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TRANSCRIPT - GR 04 11 25 ″**Polycystic Kidney and Liver Disease: How do we get from Gene Discovery to Therapy**″ guest speaker Stefan Somlo, MD, Yale School of Medicine

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Internal Medicine Grand Rounds

- Hello! There we go all right. Thanks, everyone. Welcome to our medical grand rounds today. We're delighted to have Dr. Stefan Somlow here with us from Yale to speak about polycystic kidney and liver disease. I'll take us through our Cme. Slides here.
- See? Okay, nice.
- There we go. So Dr. Samla's presentation objectives and his disclosures.
- And then for any faculty online or in the room claiming Cme. Credit your Cme. Slide.
- As this is our Philip Liverman lectureship. I'll welcome up our Nephrology division chief, Dr. Marco Cusa, to introduce the lectureship.
- Thank you, Brian.
- So thank you. And welcome to medical grand rounds. And this is the Philip Liverman lectureship, and while Shana introduces Dr. Samo, I thought I'd introduce the lectureship to you, and I think some of you already know this lectureship and others are new. But this is the Liverman Lectureship. It was established in 1986, and it's really to honor the life and legacy of Dr. Philip Liverman, who was a fellow in our division in the 19 seventies.
- So Dr. Liverman received his bachelor's degree from George Washington University. Then he came here to Uva for his medical degree, and then he left for a few years to go to Case Western for his Residency. But then he returned to Uva as a fellow in the Nephrology Division and he did research with Dr. Nazette Atook, and Dr. Atook was very much into catecholamines, and so he enticed Philip Liverman to study catecholamines in dialysis patients? so he's he is survived by his wife, Joan Liverman, his son Eric, daughter Astrid and Brother Terry, and we had a real nice dinner last night with Astrid and her family.
- So this lieberman lectureship was established after his death in 1984, and it was established by his family, and it was intended to bring the most distinguished nephrologist in the country, and you can see there's 25 of them, and the last one is, of course, Dr. Steve Somlow. So Shane is going to introduce Dr. Somlow and Steve. Thank you for coming.
- So good afternoon, everyone. It's my distinct pleasure to introduce Dr. Samlo. So Dr. Samlo obtained his Medical degree from Columbia University, and completed his Internal Medicine Residency at Albert Einstein College of Medicine. Afterwards he transitioned to Yale University for his Nephrology Fellowship, and has built his academic career at Yale, where he is now the Cnh. Long professor of medicine and professor of genetics.
- His career has focused on the study of autosomal, dominant, polycystic kidney and liver diseases with the goal of understanding the role cilia and polycystine signaling pathways play in both normal adult tissue homeostasis and in progression of cystic diseases.

- His laboratory has sought to discover and validate molecular mechanisms of Adpkd, using in vivo genetic studies in mice, making extensive use of combining alleles to better define gene interactions. In particular, his group identified Pkd 2 as well as 8 other genes implicated in polycystic liver and kidney diseases. So we are very pleased to have him speak with us today, so please join me in welcoming Dr. Sanlo.
- Thank you very. Thank you very much for those very kind introductions. You know it's a it's a real honor to be able to come here and participate in sustaining the legacy of Dr. Liverman in this in this lectureship series, and I'm also particularly honored to join the other 25 distinguished speakers that you've had before, most of whom I know, which tells you I've been around for a long time, I guess. All right. So you know, the subtitle here is, how do we get from Gene discovery to therapy? So just historically, I started by trying to find genes for Pkd.
- And then, you know, once we found them, that we had a decision point to make, and the decision was whether to find other genes for other diseases, or to continue to work on that, and we chose the latter. So I'll try to give you some insights into that. These are my disclosures. I have no conflicts here, and you saw those before. I will try to get through this today. We'll see how we do. But I was going to provide background sort of in genetics and risk factors focusing on clinical, genetic and risk factors in patients. And then I was going to switch to some of the research topics in particular. Just describe to you what polycystines and cilia, and how the genetic mechanisms work a little bit about Pkd, gene dose, pkd. protein, slash gene dosage, which is actually one of the areas that current therapies are actually entering clinical trials for the reversibility of Pkd, which I think is actually a pretty remarkable thing for kidney disease, and then, if we get to it, it will also. These are modular pieces. I'll also describe some of our work. Recent work on effectors of Pkd. This being grand round and no good, I'll keep talking. Okay. This being grand rounds you know, at Yale the the Vinnie Fogweiler, who runs, who runs grand round says you can't have any Western blots which you know, so I think I took that to heart. If not, I apologize, and the other thing is, I wanted to give a couple of clinical vignettes. I won't call them cases, but I'll just give you a sense of what Pkd patients are like. So this is, for example, is a 55 year old man who was seen in clinic for follow up for his known Pkd. He was diagnosed at age 34, when he noted abdominal fullness and flank aching, which is not atypical for Pkd. He had a family history of Pkd. So it probably he's going to have it as pretty high 50%. And then he had an Rp ultrasound which showed kidneys that are enlarged with multiple cysts, which is essentially the diagnostic criteria for the disease. At the time of presentation he was hypertensive. He was started an ace inhibitor, which this was about almost 2 decades ago this individual was seen, but that remains the 1st line therapy. He had normal kidney function, and then, by the time he's 55, his creatinine had risen to 1.8. So he's developing progressive renal failure. These are his Mris. This is his kidney over here. This is actually liver cyst here. The kidney is over here.
- Okay, the kidneys over here. So he has both the liver cystic, phenotype and the kidney phenotype. And if you recall kidneys are supposed to be 3 vertebral bodies in length, and his are about 8, and this is his family history, so dominant trait. But each individual child, from his affected father has a 1 in 2 chance. And so you can basically flip 5 out of 6 heads. And so 5 out of his 6 siblings were affected.

