

**Transcription for: "01PROOF will my genetic variant make me sick.mp4" (Uploaded File) (New Transcription)**

**Emma Mertens:** Alright, everybody. We just hit seven o'clock, so we're gonna go ahead and get started. Good evening, everyone, and welcome. Thank you for joining us as we continue the Immune Deficiency Foundation's twenty twenty six education series. Our featured speaker for tonight is Dr.

Dusan Bogunovic, who joins us to discuss genetic variants and gene expression and primary immunodeficiency. My name is Emma Mertens, and I'm the program manager for education here at the Immune Deficiency Foundation. On behalf of the organization, we thank you for tuning in to this virtual event. Throughout this series, guest speakers will provide a deep dive into clinically advanced topics from the world of PI. If time permits, this session will be followed by a Q and A with our presenter.

In offering these programs, we aim to engage and empower the PI community through education. During portions of tonight's talk, we will offer audience polls. When a question pops up on your screen, you may submit a response by clicking one of the presented options. As always, closed captioning is available. This webinar is made possible by our wonderful sponsors.

It is due to their partnership and contributions that we can offer programs like this to the PI community. Please join me in thanking our sponsors. Before we get started, a brief disclaimer, please remember that information presented during this meeting is not intended to be a substitute for medical advice, diagnosis, or treatment. We are here today as a trusted source and friend provide you with information. Always seek the advice of your physician or other qualified health provider with questions concerning a medical condition. Never disregard professional medical advice or delay seeking it based on information presented during an educational event. Alright. We are going to kick things off by asking you to answer a quick question using the Zoom polling feature. Our opening question is on a scale of one to five, how confidently could you explain the term genetic variant to a friend? And we will give about thirty seconds or so for everyone to go ahead and submit their answer.

Alright. Looks like we have a good variety of learning levels that are in our audience. So thanks everyone for participating. Alright. And with that, I am so pleased to introduce our speaker for this evening.

Doctor Dusan Bogunovic is a pediatric professor of pediatric immunology and vice chair of basic research at Columbia University. He also serves as the director of the recently launched Columbia Center for Genetic Errors of Immunity. His lab focuses on human immunogenetics and their role in disease manifestation. And his research is widely cited across the fields of immunology and genetics. Thank you so much for joining us.

**Dr. Bogunovic:** Thank you for having me. It's my complete pleasure.

**Emma Mertens:** Alright. So I'm gonna pull down my slides. We'll have you go ahead and pop yours up on the screen.

**Dr. Bogunovic:** So let me just make sure I'm sharing. Okay. So I'm gonna share this game first. Sorry, because I tried it before. There it is.  
You see it well?

**Emma Mertens:** Yep. Looks great.

**Dr. Bogunovic:** Okay. So I can start. Yeah. So good evening, everyone, and thank you so much for joining this education series. And thank you for the invitation.

I really like what IDF does and the mission. And none of this work that I'll present to you would be possible without you and without providers, your doctors, and your families. So thank you in advance for all of that. So today I'll try to talk hopefully in understandable terms but I'll also go a little bit deeper for those that like to jump into genetics a little bit deeper. So if you lose me for a second, don't worry, I'll come back.

K? I promise. So the title is decently simple and very important, and it's will my genetic variant make me sick? Right? So I'll start first by thanking people who did this work. This is my team and a lot of collaborators and a lot of government funding, actually almost exclusively government funding that has allowed for all this to work. So if you're thinking about how do we discover these things? Think about advocacy to NIH and advocacy to congressional bodies to keep funding science because without it, we can't help ourselves. Okay. So I'll start simple.

This is inborn errors of immunity or primary immunodeficiencies. You can use those terms interchangeably, and we think of them as genetic disorders that predisposed individuals to infections, inflammation, immunity, allergy, and malignancy. Right? So basically, one can have a genetic variant that they are born with or acquired throughout life that can make them much sicker than anybody else. Right?

And so far, there are over six hundred unique inborn errors in immunity or different forms of primary immunodeficiency. And they are discovered by forward genetics. Okay. So here's the first term that you may not know what it is or not be familiar with. Forward genetics and collectively, you know, inborn errors or primary deficiencies effects of about one in a thousand people.

This is all of us, right? All of us, this is actually way more frequent than we ever thought. So but what is forward genetics? Forward genetics is an approach where we in the clinic see patients and then we're like, well, they all present with infections. They all present with deficiency in their B cells, or they all present with stomach ache.

