[THEME MUSIC PLAYING]

RAY HERSHBEGER: So these disclosures are not relevant to what I'm going to talk about. I am a heart failure transplant cardiologist and before I got to OSU, I was in the trenches, directing heart failure transplant programs elsewhere. So I still come to the Tuesday morning transplant group and that's my background.

So this slide is what I always start with. Phenotype, what we observe in a person. And these are clinical studies. The clinical information that define a phenotype. A phenotype is a genetic word that we use to describe a study. And then, of course, sequence. And we use molecular genetics to identify genetic cause and understand mechanisms of disease.

I want to-- for those of you who may not be aware-- this is a really, I would say famous, well-known, paper. This is circulation 1986. The senior author is Dr. Wooley. I don't actually know Dr. Wooley. I've cited this paper many times. This was a cardiovascular genetic study before sequencing was easy.

And this is the pedigree-- if you've never seen this, it's a remarkable effort. It was done over years. And it turns out that-- let's see. I guess I don't have a-- does this mouse appear? It does. So this pedigree, the top line continues down to the bottom line. So it's a very big pedigree that goes on for six generations. And this describes lamina cardiomyopathy, which is the rest of the story. But a remarkable effort.

I hadn't looked at this paper for awhile, but I wanted to make sure that everybody at Ohio State saw this because this is truly the lineage of Ohio State. You guys have been doing genetic research for decades.

This is the appendix and this appendix goes on for another page, showing you a review of the literature. And this is part of the references of that review. And just for the trainees in the audience, Dr. Wooley read. He was a smart guy. I've never met him. I know Carl knows him and worked with him. It's an illustration of what it takes. And again, just to honor this physician, this cardiologist from Ohio State. And this review of the literature is as good as you get in 1986 about familial cardiomyopathy.

So this is a cartoon, as you can see. Of a normal sized LV that with mild side injury, turns into this dilated left ventricle with contractile dysfunction. Let me start with the story, we learn by stories. This young man, his name is David Newell. I do have permission from the family to discuss his case and his name. The t-shirt says, "OHSU heart transplant number 220 June 30, 1993". Yes, that was my first job at the Oregon Health and Science University. I was an assistant professor.

I met David one Monday afternoon after clinic and had a new consult, phoned in, sent a fellow upstairs. The fellow said you better come up. Walked into the room, David had a blood pressure systolic in the 50s, was gray, ash and pale, with some profound cardiogenic shock. I actually did something I seldom have done only a couple of times in my career. Gave him some mini boluses of epi to start the resuscitation. He'd been on Dobutamine for two weeks in the pediatric intensive care unit. He was literally dying of dilated cardiomyopathy.

Found the family, called the coordinators, got him listed within four hours for heart transplant. He was literally dying. We didn't have VADs back in 1993. And he was, actually, he looks pretty big here, but both of the-- this is Cathy [INAUDIBLE] my colleague on his left and the thoracic fellow. They're both pretty short. We didn't have a balloon pump that would fit into David because he wasn't big enough.

So we had no mechanical support, so it's the old days where it's inotropes, transplant, or death. Amazingly, we got a donor within 48 hours, got him transplanted, and this is the success story of David getting his ganciclovir infusion after the transplant. A couple of weeks and everything is fine and it's a wonderful success story.

Fast forward a couple of years. I got a call from the University of Minnesota, from a colleague I knew there, a heart failure transplant guy. He said what did David's heart show? And I said, why? And he said because his brother had sudden cardiac death and we're considering him for a heart transplant at Minnesota.

In the meantime, we'd started this dilated cardiomyopathy research project with the aims to identify and characterize genetic causes of DCM and then to translate that into practice cardiovascular medicine. And so we called in David's mom and dad, who we knew quite well because we're taking care of this post transplant adolescent, and his two older brothers, and we screened them.

And lo and behold, his father had, basically, symptomatic heart failure, but had been being treated for the last eight months by his primary care physician for panic attacks at night. Which was paroxysmal nocturnal dyspnea. The elevated neck veins and big liver and edema and had an EF from the 20s and a big heart, and had undiagnosed dilated cardiomyopathy, Cath negative, so clearly familial disease. But the funny thing was that the first cousin of David's who had the sudden cardiac death in Minnesota was on mom's side of the family.