- This is a second case which we have an ongoing study to try to define the genetics of intracranial aneurysms in Pkd, I won't discuss that today. But these are a couple of cases that were recruited to that. This is a 71 year old woman who was diagnosed at age 25 by imaging, screening. After having recurrent urinary tract infections, she progressed to Esrd at age 44 was transplanted after 9 months of dialysis at age 40, and she's now 71. She has polycystic liver disease in addition to her kidneys, and she has 5 intracranial aneurysms that were noted, the largest of which is 7 in the Mca.
- And her mother died at age 39. I don't have the detailed history, but when you see someone with a Pkd. And a family history of aneurysm who has death at age 39, although it could have been due to venal failure. It's also a consideration that you might have had sudden death from the intracranial aneurysms. We did the whole exome sequencing to identify the mutation. There's a c to T change. There'll be a small quiz on the number, the location of this thing at the end of the talk.
- And but what happens when you look at the particular coding sequence in this individual. It's a glycine to a glycine change which is supposedly a synonymous change. Now, this is the only variant found in Pkd one in this patient, and this variant is not found in nomads, or a large database of whole exome and whole genome sequences, like 500,000 plus chromosomes. So it's rare. It's a patient with typical Pkd, and it's the only thing that we found in Pkd one.
- So this probably represents a cryptic splice event. You'd have to prove that the other way to look at this is, you could look at family members and see if they inherited the exact same mutation that would probably mean it's pathogenic. But just to give you a little idea of how the genetic testing sometimes works. This is another patient for the Ica study. She's 27. She has no family history of Adp. She was diagnosed at age 14 by imaging Mra. At age 21 showed 2 aneurysms, 2 and 3 2 is kind of a minimal cutoff for what we think is potentially significant. She has hypertension, but no kidney stones, no hematuria, no other complications. She has normal renal function. But she was started. She was recommended to start tovaptan, but then discontinued it because she became pregnant. And this is her creatinine. During pregnancy, so normal renal function. This is her imaging study. So no liver cyst in this case. But she's still young and this is her mutation, which is a typical frame shift mutation in Pkd one.
- And then this is the last case I wanted to show you. This is an individual who is 62 years old. She has huge kidneys. So normal kidney volume is about 250. She's 2.7 liters, but she also has an astronomical liver volume of 8.9 liters. This is all liver here. And so you know, she basically has, you know, asked, what can we do for her liver disease. And while there is some therapy for kidney disease for the liver disease, there is not much available when it becomes this prohibitively massive. And she's, you know she's basically thin because she can't eat from the mass effect. Then the 2 options are either partial hepatectomy. If the anatomy is amenable to that or liver transplantation I will emphasize. This is a rare complication made a picture in the journal, but this is a patient that actually one of my colleagues saw in clinic near Adol, saw in clinic at Yale. So they do occur.
- So with that. Just some fun fact about Pkd, it's common. It's the most common monogenic renal disease. I'll show you numbers absolute numbers from Usrds in a minute. It's dominant inheritance so affected parent 50% chance for each child. There are 2 predominant genes, Pkd, one and 2. That result in polycystic kidney disease that progresses to end stage renal failure. There are other genes amongst

some of the ones. We discovered that cause polycystic kidney disease, but those do not progress to renal failure. So I still there's some new Kd go guidelines which I'm not going to go into which now include the gene name in the nomenclature. But I think the take home for this purpose is 77% of families with Pkd have Pkd, one mutations, 15% have Pkd, 2 mutations, 8% have no mutation detected using even current next generation technologies of those 8%. My rule of thumb is 4% are missed. Pkd, one mutations. It's a very complicated gene. I'm not going to discuss that today, but it's a little bit difficult to do. Mutation, detection, and I gave you a sense of that. When you have a glycine to glycine change, you know. How do you know it's pathogenic. And the other 4% are phenocopies.

