March 2015 Patient Education Call – Genetics and Causes of Pemphigus & Pemphigoid featuring Dr. Animesh Sinha

Dr. Sinha: It's my pleasure to be on the phone with you and everybody else today. I'm very happy to discuss pemphigus and pemphigoid (P/P) with you. Genetics and causes of P/P is something that I've been involved with for several years. As you mentioned, I got my start in Canada with my MD. My PhD afterwards was in understanding the immune system and the ways that we remain immunologically tolerant to ourselves. The immune system is our natural defense system against all of the bad guys out there – all of the pathogens, the viruses, the bacteria, the parasites, and even our own cells that have gone rogue. So, we have this incredible defense and it's made up of many components. Think of it as our military; we have the army, the navy, the marines, etc. The immune system is similar in that it has different components to the overall defense. We are able to recognize and respond to and usually eliminate different types of challenges to our body when they occur, and so we have this immune system that is poised. Normally and remarkably we remain intolerant to our own tissues, proteins, cells, and molecules. How can the immune system do this? How can it distinguish between the outside universe and our own internal component? That's the issue of self/non-self-discrimination or self-tolerance. That's what my PhD was on trying to get clues on why that is. It is still a mystery that has not been solved completely. We have a lot more information about how that works – about the genetics, the complex immune components, and the regulatory factors that keep self-directed responses in check. None the less, because we have such an incredible repertoire in our immune system and our defense some of it does spill over and does cross-react with some of our own tissues. Sometimes we get some auto-reactivity, and many times that is what we call "natural auto re-activity," that is just there and doesn't cause much harm. In some cases, in individuals this really becomes a problem. We don't just recognize the self-proteins – that recognition in certain individuals is much stronger and can lead to tissue damage and disease. That is the state of auto-immunity. There are about 100 more or so autoimmune diseases in humans. It can affect any organ in the system including the skin. Pemphigus is really prototypical organ-specific autoimmune disease. The autoimmune disease can be classified in different ways. It can be because of auto-antibodies or because of other immune cells or components that go against ourselves. They can be more widespread like Lupus in different organs or more organ-specific like pemphigus in the skin. Collectively, these autoimmune diseases affect maybe 10-20% of the population. 10-20% at least have some sort of autoimmune reactions and about 5-10% actually have disease. That is a huge number of say Americans that are afflicted with these diseases - as many Americans in the population that are afflicted with cancer. Collectively, the autoimmune diseases group is very large. It's a huge health problem. The immune system is so important to our normal functioning. It is really important to understand that in its normal state and how it works and then what goes wrong with it. I got my start in my thesis in understanding the immune system.

When I was at Stanford University we were seeing some of the genes that are very important in regulating the immune system. We were looking at those genes called HLA genes or human leukocyte antigen genes to understand their role in autoimmune disease. The sequence from genes at the time in difference diseases, but also in pemphigus vulgaris patients. We found some very interesting and new sequences that were very tightly linked to pemphigus. That is what is what still holds up today. Normally these HLA genes, you might have heard of them because it is important type them and match them when you get an organ transplant because they are involved with self/non-self-discrimination. If you have different HLA genes that are too different than you might reject the organ. Normally, they don't function in organ rejection. That is something we do artificially, but in the normal body these HLA genes are important in regulating the immune system, and they also seem to be linked to autoimmune diseases. At that time, this was in the mid-1980's, I sequenced those genes and we linked them to pemphigus. Other genes or variances of the HLA genes were being linked to other diseases like diabetes rheumatoid arthritis, and so forth. That opened up a whole line of investigation that has really allowed me to have a career in this area. We are still searching for other genes that are important. First, we try to understand how these HLA genes are linked to pemphigus and how they activate and disrupt the immune system, and how they activate certain auto-reactive lymphocytes and how those lymphocytes interplay and how they ultimately provide the autoantibodies that then go target the molecules on the skin cells that cause the skin cells to fall apart causing blisters. We have a whole line of work working off of that immune pathway and how it goes wrong. From the genetics to the immunology to the pathology of the disease. I want to say the understanding of these autoimmune diseases is called complex diseases. This means they are multi-factorial. They have genetic factors and environmental factors. Unfortunately, we don't know most of the genetic factors, and very few of the environmental factors. Environmental factors could be stress, medication, viruses or bacteria, and we don't know what they are, or how they activate disease. We have some reports and literature, but we don't have a great comprehensive understanding. Even the genetics – we know the HLA genes are important and we are doing a lot of research to figure out how they might be involved exactly in the disease. Most of the other genes we don't have an idea, because they are going to be multiple genes or polygenic. It is hard to study pemphigus because it is rare, and you need a lot of patients and a lot of DNA to do these genetic studies. We are now getting new technology that allow us to quickly sequence and type patients' DNA and to try and sort out what other genes might be important. We are very fascinated by the genetics and immunology that underlie this disease. I think my own perspective is that the only way to truly understand why people develop pemphigus and pemphigoid is through these lines of investigation. Understanding how things are functioning in pemphigus opens up the doors of understanding other autoimmune diseases or autoimmunity in general. Understanding the genes and the pathways are important. Our hope is that this will open up new ideas about how to treat disease and how to block the disease and new targets that we can pursue. Our therapies are

