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Episode name: Elevated genetic risk for multiple sclerosis emerged in steppe pastoralist populations

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Transcript

Paul: Astrid Iversen, an MD-PhD from the University of Copenhagen, discusses her recent paper on the elevated genetic risk for multiple sclerosis (MS) in certain populations. MS is an autoimmune disease that affects about 1 in 400 people in the UK, and its genetic risk has been studied through genome-wide association studies. Astrid's research focused on understanding the origin of the genetic risk for MS by analysing ancient DNA samples from Europe. The study revealed that the MS risk SNPs appeared in Europe during the migration of the Yamnaya people from the Pontic Steppe around 2,000 to 5,000 years ago. These SNPs were found to be positively selected and also associated with protection against various infectious diseases. This suggests that the genetic risk for MS may have originated as a response to the pathogenic challenges faced by European populations due to changes in lifestyle and population size.

Hello, Astrid, welcome to Immunity and Beyond, which is, you're the first guest on this podcast for the Oxford Immunology Network, so it's an absolute pleasure to have you here. And so what we're trying to do is to kind of just dig into some of the recent papers that people have published or discoveries they've made, and you've got a great one here, which we'll put the details on with the podcast so people can download it. But why don't we start with you and we'll get on to the paper.

So just tell me a little bit about your background and the sorts of things you've been involved in, in your scientific life.

Astrid: So thank you very much for being here, Paul, and I'm honoured to be the first guest in this podcast series. Well, my name is Astrid Iversen, I'm an MD-PhD from the University of Copenhagen. I did a postdoc at Stanford for four years. And I've worked in the infectious disease department in Copenhagen, and as an assistant professor in microbiology before coming to Oxford in 2002.

So I've been fascinated by viruses for a very long time and immunology. And when I first came to Oxford, I thought I would be here with my family for one or two years. That is now 22 years ago. And yes, I've worked in the field of virology and immunology ever since.

Paul: Great. Well, that's a brilliant background. And your paper, the most recent one, you've got a series of Nature papers, but the most recent one that I'd like to focus on is the one on the elevated genetic risk for multiple sclerosis emerged in steppe pastoralist populations.

So first up, I was really interested in firstly how you did it, but just explain to everybody because for an immunologist having to get into the details of what a steppe pastoralist was, and indeed what a

pastoralist is, was a small challenge. So why don't you explain what the history of the basic history that you need to understand in order to kind of make sense of the paper?

Astrid: Yeah. So before going into that, I would like to talk a little bit about MS and or multiple sclerosis, because that is possibly not a disease that everybody knows a lot about.

And also a bit about the historical background for the genetic variation that we find in Europe today. So I'll start with MS. So MS is a debilitating disease. It's an autoimmune disease. It affects about one out of 400 people in the UK today. And it's about three times more common in women than in men. So it's not a disease that gives you survival advantage, but it is a disease that is on the rise. And people have looked at risk factors for MS.

About 30% of global risk is genetic, and about 70% is environmental. So the genetic risk has been analysed in several genome-wide association studies, the GWA studies, where people have identified about 200 risk SNPs that are not found in the HLA region, and about 32 HLA risk SNPs. So a SNP is a single nucleotide polymorphism, and that is a place in our genome where one nucleotide has been replaced by another. So we use these GWA data to interrogate a huge genomic data set of ancient samples, covering the times from about 40,000 years before present to the late Middle Ages, because we wanted to understand where and when and how and why genetic risk for MS originated.

Paul: Can I just ask you, on that point, where did all these samples come from? How did they get all these samples?

Astrid: Yes. So I would like to underscore that this study is not my study as such. It's a huge collaborative, multinational study. And this fantastic data set is the result of more than 10 years of effort, primarily by Eske Willerslev, his group in Copenhagen and in Cambridge, and a lot of very determined and helpful archaeologists and historians around Europe.

So it has been an enormous effort by Eske and his group to collect this study and to analyse it. And I have not been involved in that part of it. I have mostly dealt with the genetic analysis and then, of course, with pathogen analysis. So it's a result of a huge effort.