- 90% of of you know cystic kidney disease coded. Icd, 910 is Adpd, so all the others. Arpkd. All the nephronophyses are minority of the genes, large numbers of individuals, no differences in ethnic populations, in fact, there's a finite de novo mutation rate. So as the world's population grows and there's no reproductive disadvantage that we're aware of with Pkd, the number of Pkd patients will also grow, and finally, there are no common mutations. And this is actually a problem. So every family essentially 1st order, approximation is a private mutation. So you can't design a therapy for a specific mutation, because every everybody's mutation is different. So these are little things to think about. So this is the Usrds data from I'll just click through to the end. Here, Usrds data that pulled from 2020. So leading causes of Esrd number 4 is cystic disease.
- And of course, I told you, 90% assisted 80 Pkd absolute numbers. The prevalent population at that time was around 40,000 and change. It's not going to be that different today. Out of 800,000 end stage patients and Pkd patients, according to Usrds are transplanted much more frequently than the general population.
- So this is the disease. This is, you know, if you ever get human samples in Pkd, you start with something like this. This is a 12 inch kidney, not a 12 cm kidney. So this is my little futile effort to show you to scale what they would look like. These are enormous, and this is just one kidney. And it's again. It's hard to know how this process started from here ended up here. This is the natural history of the disease in cartoon form.
- And you know, basically, as you heard from the cases around the age 20 plus or minus cysts begin to be visible and detected by routine imaging studies. And then over time the kidneys grow in size over time, you know. Initially, renal function is normal over time. The Ckd is progressive. People vary in their rate of progression. I'll get back to that a little bit later, but not atypically. This is a relatively rapid course. So this is the Gfr. Decline here. By the mid fifties the patients end up with the end stage renal disease, at which time they either go on dialysis or transplantation, for now there are some interventions that we can do. Certainly, lifestyle interventions, blood pressure monitors I mentioned, acr inhibition is 1 1st line therapies for hypertensive patients. Pain management is actually a pain for both the physicians and the patients, and it's very complicated to do in Pkd, and some of these nephrectomies are actually done for pain management not often hopefully, but they do happen to have it.
- And then, tovaptan, I'm not going to talk about at all, but it is an FDA. Approved therapy for Pkd, and if there's questions about it, I don't use it, but I don't use it because I don't see outpatients, but if there's questions about it, I'm happy to discuss it. There are 2 extra renal manifestations of adp that I think are worth noting. The 1st is liver cysts. I already showed you pictures of that. So the liver

cysts arise from the bile ducts. The liver. Parenchyma is normal. So even that person with a very huge 8 9 liter kidney liver. Sorry, had normal synthetic function and the only defect was the mass effect of the liver. So most of the complications of the liver disease are related to mass effects. The cysts can also get infected as any. Any closed space liquid containing body fluid can important features of the

- Pkd, so you know, basically, most patients will have a few liver cysts over time thankfully. Most will not have the severe disease I showed you before. It is much more prevalent. The larger livers are much more prevalent than women. It's thought to be driven by estrogen exposure. And we I won't talk about this too much. I'll show you a couple of pictures, but it can occur as an isolated disease where it's liver predominant, and there's either few or no kidney cysts.
- And that's I'll show you the mechanistic relationship. The other major complication, which is not understood genetically or clinically, for that matter, are aneurysms which occur in about it's about a five-fold increase risk over the general population. If you have Pkd. However, if you have
- Adpkd and an aneurysm, a 1st degree relative with Adpkd has about a 1 in 5 chance of having an aneurysm as well. So it's even clusters more strongly. Familially. Mra is the screening modality of choice. And you know, if you have a negative screen. The likelihood of getting an aneurysm later is actually pretty low. But again, these are hard studies to do on a population basis, and the rate of growth of these aneurysms is also not great.
- Having said that the issues are that we don't have any genetic idea of the mechanism or how to diagnose the risk for this.
- And so, you know, there's a little bit of an ad hoc approach. I mean, there are certain rules or suggestions on who should be screened. But it would be nice to have a better idea of what the genetic basis are.
- The diagnostic criteria for Ad. Pkd. Again, I'm not a busy table. Suffice it to say that there's something called an age dependent penetrance. So there's an incidence of simple cysts in the general population, and so as you get older to diagnose Pkd, the criteria for the number of cysts you need goes up a little bit, and the exclusion criteria are that you don't have, you know, certain cysts at a certain age, but ultrasound, which is what this these data are for has a very high, positive, predictive value and very high negative predictive value for cysts. So if you do, an ultrasound and a 30 year old at risk, and they don't have cysts, or even a 20 year old. They don't have cysts, they don't have Pkd, and if they have 2 cysts they have Pkd, basically. But for Pkd 2, it's a little bit less strong because Pkd 2 is a milder disease. So later in life they can still develop more cystic disease and yet not have those at younger ages. So I think this is ultrasound is the simplest test. Certainly with MRI the numbers are a little higher, because your resolution is higher, so you'll need 10 cysts rather than 2 cysts on an MRI.
- One feature of Pkd is okay. So I told you, the families all have private mutations. But does that mean that everybody in the family has the same clinical course? The answer is no, which complicates counseling. And and and, you know, sort of advising patients. And so this, this is a work from a
- Matthew Langtree and York pay in Canada, and you know they break it down here by different kinds of mutations. So these are Pkd, one. Truncating mutations. These are different kinds of Pkd, one. Mutations or missense or indels insertion, deletion. These are Pkd 2. And this is that no mutation detected category. So if you look broadly at the Pkd one mutations, the red dots represent end stage renal disease

