MIGRAINE WORLD SUMMIT

TRANSCRIPT

INTERVIEWS WITH WORLD-LEADING EXPERTS

GENETICS RESEARCH: HOPE FOR A FUTURE OF PERSONALIZED MEDICAL CARE

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Introduction (00:05): The real take-home message is that migraine research is probably the most underfunded area of research in the world in terms of the impact that it has on society and the amount of funding that it gets. And it doesn't matter which country you look at; that is a consistent finding. And so, there's a lot of migraine researchers out there that would very much like to do a lot of this research that I've been talking about, but it's very difficult to do if you can't get the funding to do it.

Elizabeth DeStefano (00:40): Imagine a future when, after you're diagnosed with migraine, you are matched with the treatment most likely to succeed based on your genetic profile. Research in the intricate connections between genetic makeup and the enigmatic nature of migraine is fundamental to that goal. What do we know and what don't we yet about the genetics of migraine and the role they play in [the] expression of our disease? We are joined by Dr. Dale Nyholt, a global leader in the field of genetic research and migraine, to enlighten us about the role of genetics in migraine and how a deeper understanding in this area offers promise for the future. Dr. Nyholt, welcome to the Migraine World Summit.

Dr. Nyholt (01:22): Hi Elizabeth. Thank you for having me.

Elizabeth DeStefano (01:25): Can you briefly summarize what we know about the genetics of migraine?

Dr. Nyholt (01:32): Sure, I'll try my best. In terms of what we know, it certainly is a work in progress. Most of what we know about migraine really had a big boost in 1996, when the first gene for a rare subform of migraine with aura, termed familial hemiplegic migraine — the first gene was found for that. And then, in 2003 and 2005, two additional genes were found that were also causal for familial hemiplegic migraine. So there [are] three different types of genes, and this is a different form of migraine than most people suffer from. This was a rare form of migraine that really was inherited through families, whereas the more common forms of migraine that most people suffer from — migraine with aura, migraine without aura — they're not monogenic; they're actually polygenic, which means that there's many, many hundreds — probably thousands — of genes that contribute towards the risk of these forms of migraine.

Elizabeth DeStefano (02:40): Does migraine have both monogenic and polygenic types, then?

Dr. Nyholt (02:46): Yeah, that's a great question. And in fact, there's even overlap between them. So, what we now know more about with the common forms of migraine is through these studies called genome-wide association studies (GWAS). And in these studies, we ... essentially what we genotype, or we measure, variations in DNA at millions of locations across the genome. And we look to see if the frequency of these particular DNA polymorphisms — commonly known as single nucleotide polymorphisms, or SNPs — if the frequency of a particular allele is more commonly observed in the migraine case population compared to the migraine control population, then we can say that these SNPs are associated with migraine risk.

Dr. Nyholt (03:33): Through these studies, we've now identified 170 — approximately — SNPs that are significantly associated with migraine risk. And we're able to essentially count how many of these SNPs that people have, so we can get what's called a genetic risk score for migraine. And what we've observed in some studies is that even those individuals with hemiplegic migraine, they tend to have a higher genetic risk score for those common forms of migraine. So not only [are] there forms of migraine that are monogenic, some that are

polygenic, there's even an overlap in that genetic risk between those. So it's a very sort of complex picture that we're only really now starting to unravel.

Elizabeth DeStefano (04:19): So, does that mean an individual can in themselves express multiple forms of migraine — a monogenic and a polygenic form?

Dr. Nyholt (04:29): Yes, absolutely. So in those very early studies — those familial hemiplegic migraine studies — there was these very nice pedigrees where there was two, three, or sometimes four generations of individuals, and you could clearly see the hemiplegic migraine going through the families, but they would also suffer from the more common forms of migraine. And some of their siblings, for example, might not have the hemiplegic migraine, but they might suffer from migraine with aura, migraine without aura. And some of those individuals that suffer from hemiplegic migraine, they also experience the more common forms of migraine.

Elizabeth DeStefano (05:09): So, if 170 SNPs are involved, is that to say that we now know that at least 170 different genes can be involved in migraine?