And then what we do is we perform whole exome sequencing, so we basically take DNA

from blood and we look at ATGC, ATGC gazillion times and we compare that to the healthy population somebody who doesn't have the symptoms. And we say, well, this genetic variant in this gene is so much more common in those people who have constant stomachache or cannot fight the viral infections. Right? So the dream and that that's how we identify these genetic variants that predispose individuals to variety primary immune deficiency phenotypes. Now, our dream and we're becoming increasingly successful at that is then we noticed we call it genotype so that is the genetic variant that somebody carries can inform us which drug and to use to make that individual better.

Right? And that is my mission life to identify genetic variants, understand what they do, and then be able to suggest a drug that can help cure that individual and have good quality of life. Okay. Now those that use Facebook know the term, it's complicated. So I'll invoke that here.

It's never that simple. As you know well, And the term that I'll describe today is called incomplete penetrance. And what incomplete penetrance means is that somebody can carry a variant. This one that is supposed to predispose individual to don't have B cells or don't have a stomachache, but actually be totally fine. That's what we call incomplete penetrance.

So it means this might happen, but need not happen. Right? So let me show you this family category. This is a made up family category. In squares, it's a boy, and in circles, it's a girl. And in pedigrees, when we joined these things, that means those are the parents. So in this family, because we carry half of our genes from mom, half of our genes from dad, we will describe any gene as wild type or mutant. The mutant is one that carries a variant. Right? So one came copy from mom, one came from dad to for this individual.

And this individual demand in this case has both wild type copies. Okay. So now they have children, and all children, and this can happen, inherit the bad gene, called that the mutated gene here in red from the dad. Right? And the wild type they inherit from mom.

But only the dad and one daughter are sick. The other daughter has some phenotype that's called partial disease expressivity. And another, in this case, boy, just like dad, has absolutely no disease. So we've known about this in genetics for decades, and we're like, well, All this means that if you have a genetic variant, you may or may not get sick. That's all we knew.

So we then we try to explain, but where is this coming from? This must have some form of []. Okay. So we understood the concept of incomplete penetrance as common ground. How prevalent, how often does incomplete penetrance happen?

Well, we don't know for sure. We think this could be as often as twenty percent, meaning that every fifth person who carries a variant might not get sick. Right? But now, this is true in families where there is some phenotype. But if you actually step away from that and look at two million people, On average, every one of us carries a known genetic disorder and

doesn't have a phenotype.

So if you think about it that way, Incomplete penetrance is common. It happens all the time. Right? If it's within the family, there is more penetrance that we know, which is how we were able to identify all of these genes. Okay?

Okay. Let me tell you not about another concept. We inherit half of our genes from mom and half of our genes from dad. What we think is that when you have DNA is that you will make RNA and then you make protein. We think that half of the RNA comes from maternal gene, half comes from paternal gene, right?

And the same goes for protein. We know there are some exceptions to that. And one is in genomic imprinting where some genes in your body, even though you inherit both copies from mom and dad, but you just express, let's say, paternal copy in your whole body. And we've known this in the seventies, there is few defined genes. So I won't talk about that. In individuals who carry two x chromosomes, so females, this happens in every cell. X chromosome, which is what makes a female female, if you have two of them, gets randomly inactivated. So you end up in each cell having just one of the two copies expressed. So concept transcriptional bursting, which I wanna talk about. And what I do wanna focus today is this concept of **monoallelic** expression.

And what that is is if you look here even though every cell genetically has maternal and paternal allele. Some cells meaning just white blood cells or just the heart cells or just the brain cells can express just the maternal allele. Despite being heterozygous in a way you become transcriptionally homozygous. Okay? Now, if we try and merge these two concepts.

At the DNA level, this family has a mutation. One wild type allele of the two copies, maternal paternal, one is mutated, one isn't. Right? You see, everybody in this family has that. Father, mother, and the son.

However, son is the only one that's sick. Father has some disease and mother is completely healthy. In canonical form of thinking about disease, we think that at the RNA level, all these three individuals are the same. They make same amount of RNA from the good copy and the bad copy. Right?

But if we now introduce the concept of **monoallelic** expression, what if somebody who is healthy, let me actually use here, pointer. What if somebody who is healthy predominantly expresses the healthy allele? What if somebody who has a little bit more disease actually starts expressing more of the mutant allele? And what if somebody who's fully diseased has toy penetrant disease actually is more mutant than wild type. So not equal anymore, like we always thought genetics works, but the mutant allele overtakes.

Right. So this was a hypothesis that we had. Now how did we identify that? So here is where I can lose you a little bit. But that's okay.