- by age in these families and overall. You see that they're fairly in their forties and fifties, which is typical. But every now and then you see some individuals who have Pkd, and yet who get to an advanced stage and don't in the same family. So the same Germline mutation and don't get Esrd. So the intrafamilial variability is actually quite significant in this group over here, which is this indel group. You have somebody who had Esrd at age 20, and somebody who made it to age 70, without Esrd. Or Getting are starting at 870. So very different. And if you look at Pk. 82, and the no mutation detected rate in general, they they occur later. They're going to be in their sixties and seventies as opposed to forties and fifties when they get to end stage renal disease? so you know, how does you know if you're seeing these patients, how do you sort of judge what their progression is going to be. And the short answer is it's not perfect, but there are some criteria. So I don't think you can. You can't see that. Okay, so you know there. There are a few things that are well known to to well, not well known, but are well associated with progression. So one of them is a type of mutation I already alluded to that the truncating mutations are going to be more severe to missense. I will show you more data on that. Another thing is, of course, you know, once your kidney function starts going down you know, it's fairly inexorable, and you can judge the rate of decline, and that's going to correlate with the severity of disease. Kidney size is going to be a big one. I'm going to show you no pun intended. I'm going to show you that one in a minute, renal. But flow! I'm going to skip. That's a cottage industry at the Mayo Clinic for now, but may very well be useful. I'll talk a little about biomarkers.
- Biomarkers are populous here, but I'll give you a couple that have interest in. There's something called a scoring system. Propic 80. I'll show you a little about that and I think those are the main ones. There are some other factors that have been known for decades like males always do worse, I think, in general, with chronic kidney disease, and certainly with Pkd first, st if you have symptoms early, so either hematuria or hypertension onset early. You're going to progress more severely, and it turns out that proteinuria is actually a marker as well. We've always assumed this is tubular proteinuria. But I don't know that we know that for a fact.
- So this was a a landmark. Study, if you will.
- That changed the course of clinical trials and clinical studies in Pkd. This was the so-called crisp study funded by the Nih. And what they did here was they did serial volumetric Mris on Pkd. Patients over a 3 year period, so one at a time 0 at entry, one year, 2 year, and 3 years afterward. And this is the same individuals. Here you can see them. And what you can appreciate is that over the time volumetric Mris can detect kidney growth, and they can do it over 3 years. It turns out it can probably do it over one year and what you all can also appreciate is that not everybody's kidneys grow at the same rate. So these folks are growing slowly. These guys are growing quickly. And then this correlated with the rate of decline of Gfr, so the bigger your kidneys were the faster your Gfr declined. So this was the basis for a lot of the basically for getting drugs approved. And it changed the course of the disease, because before that, even if you had the perfect therapy, the companies would ask you, Well, how do we do the clinical trial? And the answer would be well, I don't know. You figure that out. And they said they don't like that idea. So now they know how to do the clinical trials. And this is one thing that I do think that the tovaptan study really did accomplish.
- So I mentioned that kidney size is important. So this is the Mayo imaging classification also known as the erosevel system for the 1st author of the paper.

And what it speaks to is the fact that kidney volumes over time, the bigger your kidneys and the faster they grow the worse off you are. So male. Classification is divided into classes, one E being the largest, fastest growing kidneys, one a being the smallest, least growing kidney. So an individual at age 30, with 200 CC's. Of kidney volume, is going to do reasonably well. But someone at age 30, with one liter of kidney volume is not going to do as well.

- This is what one E looks like compared to one. A. So you just get sort of a visual on that. And you know, when you when you look at renal survival, so 10 stage renal disease. This is the one E group here. They're going down quickly. You can see by late forties it's 50% renal you know, viability, one d, 1 c, one B and one A and the rate of decline. This is just no way of showing that the rate of decline of Gfr in the A group is about 2 to 3 milliliters per minute per year, whereas in the E group it's going to be about double that. And so, you know, this is helpful. But you can see this is scatter graph. So any individual that comes to you. It's hard to give them individual. You know, recommendations based on this. You can give them some probabilistic outcomes.
- The other way to look at Pkd is by type of mutation and it took about 20 years after genes were found to actually get this study done, which is surprising. Given that there was lots of evidence that the type of mutation makes difference in alports and other kinds of diseases. So this is called Pkd, one truncating mutations. I'm just going to go through the key here. These are Pkd, one non-truncating mutations. But they're fully penetrant, which means that you have a full length protein with a change in amino acid sequence. But it basically doesn't work at all, or almost not at all and then these are hypomorphic. So they work a little bit. But they're non-truncating. And this is Pkd 2. If you look at Pkd, one truncating mutations that's got the fastest rate to end stage renal disease in this group. The non-truncating but fully penetrant ones are very similar. I mean, again, we don't have precise resolution, so we call it non-truncating, but some of them are a little hypomorphic. Some of them are not hypomorphic at all, but you get an intermediate phenotype. This is the non-truncating that basically is hypomorphic. And this is Pkd 2, so you know, just to sort of correlate the imaging classification with the mutation type. So if you look at one E, that's the most rapidly progressive one they're overrepresented for the 1st 2 types of mutation, truncating and fully non-truncating right? And that's true for one d. As well. But when you get down to one C and one B, now you begin to see representation of these other types of mutations. So there's correlation between the mutation of disease progression. But it's not perfect, right? So this it's hard to give individual level information. But someone with a truncating mutation in large kidneys has a prognosis that they're likely to end up with end stage renal disease sometime in the, you know, midlife.
- This is the pro Pkd score. So you know I mentioned males do worse. So if you're a male, you get 1 point, etc, etc. hypertension early on the big one, though you get 4 points for having a Pkd, one truncating mutation.
- And so when you add up the scores, if you score 0 3. Then this is sort of your your rate of renal death. If you get 7 to 9, this is the rate. So this is the propagate score which actually again correlates. And so we have a lot of predictive tools. As long as you can get a volumetric MRI to find out what the patients is the Mayo clinic actually is a website. You can put in the dimensions of the kidney, even without a volumetric MRI on a Ct or an MRI and get an estimate for the volume. Anyway, there are some cautions there that they're not supposed to be used for prognostication. They're

