Jane: 00:16 Hi, this is Jane Ferguson for Check, Change, Control Cholesterol. Today, I'll be speaking with Kieran Musunuru an associate professor of medicine at the Perelman School of Medicine, at the University of Pennsylvania. He and I will review new discoveries in cholesterol metabolism. So, Kieran, people have known about cholesterol for a really long time, but why is it important that researchers continue to study cholesterol metabolism?

Kieran: 00:41 That's a great question, Jane. So we've come a long way with cholesterol modifying therapy, we have the statin drugs, and they work quite well, they are well established to reduce the risk of cardiovascular disease, they've been tested in many clinical trials over the last few decades, and millions of patients are now prescribed statin drugs and do quite well with those drugs. But despite the fact that so many people are taking these beneficial medications, heart disease is still the number one leading cause of death in the United States as well as around the world. And so there's a great unmet need just among the general population, statins have a very beneficial effect, but it's clear that we need more than just statins to help patients avoid heart disease. I would also point out that there are certain patients who have an unusual condition, and an unusual disease called familial hypercholesterolemia, they're born with a genetic mutation that causes them to have extremely high cholesterol levels, sky-high cholesterol levels far greater than is typically seen in the general population.

And they can suffer very serious consequences from having such high cholesterol levels, heart disease certainly, but not just heart disease but getting heart disease very early in life, and so they have a particularly desperate need for new therapies. Statins will help them, but it certainly will not bring their risk of heart disease down to the risk that's experienced by the average person in the population, they're still at very high risk because the statins reduce their cholesterol only part of the way. There are other medications that can help bring the cholesterol even further, but the most severely affected patients, the patients with the worst familial hypercholesterolemia, the highest cholesterol levels nowadays they have to be hooked up to a machine on a weekly basis and spend several hours having the machine clean the cholesterol out of their bloodstream.
So they have serious need for new therapies, and that's why researchers like myself are continuing to study cholesterol metabolism very intensively, to try to find new ways, new therapies that can reduce cholesterol levels and help certainly these most seriously affected patients, the ones who are in most desperate need who have this familial hypercholesterolemia. But also to help the general population and perhaps someday make heart disease so that it's no longer the number one cause of death but is lower down on the list.

Jane: 03:07 So, what have researchers learned in the last ten years, and have they been able to make new discoveries?

Kieran: 03:13 So the last ten, 15 years have really seen a great advancement in the understanding of human genetics, what's in our genes and how that influences our cholesterol levels. We already know from genetic studies going back quite a while now, that the medications that we can use in patients nowadays like the statin drugs actually target a particular protein that's made by a particular gene. That gene is called HMGCR, and so we've known about that gene for quite a while, we've known about the protein that it makes, the drugs, the statins are intended to turn off the function of that protein and in doing so, prevent the body from producing cholesterol. There's a second gene called NPC1L1, and it produces a protein that's targeted by a different cholesterol-lowering drug, a drug called ezetimibe, and by inhibiting the protein made by this gene NPC1L1, ezetimibe prevents cholesterol from being absorbed by the gut, and so it's tackling the cholesterol problem in a different way.

Now what's very excited is that about 15 years ago, The Human Genome Project was completed, this was an effort to decode all of the sequences in the human genome, a that's about 6.2 billion bases, by which I mean individual letters across all the chromosomes in the genome in a human cell. And now that we have the information, we can more exhaustively and more carefully look at all of the various genes in the genome, there turned out to be more than 20 thousand of them, and we can search for particular genes that we didn't know about before that influence cholesterol levels. Now one very exciting story that has emerged from genetics is the story of PCSK9, and most everyone has heard of the drugs that inhibit PCSK9, the monoclonal antibodies that are now actually on the market and used by some number of patients. PCSK9 was first discovered in 2003, and interestingly it was discovered through the study of patients with very, very high cholesterol levels, the same disease I mentioned earlier, familial hypercholesterolemia.
It turned out that certain families in which this particular disease was affecting many family members had a mutation in this gene, PCSK9, that's how this gene was discovered, a search for the mutation that was causing sky-high cholesterol levels. Now what emerged is that this particular mutation carried by a particular family with very high cholesterol levels, this mutation PCSK9 was actually making the gene work better than it normally does. It was turning its activity up, it was making it hyperactive if you want to think of it that way, and so the protein made by PCSK9, which is also called PCSDK9 was working much better than it normally should, and it turns out that the role of this protein is actually to bring cholesterol levels up. So it actually raises cholesterol, and by having a hyperactive version PCSK9, it meant that the cholesterol levels were much higher than they should be.