And the idea here is-- and I'll use this pointer if there's anybody listening online-- this was David shown here by the arrow. Both of his brothers had some very early evidence of some DCM. I would not color them black in 2019. This was mom.

His first cousin, the guy who had the sudden cardiac death is this person. His father actually ended having sudden cardiac death in DCM in a clinical, research screening a year or two later. And so his father had DCM and so the disease that he had presented with, the disease that brought his familial disease to attention, was on mom's side of the family, but dad was affected.

So I figured, hey, you know, I'm a newbie at this. I'm an assistant professor. We just started this program. It's probably a one off. Once in my career. One in a million. I show you this pedigree because this was, really, this family is number two in our study. And this illustrates a lot of dilated cardiomyopathy genetics.

Now, we're really just beginning to figure this out. So the idea being, of course, we imagined-- and we still do-- that he inherited a nasty gene from dad, a nasty gene from mom, they came together and caused this double hit, so to speak, in David. And that led to his heart transplant.

So this was the unexpected complexity presented as simplex disease, but it's really familial. There was bilineal inheritance. How common is that? What's the genetic model? Conventional would be autosomal dominant with incomplete penetrations in his parents, variable expressivity, and a variable age of onset. Are there multiple alleles?

As we suggest here, of rare variants, or mutations, contributing to this multigenic model that could be compound heterozygosity or epistasis. Or rather complex oligogenic models, including not only rare variants, which is what we focus on, but also common variants that would be [INAUDIBLE]. Or something else, including environmental impact. We haven't looked at environment a lot.

So what we study is dilated cardiomyopathy. You know what this is. We sort ischemic from non-ischemic. We estimate that in the non-ischemic group, there's certainly over a million people in the country. Probably more, these are conservative estimates. We sort out other causes. You know what these are. It's a long list. But they're generally clinically detectable.

What's left, we call idiopathic dilated cardiomyopathy, and that's what we study. And that's been the basis of all of our studies. And, of course, if you do family studies-- that is, you find somebody with dilated cardiomyopathy, prospectively screened their first degree relatives, parents sibs, or children, you can find evidence of familial disease in anywhere from 15% to 35% depending on what the literature you read.

If you do genetic testing, you can find a plausible genetic cause and up to, perhaps, 40% of those families that have disease. Which shows that since it's familial, you're almost certain it's genetic. We don't have all of the genetic cause explained, so that is sufficient molecular testing sensitivity not only for clinical use, but also for study. But it also illustrates that further discovery is needed. So we've been sort of chipping away at all of these through the years.

So the problem is that most of what we see appears to be non-familial even if you screen the family. And, of course, what walks into clinic, as we all know, as most of the time, it's somebody with dilated cardiomyopathy of unknown cause. You label them as non-ischemic and then what do you do? You treat them and that's it. Except, the real cause is still uncertain, at least in the guideline documents. And so we have taken the approach to study all of this, both familial and non-familial, because we need the point and counterpoint.

The central hypothesis then is that DCM, whether familial or non-familial, has substantial genetic basis. I will mention this study we're doing right now, which is really to test this central hypothesis. So why we study it is shown here. Again, I'm a transplant cardiologist. I know this, heart failure, arrhythmia, embolus, morbidity, and mortality, drugs, devices, mechanical support, and transplant. That's been my career, and it's the career of some of you out here, too.

There is enormous impact on patients and families, and of course, enormous drain on health care financing. The problem is the DCM, certainly in our studies, is that it starts months or years before it finally presents with advanced disease. And in fact, our family based studies and others, too, show that in most cases, a normal heart at least in adults takes months or years to go to DCM. And then another period of time before it finally presents with symptoms. Which is when it becomes clinically evident and we can identify those subjects.

So the key point is it's asymptomatic, and they have disease, but you have to look for it to find it. So there is clinical disease, but it's asymptomatic. And of course, here's risk of disease, and the idea is that genetics is all about risk and risk information.

So if you find the genetics and you can identify risk of disease, you can do surveillance to maybe dial in simple things like ACE inhibitors or beta blockers. And perhaps prevent the progression to full blown DCM, or if you identify clinical disease with screening those family members, you can identify, start drugs, and maybe prevent advanced disease. And of course, ultimately, we're not going to put ourselves out of business we know. But we would like to actually decrease the impact of advanced disease. That's what it's all about.

