Understanding Genetics of Rare Neuroimmune Disorders

You can view this presentation at: youtu.be/b_8Ab1aMHUg

[00:00:04] **Dr. Monique Anderson:** Good afternoon, everyone, and thank you to SRNA for the opportunity to speak. This is a rather large topic, so we'll see. Hopefully, I can get through all of this within this time frame. So, let's see. I have no real financial disclosures; however, especially the latter portion of this presentation, was funded in part by the Pauline Siegel Eclipse Research Fund.

[00:00:29] We're going to start off with prevalence, overview of how genetics generally work, the genetics of each rare neuroimmune disorder, current research, and ongoing projects. Starting off here, I just wanted to give a brief view of what we're talking about when we're addressing rare neuroimmune disorders. That's going to be idiopathic optic neuritis, acute demyelinating encephalomyelitis, MOGAD (MOG antibody disease), acute flaccid myelitis (AFM), idiopathic transverse myelitis, and NMOSD (neuromyelitis optica spectrum disorder).

[00:01:08] And you can see that generally these are usually less than 10 per 100,000. So, quite rare disorders overall. And then, this was actually something that was emphasized during my training in both grad school and medical school, is an overall view of how we see autoimmune disorders, and this is actually something that can be applied to neuroimmune disorders as well, where you have an interplay of genetics, the immune system, and then also environmental factors.

[00:01:38] For each disorder, how this actually interplays is different, but it's just an overall view. And of course, like today, I'll be focusing moreso on the genetics. So, before we dive into that, I'm going to try to break down what we're thinking of when we say, 'genetics.' So, for genetic mutations, you'll see some things in the literature and just in media where they describe point mutations.

[00:02:04] When we're looking at a DNA, the genetic code, there are four letters. Basically, you can get a mutation if there's a change in one of those letters. It changes the code, and that means that what protein, what RNAs are made from that, the building blocks can basically then change. This can also happen with an insertion, if there's another letter that's put in; if there's a deletion, if there's a letter that's taken out; if there's a duplication.

[00:02:30] So, it's all very, very exact in those ways. And then, you have other things like translocations, where a gene that was on one chromosome, all of a sudden, is in another area that it's not actually supposed to be.

And similarly, if the gene is flipped around in its orientation, that also changes how basically it can potentially function.

[00:02:55] Then separately, you have germline versus somatic mutations. So, what that just means is that if there is a mutation that occurs within the genetic code in either sperm or like an egg, that's something that can actually be passed down to children.

[00:03:12] Whereas a somatic mutation, which is usually a mutation that's occurring basically anywhere else, any other cell within the body, that can also still potentially lead to disease or an additional disorder; however, it's not something that's going to potentially be passed down to additional generations. And it's a similar principle to what occurs with de novo.

[00:03:34] So, essentially, all that means is, 'new,' that's something that has not been seen in your family before, this is something that's new to you, or inherited, which is actually something that you inherit from previous generations. All of these can increase or decrease the likelihood of developing a disorder. So essentially, you'll see things where people describe predisposing or basically protective.

[00:04:01] So, that's pretty much the exact same terminology where there's a mutation or a change that's occurring that puts you more at risk for developing a disorder versus less at risk. Then, you can also see changes in terms of how well you actually recover from injury, and also the severity of that injury. That's also still linked to genetics. So, that's a very broad overview of genetics. I just want you to keep that in mind as we go through this talk.

[00:04:35] So, where this actually began was actually looking at genome-wide association studies. And this is just a large schematic. This includes most of neurologic disorders. But when this was first started, a lot of the emphasis, especially in neuroimmune disorders, was on multiple sclerosis. And you can see here that there are a few genes that were continuously coming up, and these were ones that were immune-related, and I'm going to describe these in the next slide.

[00:05:04] So, the human leukocyte antigen or HLA is located on chromosome 6. There are approximately 165 different protein coding genes in this area, which are all immune related. And if you look basically at this figure that's here on the right, when we're talking about Class 1 -- honestly, you don't need to remember the names of all of these different HLAs, just a general sense of, 'Okay, this is immune related.'

[00:05:19] Class I is present on the cells, pretty much every cell in the body except red blood cells. This is the body's way for the immune system to regulate anything that's inside the cell, so essentially preventing things like cancer or viruses, so anything that's going to essentially attack or damage the cell from the inside. Then, you have Class II molecules, which are actually our body's way of being able to protect us against things from outside the cell.

[00:06:02] So generally, it's how our immune system is educated against basically extracellular infections, and it's very important and these Class II molecules are only present on immune cells. So, given a bird's eye view, now we'll dive in. So, when you're actually looking, and as I mentioned, the initial studies that were done with these genome-wide association studies had looked at more specifically multiple sclerosis, but there have been additional ones that have been done with neuromyelitis optica.