For some that I don't lose, enjoy. If we take anybody who has a heterozygous mutation, this

individual here had a de novo mutation. De novo mutation happens where your parents don't have the mutation, but somewhere as you were becoming a human on your way to birth. You acquired a mutation. It's called a somatic mutation in development, and that can be, in this case, designed meant that fifty percent of this individual's body carried the genetic mutation at DNA level, and the other part was wild type.

So if you carry any heterozygous variants, meaning your copies from mom and dad are not the same, you carry one variant and one allele, we can look at every single cell and look at RNA, so not DNA, but RNA from every single cell. And if you're heterozygous, this is the wild type allele, this is the variant allele, you'll be essentially double positive. Right? The more your sequence, the more biology works as it should. You have equal level of expression on maternal and paternal genes, right, and the RNA.

Now in this individual, we did the same thing, but it didn't make sense. And let me explain what happened here. Fifty percent of these girl's cells were wild type, so we should express fifty percent just the wild type allele. K? However, the other fifty percent of her cells carried the mutation, so they should be double positive.

They should all be here. Yet they're not. They were expressing just the mutated allele. So we were staring at this. And I'm like, but this makes no sense.

This is not how biology works. Right? But it is this single patient and the family and the doctors, providers, and the research team that I worked that allowed us to form the hypothesis that maybe this skewed expression of maternal and paternal allele is what allows for this phenotypic variability in inborn errors of immunity. Right? What if we shouldn't be just looking at DNA?

What if we should be actually looking at RNA? Okay. So how did we start studying that?

Well, we are like, okay, let's see how generalizable is this phenomenon that we've described in the single patient. So we took healthy New Yorkers blood, those kind individuals that go to the blood bank and donate blood, and decided to essentially through a series of assays.

I'm not gonna go into details that. We take a single cell from their blood and make million cells out of it. And we do that multiple times. And what that allowed us is to study this phenomenon. Okay?

So when we expand these t cells from individuals who donated blood, we sequence every single gene and determine which is maternal, which is paternal copy. This is a DNA level. At the same time, we sequence their RNA and then compare to DNA and see if RNA is fifty-fifty just like DNA should be, right? Because that's Mendelian genetics, that's how we were taught biology. And if they aren't to mark them, computational pipeline and many different methods to determine that.

Okay. So Which primary immunodeficiency genes undergo **monoallelic** expression was the first question? So where should be looking? Which genetic deficiencies this might

happen in or not happen in? So at a time, there were about four eighty five primary immunodeficiency genes.

In these ten New Yorkers, we could assess about three fifty of them because they needed to have a variant that's different between mom and dad, and they needed to be expressed meaning RNA has to be there in the cells that we tested. Okay. So let me orient you on this graph. It's pretty looking and there is meaning to it, I promise. So on the top here, you see donor.

So this is a healthy New Yorker A and we have these two different T cell clones. This is healthy New Yorker D and we have six and so on. If something is red, that is completely maternal. If something is blue, it's completely paternal. The bigger the circle, the more we trust the data.

Okay? So if you look at donor f here, this is a known imprinted gene, means that the color should be the same in all clones, and it is. So that made sense and we're like, okay, our assay is working. So this is what we call in science, positive control, so we know we can anchor to some truth from the past. Okay.

And then we did the experiment. So let me orient you to this one. So now this is again a donor f. And this is the gene JAK1 that inspired all of this work from that girl's single experimented within. And you can see in all the clones from these individual everything is equally expressed between mom and dad, just like we thought.

But if you go to donor C, you can see that half of the clones are expressing just the paternal gene and not maternal, which means this happens in healthy individuals they just don't carry a mutation that can cause disease. So this is a naturally occurring phenomenon. And if you look at donor f in another primary immunodeficiency gene, PLCG2, and I'll come back to it. You can see that one is paternal One is maternal and the other ones are biallelic. So we started looking at all primary immunodeficiency genes.

And you don't need to understand this. There's just a lot of them. And if we actually zoom out at a time, this is only a year and a half ago that we published this, we said five percent of all genes in our body. We have twenty thousand of them. Can undergo this **monoallelic** expression, autosomal random **monoallelic** expression, ARMAE, and about four percent of genes that are known to cause inborn errors of immunity.

But we assessed that only in about ten New Yorkers, and on average, we had two, three clones from them. So somewhere about twenty to thirty clones, we we could decide that. So the past year and a half, we expanded this by a logarithms. So now we have over two hundred clones. And the results have changed in a way that they say that about fifty percent of all human genome can undergo **monoallelic** expression.

