this change must be important, because the fact that it's conserved so highly among other animals suggests to us there's something really important about it.

[00:15:34] So, we went about modeling this, and the first thing we did was, we just knocked out the protein altogether. We knocked out the gene, and we said, "Well, what does a mouse look like if it doesn't have the VPS37A protein at all?" And as I mentioned, that's not compatible with life. There were zebrafish that had, not the gene completely knocked out, but a specific mutation. And in those zebrafish, there was a dysfunction in their tail function, sort of like a spinal cord paralysis. They couldn't move their tail fins correctly. So, the next thing that we're doing is making specific mutations in this gene so that the mouse can survive, but then we can try to figure out what's important about that specific mutation. So, you'll have to log in next year to hear that information. And so, I'm happy to take questions at this point.

[00:16:27] **Roberta Pesce:** Yes. Perfect. Thank you so much, Dr. Levy, for this talk. We have a couple of questions that came in. What is the role of diagnosing and treating comorbidity of B-12 deficiency, especially due to comorbid MTHFR gene mutation, in the overall treatment plan of neuro-diseases?

[00:16:47] **Dr. Michael Levy:** That's a good question. There are people who have vitamin B-12 deficiency, and as a result, they get a spinal cord myelopathy. It's a disease condition that looks like transverse myelitis, but it's not due to inflammation. It's due to a deficiency of vitamin B-12. And we always look, when a patient comes in with a spinal cord problem -even if we think it's transverse myelitis - we always look for a vitamin B-12 deficiency. It's such an easy thing to correct. You don't want to miss that. And then there are some people who have both. There are people that come in, and it looks like they have a transverse myelitis, but it also looks like they have a low vitamin B-12 level. And so, as I said, because it's so easy to treat, we don't want to miss it. We always treat presumptively. If there's any reason to suspect that you're not absorbing your vitamin B-12 and that's the problem, then we'll just give you a muscular injection weekly. And if it's just that you're not taking in as much vitamin B-12, because maybe you're a vegetarian, and B-12 is only found in non-vegetarian diets, then we'll just give it to you as a supplementation. But it's always important to maintain your B-12 levels, so as not to exacerbate anything else that you have going on in your spinal cord.

[00:18:03] **Roberta Pesce:** All right. Perfect. Thank you. We have, they're coming in as we speak. Can you get tested for this gene?

[00:18:13] **Dr. Michael Levy:** Not yet. Well, no. I'm assuming it's the VPS37A.

[00:18:19] **Roberta Pesce:** Yes.

[00:18:19] **Dr. Michael Levy:** And last year, we started collecting DNA from patients on a research basis, and the community was so wonderful. We're so grateful. We collected, I think, 137 samples, and so we really do have our hands full trying to figure out how this gene works. We're not really doing the test as a genetic test for patients, but we do have the ear of the Quest Diagnostics folks, and so if they are interested in providing this test on a clinical basis, then that would be available nationwide.

[00:18:53] **Roberta Pesce:** Great. Thank you so much. Another question that came in: Is exosome treatment worth trying for TM?

[00:18:55] **Dr. Michael Levy:** Exosome treatment? I'm not sure what that is. Maybe that person can just write in.

[00:18:58] **Roberta Pesce:** Yeah. We'll have them clarify, and meanwhile we'll go on to the other question that is: Should genetic counseling be done when grandparent has TM and father has MS?

[00:19:20] **Dr. Michael Levy:** Oh. I would say it's worthwhile, at least, to understand what the risks are. Certainly, if a father has MS, your risk is about 3 percent of having MS, not specifically transverse myelitis. The genetic

risks involving more than one family or generation definitely increases the complexity, and maybe a genetic counselor would be helpful there.

[00:19:49] **Roberta Pesce:** Okay. Perfect. Thank you. And then, what supplements would you recommend for MOG patients who had ON?

[00:19:57] **Dr. Michael Levy:** So, I assume the idea there would be to find a supplement that would help in the healing process and the remyelination, and there's no specific supplement we would recommend. I would say that vitamin D is recommended to everyone. Anyone with a demyelinating disease seems to respond well to vitamin D, so we recommend that, even without specific data. But if you're asking what could help in the recovery process, we don't have any specific supplements, just a healthy, balanced diet.

[00:20:29] **Roberta Pesce:** Okay. Perfect. Another one. Dr. Levy, we're going strong here. Do we think there are other genetic mutations that have not yet been discovered?

[00:20:38] **Dr. Michael Levy:** Oh, I'm a believer in that. If you just look at this one gene that we're studying, we found already two or three mutations that have never been described before. There are databases with millions of people who've had their genomes sequenced, and yet we're finding people with transverse myelitis. Just with this specific gene, there are already two or three mutations that have never been described. It seems to be very specific to TM. So, I'm sure there are more.

[00:21:06] **Roberta Pesce:** Okay. Yep. And we just heard back from the person that was talking about exosomes. The person said it's type of stem cell treatment.

[00:21:15] **Dr. Michael Levy:** Okay. So, exosomes, in general, are... They're blebs of ... that come from cells. They're blebs that have a lipid exterior, and inside it could be something else. There are many different things you could pack into an exosome. I'm not sure if they're thinking about maybe packing in stem cells into the exosome and then maybe trying to use the lipid bubble of the exosome to deliver it to a certain location, like spinal cord in the case of transverse myelitis. I have to admit I'm not familiar with the trial approach like this. I'm happy to take a look though and make a more formal comment.