Now, what's interesting is just a few years later, in 2006, it was realized, it was discovered that there are people in the population who actually have mutations that turn off this gene, turn on PCSK9, and so because the role of the protein is to increase cholesterol level, if you have a mutation that's turning off the activity of the protein or turning it down, it causes you to have lower cholesterol levels. And these mutations that reduce the activity of PCSK9, and actually are pretty common in the population, something around the order of three percent of people have this mutation. And what emerged, starting in 2006 and in the years that followed is that people who have these mutations that turn down PCSK9 actually not only have much lower cholesterol levels, they actually have a substantially reduced risk of heart disease, as high as 90% reduction of risk compared to the average person.

So this is a kind of mutation that makes these people, if you will, genetics superheroes, they've been born, they've inherited these mutations, and they actually experience good benefit from these mutations, these are good mutations to have. They've won the genetic lottery, in a sense, and so that got a lot of people excited because now we had evidence from naturally occurring mutations in the general population, an experiment of nature if you will, showing that these mutations that turn down this gene have a very beneficial effect. And then what was even more exciting is studying these three percent of people in the population who have these mutations, they actually don't seem to experience any particularly severe adverse effects of having these mutations. They have the benefit, they have a reduced risk of heart disease, but they don't actually seem to have any ill effects from having the mutation. And in fact, what emerged
after 2006 over the next few years is there are actually very rare people in the population who have multiple mutations in this gene and actually make no PCSK9 protein at all, they entirely lack PCSK9 and have very low cholesterol levels.

But they're very healthy, they live to a ripe old age, they have children of their own, clearly this gene is a good target if you're thinking about developing a new drug, because we know from genetics, we know from studying live breathing people that turning off this gene should be protective, it should be all gain with very little to use because these people don't have any adverse consequence. In fact don't even need it to live and to thrive, and so that has led to the production, the development of a variety of drugs that target PCSK9, there are two monoclonal antibodies that were developed and then were approved by the U.S. Food and Drug Administration in 2015, have been on the market, have now been tested in large scale clinical trials and have been shown to reduce cardiovascular events in patients who are taking these drugs. So from 2013 to 2015 marks the discovery of the PCSK9 gene to the availability of novel drugs that target this gene, the availability for patients.

Jane: 09:48 The PCSK9 story is really just so fascinating, and I think PCSK9 inhibition is a really beautiful example of an important advance that has been made. But, have there been any other discovery's sort of similar to the PCSK9 story that are promising for the development of any new therapies?

Kieran: 10:05 That's another great question, Jane and in fact, the answer is an unequivocal yes. There are other genes that share very exciting features similar to PCSK9, by which I mean that there are naturally occurring mutations, good mutations, in the population in these certain genes that result in the reduction of cholesterol levels, or the reduction of triglyceride levels, that's the fat content in the blood of course. So we're not just talking about cholesterol now, but also triglycerides, without having any serious adverse effects. And so one such gene is a gene called APOC3, or A-P-O-C-3, this is a gene that came to everyone attention in around 2008 or so, and what was exciting about this gene is as with PCSK9, it turned out that there were people in the general population who had mutations that turned off this gene. In fact, it was discovered, this particular mutation in question was discovered in a group of Amish individuals in Pennsylvania. It turned out that when the Amish migrated from Europe to what is now the United States, it was a relatively small population and there was either one or a few people who had this mutation in them, and then the Amish population being
a relatively small population, a relatively isolated population over the ensuing several hundred years, this particular mutation became relatively common in this population.

And from studying these Amish individuals, it became clear that these mutations in APOC3 resulted in the reduction of triglycerides, so now we're not talking about cholesterol but rather triglycerides, the fat content in the blood once again. And they seem to have less heart disease, they seem to have less coronary artery disease compared to the general population, compared to individuals who didn't have the good mutations. And so, that was very exciting because again it sounds like PCSK9, you benefit from having the mutation, from studying these people it seemed that there were no real adverse consequences from having these mutations. And then some years later, a group of researchers who were studying families in Pakistan identified the same mutations in APOC3 but were actually able to identify a complete family in which every single person in the family, mom, dad, and many children all entirely lacked APOC3. They had multiple mutations in APOC3, they have very low triglyceride levels, but their entirely healthy, indeed there's a whole family of them.