Dr. Nyholt (05:21): Yeah, that's a great question. So, we know that there [are] at least that many. And so, there was a study that I referred to [that] was published last year, and another study was published around that same time. They found a large number of variants that overlapped with those 170, but they essentially found about another 10 single nucleotide polymorphisms. So we now know there's probably about 180 variants that are significantly associated with migraine. Now the next step is then saying, "Well, what's the functional consequence of those single nucleotide polymorphisms?" Try to then tie that variant to a gene in some functional unit and what it might do to that gene. Does it increase the expression of that gene, for example, or does it reduce the expression? And so at the moment, most of those SNPs are really what we'd call statistical association. We can do a number of these sort of in silica genomic analyses where we can find what's the most likely gene that that variant is influencing.

Dr. Nyholt (06:25): But there's still a lot more functional work to be done to actually nail down what the gene is. And in terms of going back to your original question, we know that there [are] at least that many. What we've learned from studying other complex traits where we've been able to analyze much, much larger sample sizes, we can probably estimate that there's probably hundreds of different genes and perhaps even thousands of genes that influence migraine risk in some way or another. It doesn't necessarily mean that an individual who suffers from migraine has all of those genetic risk factors. It just means that they've got enough of them to push them over the threshold where they then start experiencing the symptoms of migraine today.

Elizabeth DeStefano (07:11): So does this explain why we so commonly say and hear that migraine is a very complicated disease and so different from one person to another?

Dr. Nyholt (07:22): Yeah, absolutely. And in fact, when we first started doing these studies, a lot of the clinicians and the genetic researchers, we had some really interesting discussions. Because a clinician will tell you that a migraine sufferer, when they first come into their clinic, will suffer certain symptoms. And then, over the course of their treatments and just over the course of five years, 10 years, the migraine that they suffer in 10 years' time can be quite different to the symptoms that they experienced 10 years beforehand. And so, it's a very dynamic disorder; it's very complex. And when we say complex in the disease area, what we

mean by that is that there's genetic factors and there's nongenetic factors. And a lot of people experience what they believe perhaps to be triggering factors as well, when sometimes they eat certain foods or have certain drinks. Certainly, women report — up to 60% of female migraine sufferers report — a relationship with their menstrual periods and migraine headaches. And so there [are] a lot of different factors that contribute towards migraine. And we are really just starting to unravel what all those different factors are because it's so complex.

Elizabeth DeStefano (08:43): Is it possible to say, from your perspective, what percentage or to what proportion genetics are involved in migraine?

Dr. Nyholt (08:54): Yeah, we can say that quite robustly in that we've done a lot of twin studies before the era of doing these genome-wide association studies. And in these twin studies, they're based on a very robust and simple premise that when you have identical twins, they will share 100% of their genes and their genetic risk. And when you've got nonidentical twins, or dizygotic twins, they share 50% of their genetic risk. And so, there's pretty good, sound evidence that about 50% of the variation we see in the population is due to genetic risk.

Elizabeth DeStefano (09:33): So, is that to say that if someone lives with migraine, that there's a 50% chance that they have a relative or a parent with migraine? Or does that tell us something else?

Dr. Nyholt (09:48): Yeah, that tells us something else. And so, this is where it gets a little bit complicated when we talk about genetic risk and we talk about heritability. So the best way to think about heritability would be, let's say if you have a look at the population or you've got a room full of people that — who has a migraine and who doesn't? That variation that we see there, about 50% of that is due to genetic risk factors — as opposed to a relative risk. So that is, if you are, say, an identical twin, the chance or the increased risk you've got of your co-twin having migraine if you've got migraine might be about 3.6, for example — so 3.6 times the risk than if your co-twin didn't have migraine. And so it does relate to that increase in, "If I've got a first-degree relative, I do have a much-increased risk of having migraine myself." But it also works the other way around: If you've got a first-degree relative who doesn't have migraine, you are less likely to have migraine. And so it's a measure of increased risk in terms of, yes, some of that increased risk is due to genetics. But the 50% — that doesn't directly relate to how much extra my risk is; we have to do slightly different studies for that.

Elizabeth DeStefano (11:10): And if someone has migraine disease without a direct relative known to have migraine as well, is it possible or therefore very likely to say that their parents or a parent had genes or have genes that code for migraine but didn't express the way they do in their offspring who has migraine very clearly manifested?