This is where we are experimentally. We are about trajectory at about thirty percent but we are projecting then we'll get well over fifty percent of genes. Suggesting that we are discovering a naturally occurring biology. It happens in all of us irrespective of not if we

have primary immunodeficiency. Just in the primary immunodeficiency, it may matter. And I'll get to that in a second. Okay. Does this change with age? So Mitch, a PhD student in the lab took blood from kids who are zero to two years old, individuals who are eighteen to thirty five, and those who are sixty to sixty seven, and you can see there is growth clearly with age, there is a [higher propensity for monoallelic expression] in some of our cells, some cells in our body, both and then some genes are completely maternal or completely paternal. If you wanna be funny about this, you can say, Finally, I can explain why people are telling me that I'm just like my mother.

Right? Okay. So we assess this over time, and this is stable for weeks in culture. So this is not something that fluctuates. This is something that's relatively stable.

We think we can influence it and change it, but it's relatively stable. I'll go into the mechanism of it. Suffice to say, if we perturbed if we change inside of the cell and how DNA is wound up. That'd be simple like that. We can actually reverse this phenotype.

Something that was [] can now become biallelically expressing. Same thing something that was biallelically expressing here, if we play with how we organize DNA, I'll stop at that. We can actually change it back to being just reference fully. Okay? So we are beginning to understand the mechanisms that can help us manipulate this, which we hope can lead to therapy.

Okay. Back to our patient that inspired us. This patient had a mutation in JAK1 that was leading to gain a function. And what that means, this patient had a lot of inflammation. Right?

So when we experimentally proven this with this assay, this is the patient mutation, this is the wild type in the patient mutation in absence of any signaling, you can see this sharp black band that almost doesn't exist here, which told us that this was a gain of function. This is spontaneously hyperactive spontaneous inflammation in these patients. This is biochemical evidence that this is true. And here is again a family. Let me take you through this family.

So this is an incompletely penetrant family where the grandfather, mother, and the granddaughter all carried mutation. You see, wild type m, wild type m, but only the mother is sick. And we're like, well, how's this possible? Right? So we tested our hypothesis.

Again, you see a big black band saying that this is bona fide a gain of function, meaning that This happens in the daughter's cells. This happens in the grandfather. He's eighty two, and he's fine. How is this possible? This hyperactivity.

Right? What is taming it? Okay. So here is the healthy carrier, so this is the daughter and this is the mother. If you look at RNA of a gene that is heterozygous, meaning wild type came from mom and it's okay, came from dad and it's mutated, right?

It should be fifty-fifty percent if this is biallelic expressing. So this is not fifty-fifty, and this is not fifty-fifty. So the healthy carrier was suppressing the bad allele to allow only twenty two

percent expression. And even the mother who had a disease is doing the same at thirty six. So we're like, oh, there are signs here.

Right? So then what we did is we took blood from these individuals and isolated B cells monocytes NK cells and T cells. And what this graph is showing is the percent of JAK1 mutant allele expression. Again, something that's called [] should be flat at fifty. And you can see overall suppression of the mutated allele, right, with the exception of maternal T cells.

So they are the runaway bad guys, if you wish, where the mutant allele who we know biochemically's gain of function was leading to disease. So this was the first example that even though the whole family has the same genotype. And we know this is a disease causing allele that we have to introduce a new term, and we call it instead of genotype, transcriptotype because it's a transcript, it's RNA that leads to the protein production afterwards, that was different. So genotype is fifty fifty, but transcriptotype in this case is eighty twenty, which causes disease but not in the patient because it's flipped. Okay.

I'll give you two more examples or three, and and and pose a little bit of a theory and then we can go into q and a. So this is a PLC Gamma two that I showed you as can undergo **monoallelic** expression in healthy New Yorkers. None of these healthy New Yorkers could carry any deleterious variant. This is just normal variation, which is normal. The difference between me and any of you here is in about twenty thousand these variants that we carry. And that allows us to be us. Otherwise, we'll be all identical twins. Right? That's the point of diversity in genetics. And this is a very complicated family, but the point is affected carriers here in black were carrying the mutation, and these two cousins were all both sick, but once B cells were making immunoglobulins and the other person's cousins who had the same genetic makeup were not making immunoglobulins.

Right? So this is that variable disease expressivity, much milder disease, but still not quite okay. So we did this test. And let me show you the results. So in the wild type wild type PLC gamma is gonna be on the x axis, mutated one is gonna be on the y axis.

Healthy donor is in blue, If you look at healthy donor and you measure how much RNA is coming from wild type, well, all RNA is coming from wild type because there is no mutant. This is a healthy donor, both copies of wild type. Now if you take patient a, so that is the patient that has normal IgG levels. This patient is heterozygous, so it carries both the wild type allele and the mutated allele whether or not the cells are fluxing. Right?