And so this has got people very excited about APOC3 a potential drug target. I'll mention on other gene, this is a gene called ANGPTL3, and what's particularly unique about ANGPTL3 is that once again, like PCSK9, like APOC3, there are people in the general population who have mutations that turn off this gene ANGPTL3, but it's a double whammy. Not only does it reduce cholesterol it also reduced triglycerides in these people with these mutations, so these are particularly good mutations to have because it means both the cholesterol and the triglycerides are down. And it's been established that people with these mutations are protected from heart disease, and in fact, there have been individuals identified who entirely lack the ANGPTL3 protein, the protein that's made from the gene, and they’re entirely health and once again they live to ripe old ages, and they have children and no adverse consequences. So we're really seeing more and more of these genes come out of the woodwork where we have genes like PCSK9, that effect just cholesterol, we have genes like APOC3 that effect just triglycerides, and you have genes like ANGPTL3, which actually give you more bang for the buck so to speak, reduce both cholesterol and triglycerides.

And it's quite possible that there are more waiting to be discovered.
Jane: 14:13 Wow, so those definitely sound really promising, are there any drugs that are in development based on ANGPTL3 or APOC3?

Kieran: 14:20 Well exactly as you would expect, the discovery of these genes and the understanding that these good mutations in these genes protect against heart disease without any adverse effects, have gotten companies very interested in developing therapies against these genes. So with ANGPTL3, there are at least three different types of medications that are in development, there's a monoclonal antibody against the protein product of the gene that's been in clinical trials and looks very good in patients. It reduces both triglycerides and cholesterol levels, there's an antisense oligonucleotide, this is a molecule that's delivered directly into the liver where this ANGPTL3 protein is produced, and actually turns off the gene directly within the liver which is sort of it's home if you will. It's where the action is, and by turning off the gene itself in the liver it prevents any protein from being made at all.

There a similar molecule called Small interfering RNA or an siRNA against this gene that's also in development, and just as with antisense oligonucleotides, it's delivered directly into the liver and can turn off the gene at the source. APOC3, very similar, there's a monoclonal antibody that's being tested, there's an antisense oligonucleotide that's being tested, and there is an siRNA that's in development. So both of these genes are the intensive subjects of research and drug development.

Jane: 15:47 So, Kieran, it sounds like many of these new genes are related to triglycerides, but most of the focus with therapies so far has been on cholesterol. So, do you think triglycerides are the next frontier for this field?

Kieran: 16:01 It's very much looking like that, so as you said, many of the existing therapies target cholesterol, statin drugs, ezetimibe, a few other medications that are available. And so that's where most of the action has been, and there's been a lot of benefit from targeting cholesterol no question about it. But the human genetics really seems to indicate that triglycerides are also a very important causal risk factor for disease, that it's contributing to a similar degree as cholesterol. And so, it makes a lot of sense to try to target triglycerides, now we have to be very judicious, very thoughtful about how we target triglycerides because its possible that some medications that reduce triglycerides by some mechanisms may protect against heart disease, but other medications that reduce triglycerides by entirely different mechanisms may not have the same
beneficial effect. This is still very much being worked out, but what we know from the human genetics is that these certain genes, the ones that I've mentioned as well as a few others, APOC3, ANGPTL3, we know that if we can make medications that successfully target these genes and the proteins that they make, we know that reducing triglycerides in that way will protect against heart disease, and we know that because again these experiment of nature.

People who are born with mutations that turn off these genes, experience these combinations of good effects and lack of bad effects. And so I think there’s going to be a lot of activity in the triglyceride space going forward, I would point out that just a few months ago at the American Heart Association scientific sessions in November 2018, the results of a trial called Reduce It were announced. It tested a particular medication, if you want to call it that, called icosapent ethyl, this is a highly purified form of an Omega-3 fatty acid, at a very high dose in pill form that patients can take as a medication, and what this trial showed is that this medication whose primary role is to reduce triglycerides in the bloodstream, this medication worked exceptionally well, better than just about anyone would have predicted. It greatly reduced cardiovascular risk, it actually had a mortality benefit, it reduced death from cardiovascular disease and other causes. And it was a dynamite trial and it really speaks to the potential of therapies that primarily focus on the reduction of triglycerides.