Dr. Nyholt (11:34): Yeah, that would certainly be one way in which you could interpret it. And so maybe another way to help talk about that is that I talked about those monogenic forms of migraine, familial hemiplegic migraine. There's also hemiplegic migraine, where there isn't a first-degree relative. There doesn't seem to be this form of migraine that is traveling through their families. So somebody can have what's called a spontaneous mutation, and they could then express this hemiplegic migraine form of migraine. So that's a monogenic form. In terms of the polygenic forms, well, you see these common variants; they're just out there in the population. And so your parents might individually not have inherited enough of these common variants for them individually to have migraine, but then when you inherited their DNA

together, you just might've had a combination that pushes the sibling or the child over the threshold to get migraine. So it again adds to that complexity of migraine.

Elizabeth DeStefano (12:40): So, if we have now mapped the human genome, why don't we have a cure yet for migraine or every other disease, for that matter?

Dr. Nyholt (12:49): Yeah, that's a great question. And I often think back when we were first doing these genome-wide association studies. And when we'd first mapped the genome, there was a lot of talk in the media, in particular that, "Oh, now that we've solved the genome that all these genes are just going to fall out and we'll understand all these diseases," etc. And oh, if only it was so simple. Unfortunately, even when we find some of these genetic risk factors, finding out what the biological consequence of those genetic risk factors are, there's a lot more work to do. And then I think what's the really fascinating thing is that the more we learn about ourselves and biology, that we understand less. And that there's all these compensatory mechanisms as well, where you might have a particular group of people that have these genetic risk factors that could be causing these genes to be aberrant, but then there could be another set of genes that help counteract that effect.

Dr. Nyholt (13:50): And so some people could have the same genetic risk factors but still not actually have migraine because they've got a different genetic makeup that's actually driving a compensatory mechanism that's stopping them from getting migraine. So it's essentially a very sort of heterogeneous picture that we are dealing with. And so, in order to find these genetic risk factors, we need very, very large sample sizes. So, one of the first GWASs that we did was in just 60,000 migraine cases compared to a little over 300,000 controls, and compared the DNA between them. And in that more recent one I mentioned, where we found about 170 SNPs associated with migraine, there was 100,000 cases compared to over 700,000 controls. So we need these really, really large sample sizes because it's such a heterogeneous mixture of people that they don't all have that same genetic risk factor. So we need these really large sample sizes.

Elizabeth DeStefano (14:55): You've spoken quite a bit, which we thank you for, about what we *do* know about genetics in migraine. What *don't* we know about the genetics of migraine?

Dr. Nyholt (15:06): Yeah, that's a great question. So, things that we don't know: We really don't understand, "Well, what's the difference between migraine with aura and migraine without aura?" These two very common forms of migraine — they're the forms of migraine that we've been analyzing in these large genome-wide association studies. And most of the variants that we find appear to be contributing to the risk of both of those common forms of migraine. We've done some in silica analyses where we've tried to sort of work out, "Well, this variant looks like it's more likely to be only associated with migraine with aura. These few here seem to be only associated with migraine without aura," but we're really talking about perhaps a dozen of those SNPs out of those 180, 170, that were found. And then the other thing that we don't know is that, well, migraine is quite heterogeneous in its symptoms, where you can have a pulsating headache and you can have nausea and vomiting; you can have the migraine to be on one side of the head, on the left, some on the right, some people have it on the left; and then they might also have it on the right-hand side.

Dr. Nyholt (16:15): And so there's lots of clinical heterogeneity, as well. And we are yet to start mapping these genetic risk factors onto those specific symptoms. And that's an area of research that many of us are very, very keen to do. But again, it's complicated in that, in order to find such relationships, well, we'd need very large sample sizes because we'd then be concentrating

on very specific groups of people. And so we'd need even more of those individuals to do that. So there [are] some of the questions that we're really interested in doing.

Elizabeth DeStefano (16:51): What are the most recent discoveries in migraine genetics?

Dr. Nyholt (16:58): Well, some of the most recent studies, I think, have come from those genome-wide association studies. So, some of the things we've learned from that are that the genetic risk factors are not all neuronal. Some of the genetic risk factors definitely have this sort of vascular component, as well. And so people used to think that, well, as vascular mechanisms that were involved in migraine, that was secondary to neuronal mechanisms. And we've now got some genomic evidence that indicates, well, some individuals are likely to have a very sort of vascular mechanism behind their migraines, and other individuals will have a more neuronal mechanism. And that really feeds nicely into what the general consensus is, that migraine is a neurovascular disease. And so again, that sort of feeds into there's not probably one form of migraine. There [are] actually many roads to individuals that have a migraine.