It's a functional output of B cells. No need to get into it. However, the cousin that cannot make him in the globulins, cells that are not fluxing, they shut off the wild type allele. They only have the mutant allele expressed even though genetically wild type is just there. But it's not doing a job.

Okay? So this was the second example. The third example we have is from three different patients that have variable disease penetrance that all have different STAT1 gain of function

mutations. K? And I say in essence, if you look at that something that is red is completely mutant, something that's blue is completely wild type.

You can say the sicker the patient is the more mutant expression exists in different cell types. And I'll give you a last example. This is a family where dad was okay. And the kid is not okay, had severe mycobacterial disease. We sorted all these different white blood cells. They all have a special function. Again, if something is wild type, it's completely If something is blue is completely wild type, if something is red, it's completely mutant, and the bigger the circle, the more we trust the data. If you look at a healthy control, everything is blue as it should be, it's wild type. Right? There is no mutant allele to be had.

However, when we looked at the dad and every cell carries a mutation at genetic level, we couldn't detect a single mutated transcript at the RNA level. So that cells have shut off the bad allele. Which is why that was healthy. And if we looked at a sick carrier, the sick carrier is actually also shutting off in T cells and NK cells, the bad allele, but in B cells and in monocytes, it wasn't able to And we know that mycobacterial disease comes if you monocytes are not functioning properly in terms of that one function. So this made perfect sense.

Okay. What have I told you? **Monoallelic** expression occurs naturally in all of us. Right? In healthy New Yorkers in disease patients can help some.

Right? Why? I don't know that we have an answer, but we have formed a hypothesis which we call personal evolution theory. What do I mean by that? What if we inherit and we do half of our genes from mom, half of our genes from that?

And what if early on in life, we are mostly biolytic, but human biology has allowed us to test which maternal or paternal gene might actually be better for us, right, as a form of a fitness. And as we change an age through life, we slightly morph where we need it. It can be in the lungs. It can be in white blood cells. It can be in the brain where specific genes are better if they're maternal than paternal.

So over time, we personally evolved into the best versions of ourselves to the extent that our genetics allows us to do. And that can also happen perhaps even with infection. Infection can shape something. So we're actively testing all of that. It's a little bold hypothesis, but we have to think.

A little bit more nimble. What have I told you? **monoallelic** expression can render explanation to some incomplete penetrance cases. Meaning, if you have a variant and you're not sick, well, perhaps this is the explanation for it. It might not be in all cases. We simply have to test each and every individual and their family member to see. Right now, we are at about I think ten genes that we know can undergo this and can explain **monoallelic** expression, there is five ninety more to go. We are working on it, so it's gonna be a process. So in a way a family member that has a variant may not get sick, but this is not for sure. We need to monitor it.

So from what we see right now is that one and done genetics may not be the future. It's a propensity and likelihood of developing disease. Some genetic diseases are highly penetrant. So we'll know that, yes, this will happen. We gotta treat it.

Some or not. And it really depends on the gene and the genetic variant to do it. We need to monitor it. And I think we'll need to monitor that something like we do with thyroid problems. Right?

Maybe six months, every year and see whether the mutant allele and wild type where they are in all of this. And it is complicated. We are working on it. It's very exciting time in terms of what we can do, where the technology has taken us, and how much of it we can feedback to patient treatment. I will stop at this point and I'll happily answer any questions.

**Emma Mertens:** Alright. Thank you so much, Dr. Bogunovic. Okay. Let's see. I'm gonna go ahead and move my slides back up.

**Dr. Bogunovic:** Good day. Stop the share. Yeah.

**Emma Mertens:** Yep. We're good. Alright. So with that, we'll go ahead and dive into q and a. Friendly reminder to our audience members, if you'd like to ask a question, go ahead and pop that in the Q and A box on your toolbar, on your Zoom screen, and we'll go ahead and get started.

Alright. I know you touched on this a little bit in your in your last talking point. This individual wants to know if incomplete penetrance is so unpredictable, How do the doctors decide who needs closer monitoring versus who is okay to just keep on with the life?

**Dr. Bogunovic:** Yeah, that's a very difficult question. So far because mostly we don't know still. Than we do know. We know that for some genetic disorders, the penetrance is extremely high. So this is where your provider, your doctors likely going to be way more aggressive.