This is a poster child for genetic medicine, or precision medicine, whatever you want to call it. There are other cardiovascular conditions like this. The channelopathies, familial hypercholesterolemia, and others, where you can imagine early detection with intervention, FH, start a statin, makes a huge difference.

Channelopathies may take a different approach, drugs or devices. In dilated cardiomyopathy, of course, we're trying to prevent transplant and all of that. You know this and I will not [INAUDIBLE] here. We have put categories of dilated hypertrophy and arrhythmigenic cardiomyopathy, I think, is still a useful approach. And we use this approach to sort our genetics, too.

The gene ontology for HCM is pretty straightforward. If you find genetic cause, 80% is between two genes-- MYH7 or MYBPC3. And then if you add in the troponins and tropomyosin, you get another, almost, up to 98%, 99% of cause. So hypertrophic cardiomyopathy is largely a mendelian disease, single gene, that's relatively straightforward. And of course, now we considered it a genetic disease of sarcomeric proteins.

ARVC, these are proteins of the desmosome. And this is a picture of the desmosome, this connection between cells that have a variety of different functions, and when it's disrupted, cause arrhythmias. And ARVC is considered a genetic disease of desmosomal proteins. Complicated, not as straightforward is as hypertrophic cardiomyopathy, but at least from a genetic standpoint, relatively straightforward.

This is the DCM gene ontology. And since this is OSU, and since I know all of you, I'm going to [INAUDIBLE] each of these genes in exquisite detail. I won't, trust me. But you can see the ontology is really quite diverse.

Genes of the sarcomere, the cytoskeleton, the Z-disc. Lamin is a gene of the nuclear envelope. It's not in anybody's short list for why it would be causing DCM, but still remarkably interesting. The presenilins, we contributed. Phosholamban, a calcium handling protein, a transcription factor. RBM, a 20 and RNA binding protein-- fascinating gene. Really remarkable. BAG3, we also helped contribute this. A co-chaperone of heat shock proteins. Really, very interesting important gene for DCM. Ion channels, SCN5A, and others. And there is some crossover, of course, ARVC and DCM.

This is a figure we had published now a few years ago. DCM is in the middle. The lines show the connections to the different genes and then the other phenotypes with these dotted lines, also showing some connection now. The evidence for each of these genes, certainly, for hypertrophic cardiomyopathy is not-- that is, some genes are far more relevant. But this does give an illustration of the genetic complexity and crossover.

Down here is lamin. Lamin causes nerve muscular disorders, Emery-Dreifuss muscular dystrophy, and also causes syndromic disease. It's a fascinating talk unto itself. The channelopathies, of course, have a genetic component. SCN5A connects all of them, both ARVC as well as DCM and the channelopathies. So some complexity here.

So can we get to precision medicine with this? The issues of testing this is some clinical background. The sensitivity of testing for DCM, if it's familial, 20% to at most 40%. Non-familial appears to be less.

And using the American College of Medical Genetics 2015 standards, which was published, which we use now for genetic interpretation, they're more stringent, they're more conservative. It's harder to get to a likely pathogenic or pathogenic variant when we're doing genetic testing. So the realities of DCM genetics, the term locus heterogeneity. Many genes, I've shown you those. That's what we deal with. And then allelic heterogeneity, these are many variants, many different mutations, even within these genes.

Then the problem is that when you test somebody, you find a mutation that's never been seen before. It's called a private mutation or something novel. And then it takes a bunch of evidence, by ACMG standards to actually move that variant from a variant of unknown significance to path or likely path, it's just complicated, takes work.

There are guidelines. I was pleased to be part of Heart Failure Society of America practice guideline. The first edition of this was in 2009. The Heart Failure Society wanted a re-up of this, so we redid this in collaboration with the American College of Medical Genetics. The guidelines in one slide. Comprehensive family history, family member clinical screening, so with each diagnosis of cardiomyopathy, it is recommended that you do the at-risk family members, clinically scree them.