Dr. Nyholt (17:55): The other thing that we've really learned is that by leveraging these genetic risk factors and doing some of these studies called post-GWAS studies, where we essentially say that, well, if we've got these genetic risk factors for migraine, we can start looking at other omic datasets. And these essentially can be genetic studies that have found these SNPs associated with protein levels or metabolite levels. And we've been able to look for relationships between the migraine risk SNPs and these SNPs that are associated with metabolite levels or protein levels. And through these what we call multi-omic integrative studies, we've been able to sort of link these genetic risk factors for migraine with having a high level or low level of particular metabolites and protein levels. And so, this is really getting into some of that really exciting area where we're starting to attach a functional consequence to having a migraine. And we're identifying particular metabolites or proteins, which we could then develop methods to change those levels of those metabolites and proteins, which could then be used to treat in an individual with migraine.

Elizabeth DeStefano (19:11): Am I correct in understanding that those studies are suggestive of the idea that less or greater than ideal levels of proteins or metabolites are contributing to that individual's migraine disease?

Dr. Nyholt (19:25): Exactly. And so, some examples could be that where some metabolite levels that were found to actually causally increase through some statistical analysis that we can do. That lower levels of a particular omega-3 that's known to reduce inflammation were causally related with migraine headache and another metabolite, which is a chemical that blocks an anti-inflammatory molecule. And so this fits nicely into the hypothesis that part of the migraine mechanism is due to these inflammatory processes in the brain, which causes this sort of cascade of effects, which causes the migraine headache. And so other things that we've also found are particular protein levels, where lower levels of these proteins can cause a migraine in a particular subgroup of individuals. And then we can do more simpler things, as well, where we've found that, well, some blood sugar levels — there seems to be a relationship between particular glycemic traits and migraine headache. And this fits nicely into some of the observations that have been found for quite some time, where there's people who go for a period of time without eating, that they can trigger a migraine headache. And some people get a migraine first thing in the morning.

Elizabeth DeStefano (20:51): What is epigenetics and its importance to migraine?

Dr. Nyholt (20:57): It's one of those questions that, if you ask different people, you'll get different answers. And epigenetics is essentially, it's a way in which genetics — that genes can be influenced by nongenetic factors. And so even hormonal levels or even stress could have what's called an epigenetic effect, where it could increase or decrease the expression of a particular gene. We're still learning more and more about what these mechanisms actually are. And one of the particular forms of epigenetics is DNA methylation. That's probably what's been examined the most, where whether or not a particular molecule is methylated in your genome can influence the expression levels of a nearby gene. And so epigenetics is thought to be one of those ways where these sort of gene-environment interactions could be mediated, such as the blood sugar levels or certain foods, and stress can then influence our genetics — that is, our gene expression — which can then influence these pathways and biological mechanisms.

Elizabeth DeStefano (22:17): So this is sort of the intersection of what we're born with, but how what we're born with is expressed or behaves based on how we behave and live and other external forces separate from our genetics.

Dr. Nyholt (22:33): Yeah, that's a nice way to think of it. And when I talk about DNA methylation, that's just one form of these epigenetic mechanisms that are believed to be out there. There's also histone and other mechanisms. And then the complexity of gene expression as well, where there's different splicing sites and isoforms, which are different forms of genes that people can have.

Elizabeth DeStefano (22:56): In your editorial in *Personalized Medicine*, you explained that genomic-wide association studies — which you've mentioned here today — that identify predictive genetic risk factors for migraine have limitations. And that this is due to the power of those studies because of the thousands of genetic variations involved in migraine disease. You've mentioned the large volume of individuals that would be required to power studies that could kind of push the needle. You've also just mentioned technology, as well. What would it take in total, really, from a research perspective to advance beyond where we are now toward an era of personalized medicine and migraine?

Dr. Nyholt (23:40): Yeah, great question. So, I think from a genome-wide association perspective, if I may start there, in that if we've done studies with 100,000 migraine cases, what we really need to be looking at is probably 10 times that: 1,000,000 cases, and then we'll start really identifying many, many more loci. We know that this will work. Looking at some of the other very large studies that have been done for more easily ascertained samples, for example, a lot of the knowledge that we have through complex traits — through traits such as height, which is the classic polygenic trait — it's easily measured, and we can do genome-wide association studies with a million-plus individuals.