Paul: Yeah, well, an excellent result. So as you were saying, they collected the DNA and analysed the SNPs. And then you're interpreting essentially the evolution of those SNPs as the populations have mixed within Europe over the last few thousand years.

Exactly. And to that point, I would like to go back and tell you the story about how people have mixed in Europe over the last 10,000 or 14,000 years. So in the beginning, the population in Europe consisted of Western hunter-gatherers and Eastern hunter-gatherers. And these populations were pretty separated and lived like other hunter-gatherers, a lifestyle where they consumed meat and fruit and berries and fish and all kinds of different food elements. Then two technological advances outside of Europe really shaped the history of Europe to this day.

One was farming, which evolved in the Fertile Crescent in the Middle East about 12,000 years ago, and spread to Anatolia about 9 or 10,000 years ago. So Anatolia is a region that is now pretty much covered by present-day Turkey. So the Anatolian Stone Age farmers started to spread in a South-North gradient throughout Europe, with farming arriving for example in Norway only 4,000 years ago. And farming and the Anatolian farmers spread and to a small degree mixed with the original hunter-gatherers, primarily the Western hunter-gatherers, but not to a very great extent.

So the farming lifestyle was characterized by people consuming a lot of grains, which resulted in great population increases, but also when you look at the skeletons, it looks as if it wasn't a super-

healthy lifestyle compared to hunter-gatherers. They seem to have not been taking in an optimal diet, as the skeletons look as if they are a bit weak, and their height decreased. But there were a lot of them, and with farming and farming practices such as using human and animal waste for fertilizers, and the increase in population density, new diseases were able to spread. Crowd diseases like probably Flu and Measles and Rubella, diseases that really probably did not affect the original hunter-gatherers to a very great degree. Also parasitic infections were likely far more frequent because of the use of waste as fertilizers. The other technological advance happened on the Pontic Steppe, which is a region that is now covered by Ukraine, Western Russia and Northern Kazakhstan. In this area, people tamed horses and they traded wagons, and that enabled them to herd greater flocks of sheep and cattle and goats, and also enabled them to acquire a nomadic lifestyle.

This very successful lifestyle also resulted in population increases. Their diet was quite particular. It consisted of meat and dairy products, and they seemed to almost actively have avoided grains as a food source. These people were about 10 centimetres taller than the Anatolian farmers, and when you look at the skeletons, they seem to be more robust and strong. So about 5,000 and 6,000 years ago, the Steppe people expanded and started to migrate both westward into Europe and eastward into Central Asia.

And one of the big migrations happened about 2,000 to 5,000 years ago, where you can see the Steppe people, in particular one group that were a bit intermixed with eastern hunter-gatherers, called the Yamnayas, sweeping into Europe and in the span of 1,000 years, in some places completely replacing the farmers, and in others, intermixing with them. So, for example, in Denmark, you see over 1,000 years the hunter-gatherers being replaced by farmers, and then about 1,000 years later, a quite rapid replacement of farmers and intermixing with them over 500 to 1,000 years.

And this latter migration of the Steppe people seemed in some places to have been pretty violent. So, the Steppe people didn't have such a great impact in southern Europe as they had in northern Europe.

Paul: Okay, so that is the story of how European genetic history could be kind of traced back. So, that's based on genetic and archaeological and historical data that what you just described.

Astrid: Yes, so the populations are defined by genetics. And of course, as these genetic data correlate extremely well with archaeological data, we sort of have a, not perfect, but some picture of what they did and how they did it and what they were capable of.

Paul: Okay, so that brings it all to life. So now, how does that link to MS? And what's the kind of main message regarding MS from all this work?