It's the old adage, you only find what you look for. Actually if you look for it in families, it's amazing what you find. So again, refer as needed, especially syndromic disease or children. And we have no hesitation at our cardiovascular genetics clinic. I'm a cardiologist, heart failure, transplant guy, not a geneticist. So if there's something that I say I don't know what this is, we punt to a geneticist. And genetic testing is recommended. Genetic counseling for all of the above.

I'm going to skip number six. Medical therapy based on phenotype, device therapy based on phenotype, and then ICD therapy prior to and ER less than 35% in high risk patients. That was put in the 2009 guidelines as for the physiologists, because we know lamin cardiomyopathy, that big pedigree I showed you of Dr. Wallace back from 1986. Those people die of arrhythmias before their EF sometimes drops into the '30s.

This was the updated one in 2009. It was genetic testing should be considered. Genetic testing is now prime time. It's easy. I mean it's easy at least if you can send it out and get results back in a few weeks. And then this was a new finding or a new recommendation at the request of the American College of Medical Genetics. It's called secondary findings. You are spared from this now. But with the advent of exome sequencing and sequencing all 20,000 genes for clinical purposes, you can come across things and genes, which you weren't anticipating finding things.

There's an ACMG 59 list, and about half of those genes are cardiovascular, channelopathies, cardiomyopathies, aortopathies. And if a variant comes back in one of those genes, it's just called a secondary finding. And then they come to us and we have to figure out in a patient who has no phenotype. So it's a new area, and it's important. We gave some guidance, of course with no data, but so it's essentially opinion, but it's an important thing. We all may be seeing more of this in the years to come.

We do have a Cardiovascular Genetic and Genomics Clinic. I have two fantastic cardiovascular genetic counselors, Laiken Peterson, Elizabeth Jordan, and I also recognize Garrie, who comes and helps when I'm out of town. And Garrie is helping with the study and helping with a lot of things clinical. So we're pleased to serve course the bread and butter cardiomyopathies, the aortopathies, FH, channelopathies, Dr. Weiss takes care of those with Elizabeth.

And we also see other less common conditions, primary pulmonary hypertension. We do see some sudden spontaneous coronary artery dissection. It's really not a panel testing, but there are research referrals and some testing available. And then it's easy to refer to us, [INAUDIBLE] so.

Let's do a case. This is a real case, 58-year-old female, and you're the cardiovascular consultant. She presented two years ago, at that time, a bradycardia, intermittent junctional heart rate PR interval of 300. Atrial tach, six sinus, had a pacemaker negative angiography, significant MR, but her heart function was OK. You consult her for second opinion, because now her EF is falling, she has worse MR, onset of heart failure, so it's trouble coming, and surgical mitral valve repair into consideration, what should you do?

So this is the guideline. Get a family history of three generations. And then screen those at risk relatives. And this is her family history. She has an older brother 63, stroke at 50, a pacemaker, an implantable defibrillator, 66-year-old brother, bradycardia, pacemaker, and an ICD, and a 71-year-old sister, bradycardia, atrial fibrillation, a pacemaker, and ICD. So it smells like there's arrhythmia disease there.

So is the family history relevant? Yes. Could you have a genetic cardiomyopathy? Yes. Is it relevant to help decide about a surgical intervention? I've been showing this case for years, I have no idea of that question, but it's something that may become more relevant as we have more genetic information going forward coupled with outcomes. Would you recommend the MVR with a genetic cardiomyopathy?

What cardiovascular genetic information would inform the case? Family clustering of pacemakers and DCM. This is lamin. And of course the EP guys now, they sniff out lamin like that. In 2019, actually in 2010 they did, or even in 2000. Actually it was published in-- The first lamin paper was new to the Journal of Medicine, I guess in, let's see, in 1999. So yeah, this was well known, so.

So lamin is the most common gene defect after titin and dilated cardiomyopathy. And if you select out DCM plus conduction system disease, it's way up. It's probably 30 cases, so. So what do you do now. Well, ideally you would test her. And she actually did have a MVR. She actually got sicker. I saw her at 60, she needed a transplant. She was a good candidate. She was transplanted, doing well. What did we find? Lamin mutation.

I telegraphed this diagnosis, of course. Unfortunately, we never got the family members to participate. So we assume that they also carry lamin variant, but she certainly did. And she was quite sick and had quite advanced disease and needed heart transplant. So in summary, genetic testing wasn't available when she underwent MVR. And I think that all of you would sniff out this genetic cardiomyopathy, even taking occasional family history.