Dr. Nyholt (24:29): When we're doing studies of that size, we're starting to capture almost all of the genetic variation — the common genetic variation — that's out there to be captured. So I think that's a good starting point that if we could get there — but then integrating that with these other omic data because we can also leverage and so we can improve and increase our power by integrating this genome-wide association data for migraine risk with the genetic factors for these other omic levels — when I'm talking about the metabolites and the proteins and things like that — that can improve our power, as well.

Dr. Nyholt (25:10): And then, once we start having enough of these variants, we can really then start to piece together what are the biological pathways that are being affected here with

migraine headaches. That's the basic science that we can do to really understand migraine. But I think it's worthwhile mentioning that along the way in which we do that — and even in that study I mentioned last year — what was particularly exciting about that was that for the first time, the loci that had identified — two of those loci were genes that are targeted with two recently developed migraine drugs. And so the calcitonin gene-related peptide, the CGRP that's encoded by these two genes, CLCA and CLCB — one of the migraine risk factors was essentially at that risk locus.

Dr. Nyholt (26:05): And another of those 170 risk SNPs was at a serotonin 1F receptor. And that's the target of ditans, which is a recently developed drug that's related to the serotonin pathway and gepants. And so here we've got a proof of principle, so to speak. We are doing these large genetic studies. We are finding these targets that are being already targeted by drugs. What that then means is that, well, of these other genetic risk factors that we've already found, that they're very likely to be viable targets for new therapeutics for migraine. And so it's not a single street that we're driving down here. There's lots of sort of detours that we can take along the way. There's lots of different things that we can be looking at. The real take-home message is that migraine research is probably the most underfunded area of research in the world in terms of the impact that it has on society and the amount of funding that it gets. And it doesn't matter which country you look at; that is a consistent finding. And so there are a lot of migraine researchers out there that would very much like to do a lot of this research that I've been talking about, but it's very difficult to do if you can't get the funding to do it.

Elizabeth DeStefano (27:38): What findings in this arena can we expect in the next five to 10 years?

Dr. Nyholt (27:43): So, I think we can expect to see more genetic risk factors being found. That goes without saying. Sample sizes are increasing slowly and through collaboration, but there's not going to be a sort of an exponential jump unless there's a big funding boost. And I know that, say, for example, in the U.S.A., there's work to try and get migraine special funding announcements where people can target and get that funding. I think in the near future what we're going to see is more of these integrative multi-omic studies where, because we have found now 180 loci, I know that there's work ongoing and probably there's an extra, maybe 30 loci, that have tentatively been found. We are going to be then trying to characterize those loci and find out what genes, what proteins, what methylation levels are being influenced by these genetic risk factors. And then that's going to lead to much more knowledge about biology. It's going to lead to new drug targets, and it will lead to the development of new drugs.

Elizabeth DeStefano (28:51): Is there anything else that you'd like to share with our community, Dr. Nyholt, on the genetics of migraine and their application towards the future that we all hope for — of an era of personalized medicine and migraine?

Dr. Nyholt (29:06): I guess what I'd like to try and say is don't give up hope. The more findings that we can have, the more media that we can have on it will just improve each other's understanding of their own migraine headaches. I think getting together and speaking with other [people with migraine] can be really helpful and beneficial, as well. The more you learn about it, the more something will resonate with you and say, "I might try that, and that might work for me." I think that it's important to not give up, be proactive, and seek advice if something's not working for you. Try and get something, try and change something.

Elizabeth DeStefano (29:45): Where can we learn more about you and the work that you do?

Dr. Nyholt (29:49): Sure. Well, I think you could travel to my webpage, and [the] Statistical Genomics and Epidemiology Laboratory (SGEL). I certainly have some of my papers and literature [that] should all be publicly available on my university's EPrints page. But often just Google "migraine." Or Googling my last name is often the easiest way to go about doing it, and maybe we could put some resources or some links [from] there.

Elizabeth DeStefano (30:16): Dr. Nyholt, thank you so much, not only for being with us to share incredible information on this topic, but also for the very important work that you do in genetics and migraine. We greatly appreciate you.

Dr. Nyholt (30:29): Well, thank you, Elizabeth. I very much appreciate the opportunity to talk about migraine research.