quite general and non-specific. We either use prednisone to knock the whole immune system down, with rituximab to knock the b-cells out, which makes the auto-antibodies a little more specific and it seems to be working well in many patients, but it is still not as specific as we would like it. That's why our goal of doing all of this work is not just because we are fascinated by it, but because we want to leverage this information to help us better understand the details of why disease happens and through that come up with therapies to treat people and to maybe cure people.

Question: I'm taking IVIG for mucous membrane pemphigoid and I'm curious how my IVIG interacts with my genetic situation. Am I swapping my other genetic information with those from the outside? What are the mechanics from the IVIG if you can explain that please.

Answer: IVIG or, intravenous immunoglobulin therapy, is a treatment that is used in a number of autoimmune conditions including pemphigus. Basically it takes pooled serum from hundreds of thousands of people, and they harvest the immunoglobulin fraction (those are the proteins that are part of the antibodies that are created as part of our defense system – produced by B-lymphocytes) and for some reason after given as an infusion over 2 to 3 days for a month it works to rev up the immune system or regulatory systems in our body that chew up the over abundant proteins. There are theories and people are still working out the reasons why IVIG works. It seems to work in a certain number of patients. It seems to help especially in disease that is rapidly progressing. So, somehow there is a down regulation or chewing up of the antibodies that are causing disease. Now where genetics gets involved is that the proteins themselves doesn't really interact with your genes at the DNA level. Your genes stay the same in every cell in your body. Where it may have effect is on the genes that are express. Only certain genes are turned on at a single time, and that changes depending on cell type or the condition of your body's response to something. So, it is possible that IVIG affects your gene expression with some of the genes involved with the immune system. The other ways that this may interface with genetics is it may be that a certain subset of patients may have certain genes that make them "responders" or "non-responders" to this treatment or any other treatment. We know overall that only 20-30% of patients respond to any sort of treatment, and why is that? Perhaps having different genetics makes you more likely to respond to a certain medication. Maybe that is why we see in pemphigus that not every patient responds to every treatment. This makes it very difficult to have a concrete system or consensus of how to treat patients. What we hope to do with future studies is to stratify patients based on their genetics and match them to therapies that they will respond to. So that we don't waste time that we don't lose time and expose people to adverse side effects. Every drug has a side effect profile, so if we know that this drug is unlikely to work with a patient with certain genetics then we certainly don't want to give that medication to that patient. That's how genetics can play into therapy. A certain drug can affect the gene expression profile - those genes that express more highly or less highly than normal and that may affect how the immune system functions. Also,

the genetics or which patients are more likely to respond and benefit from a certain drug versus those patients that won't.

Question: I was told that my children don't have to worry about getting pemphigus vulgaris because there is no familial genetic component to PV. Now her sister has been diagnosed with pemphigus vulgaris. The fact that my children will not get PV seems false. Can you please clarify the situation regarding my children?