Right? And if the penetrance we are onshore may or may not develop, then that goes into a monitoring phase you know, first line of drug response, if you're doing well, well, let's not change anything. So there is a lot of astute experience that goes into these decisions. Hopefully, in the next few years will be infusing a little bit more diagnostics of these forms as we learn which genetic loci, which genes that if mutated may or may not undergo this form of regulation, which can explain this incomplete management.

**Emma Mertens:** All right. Thank you. All right. Our next question, can you explain MAE again? Some folks, I think someone mentioned I'm a ADHD and I'm having trouble explaining that.

So if you could if you could dive into that a little further, I think, folks.

**Dr. Bogunovic:** Yeah, absolutely. You can think about MAE as, you know, any gene that inherit one copies from mom, one copies from dad. Right? And every our cell in our body has the same genetic material. Right?

And then that gene becomes RNA. That's the process of, you know, very basic biology and that an RNA becomes protein. Right? And we keep going through that cycle through our life where we thought that that is equal on essentially all genes. What we are discovering now that That is true, of course, but some cells, maybe brain cells.

In this case, t cells in the white blood cells. Some cells can commit to being just maternally expressing or just paternal expressing. Right? So if you think about it, you can think about it as a transcriptional [**mosaicism**]. I hope this helps.

**Emma Mertens:** On that note, since you bring up **mosaicism**, can you tell us a little bit more about what that means?

**Dr. Bogunovic:** Yes. So **mosaicism** is on genetic level means that you acquire genetic mutation, []. So basically, just your t cells have the mutation and nowhere else but that's in a DNA level. Right? And what I'm talking about is that all your cells are genetically the same, but they just express the mutant allele in t cells.

Right? So this expression would make it an RNA **mosaicism** versus the DNA **mosaicism**.

**Emma Mertens:** Alright. Thank you. That's something I've been reading more and more about in research. So, ma'am, it's it's all very fascinating.

**Dr. Bogunovic:** Yeah. They're excited.

**Emma Mertens:** I'm glad you brought that up. Alright. So this person says, thank you for a great talk. And they'd like to know Is there something that can happen in someone's life or some sort of environmental trigger that might cause a mutation to kind of flare up or become more active at one point or another in their life?

**Dr. Bogunovic:** Yes. We think so. We think so. We are actively testing that in the laboratory now to see whether external stimuli so environment can trigger basically disease to be worse or vice versa for it to stop. It's mostly unknown, but we believe that can absolutely happen.

**Emma Mertens:** Sure. And kind of a follow-up question to that. So going back to one of your graphs that you showed during your talk, there was a chart that was kind of comparing age groups. It was, like, zero to two, eighteen to thirty five, and then older folks. Based on that, do you think that that could be a potential explanation for why some individuals with primary immunodeficiency don't receive a diagnosis until later in life?  
Absolutely.

**Dr. Bogunovic:** Absolutely. We don't have formal evidence for that because we would have to monitor that same individual. I have a couple of families where we are doing that right now. From early childhood no symptoms to beginning no symptoms and how many changes, but this is highly indicative that that is a good hypothesis and that truly may happen.

**Emma Mertens:** Okay, very interesting. Alright. So going back to another one of your graphs, you showed one that was kind of mapping out all the recent genetic discoveries and how we kept you know, discovering more and more of these genes and these variants each year. And in fact, when you said six hundred different types of inborn errors of immunity, I thought we were still at five fifty. So there's already been a whole whole bunch more discovered just to make that make that statistic inaccurate. On on that note, does that mean that we are constantly just discovering new genes and new forms of primary immunodeficiency, the more that we dive into the genetics.

**Dr. Bogunovic:** We are – we are – I don't know if we are at the plateau yet. We keep discovering more. I don't know where we'll stop. But absolutely, it it appears that, you know, every year we are adding fifty to a hundred new ones at the very least.

**Emma Mertens:** Yeah. Fascinating. Alright. Thank you so much. Alright. Going back to another thing you said on one of your slides, you mentioned that half of known inborn errors of immunity genes are accessible in the t cell system. And you said that half of PI genes can be tested that way by testing the t cells. So what about the other half of primary immunodeficiencies? Yeah. Where where are we finding those? And are we just missing information on those?

**Dr. Bogunovic:** Yeah. We are mostly missing information on it right now. But we do have technology where we can look at non T cells. We can look at monocytes. We can look at B cells. We can look at plasma centered and dendritic cells. All the different cell types. And we are doing it as we speak. So we have information now on many other cell types and many other genes that I didn't have a chance to show you today.

**Emma Mertens:** Fascinating. Thank you so much. Alright. This next question, I don't know if you can speak to this, but one of our audience members is a individual who sees doctor Orange, and I think they saw his name on your on your slide that he as a collaborator in your lab. I'm not sure if you can speak to this, but she was wondering if you can share any updates on his work or if you are familiar of anything he's working on related to functional, natural, killer cell disease. And if not, that's okay.