supposed to be used for selecting patients for clinical trials, and perhaps for a possible endpoint. When you do when you do a when you, as a surrogate, possible surrogate at a point where you have an accelerated approval process going on. So this is the biomarker thing I mentioned. So it turns out that not surprisingly one A and one B is relatively rare. One E is not super common, but one C is the most prominent category. That's that middle category, and you can figure. Some of them are going to be closer to B, and some of them are going to be closer to D. So how do you stratify those. So this is a recent paper like just last month. By Ron guns of group in Neymen.

- 9 million, I think. And what he's looking at here is basically, this is sort of the predictive value of all the other things that we talked about sort of prop score all those things if you add to that, and I did this last time, and people couldn't read it. So I have a big version of that. So if you add to that urinary Mcp one and serum copeptin levels, then you get a better discrimination. So this is relatively new information. These are not yet standard in use. But we'll see how this plays out, because you might be able to stratify who amongst the one C. If they have high urinary Mcp. One and high Copeptin, they might be more on the one d. Side of one C as opposed to. If they had low, then they might be closer to the other one. So you know. So there is good work going on, and we're hopefully going to progress.
- One more interesting finding that was published a couple of years ago by Christoph Karolik from Columbia. I would say. It's not surprising, but it's impressive.
- Is he developed genome, wide association, score, risk score, Gwas score or polygenic risk score for chronic kidney disease. So this has like, I don't know, 500 loci, some large number of loci, and the more you have the worse off you are! And so he divided the people. The Polygenic risk scores into tertiles, and this is in the general population. He put the individuals with the intermediate tertile, polygenic score, as you know, having average risk in the population. Those with the lower tertile had lower risk of having Ckd. This is not new Pkd yet, and those with the higher had a higher rate. He applied this polygenic risk score to patients with Pkd in the Uk. Biobank and it turns out that those who had the lowest tertile score here had an increased risk, obviously because they have Pkd, they had about 3 fold increased risk relative to the general population intermediate score no Pkd. But look at the people who are in the upper 2 tertiles. They had a 30 to 50 fold increased risk to progression to Esrd. So this suggests, you know I don't think this tells us mechanism, but it tells us, if you have genetic background, if you will, that is predisposing you to chronic kidney disease. And on top of that you have Pkd, then those factors will interact and will potentially increase your risk of pkd, so there's a lot of sort of predictive tools. And that's important. Because you want to select people. You know, as you develop therapies, you're going to want to select people who are at highest risk to undergo therapies, and this will give you a better opportunity to balance the risks of the therapy versus the benefit of the outcome.
- So that's the that's the background part. And now we're going to switch to cartoons. So polycystins and cilia. And by the way, polycystins were found about a decade before we recognize that they're in cilia. So there's a that was an interesting time, so polycystine one in cartoon form looks very nice. But in real life it's kind of a beast of a protein. It's 4,302 amino acids long, which in the global scale of things is out there. It's very large. It's got 11 membrane spans in this huge predicted 3,000 amino acid, extracellular loop.