Answer: I think a lot of these terms are used a bit confusingly – that pemphigus is not a familial disease. I think when they say that – what they are meaning when they say that is that there are certain diseases where there is a single gene defect, so like sickle-cell anemia for example. You have a gene that is disrupted and it causes a problem in the shape of your blood cells and there are a number of diseases like this where they are inherited or familial that can be passed down from generation to generation. Sometimes you need one compliment of that gene to cause disease and that's called dominant. Sometimes you need two allotments of those genes – one from your mother and one from your father to get the disease and that is called recessive. Pemphigus doesn't work like that. It's not a single gene, so there's not a simple medallion. If there is a single gene we can track that more easily, and there are a number of diseases in humans that are like that. With complex diseases where there are going to be multiple genes where they aren't monogenetic, but polygenetic and we don't know what they are. So, you need a combination of these genes. Remember there are environmental factors too that come into play. Therefore, just inheriting a gene like the HLA gene that we link to pemphigus doesn't mean that you are going to get disease. You need all of the genes, and the problem is that we haven't identified all of the genes. Even if you inherit all the genes, meaning an identical twin, there is only a 20-50% chance of inheriting the disease. There are also the environmental factors to consider. The chances of a son or daughter getting the disease is very, very low. I don't even know if there is an accurate estimate in pemphigus. In our own studies with have 200-300 patients in our database and I only think we have siblings in 2 to 3 cases, but sometimes none the less we will see a sibling, or a parent, or a 1st of 2nd generation relative with the disease as well. It's just rare. I would say that pemphigus definitely has a genetic basis, but it's complex genetics that has multiple genes involved, so the chances of a child getting this disease if the mother or father has it is extremely low, but it is possible. It can occur in families it is just not common.

Question: I was given the shingles vaccine and then began seeing pemphigus symptoms. Is there any correlation of the shingles vaccine and my diagnosis?

Answer: Vaccines work by introducing a protein related to some sort of pathogen that then primes or boosts your immune system to build up its defense to that molecule or virus/bacteria. In doing so, it can skew the immune system and perhaps in a genetically

susceptible human being who has the genes that would allow for the development of pemphigus. Tipping the immune system even further may bring the patient closer to disease development. I don't think we understand all of that. We had a patient years back where we correlated the shingles vaccine with the development of bullous pemphigoid, and I imagine there is a few cases where that has happened in pemphigus vulgaris. We don't know if and how genetics plays into that at all. We just don't know all of the effects that genetics have on the immune's response to a vaccine, but that is the only way where I could see that there is a link.

Question: I had read that pemphigus is more common in Jews of Ashkenazi descent. Is there any connection?

Answer: This really ties back to my earlier work. These HLA alleles (alleles are just variants of the gene) are very diverse or polymorphic. We all have HLA genes and a bunch of them on our chromosomes. There are hundreds of different variants of those genes in the population. So, my HLA genes are going to compare a lot differently to yours. Of course, if we are related they are more likely to be closer at least on some of the alleles. It also happens that certain populations have more certain variants of alleles. It happens that in the Ashkenazi Jewish population that they carry a lot of this one particular allele (0402). Probably because of in earlier times it was more common for interbreeding to occur, so the Ashkenazi Jewish population was probably more close knit. They had less diversity. They kept a lot of genes within their population that didn't get diluted out. Most people who have the 0402 gene don't get pemphigus, but most people who have pemphigus, especially if they are Ashkenazi Jewish carry this gene. There is another one called 0503 that the two of those together about 90% of the pemphigus population have those two genes. It just so happens that it is very common in the Ashkenazi population. In fact, even when we see people here and if they are 0402 positive they may have some link to the Ashkenazi population somewhere in their lineage. So, there is a very strong link to the genetics in some populations. Certain geographical populations are linked to certain HLA genes, and certain HLA genes are linked to certain diseases, and that is why you see more cases in the Ashkenazi Jewish population.