**Dr. Bogunovic:** That is totally okay. So Jordan is a phenomenal doctor and Jordan is a phenomenal scientist. Right? So it's really he's a remarkable individual. There are many new things that he's working on in the context of NK cell deficiencies. He's probably the father of the field of NK cell deficiencies, if not the most important person. There, so keep following his work, keep following his publication record. There is a lot more coming. I was privy to it a few months ago, and I just love what he does. So you're in good hands.

**Emma Mertens:** Awesome. Alright. Next question. Let's see. Okay.

This individual wants to know, as we are identifying more and more genes and learning more about these mutations, how often should we repeat genetic testing?

**Dr. Bogunovic:** It's very important. So if genetic testing was done ten years ago, you should absolutely repeat it. Right? If genetic testing was done last year, depending on what type of genetic testing you got done, you may need to repeat it. Maybe sometimes it's just worth reanalyzing because Okay, let me get a little bit more complicated than that.

Because when we deliver results to the patient, we want to have some form of absolute certainty that what we are saying is correct. Right? You don't want to come to somebody and say, hey, you have a mutation and like, oh, that was a mistake. Right? It's horrible, that's unethical, that may imply some form of treatment that's not needed.

So the bar to identifying and communicating that there is a mutation is extremely high. As it should be. Right? Well, that means that when you have an approved, you know, insurance paid or not paid, but a clear approved genetic test, that is very restrictive. In terms of what's allowed and it's actually state per state kind of diagnosis because of how it works.

So often there is much more data than is allowed to be reported because of those []. It's worth discussing with your doc how the test was done, whether the simple reanalysis of that test may lead to potentially new diagnosis or if there was no diagnosis unremarkable test is what we say is when we're like, well, we didn't quite find anything. And not quite finding anything is still the case for seventy five percent of individuals. Right? So especially those without diagnosis, I would go back and just politely ask, well, what was done?

Is this whole genome based? Is this whole exome based? Can we reanalyze that? Is there a research study I can be a part of? Because if you're part of the research study, then officially you can't get a result because it's not that clear a certain result, but I can suspect something, communicate that to physician, and then physician can order specific test via medical genetics lab that may be not a panel that exists.

So again, it's complicated, but it's really genuine on on the side of providers, inside of the researchers to truly try and help the patient in the most ethical way we can.

**Emma Mertens:** Thank you. Kind of a follow-up piece to that. This individual shared that they strongly suspect that their child has a PI, they themselves are a patient, and you mentioned getting involved with the study, you're getting involved with the research. How would you propose that someone go about doing that? Starting from scratch?

**Dr. Bogunovic:** Yeah. So, as you know, our community on patients with primary immunodeficiency are riddled with this diagnostic odyssey. Right? Even to get a diagnosis. It takes years and and and it is difficult.

And that's why IDF exists and that's why a few other foundations exist to to try and shorten that period because the sooner you get some treatment, the better you are, the better quality of life it is, and and that's what it is. So I would talk to your provider and say, hey, you know, what are my options? Right? I got a treatment and let's say you are feeling better. That's best case scenario.

You can get a treatment and still not feel well. Right? And then you can, okay, well, what is there that I can get a diagnosis. And they will tell you, we can do this primary immunodeficiency panel, which is now used extremely well. We can do multi inflammatory panel.

And then if you keep getting negative diagnosis there, there's, again, most likely, the thing that you will get. You can say, okay, is there a research study I can be a part of? Right? And there is quite a few different research studies that you can be a part of. There are lists of people, including Dr.

Orange, including MyLab, including many other people who here at the seminars that have ethical board approved research studies that if you fit, you can be a part of. Right? So I would ask your doc, who is that? Where can I do it? And they'll tell you, they're usually well learned, well intended individuals.

And if they don't know, then you have IDF. Right? Ask IDF of who should I talk to about this? And IDF can help you and direct you. And it might be a little bit of meandering, but it might not be.

It might be, you know, simple thing. The best is if you're close by to the center that does it, but it may not be the case. We can FedEx ship things overnight and then there is many ways to do it. We can phone consent. We can video consent.

It's much easier than ever to do it these ways.

**Emma Mertens:** Alright. Awesome. And on that note, if someone just had questions or they wanted to explore what studies are out there or is there a study for my diagnosis, where would you suggest that they get started if they just wanted to do some kind low stakes independent research on their own. Where might you direct them?