Astrid: So, what our study showed was that most of the MS risk SNPs actually seem to have appeared in Europe coinciding with the Yamnaya migration. So, they seem to be on a genetic Yamnaya or Steppe population background. And they also seem to be positively selected, many of them, which is not, you know, you sort of think, well, MS is a debilitating disease. Why are these SNPs positively selected? What pleiotropic effects might they have? Or maybe I should say, what other things do they affect? And we looked into this using the fantastic data in both the UK Biobank and the FinnGen Biobank. So, FinnGen is similar to the UK Biobank, but in Finland. So, it has quite a lot more infectious disease data studies in it than the UK Biobank. So, combining those two sources were very helpful. And what we found was that a large proportion of the SNPs were actually also

associated with protection or better outcomes of various infectious diseases and pathogens. And that was both bacterial and viral and parasitic pathogens. And to us, that suggested that one of the reasons that these MS risk SNPs were positively selected could be because they offered protection against a range of pathogenic challenges that the European populations encountered following changes of lifestyle. Like in small hunter-gatherer groups, chronic viruses like EBV and CMV can spread and spread between populations, but the population sizes are simply too small to support spread of crowd diseases like influenza and measles.

Now, if you have great population increases, then certainly new diseases are able to spread through a population much more readily. That is one thing, but also the changes in lifestyle, like farming, using waste as fertilisers, living closely together. You also get exposed to new pathogens or old pathogens to a much larger extent than you would otherwise. And as far as the steppe people, as pastoralists go, well, they lived very closely with the animals, and they consumed meat and dairy. So they were most likely exposed to potential zoonotic transmission to a much larger degree than the other populations.

Paul: Do you think there was one in particular, or is it just a general effect? Because when you think of HLA, it's often linked to protection against a particular disease, so B57 and HIV and so forth. So what do you think HLA is doing, the risk allele, the 15:01 is doing for the disease in particular?

Astrid: Well, the greatest HLA risk allele is HLA. You have seen 15:01. And it's, well, one study has linked it to protection against TB in European populations, but not in non-European populations. However, it is important to understand that there are severe limitations to what we can see. And those limitations are based on the fact that in order to interrogate infectious diseases, we need GWAS studies to aid us. And there are no GWAS studies, of course, for some pathogens that were extremely important in the past, like plague and smallpox. They just don't exist, so we can't see them. And also some of the GWAS studies on measles, for example, are very small, because we vaccinate against measles and rubella. So those GWAS studies either do not exist or are very weakly powered for obvious reasons. So we are limited to what we can see. And as far as TB goes, that is also very limited, because TB is not really very frequent in Europe today. It was completely different just 120 years ago or 100 years ago, where a huge percentage actually died of TB. But today, when you have TB in Europe, it's very often imported from outside Europe. So the genetic background is not homogeneous. So it's hard to do those studies today.

Paul: But what would your guess be? I mean, this is a podcast rather than a kind of part of the paper. So I mean, I'll tell you, my guess could be you kind of make a big play about the zoonosis. And then when you think about that nowadays, flu springs to mind. I mean, people are living with chickens and cows, and you think and pigs and so forth. Well, cows less, but increasingly a problem. But chickens and pigs, you think of flu reassortment, and so forth. Do you think that's it? Or do you think it would be something else? You mentioned TB. Is that your top pick?

Astrid: Well, I would say most of those genes, including 15:01, are found in the Yamnaya or steppe background. So if you think about the way they lived, and what they consumed meat and dairy products, you would think about what is transmitted. They did not have pigs at all. So you need to think about cattle, goats, sheep, and dogs. And then you have to think about what's transmitted in milk, non-pasteurized milk. So definitely, yeah, TB could be one. And bacterial infections that are transmitted through milk. Listeria would be one. But really, we don't have the GWAS studies to really prove that this is the cause. But we can say that what the likely subjects...

Paul: Okay, you can narrow it down a bit, but you can't pinpoint it.

Astrid: Well, we don't have GWAS. I mean, if we have that, that would be wonderful. But what amazes me to a certain degree is that we're actually able to see these many protective effects also against something like measles, and against parasitic infections, even with those limited GWAS data.

Paul: Well, there must be a strong effect in that case. But then you also produce a bit of data on rheumatoid and risk. It's different HLA. And it seems to come from a different, historically different part of genetic makeup as European.

Astrid: Yeah, the Western hunter-gatherers.

Paul: So but would not the same issues apply? Presumably, the HLA there is also being selected. Why don't you explain what you found and why it's different from MS and the MS risk allele?