And whether she might have just avoided that MVR, I don't know. It's a complicated case. We have CMR experts in the audience, I see, so they can figure out everything for us. But and couldn't she avoid a second thoracotomy? And also then, there's the thing of specific therapy. And actually there is a very small clinical trial of lamin-specific clinical therapy that is ongoing. And whether that will ever come forward to be the real deal is still uncertain. And of course there's gene therapy.

Let me stop. It's too long already, but if you haven't seen the*New England Journal,* there's a fascinating case of a child who had intractable seizures that has Bannon's disease, which is a rare disease, recessive disease, but they did gene therapy. And basically it helped. And then there is a publication in *Nature* just a few days ago, that is really a major advance and CAST CRISPR technology, so that you can literally edit out any base, edit out or in any base. It's a major advance.

Gene therapy is-- this isn't a gene therapy talk. That's a whole different story. It is happening. And it certainly will happen in the careers of the cardiovascular trainees here. And I will probably see it in my lifetime. I'm not sure I'll be participating in clinical trials, although, we may be closer than we think. And then obviously that would be one would have to get this gene therapy in before the horse is out of the barn, before the heart is big and you have an EF of 20%.

But imagine it. It will happen and that's ultimately what we're swinging for the bleachers. You've got to do this if you're going to do research, so. So if DCM results principally from genetic cause, and most of it appears to be sporadic, then what are the genetic patterns of disease?

So the usual patient is shown here in a very simple pedigree to unaffected parents and a subject that comes in with dilated cardiomyopathy, shown by the black circle there. And genetic possibilities are a de novo mutation, started with this subject, or what we call reduced penetrates. One of the parents carried the mutation, but it was seen in the offspring.

The other possibility is bilineal inheritance. I already showed you one story of that, where there are reduced penetrants variance from both sides of the family that sum up and cause the phenotype of interest in the offspring. And we call these monogenic or oligogenic. Oligo, meeting a few variants rather than one. Now, many of the times, it's not that simple. And so you have a family, and you have somebody affected, and you find a variant of unknown significance, but it's in one of the parents and maybe a sib, they have no phenotype.

Then you do some research and you find another VUS. And you have a couple of VUSs in at one subject that's sort of scattered throughout the family. And of course, it's seldom black or white. It's usually shades of gray. And that's what you have to deal with. So this is the reality that we deal with most of the time with DCM genetics.

So what's the evidence that IDC or simplex disease has a genetic basis? This is data from our studies. This is Sanger sequencing. I won't perseverate on this, but basically we found non-familial versus familial cause, about the same. This happened in the first Sanger sequencing study back in the 2000s.

And when we summed all of our Sanger sequencing studies, it still came out about like this. We didn't have-- The caveat is though, that while all the IDC probands had an extensive family history taken, almost none of them, or at least not on a systematic basis, they didn't have the family members clinically screened.

Turns out the family history is very insensitive. You have to screen the family members to actually find the disease, because DCM is asymptomatic, till it finally presents with late phase disease. So that's the problem. What's the evidence that DCM is oligogenic? Want to go through this very quickly.

This is data from the Laboratory Molecular Medicine colleagues in Boston. They had published 124 cases. And basically, in their path or likely path, and what they call the high probability view at 7.3%. If you take all of this and add it up, it's 44%. And then they had 319 cases with at least one path, likely path, or VUS. And that was 35%.

This is personal communication. It's actually a published study, but you can't dig this out of the paper. And Missy and Birgit gave this data to me. So there's some clearly evidence. And this was the best study in 2014 when it was published. Here's a more recent study. This European study of 639 DCM patients sequenced radio for genes, half sporadic, half familial.

They found that 38% of patients have compounded combined mutations and 12.8% have three or even more. Now, the rigor that they use to assign variance, we would increase the rigor of that substantially. But nevertheless, I mean if they're only half right, it's still substantial, substantially more than one pathogenic variant per subject. This is some legacy data, exome data from 412 subjects, 175 pedigrees, as shown.