- It undergoes a number of proteolytic events shown by these little arrows. The one that's best characterized is the so-called GPS cleavage. So this is an autoproteolytic cleavage event which means there's no protease involved. It just basically cuts itself the way it organizes it. And you know this leads to that large extracellular domain. And then this intermembranous domain, and the 2 of them remain non-covalently associated. So they travel together for at least a certain period of time. And then this complex interacts with polycystem Ii polycystine. 2 is a member of what's called a transient receptor potential Channel family. These are sensory channels. So if you eat hot peppers and it's hot. That's the capsaicin receptor. That's a trip channel, as is the menthol, a cool receptor. There are many others. So the idea that this complex forms a receptor channel complex on primary cilia and the primary cilia I'll show you in a minute is actually reasonable.
- And 25 years after the fact, it's still not proven. So reasonable concept very hard to prove. Now, the cilia are a particular kind of cilia, the primary cilia refer to non-motile cilia. They are single organelles. This is the apical surface of a rapid rabbit collecting duct. And there's that cilium is just sticking up over there. That bright thing the unique features of cilia are that everything that goes into the cilium is produced in the cell body. There's no protein synthetic mechanisms in the cilium.
- There is essentially a gatekeeper. It's called a transition zone, which regulates what gets in, how much gets in, how fast it gets in, how fast it gets out, etc. And so this is a very restricted compartment. That likely is a sensory organelle, I mean. Why would you stick an antenna into the lumen of the nephron if you didn't want to send something and it's built by a process called intrafagell transport, which is a convenient way to attack cilia when you want to get around to it. I only show you this because it's pretty. This is a Pkd one with a certain kind of epitope tag. These are live cells, and you can fluorescently label Pkd, one in the living cells, and there they are. In Cilia. The red is a different is a marker, but the green one is the Pkd one. So it's there, at least in overexpressed systems. We now have evidence that the endogenous protein is there too.
- This, if you read a review on Pkd, and this was an older one, but it only gets worse. This is what you see and there's a lot of information here, but I'm going to focus on the I study polycystine one and 2. So this is like a Where's Waldo? Where's Waldo? Moment? Where's Pkd one and 2. So it's in the cilium. And how does it relate to all these beautiful pathways that it's tied to? Well it used to be thought that somehow it changed cellular calcium that's been debunked. So that's not the case. So basically, what we have is a black box. We have polycystems in cilia, and we have all sorts of stuff that changes in the cell. And we don't know how that's connected. So I actually prefer this more recent review, which basically summarizes what we actually know, which is that polycystem 2 forms a homo tetrameric channel. So 4 subunits. It's robustly expressed in the er it's also expressed on the primary psyllium. It passes mono and divalent cations. We used to call it a calcium channel. Now, it's like this debate about that. It's probably voltage gated to some extent. In addition to that it is hypothesized. And there's some data to support this, that there's a separate complex that is one polycystine, one and 3 polycystines, 2. So heteromeric. Complex. This is that cleavage event I mentioned to you, and these are also obviously in cilia, and then the rest of it remains that black box.
- So how do you look into the black box?
- Well a couple of things you have to. You want to ask. The 1st thing is, how does cysts form right? Because I show you the kidneys are normal at age 20, at age 50.

They're a mess, but they're normal. At age 20. Your genome hasn't changed, or has it. So the reason that this happens is because cilia is a Pkd is a two-hit disease so important. I didn't used to show this. I used to show this. 20 years ago. I stopped showing it. I'm showing it again now. All segments of the nephron get rise to cyst. The reason I feel it's important to say that is because what's tolvaptan tolvaptan is the Vasopressin 2 receptor antagonist. Where's the Vasopressin receptor 2 expressed collecting duct and so all of a sudden there's been some, and collecting duct cysts do grow faster than proximal tubal cysts, but all segments, these are human kidneys that are microdissected, and all segments of the nephron give kidneys these early cysts. All segments give rise to cysts. They do grow at different rates, but I think we have to keep that in mind, because when you think about therapy, you don't want to just target collecting duct cysts and ignore the others.

- So now, back to the two-hit mechanism. So normally, this is a normal nephron. If an individual has Pkd, he or she inherits one mutant copy of Pkd one and one normal heterozygous state all of a sudden something happens to a cell along the nephron, a somatic second hit mutation. So now you lose that normal copy. And once that happens this, these cells begin to change and begin to change, and they begin to sort of grow out cysts. And here's a picture from one of our mouse models, and you can kind of see this is a pas stain. So the red stuff is proximal Tubal brush border. This is probably a collecting duct because it has no brush border. It's in the cortex, and you can kind of see that this is happening just as in the cartoon. And then these cysts grow, and eventually, once these cysts grow enough, they get walled off from the nephron, the nephron either drops out or is no longer connected. So then these cysts have to have several features. One is they've changed shape. They've gone from a really nice columnar epithelia that we have in the kidney with beautiful mitochondria to kind of these really flattened out cells, they still have tight junctions. But now the mitochondria are a mess.
- They have low, level proliferation. It's not a primary proliferative disease. You don't get big clumps of cells. You just get these, you know they do get the expansion they have to secrete into the Lumen. Right? How do you get fluid into the lumen? Once you form a cyst like this, it has to be secretary. The nephron is normally a resorptive, so it's changed its properties. There are other features that occur, so inflammation we talked about that all these secondary features that happen, and fibrosis, and so on. So how do we know about this disease? Well, this particular model? I just want to show you very briefly how we came to this.
- We're only going to. We're going to skip the last part looking at this. But anyway, so we made we try to make a knockout many years ago of Pkd, and the way you may make a knockout is you put something into an Exxon that you don't want that you want to disrupt the Exon, and you replace the normal exon. Well, in this particular case we didn't replace it. We added it in. And because of that this, this system is able to undergo a process called either inter or intragenic homologous recombination. It doesn't make a difference. Basically, this allele is metastable. It can undergo a somatic second mutation to either form a complete loss of function, allele or revert to a wild type allele. And the issue is that humans have 30, 40 years to have a second hit and grow cysts. Mice, I give them like 3 months. So the time scale is just different. And so there's, you know. So if you just go with the normal mutation process. You'll have one or 2 cysts. But with this mouse you get pretty significant cystic disease. And so you can appreciate this mouse's kidney. Looks like if I took that initial kidney I showed you in the section through. This is what it

looks like. Big cysts, normal areas of parenchyma around it like over there. This is the liver actually in this mouse. So these mice also get polycystic liver disease.