[00:06:35] And then, on top of which there's been an expanding view of the role of the immune system and other neurodegenerative disorders. So, there are quite a few genes that are up here. This is not for you to

memorize or remember. It's just an idea of how many different potential mutations can actually occur that are associated with. And you can see, again, predisposing versus protective.

[00:07:01] So now, I'm going to dive more into the rare neuroimmune disorders and, basically, what I was actually able to find in terms of genetic linkages within the literature. So, with idiopathic optic neuritis, there are quite a few genes, and I'm just going to hit on a few of them.

[00:07:20] One of them that actually came up was APOE4, which may not be as familiar to optic neuritis, but is something that's usually seen associated with Alzheimer's disease. It is a cholesterol metabolism gene. So, it's quite interesting that it's actually seen to have increased levels present in optic neuritis patients as compared to controls.

[00:07:43] And then, basically, there were additional genes like VEGFA, which is a vascular endothelial growth factor. This is basically a gene that actually helps with the proliferation of blood vessels. And essentially, what they were able to say were two separate alleles, so this is essentially like a different variation of that gene. Two separate alleles basically were seen associated with optic neuritis.

[00:08:11] Again, not something that you really need to be very aware of, but just something for you to know, just the overall variety that you're seeing within these disorders. These are still even more genes that are actually associated with optic neuritis. Several of them are immune associated including these interleukins IL-6, IL-1, RAGE, and MMP are probably less familiar to you, but these are also important in the immune system.

[00:08:38] MMP, in particular, is actually important for the trafficking of immune cells into the central nervous system. And then, HLA-DR2, which is one of the HLAs that I mentioned earlier, is actually more closely associated with MS. The ones here on the left are more predisposing risk alleles, whereas the one on the right, cholesterol ester transfer protein, is actually one that seemed to be more protective.

[00:09:09] And I thought it was interesting because this actually happens to be a protein that is involved in a separate pathway for the formation of extracellular vesicles or exosomes, which I will be touching on later in this talk. And then, still elaborating further, there are even more genes. And I'll just point out here what definitely became emphasized as I was going through all of this information is that, the reason why you're seeing so many different genes not only is because of the multi-factorial nature of these disorders, but also because a lot of times will depend on the population that you're looking in.

[00:09:51] So, some of these studies occurred basically in Europe. Some of these studies actually occurred in Asia. With that, you're actually going to see a lot of variations and also different associations of risk alleles within those populations. I'll mention the SLP1, which actually happens to be also described as VPS33. It's another protein sorting protein. Not really important for you except for the fact that I'm going to touch on this a little bit later. Then you have complement receptor 3. We know of complement more so in relation to neuromyelitis optica, but here we're also seeing the receptor here involved with at least risk for optic neuritis.

[00:10:37] And then, integrin subunit alpha 4. So, again, a lot of immune-related genes. So then moving on to ADEM, there were actually no true linkages that were seen at least in the adult population; however, there were some linkages that actually could be seen with HLA-DQB1*0602 and the DRB*1501 as well as DRB*1503. And this seemed to be like a slight association with monophasic ADEM.

[00:11:14] And then, we have some additional variations that seem to be more so associated with pediatric patients with MOG positive ADEM, but, again, these all were more so immune related genes. So, I know I'm

throwing a lot of genes out there. Again, not something for you to memorize. It's just letting you get the landscape of how varied this is.

[00:11:35] And then, this is just pointing out, again, those three HLAs, that I had mentioned earlier, that were more so linked with at least these patients as compared to healthy controls. And this is another point for me to point out another issue or caveat with some of these studies is that, especially when you're looking at some of these rare disorders, a lot of times they don't necessarily have large populations. And one of the things that are usually necessary for helping us to associate potential genes, gene mutations with risk is actually a larger population. So, just put an asterisk next to some of these.

[00:12:21] So, moving on to acute flaccid myelitis, I had to place this slide here, but just to let you know, unfortunately, I could not find anything within the literature of any genetic susceptibility noted to date. My understanding is that likely this is due to not only the rarity of this, this usually occurs about one per million, at least, during those outbreaks, and that only occurs generally every two years. So, it's a very small population. So, potentially, as time moves on, they may be able to do more genetic studies within this population.

[00:12:57] Then moving on to MOGAD. And similarly to what was actually seen with ADEM, generally, the immune associations or the risk alleles that are associated with it are more so seen within the pediatric population, and they found actually DQB*0502 and DRB*1602. These particular variations, these alleles were associated with pediatric onset as well as with the severity of disease.