Astrid: Yeah. So what we found was that the risk of rheumatoid arthritis actually decreased over time, whereas we found that the risk of MS increased. So rheumatoid arthritis risk genes are often found on Western hunter-gatherer backgrounds. And we also found that these risk genes were associated with protection against a range of diseases. However, with rheumatoid arthritis, one of the key suspects for triggering it is flu. And it is possible that this protection against various bacterias and parasites that hunter-gatherers encountered were beneficial to them. But it is not… to have the risk genes is not enough to get the disease. And also for rheumatoid arthritis, genetic risk is less than environmental risk.

So you need to have a trigger. So even though you have these risk genes, in the Western hunter-gatherers, it's not certain that they ever or that many of them even got rheumatoid arthritis, because the triggers weren't there. However, when you have this huge mixing period, 4-5,000 years ago, where the population increases, then suddenly you have a spread of new diseases. And for a hunter-gatherer, their immune system is probably optimized to combat the pathogen challenges they encounter. Those challenges are very different from what they would encounter once you have this mixture of populations, and you have a mixture of lifestyles, farming and pastoralism, and suddenly you have crowd diseases. So it is possible that suddenly, when new diseases start to spread, that you suddenly have a trigger for rheumatoid arthritis, or several triggers that weren't there before. So that might be part of the reason that the risk for rheumatoid arthritis decreases, simply because now people are triggered, people get ill, and having arthritis is not a survival advantage. So suddenly you might have a trigger, and they might get ill. But I mean, just having the risk genes is not the same as having the disease, that is very important. It's just a susceptibility.

Paul: Okay. So different risk genes for different diseases come from different parts of our history with different selection forces upon them, I guess, would be the kind of conclusion from that.

Astrid: That is beautifully put, Paul. And at least for the diseases we've looked at so far, true.

Paul: I was fascinated by lots, but I think we should probably not dwell on all the details. Well, I want to ask you a couple of other questions, which I think I'm going to ask everybody in the series. So what were the reviewers fussed about? And maybe it just went in without any real comments, but was there a pushback on, because some of it's speculative, I guess, did they want more or less speculation, they want more robustness in the statistics, or what were they bothered by?

Astrid: Well, I think the reviewers were really helpful, I would like to say that, because they did have a lot of questions and wanted us to address some of the genetic analysis and the selection methods and so on and so forth. And I think the paper really benefited from the, I think it was two rounds of reviews.

Paul: They didn't want you to go back and sequence a whole lot more bones from the Middle Ages or something?

Astrid: Well, I mean, you know, everyone always wants more data, right? But they did understand that when you look at ancient DNA, you're limited by the samples, right? And it's just not... whatever samples you're able to get hold of depends both on people's burial rituals - obviously, if bodies are burned at a certain state and time, then there is nothing left to find. Whereas if they're buried in a hole in the ground and there's a mount on top of them, then they're more easy to spot. So what you're able to find is what you're able to find, basically.

Paul: Do you think we got to the end of that or is there plenty more to discover in that area? Or have we kind of reached the limits of what we can pull out from that sort of study?

Astrid: Well, I think, well, firstly, archaeologists find samples all the time, right? And secondly, DNA technology gets better and better. So whereas now we, I think it was only one out of every five or six samples where we could find DNA and sequence it to a quality that enabled us to analyse it. Well, certainly, probably we will be able to go back to some of those samples that do not work now in a couple of years and maybe get out some more material. So I think the more samples you have, the less grainy the picture will be.

And also, increasingly, there are coming GWAS studies and larger GWAS studies. A couple of days ago, a huge influenza GWAS study came out, which will be interesting to look at. So in that respect, I think there's plenty more to understand.

Paul: So the News and Views, which I'd encourage people to read, which is beautiful, has a couple of questions at the end, which are probably better than what I could think of. But they recommend that you look at this in, because this is for the N equals one experiment in Europe. And I think they're recommending that you look at it elsewhere. Is that ongoing?