**Dr. Bogunovic:** You know, I must say that patient groups are extremely smart and very sophisticated and well read. [] Use IDF, use all the resources, find who the emails are, especially if you have, you know, genetic variant and you don't know what it does. Right? If you have a generic variant that you don't know what it does and you're suspicious of it, we have a program. You can look at it, look it up.

It's called New York Royal. It's New York Royal dot or g or dot com, I don't remember right now, you can look up. And that's something that usually with physicians, we get referrals and we are trying to curate in a reasonable fashion, which of these variants might be causing it, which might not be. Some we say, well, this is highly unlikely, and some are like, okay, we need to test these. So that's one resource.

The resource, there is another consortium called NYSER. Consortium. They're doing wonderful work. You can also get involved in undiagnosed disease patient programs. And there is quite a few centers around the country that you can find.

They're not focused on primary immunodeficiencies only. They're focused on all genetic disorders.

**Emma Mertens:** Right.

**Dr. Bogunovic:** So as always, trust yourself, get a second opinion, get a second genetic opinion and keep back doing it because we keep discovering more and more things.

**Emma Mertens:** Oh, thank you.

**Dr. Bogunovic:** That's a fair assessment, I think.

**Emma Mertens:** Thank you. Alright. A couple more questions and then we're gonna wrap up. This person asked about a specific variant, but I'm gonna make this just a little more general. If an individual has multiple kids in their family, they have multiple children, and one child is sick with an that has a known variant, but the other child is not displaying any symptoms.

It doesn't seem like they have the variant. Would you suggest getting the well child tested to see if they have the variant as well?

**Dr. Bogunovic:** Absolutely. I would. And, you know, it's a peace of mind if they don't have. If they do have, then there is the opportunity to discuss options with a doctor. So let's go, you know, best case scenario, and let's go worst case scenario.

Right? So worst case scenario is you do nothing and the child gets so sick that it's very hard to help the child. Yeah. As much as yet to help the child when they're healthy. Right.

And if this is a highly penetrant disease, meaning, most likely, kid will get sick. Even though it's totally fine right now, this is a time to act. Outcomes are so much better if the kid is healthy when the intervention happens. Right? Mhmm.

If this is a variant, then we're like, well, sometimes they do, sometimes they don't, then, you know, you monitor. You're like, okay. It's a little bit like when you have a funny mole. Right? You go once a day, once a year and see, okay, is it changing?

It's not changing? Are we going to remove it? Maybe not? Maybe yes. And then what's going to change a little bit?

Let's remove it. Right? So I think you should be in an earnest discussion with your doc about how to approach it. But for me, knowing is always better than not knowing. No. I understand that some people may feel differently and that's okay too.

**Emma Mertens:** Thank you. All right. This will be our last question. You mentioned whole genome sequencing in your talk. We actually just recently back in March, we had two principal investigators from the Bridges initiative who kind of gave a overview of whole genome sequencing and the potential implications for that being utilized in newborn screening, which is very exciting.

But anyway, I just feel like that's on the brain of many of our community members, so I want to close with this question. This individual says, Dr. Baghunovich, what advice would you give a family who is navigating whole genome sequencing for the first time? They're very new to the world of inborn errors of immunity. And they're currently searching for answers for their ten year old.

Is there anything that you wish families knew going into this process or any sort of specific questions that we should ask as the results are interpreted?

**Dr. Bogunovic:** It's a very good question. I understand it it can be overwhelming. And it can also, you know, feel invasive, like, oh, who's who's looking at my genome, what does that mean? Is that, you know, is the insurance company gonna punish me because of that? Right?

I think you should discuss that with medical genetics and you should discuss that with your doctor. In general, I'm of the opinion that especially in the cases where there is a phenotype meaning there is a ten year old with a disease If it were my child, I would unabashedly go for genetics and seek an answer. And seek an answer at one point, seek an answer at another point just to be sure, like, have a couple opinions and see where it is. It's complicated because depending where you live, depending where insurance you have, some insurance covers that, some doesn't. There's also unfortunately some cost, often none to be associated with it.

So it's just something to navigate. Well, but I would go towards genetics. I would go towards diagnostic tests that are approved, but I would also go with those come negative towards research panels that we just talked about that may be helpful depending on the phenotype, depending on who studies it, till we get to the bottom of it.

**Emma Mertens:** Alright. Thank you so much. With that, we're going to wrap up our Q and A this evening. We would like to give a wholehearted thank you to Dr. Dusan Bogunovic for joining us this evening.

We so appreciate the time and effort that you put into your talk and all this excellent information that you shared.