[00:13:26] And again, as I mentioned, no risk alleles were associated with adult onset. One thing I did want to point out, which I thought to be very interesting, because I know that I've been describing some of these genes and the potential associations with the immune system and that might be what's increasing or decreasing the risk, for this paper, they actually went in and looked at this HLA. So again, this is how your immune system is presenting proteins or antigens to the immune cells to direct for activation and attack.

[00:14:00] Here, what you can see is actually, we're doing a computer configuration of this particular MHC, so how it's being presented. The MOG peptide, that portion of MOG, actually fits quite nicely within the groove. This is giving an idea that, okay, this particular allele, this particular variation of that HLA, is potentially presenting the MOG protein more efficiently than others. So, maybe this is how it's actually involved in the disease.

[00:14:38] And as I mentioned, again, a lot of this will vary based off of the population that you're looking at. So, the one that I just described was a population within China. This is another study out of China, where they actually saw a prevalence of RNASE2, T2, BANK1, and TNIP1, all of which are, again, immune associated. So, you're going to see that repeating theme.

[00:15:04] I guess just as expected, you're seeing a lot of immune-related gene mutations within some of these rare neuroimmune disorders. However, there were none of these variations that were actually seen within studies done at the Netherlands and UK.

[00:15:21] So, moving on to NMOSD. Unlike MOGAD, we actually do have both familial and sporadic forms. So essentially, where inherited versus that, again, de novo, as I was describing earlier. And with this, and again repeating theme, just based off of the population that had been studied, there were several HLAs that were associated with increased risk for NMO.

[00:15:48] I'm not going to spend too much time on this. Just to mention that the HLA-DRB1*03, which is, I believe, like, the one that's been most closely associated with NMO, initially, at least for the association was seen within Afro-Caribbeans.

[00:16:05] And then this is, yeah, I know a lot, but I did think this was interesting in the sense of some of the non, I guess, classically immune-related, or at least that don't necessarily scream immune-related when you're initially looking at them, are indeed still involved with the risk for NMO.

[00:16:25] And so, I wanted to point out the CYP27B1, which is a vitamin D activator, and then, of course, we think of sometimes the potential risk that's associated with vitamin D metabolism or low vitamin D overall.

[00:16:40] Then, thyroid hormone receptor, the CD226, which is an activating molecule for NK cells and T cells. So again, these are not necessarily the HLAs, the ones that are most traditionally seen with immune disorders, but they're still immune-related.

[00:17:01] So, this is a lot of information. Again, not for you to remember. Just pointing out, again, through all of the different populations, the several different phenotypes and alleles that you're actually seeing associated with NMOSD. And this is even more of that. But I did want to at least point out there's a more recent paper that had actually been published where we actually were able to see that there were T cells that were specific to aquaporin-4 with this HLA, and that was actually of importance.

[00:17:37] So, it's tying again to what I had shown earlier where they were able to see a MOG peptide basically bound within that HLA. So essentially, meaning that the immune system is in fact recognizing the protein of interest, and that's similar to what's occurring here. So that's, again, giving us a clue that this might actually be what's causing some of the autoimmune response that's occurring in the disorder.

[00:18:01] So now, to end with my -- I don't want to say -- my area of interest, idiopathic transverse myelitis. Again, this is a disorder that's felt to be more so sporadic and not familial. However, before joining the Levy Lab, there had actually been a paper published through genetic analysis of patients with transverse myelitis, and a pair of siblings with idiopathic transverse myelitis were found, and they had a shared unique point mutation in VPS37A.

[00:18:37] And this is essentially the springboard for a lot of the studies that I have been doing. And interestingly, this same mutation was also found in a separate unrelated patient with transverse myelitis, and it is not found at all in basically the rest of the population. So, with that being the case, we thought potentially that this was of some importance to potentially increasing the risk for developing transverse myelitis.

[00:19:05] And the reason why I became interested in this is because I realized from my prior work that this was actually a gene that's involved in the endosomal sorting complex required for transport. All this, and I'll just highlight it right there in red, it's a very small part of this entire complex. But the reason why it's even important is because this is a complex that helps to package antigens or proteins into exosomes or extracellular vesicles.

[00:19:38] And the only reason that any of you probably would ever care about any of this very technical stuff is because exosomes are actually another way that the immune system actually communicates. So, this is something that has been seen previously where you can actually get the packaging of proteins present in these vesicles, and they can actually go on to activate the immune system.

[00:20:02] So, now the question became, is this actually something that's occurring in transverse myelitis? And that's essentially the work that I've been doing. And so, I'm going to run very quickly through this. I don't want to get too technical, but essentially, what I am showing here is that I'm actually seeing increases in the amount of these exosomes that are being released from the cells that are expressing this transverse myelitis mutation in VPS37A.