And basically, if you look at the number of variants and the number of probands, and the percent of probands, and you sum it up, comes out to about 29%. This is unpublished. This is not a final deal. It needs to be published. But the goalposts for how you define a variant's path or likely path it's been moving over the last five years. And it's complicated But it certainly is more than 1% or 2% or 3%, which is the case in hypertrophic cardiomyopathy.

So this is a more recent paper, just published, I think, last year. Five families actually non-segregating lamin families. Each family had also a second rare variant identified in a known DCM gene plausibly relevant. Dan Kinnamon, a statistical geneticist in the group, did a formal analysis, and showed that these additional variants are biologically relevant. And I will not go through the data on this. It is published. But it's the first data that I know about, in a formal statistical approach, that actually shows that multiple variants are relevant to dilated cardiomyopathy.

This is Dan, at a poster. This was last week at the American Society of Human Genetics. And this is now a much larger set of data from over 100 families, a preliminary data cut from the precision medicine study, which I'll mention at the end. And preliminary data, 114 probands, 234 family members. Basically, a much more rigorous and larger study that shows that these multiple variants are biologically relevant.

Again, you have to be in the genetics universe to understand that most VUS, and certainly cancer are thought to be probably mostly benign. And it may well be the case in cancer, but in cardiovascular disease, at least in dilated cardiomyopathy, it looks like that is certainly not the case. The conclusions were then family members carrying more of the probands VUS alleles, they had a worse LVF and larger heart. And again, those may be biologically relevant.

So might an oligogentic architecture explain in part simplex DCM? Family based illustrates this. I told you the story of. David here he is again. And I showed you this pedigree. The problem is that this black and white approach for effective status is simply inadequate if you get into complexity. We need more phenotype detail.

And so in the paper I showed just last year, we decided to categorize, increase the phenotype detail, category 0 through 4, where 0 is no cardiovascular disease, category 1, evidence of myocardial dysfunction, but not meeting formal DCM criteria. Number 2 is asymptomatic DCM. It's what we see in a lot of our family members when we find them. Symptomatic DCM, treatable and stable. And then advanced DCM, or DCM-related death, transplant VAD, DCM-related death, or an ICD 4 symptomatic disease.

So this is the same pedigree I showed you of David. David at 14, critically ill with heart failure, emergent heart transplant. He lived to 22, died of post-transplant complications. His next brother had heart failure in his 20s, had a heart transplant at 31 years of age, still survives, had a second transplant. He's at the Oregon Health and Science University still. Doing actually quite well.

The next older brother was fully asymptomatic, really has, at most, has had borderline LV enlargement, has had a little bit of atrial tachycardia. But essentially, asymptomatic, and it weren't for this family studies, you'd never know that he has anything wrong with his heart.

Dad, I took care of him in clinic. He was diagnosed at 44 in the research study. He died of heart failure at 57, never wanted to transplant, never was really flamingly sick, just kind of a chronic heart failure guy. And then mom has never been symptomatic. She's had borderline left ventricular enlargement. She's had a dozen echoes over the last 20 years. And she's just always sort of on the cusp of showing some LVE and sort of a lowish, yes, but really asymptomatic.

The uncle was asymptomatic at research screening, had sudden cardiac death at 43. Luckily in church on a Sunday morning, he was resuscitated, has a [INAUDIBLE] in place, is alive with minimal symptoms. And the cousin who had sudden cardiac death at 17, really was incredibly responsive to treatment. So this is the family. With the Sanger sequencing study, I'm going to skip this. We didn't find a lot. Spent a lot of time and effort.

We moved on to exome sequencing, and what we found was an FLNC positive variant on mom's side of the family. FLNC is a really interesting and relatively recent DCM gene. I won't go through all of this, but in dilated cardiomyopathy, there are truncating variants with prominent ventricular arrhythmia, and sudden cardiac death, and relatively less LV dilatation, and much less heart failure, which fits with mom's side of the family.

What else did we find? And we think that FLNC variant explains this, because of the segregation and et cetera, the phenotype. What else did we find? We found a titin truncating variant on dad's side. Dad and all three of the brothers had this titin truncating variant. We know the titin truncating variants are certainly important DCM, but they're difficult to assign pathogenicity.