Question: All of my family has autoimmune diseases. My father and I were both diagnosed with pemphigus a month a part from each other. Do certain genes have a high pre-disposition for autoimmune diseases?

Answer: Yes, there is something called the common gene hypothesis that states that there is certain causal factors, especially genetic factors, that are shared amongst different autoimmune diseases. If a person has any given autoimmune disease they are more likely at risk to develop another one or have a family member with an autoimmune disease. Not a high risk, but higher than what would be normally expected in someone who doesn't have an autoimmune disease. That right away tells you that there is something that links this and it is

likely through genetics. When we see patients we always take some demographic information, and we had collected a lot of data and have now published it concerning what kind of autoimmune diseases are common in patients who have pemphigus. First of all, absolutely, about half of pemphigus patients have some first, second, or third degree relative with another autoimmune disease. We can rank them as well. So, thyroid disease is most common and then there is diabetes and so forth. If you have an autoimmune disease you are more likely to have a second autoimmune disease as well as your blood relatives. The risk is low in any case, but it is still higher than in those without an autoimmune disease. In our own lab we have dived further into this and found that there are also disease clusters. The cluster of autoimmune diseases that we found are pemphigus, immune thyroid disease, diabetes, and rheumatoid arthritis. Right now we are doing a study to look at all of the genes that have been linked to those diseases and looking at those linked genes in the individual disease and see which ones are shared across all of those diseases. This would lead us to the underpinnings of a common gene cause. Say we find 3 or 5 genes that are common within all diseases in that cluster then we would know those genes are pre-disposed to autoimmunity. You have a very interesting family, and I would encourage your family and all of the listeners to contact us to donate blood. Whether your blood relatives have disease or not, because that is the only way that we can do these studies and learn more about the genetics that are relevant. We really appreciate these samples so that we can extend our studies.

Question: How can patients go about donating blood samples?

Answer: We collect blood at our lab for different reasons. We can extract the DNA, and then we can do our genetic studies from that. We can extract the RNA and that tells us what genes are actually expressed in the blood. Those are usually expressed towards the immune system, so this gives us more clues about the pathways of the disease. We extract the serum, and there we can look for certain proteins and autoantibodies as well as other immune mediators called cytokines that help to regulate the immune system. For blood donations we are looking for patients and blood relatives with or without disease. To donate you can email my team at krs2002@buffalo.edu or call (716) 842-2118. Just tell us about your willingness to donate blood and we will find a way to get a kit out to you and send back blood back to us. We are very dependent on these samples to try and figure out what is going on.

Question: Is it possible to have one form of pemphigus and pemphigoid and then get another?

Answer: Unfortunately, I don't have an answer for that. It is possible to have mucosal lesions and then to get skin lesions. This is another aspect that we want to study. Why do some patients only get skin lesions, why do some only get oral lesions, and why do some get both? With more research we hope to have these answers.

Question: Would it still be beneficial for me to donate blood even though I am in remission? Also, if we donate blood can we get results back?

Answer: Yes, your contribution would still be very beneficial for two reasons. The first being that your DNA does not change. Second, we are very interested in your immune response now that you have gone into remission. We can give you certain results. We can give you your antibody titers and our HLA type. The other results aren't really meaningful individually. For example, your gene sequence or your gene expression, because we don't know yet what that means specifically.

Question: I am getting Rituximab infusion next week. Is that harmful to your body?

Answer: Rituximab is an antibody against the B-Lymphocytes, and those are the cells that produce antibodies in our body. Which are important for our immune defense. In pemphigus, some of those cells are directed against important components of skin cells and keep them attached. So, when those skin cells are detached they disrupt the adhesion or contacts of skin cells and they fall apart and you get a blister. So the rationale behind Rituximab is that if we can get rid of the cells that make the antibodies then we can stop the disease. Of course, you may be knocking down more than just the b-cells that make the antibodies that are important to pemphigus. We still need to do long term studies. So far, it has been safe. It is hard for me to answer for your case specifically as I am not your doctor, but Rituximab has been effective.