[00:20:31] Then, separately, I actually have a cell line that expresses aquaporin-4. So now, when I'm looking for an antigen of interest, aquaporin-4, I'm able to see increases of that protein in the exosomes that are released from those cells. So, this is building into the picture of, "Okay, is this then meaningful, and is this meaning that there are more of these antigens, these proteins getting to the immune system, and then basically causing them to be activated and leading to eventual injury?

[00:21:09] We're not stopping here, though. This is just one mutation that we are currently studying. We've actually been able to find additional mutations that do appear to be linked with increased risk. And so, what you're seeing here basically is IST1 and CHMP1A. These are also additional proteins within that S cart, that same pathway that helps to create exosomes. And then, you're also seeing some additional ones as well. This is actually work from Dr. Taka Mikami, who's also within the Levy group.

[00:21:47] So, what can we do going forward? As I mentioned, I just wanted to point out ongoing research. We do have survey that's still ongoing but wrapping up, where we're looking at residuals of transverse myelitis specifically in MOGAD. However, this is something that, once we are going through the data, we're going to also apply the survey for idiopathic transverse myelitis and also send that out for additional information.

[00:22:17] One of the things that's probably the most helpful is for us to actually have either whole exome sequencing or basically any additional genetics that have actually been done for patients, and that's something that we're actively doing within the lab. So, in conclusion, I don't know if I was able to put forward this idea that for these disorders, it's frequently quite multi-factorial.

[00:22:47] You're usually not going to see a singular gene that's responsible for developing that disorder. A lot of times, it's several that may be involved, and each person may have a different one or may even have one or more. Usually, genetics can potentially create a background where you are at increased risk for developing disease, and then often, there may be multiple genes and factors at the place I mentioned.

[00:23:15] And then, however, the genetics can actually help guide us into potential therapeutic targets for disease, and that's what we're working towards. So, I'll end with acknowledgements, including everyone in our lab: Dr. Levy, Dr. Mikami, Shuhei, as well as Natalia and Hui-Fang, who really helped with the latter portions of the project. And then, I'm going to put my email and our contact information just because if you happen to have questions or you say, "Okay, actually, I've had some of this testing done. Can you help me look through the information?" I'm very happy to. I'll, of course, end there, so.

[00:24:25] **Audience Member 1:** So, when you have the genetic sequencing done, a lot of times they can take it and do your traits and things through ancestry and things. Can you use that genetic sequencing?

[00:24:37] **Dr. Monique Anderson:** Yes. Actually, there's a way for you to go on to their website to download the entire sequence.

[00:24:42] **Audience Member 1:** Got it. So, you can work with that?

[00:24:46] **Dr. Monique Anderson:** Yes.

[00:24:46] **Audience Member 1:** Okay.

[00:24:51] **Audience Member 2:** So, did you want us to send you if we do have ancestry, to send it to you?

[00:24:56] **Dr. Monique Anderson:** So, I believe there was a survey that had been put out for just asking who had gotten the ancestry, some of that DNA done. I thought there was one that was put out by Gabriela. So, if you happen to respond to that, that would actually be a useful way for us to be able to get in touch, or you can just also just email me.

[00:25:18] **Audience Member 1:** Gabriela?

[00:25:19] **Dr. Monique Anderson:** Gabriela Romanow, yes.

[00:25:24] **Audience Member 3:** Hi.

[00:25:25] **Dr. Monique Anderson:** Hi.

[00:25:26] **Audience Member 3:** Actually, a couple times, we asked a few docs to test the MHC, HLA genes. And if they didn't laugh, they said that they straight up wouldn't do it. There's no protocol for it; they wouldn't know what to do with it. Even a couple docs said that it's only for transplants, not for anything broader immune system-related, which, of course, we know that that's not true at all. But do you have any advice as to how to interact with doctors in that regard?

[00:26:01] **Dr. Monique Anderson:** Yeah. So, even for our group, how we're approaching this is definitely different. So, I will say that the testing for HLA, especially, as I showed you, there are 165 different proteins. So, it's a pretty large undertaking to actually do the analysis, especially within that region, which is probably why people are a little bit reluctant and they usually won't do that unless they are working up for transplant.

[00:26:26] So, our approach to this has also changed, where we are trying to work with the group at MGH now to tailor the order set so that we can actually get the proper testing. Because, again, the ones that are usually in the system that we're able to order are specific to, basically, like, solid organ transplants. So, it's one of those things where you may just need to continue speaking with your doctor and see whether or not they're able to help tailor some of the orders that are actually available to be able to do that sequencing or, essentially, do the whole exome sequencing.