Astrid: Yeah, in one place. But it's still in the early days. One of the limitations is that, well, you have to have samples, but you also have to have something similar to the UK biobank. Like there is a beautiful biobank in Japan, for example. But in many places in the world, they don't have that kind of data. So it's really a question of both having the samples, but also having data on the populations that you're looking at. And hopefully, there will be a drive to get...

Paul: I think people will be encouraged. And the other suggestion they make, and you touched on it, is that the suggested association between infectious disease and MS risk needs to be confirmed. And you've sort of indicated that GWAS will be an important tool. But let's say you've got a GWAS hit, but you've already got some hits. But where do we take it from there? Because it's still an association. So is there a sort of pathway to proving something mechanistically that you've got in your head?

Astrid: Well, I think one important thing coming out of this is the realisation that our immune response setup is not optimal to the way we live today. And I think that is important, because what our setup is, it is balanced to combat both virus, bacteria and parasites. So because parasitic infections elicit Th2 response, anti-inflammatory responses, and those anti-inflammatory responses mute pro-inflammatory responses, in order to combat a given pathogen or virus or bacteria in the past, you would have to have selection for a certain level of strength of pro-inflammatory responses. Because you would have to counter the effect of the anti-inflammatory response that mutes it. So if you take away the anti-inflammatory response, suddenly the selection is actually enabling you to make a too big pro-inflammatory response. So today we probably overreact to bacteria and virus infections, because we have nothing that mutes it. So a lot of infections will likely give you too much

inflammation in the body. And inflammation is a good thing if you're infected with something. But it's not a good thing to have heightened levels of inflammation in your body, pre-disposes, as you well know, to a huge range of diseases.

So I think, I'm not suggesting that everyone should go out and get a parasite infection, but I do think that it does suggest that maybe we should try to look for ways to rebalance our immune system. That we should try to look for some way to mute our pro-inflammatory responses, once they're no longer needed to combat the infection. And that one of the ways could be exploiting what parasitic infections does, and maybe...

Paul: Sort of more mimicking the steppe pastoralists' exposure, if you could do that in a more controlled way.

Astrid: Yes, and there have actually been a couple of studies where MS patients have been treated with parasitic worms. Unfortunately, those worms were from pigs, so not optimal.

Paul: Oh, they didn't know their history.

Astrid: No, they did not know that the steppe people did not have any association with pigs. So maybe looking at parasites in cattle, sheep, or goats, look at the antigens, maybe exploit that in some kind of immunotherapy where you can mimic some of the responses that these elicit. I do think that would make sense to try to rebalance, recalibrate.

Paul: Do you think if you've got a background which is more of a hunter-gatherer, then you should try and rebalance yourself more to a hunter-gatherer kind of exposure, and if you're more steppe pastoralist, do you think it doesn't need that kind of nuance, because we're all so mixed up anyway?

Astrid: Well, I mean, I think it's impossible in the way we live, right? We do still have bacterial infections, and we are bombarded with virus infections, especially respiratory infections, and I don't think that unless you live in a very extreme way that you can really limit your exposure to modern-day pathogenic challenges. So I do think, however, that it does suggest that it would be a good idea to try to limit our exposure to respiratory pathogens, and I mean, we are currently in the fifth year of the SARS-CoV-2 pandemic, and we know that we are able to fairly easily clean the air more than we do today, right? We know that ventilation with the right filters, we know that mobile HEPA filters can reduce the number of pathogens in the air, not just corona, but also flu and RSV, and I mean, we do not need to be exposed to the same level of respiratory viruses all the time as we are now. So I do think that should give us fruitful thought that maybe we can do some simple things just to limit the times that people are infected by respiratory or airborne pathogen every year. It does not need to be this way.

Paul: Well, we have gone a long way from the steps of the caucuses to the future of the HEPA filter there, so I think we should stop at that point. Thank you very much for being the first guest, and hopefully this podcast will be freely available to the community. Thanks very much to Sally Pelling-Deeves who is helping put it together, and any comments, any volunteers, especially if you have made a discovery that you would like people to hear about, we are very keen to hear from you. That we spread the word, so please do put yourself forward, and we'll be in touch. Thanks very much.

Astrid: Thank you, Paul, for inviting me.