- They actually get pancreatic ductal cysts. So we described these. And then clinicians went back and looked at patients. And about 10% of Pkd patients have ductal cyst pancreatic ductal cysts. They're not clinically significant.
- So we're going to talk about the dosage phenomenon. So I mentioned to you that we initially, we found these patients who have polycystic liver disease. But if you look at their kidneys they're normal. And so we thought, gee, this is a good model to find some genes and maybe give us some other members of the Pkd pathway, right? Because we didn't understand what Pkd genes were doing. And this just gives you an appreciation for the mass effects here. So eventually we found 8 genes or so. And where are these genes? What insights did they give us? Well, the insight they gave us is hmm! They're all in the endoplasmic reticulum. So all of this is the er here. So this is nucleus transcript over here. This is the endoplasmic reticulum. So it turns out. They're all involved in the biogenesis, maturation and folding of proteins that either go into the secretory pathway or integral membrane proteins.
- And so all of these phenotypes resulted from that, and that was kind of curious to us. And so, not having a broad sense of biology, we thought, well, gee, this must be messing up the biogenesis of Pkd, one polycystine one, and that's why these patients get cysts. Now, there are other. There are thousands of other client proteins for these systems, but none of them manifested anything that we can pick up, at least not on an ultrasound. So I think that's what was happening. So how do we show that?
- So we made a mouse. So the rest of this talk is going to be about mice. We made a mouse, and initially we made a mouse where we knocked out one of these polycystic liver disease genes. This is the glucosidase. 2 beta subunits involved in protein quality control in the endoplasmic reticulum. And when we did that in the kidney, we knocked it out in the kidney. We got kidney cysts. So okay, that's good. Because we started with a gene that we discovered in humans. That's liver cystic. It causes kidney cysts. So at least we're on to something that may be related.
- And then we reasoned that if it's involved in the biogenesis, then if you reduce the starting material for the biogenesis, it should make the disease worse. Right. If it's rate limiting already. In other words, you don't make enough of the good folded protein. If you start with less messenger, Rna, then you'll make less of the good folded protein and should get worse. So we mated these mice onto animals that were heterozygous for Pkd. One. So only one copy of Pkd. One rather than 2. And these mice, as I mentioned, you don't really get cysts in real time.
- However, when we combine these alleles, we got a much worse cystic phenotype. So that's consistent with the fact that less starting material, inefficient process, worse disease. And it's Pk, 81 dependent. We did the same thing with Pkd 2, and we got intermediate worsening, but not as bad as this. But then we did one other thing. We said, Okay, well, what if we do the opposite. What if, rather than reducing the Pkd one, we actually added 3 copies of Pkd one. So this is actually this mouse here. It's heterozygous for pk. 82. But now we've added 3 extra copies of Pk. 81.
- And all of a sudden the cysts go away.
- So, in fact, it is related to Pkd one dosage and Pkd, one is the rate limiting component of this process. And to prove that we, you know, when we went back and did the Pk, 82.