And so I think we have statin drugs, we have ezetimibe, we have good drugs that work against cholesterol, now we have the prospect of drugs that work on another risk factor for disease, triglycerides. And I think you're going to see very good drugs targeting triglycerides come out onto the market in the coming years, and I think what's particularly exciting is the prospect of being able to have combination therapies that reduce both cholesterol and triglycerides in tandem.

Jane: 19:05 Wow yeah, that’s definitely really exciting, but out of all of these therapies that we were talking about, they’re all either pills that you have to take every day, or injections that you receive every few weeks or so. But since we've been talking a lot about genes, is there any possibility of doing something like gene therapy to permanently change a patients' cholesterol or triglyceride levels and reduce their risk that way?

Kieran: 19:26 Yeah that's a wonderful question. So the statins, of course, are pills, a lot of the other drugs I've been talking about, the
monoclonal antibodies, the antisense oligonucleotides, the siRNAs, they all have to be delivered into the body via the bloodstream, so that means injections of one sort of another. Whether it's intravenous injections or subcutaneous injections, and so those are less convenient with respect to the dosing, they're injections that you have to take every few weeks and potentially going forward maybe every few months, but it's still something you have to take over, and over, and over again. And since heart disease is a chronic disease, if you really want to get the full benefit that means taking it over and over again for the rest of your life. Now there is the possibility that you could find a way to target one of these genes we've been talking about, whether it's PCSK9, or ANGPTL3, or APOC3 or yet some other gene, or it's protein product, there's the possibility of finding a way of permanently turning it down.

Now again, I go back to the people in the general population who are born with these naturally occurring mutations, they're in a very beneficial position because they were born with their gene permanently turned off, and so their cholesterol levels or their triglyceride levels, or the combination of the two are permanently reduced compared to average people in the general population. So the precedence is there from human genetics, so then it's a very natural question to ask, well can we find a way to reproduce that in average people? These people have won the genetic lottery, they have these good mutations, can we reproduce the effect of these good mutations in the general population and really protect large portions of the population, the people who are at risk for heart disease, can we protect them from heart disease through one-shot therapy whether you think of it as gene therapy or you think of it as a vaccination, the idea is that you would receive it once and then you'd be done, and you would have enduring and possible life long protection against cardiovascular disease.

And so one such approach is something called gene editing, and many people listening to us right now are familiar with CRISPR or have heard about CRISPR in recent years, this is a technology that allows researchers and eventually physicians to turn off genes in the human body. And so the idea is you could use this gene editing to turn off one or more of these cholesterol genes or triglyceride genes and then the idea is because you're permanently turning it off in the genome, in the human body, then you would have lifelong reductions in cholesterol levels or triglyceride levels and protection against cardiovascular disease. So that's one possible approach, there's a different approach that other researchers are actively pursuing and this is really
more of a classic vaccination approach. And here the idea is, rather than targeting the gene, you target the protein product of the gene, and the proteins that are made by all of these different genes we've been discussing, they all end up in the bloodstream and they're circulating around in the blood and they have their effects on cholesterol in the blood, or largely in the blood.

And so that's why monoclonal antibody therapies can work on these gene products, these proteins because they're accessible in the bloodstream. And so you can imagine making the body produce antibodies, make its own antibodies against these proteins, and the idea is you would use a vaccination, you would use a piece of the protein or something that looks a lot like one of these proteins, and use it as a vaccination. Inject it, have the body's immune system respond to that piece of the protein or whatnot that's now been introduced into the body and then create an immune response that will then cross over and target the naturally occurring protein product. So imagine instead of having to take an injection every few weeks of monoclonal antibodies again PCSK9, what if you could have your own body make its own antibodies and do that for the rest of your life, so you never need to change injections, you would just have your body do the job of making antibodies.

So you can imagine vaccinating someone so that they're making their own antibodies against PCSK9, or APOC3, or ANGPTL3, and getting the therapeutic effect that way. So I think there are some very exciting things that are on the horizon, so stay tuned.

Jane: 23:49 Yeah, I mean that's just really cool and so fascinating, I think this is such a promising and exciting field. So thanks so much for talking about this, and giving us that sort of history and future.

Kieran: 23:58 Thanks so much, Jane.