And in this pedigree, we don't think this titin truncating variant is likely to be causal, because of the lack of DCM in the older sibling. So again, this brother has the titin variant, but essentially has nothing, or imperceptibly disease. What else did we find? We found a SOS1 variant. SOS1 was in dad. It was in David and David's older brother. The oldest brother did not have it.

What is SOS1? It's associated with Noonan syndrome, which Noonan syndrome, for those who do congenital heart disease, know what this is. Short stature characteristic facies, classically hypertrophic cardiomyopathy. Most of it is PTPN11. Some are KRAS, if those are negative 20% in SOS1. This is a RAS guanine nucleotide exchange factor. And this variant is absent in the large databases.

And we have extensive unpublished data, in fact, postdoc in the wet lab has been working on this variant and other SOS1 variants. And this manuscript is ready to be submitted. It shows that it does cause a functional effect. So the idea here is we propose the SOS1 variant explains why the oldest brother had minimal disease. He only had the titin variant. The lack of the FLNC variant explains the later onset and more benign course in David's father.

The FLNC, titin, SOS1 explains the greater disease in David and his next older brother. But why did David have a much more malignant course than his next older brother? Well, we also found a CAM kinase 2D variant that we think is a protective variant. We always think of variances as being nasty and deleterious. It can also be protective. It's a very difficult thing to sort out.

And it turns out that CAM kinase, two delta for those of you who work in cardiovascular research, this is a very well-known protein, and interacts with fossil and band. And there's actually abundant data in animal models, and also decreased CAM kinase two delta models is suggested to be protective. So this isn't a novel idea necessarily from us, but it fits with the available data.

So we hypothesize that this variant is protective. And it helped dad to live to 57. David's next older brother had the protective variant for later onset. David did not have it, so he had earlier onset and more malignant disease, And David's oldest brother didn't need it, because he didn't have anything nasty to cause DCM anyway. At least that's the going hypothesis now. Now what kind of data do you need? You need real data, not inference.

And so Sarah Koenig and Peter Mohler's group has amounts to building with this variant to try to prove that this actually may have protective effects, to prove this and finally explain this family. Am I making sense so far? OK. You could do some kind of a crude scoring, and you come up with David having the worst case, and this guy has protective variant, and dad doesn't have as much, and this guy has almost nothing.

And so it's not simple any longer. It's more complicated. It will likely get more complex until we understand where monogenic meets oligogenic. Of course, to figure this out, you have to test key hypotheses. And again, I've shown you this slide.

To test that hypothesis, we have put together this study. It's a multi-site study. We started enrollment June 2016 with 12 sites. We've expanded now. We have 25 active sites, shown here. It's a great group, really. Invested investigators, a couple of family member only sites, because this is a family-based study and we have some family members between Omaha and Seattle or Palo Alto, and so Utah can help get family members. We'd have nothing in the Upper Midwest. So this is the group.

So that grand hypotheses, what's the proportion of familial DCM? The hypothesis is the third of it is familial. The second hypothesis is cardiologist may not appreciate that familial DCM as a genetic basis, much less consider the possibility of genetic cause for non-familial. So the hypothesis is it's all kind of genetic. Third hypotheses, communicating DCM genetic risk from probing into family members hasn't really been studied. And of course family members have to receive and understand that risk via the probe and to pass it on.

So we have a hypothesis that a tailored intervention, a communication intervention will help improve this communication. The fourth is that IDC has been suggested to be more common, morbid, and lethal in African ancestry compared to European ancestry. But we have almost no African ancestry data, pedigree or genetic data. Been attributed to hypertension, other environmental causes.

And the fourth hypothesis is that similar proportions of DCM of either European or African ancestry have identifiable genetic cause. So diversity is essential. I became aware of this back with the exome sequencing project. And then we created a plan to add diversity. Takes extra time and effort, and of course, we all know that white guys are the easiest, and most studies are white guys. And this is, of course, then the sex disparity has been noted upon. Minorities, of course, have had historical reason for concern.

This is data on GWAS 2009, 96% percent European ancestry data. 2016, still 81% European ancestry. And most of this is otherwise, Asian. And then even 2018, this is GWAS data, not rare variant data, which even worse. 78% European, if you look down here, the African ancestry and Hispanics are 3.7% contributing. Yet, if you look at the associations that is what you find when you add diversity. The non-European diversity added 46% of findings, even though it was only 22% of the GWAS associate.