[00:22:01] **Roberta Pesce:** Okay. Thank you, Dr. Levy. And I think a final question would be: For the nutrition study, how are you controlling for other factors?

[00:22:09] **Dr. Michael Levy:** It's so difficult. Diet studies are notoriously difficult. The most reliable ones are where you basically lock up the participants in an apartment, and you really monitor everything that they're eating and taking in and putting out because you can't... It's very difficult to control for everything. Even when you ask participants to eliminate dairy, they may go out for pizza thinking it's cheese but not specifically milk. It's... Diet studies are very, very difficult, and so controlling for other factors is a major challenge in these types of studies.

[00:22:47] **Roberta Pesce:** Yep. Yep, indeed. And we received a very good question here. "If we did a 23andMe or another genetic service, can we see if our DNA has the mutation?"

[00:23:00] **Dr. Michael Levy:** It depends. Some of them will let you download it. I believe 23andMe... I did one of them. Ancestry.com lets you download your entire genome, and then you can upload it to a service called Promethease. I think it's $12. And they'll give you all the mutations that you have. Now, it's a little

risky to do that, because you may find that you have 100 risk factors for dozens of diseases, and it's hard to put it into context. And Promethease is really just a research tool designed for researchers, so I don't really recommend that patients do it because it might be hard to understand the context. As I said, even I did it. I uploaded my stuff, and I have hundreds of different risks, and it's hard to put it all into context, so it's not an easy thing to do. So, for now, I would just say hold on to your DNA, and let's wait for more validated studies before you check them out.

[00:23:59] **Roberta Pesce:** Yep. Okay. I'm just going to keep going for a couple of minutes...

[00:24:02] **Dr. Michael Levy:** Sure.

[00:24:02] **Roberta Pesce:** ... if you're okay with that, Dr. Levy. Okay. This is a similar question that we already touched on, but slightly different. If I test positive for the gene mutation for TM, should I have my family members tested to see if they are at risk?

[00:24:15] **Dr. Michael Levy:** That's a very difficult question. When we proposed out our Institutional Review Board that we wanted to test patients, they said, "What are you going to do if a family member tests positive? Is it something that you would inform them about, or is it something you would ask them if they want to learn about? And then how penetrant is it? In other words, if you test positive, how likely is it that you'll have the condition?"

[00:24:36] And because we don't have the answers to those questions yet, we're really just in the learning stage where we do the best we can. We offer the patient the information they really want to know. And then, because we don't know things like penetrance yet, we can only make guesses. For this specific mutation in VPS37A, there are certain mutations where every single human being in the world that has this mutation also has TM. But they must have existed before they had transverse myelitis with the same mutation, so maybe it's just a matter of finding enough people with the mutation to really understand what the risk is.

[00:25:17] **Roberta Pesce:** Right. So, then I...

[00:25:18] **Dr. Michael Levy:** I know that's a cop-out answer. I acknowledge that.

[00:25:22] No, no. This is good. Thank you, Dr. Levy. And the final one, I believe, "I want to know how I can be tested for selective IgA deficiency given that I've been on Rituxan for 15 years, have hypogammaglobulinemia, and my IgG levels were not tested before starting Rituxan."

[00:25:40] Okay. You almost certainly don't have selective IgA deficiency if you've been on IVIG for a while. Or wait a minute... No, it doesn't look like you have been. All you have to do is get your IgA levels tested. It's a very common test. We get IgA, IgG, and IgM levels checked on our patients all the time. And IgA deficiency is very rare, but you either make some or you don't. And even if you're on Rituxan for 15 years, you should have a little bit there, unless you're truly deficient.

[00:26:20] **Roberta Pesce:** Okay.

[00:26:23] **Dr. Michael Levy:** Yeah.

[00:26:24] **Roberta Pesce:** Okay. This is the final ... They keep coming in, and I just ...

[00:26:28] **Dr. Michael Levy:** That's fine.

[00:26:28] **Roberta Pesce:** ... want to make sure that we're covering them. This is the last one I'll take before I move to our next talk. "Is there any application of banked stem cells, cord blood, for MOG patients?"

[00:26:41] **Dr. Michael Levy:** So, embryonic stems cells are thought to float around in the placenta and in the cord blood after birth, so we offer to bank them, more for the purposes of hematologic malignancies, like a lymphoma, where we can turn those cells into immune cells. For MOG cells, I imagine the implication is, can we turn those cells into myelin-producing cells? Maybe one day, but nobody has tried doing that yet from banked stem cells. Remember, it has to be from your own body, so I don't know... People my age, certainly, we didn't have the opportunity to bank our stem cells. But if you have a child, and you banked their cord blood, and now they have ADEM and MOG, maybe there is potential there to develop that into regenerative stem cells.

[00:27:33] **Roberta Pesce:** Right. Well, Dr. Levy, thank you so much for taking this many questions. We truly appreciate it. And, of course, for your ...

[00:27:40] **Dr. Michael Levy:** Happy to be here.

[00:27:40] **Roberta Pesce:** ... incredibly comprehensive talk. So, thank you for being here, and we'll see you later today...

[00:27:45] **Dr. Michael Levy:** Yes, ma'am.

[00:27:45] **Roberta Pesce:** ... for your talk about COVID. Thank you.

[00:27:47] **Dr. Michael Levy:** See you later today.