The bottom line is, scientifically, this is really important. It's also important for other reasons too. But and for that reason, we decided to add-- do half and half, half white, half black. We had a supplement from the Genome Institute to add 100 Hispanics so we've added 100 Hispanics. So a phenotyping aim, a sequencing aim, and then a communication aim shown graphically here. We phenotype families, we recruit families. The innovation of this study has been training my colleagues around the country heart failure transplant cardiologist how to do family-based studies. It's different than doing just a clinical trial, which we've all done dozens of.

We then sequence the affected members of the families. Exome sequence. We return genetic results in a randomized study to assess a communication intervention. This is the study design for the Aim 3. The program is randomized to a communication tool at baseline. That is what we call family heart talk. It explains DCM genetics with pictures and such and diagrams, And what should my family and I do, and how to talk with your family members about DCM. That is the intervention.

And then we recruit family members. And then the first endpoint is the number of family members clinically screened. So we do count bodies, but hopefully they're all alive. These are people that come in, family members that come into the study. And to see if that intervention is effective. We return genetic results, then we look at family member adherence. If a website turns to Spanish because of the Spanish supplement.

This is our data report as of September 30 we have 1,142 of the 1,300 recruited. We have 1,725 family members. And we have our European ancestry probands are done. Our Hispanics are essentially done, but the lag of courses is the African ancestry, and we're continuing to recruit out our African ancestry probands of families.

This is the enrollment curve. I will not perseverate here. And the family member enrollment has been blessedly on track, so the NHLBI is satisfied. This is the big picture plan. I show this here, you will understand that we're in this active enrollment phase. We are adding ancillary studies. We want to finish this study by 2021. And then we do need to follow on study. We'll have all these family members with all this genetic information, and their phenotype data, echo data, and we need to see what happens then with them as they evolve with their genetics over the next ideally 5 or 10 years.

We have added to ancillary-- Actually, we added one ancillary study to do some speckle-tracked imaging of the family member echoes. This is being done in collaboration with Sanjiv Shah, Jane Wilcox at Northwestern. Then we have another one out for CMR where we went to nine sites to pull in family members and to see more studies, those that have genetic information, to really find very early evidence of DCM before, again, there's obvious systemic dysfunction and left ventricular enlargement.

We're planning the next study. We call it DCM Discovery. It's a very ambitious goal. We need much larger numbers. The aspirational goal is to get to 10,000. We have 1,300 in our current study and roughly 700 in our legacy study. We need a much larger study, much larger sample size, and negotiations have begun within NHLBI and others to see if we can derive some additional support. That's in prep and pilot phase. And we've done a lot of work for this, including having a single IRB and enlarging our infrastructure more scientific personnel.

So in summary, what we know, DCM drives a significant portion of heart failure, drives considerable rigidity mortality cost. We understand Mendelian aspects of DCM genetics. Some of it is Mendelian and it makes sense. What we don't have all figured out yet is complexity, that complexity beyond Mendalian and the boundaries of that, and how to figure that out and how to move ahead.

So the way forward is much larger cohorts, very well phenotyped. I'm a phenotype or I'm a clinician. That's what we do, this is exceedingly important. Just to say I haven't said it, I don't think during this talk. I'm a transplant cardiologist. I happen to do genetics. But phenotype in your clinical assessment is at least as important, if not more important, than the genetic analysis.

Genetic sequencing is almost a commodity. You have to have smart people to do that part too. But really it is really fussing about what a patient looks like, what they have, and then getting all that data down into databases and really doing a great job. So but we need well phenotype, much larger cohorts, robust analysis, and then of course functional studies. I mentioned one in our wet lab and the one association with Peter. We do need to take that than to the next level to prove all of this stuff.

Let me recognize all these sites and site investigators Garrie Haas is the OSU site PI, and it's done a fantastic job. OSU is-- I haven't shown you the data, but we're leading the pack in terms of enrollment. And give Garrie some credit. And then others around the country. This is really an outstanding group. We have collaborating investigators around the country too, clinical research team here, informatics computational team, recognizing them, varying analysis. We have a publications presentations committee, others, and this is the most recent picture of everybody and there's our website.