- The extra copies of Pkd, 2 don't help things at all, because basically it's Pkd one's rate limiting. So that kind of proves that the dosage makes a difference. Now, the issue here is that here the Pkd one genes are normal, and what we're messing up is in these patients with polycystic liver disease we're messing up is the biogenesis.
- So we now more recently did another study where we took a basically what's called a hypomorphic allele. So this is a point mutation in Pkd one that came from a patient, and we just made the same mutation in the mouse and when you look at it. I mean, I won't go through the details, but you get assisted. Kidney. However, when you use a transcription factor called Xbp. One, to turn on a panel of chaperones. So these are just this is a natural cellular response. It's not a molecular chaperone. It's cells chaperone response. When you turn on the chaperone response. You can improve the kidney disease. So this is now a human point mutation, and you can turn on if you turn on chaperones which help it mature better, you get an improvement in the phenotype.
- So this actually has made its way to clinical trials. If you guys know about cystic fibrosis, you know that there's correctors out there for Cftr. And so that same company is interested in correctors for Pkd.
- Now, the difference in Cftr and Pkd is that, you know, Cftr, 70% of them have a delta, 508 mutation. And there's some other mutations that are common. Not so in Pkd, so they're looking for correctors for class effects. Another drug which may be going to phase 3 just past phase, 2. Clinical trials is an antimer. So an antisense oligonucleotide targeted at mer. 17. This is work from vishal patel at ut southwestern and the idea here is that the microrna is upregulated binds to the Pkd one gene, and results in down regulation of the messenger Rna by degradation, by the Argonaut pathway.
- So if you use an antimer, so you block the microrna from binding the messenger Rna of Pkd one. Then you basically increase the steady state levels of Pkd one and by doing that they seem to be able to slow disease in the phase. 2 clinical trial. I'm not going to comment on anything to do with the drug trial. I don't know, but the principle is there that you're increasing the messenger, the steady state messenger Rna level, even though some of it is mutant. And you may get a benefit.
- So this. Actually, you know, a few years ago, when I gave this talk, how do you get from a from a gene discovery therapy? I said, well, you don't. And now I can say that you know at least some of this stuff has has made its way through.
- So in the last sort of 10 min to to maybe start a little bit. Stop a little bit sooner. We. We asked the question of if you could do the best possible therapy.
- What's the best possible? What's the best outcome you could expect? And what's the best possible therapy? Well, if loss of the gene Is causes the disease, then replace re-expressing. The gene is your best possible therapy. Go back to, you know. So a little more mouse genetics or whatever mouse engineering.
- Okay? yeah, it does not like these slides. Okay, here we go. Good. So I don't know if you can actually see that. But suffice it to say, this over here is a normal Pkd, one allele. So it expresses normally, but it's fluxed, which means that we can conditionally inactivate it.
- This one here is a null allele. So basically, it doesn't work. So this is the Heterozygote state that all patients and mice inherit.
- But now we've added another copy of Pkd one here just on a random chromosome, and this copy is silenced so that it it cannot express right now. But this one can be turned on, and it's a different recombinase than this one. So we can use the cre

recombinase to inactivate this one. And now we have no functioning copies of Pkd one. And when we do that we use doxycycline to induce it, we get cystic kidneys, and then we can use tamoxifen as a drug to induce the activation of this allele. So now this one gets turned on, and we can now reverse the disease.

- So that's that was, that's the mouse and it actually works. So, you know again, these are just aggregate data. I'm showing you that I'm not. I'm not giving you like the best picture I have. But this is the 13 week kidney. And so this is a normal kidney. And when we knock out at 4 to 6 weeks and look at the kidney 13 weeks, it's bigger. You can't really see cysts here so clearly low level. But you can see it here right? There's a lot of dilation tubules, if nothing else.
- If we let the mouse go 3 more weeks, the kidneys are more cystic, and you can see that here as well. If at 13 weeks we turn the gene back on then by 16 weeks. Sorry by 14 weeks. The kidney is already smaller, and it looks like this. So you've gone from this to this in one week after turning the gene back on, and 3 weeks later you go back to a normal appearing kidney. Now, these are not very cystic when they start out you can see there's lots of dilations, but they're not very cystic. And so this represents, you know, reversal of disease, and not something that we thought the kidney could do the other way to look at this is in mice is to do serial, Mris. They're not quite perfect, but they're good enough. The mice don't seem to cooperate with the motion requirements. We make them sleep, though, so that's okay. Anyway, I'll show you the extreme example. Here are the ones that are more representative. But here's an extreme example. This particular mouse had a huge had a huge pair of kidneys at 13 weeks. These mice are congenic. They're essentially twins. But yet there's variation in how this disease progresses, which I don't know how that happens. But it happens in the mouse, too.
- These are huge kidneys. 3 weeks later the kidneys are smaller and 9 at 3 weeks after that, 19 weeks of age, they're smaller still. So these kidneys actually shrink. And then, you know, having said that when you start out with really cystic kidneys, you can still get reversal. But it's not perfect. So here's the other experiment that we did where I can show you some histology, which is last time I told you we started at 13 weeks. This is the 16 week kidney. So it's more cystic, right?
- And I can show you the histology here. Hang on for a second. So it's more cystic. This is the starting point. And now, if we re-express, we end up with this kidney here so definitely goes down in size and so on. If we don't re-express. It stays the same. But when we look at the re-expression now, it's not as beautiful as the other kidney I showed you. There's some. This is a trichrome stain. There's some fibrosis here, and there's some inflammatory infiltrates. There's also a lot of normal appearing tubules. So these kidneys actually you know if you have scar and bad inflammation. It's not going to go away, but it will reverse a lot of the cystic disease. And then, you know, pictures are nice, and kidney size is good, but does it affect kidney function? So the answer is, yes. So here these are 16 week old mice. The normal Gfr in mice is here, and these are reduced about 60% kidney function. When we do re-expression, almost every one of them, except one, had improvement in Gfr at 19 weeks.
- So not only does the structure of the kidney get better, but actually the function also improves and then the last one is this. So? This is a nuance of the the way these models work the cre recombination. You know you have a promoter that drives it across. Let's say the whole nephron. So you have simultaneous inactivation of all of the copies of the gene along the whole nephron.

- That's different from the cartoon I showed you for the 2 hit where it was like one cyst, and then it kind of grew out. But here's this model which I already explained to you, and so we did Mris with re-expression in this model. So these cysts, you can actually see individual cysts on an MRI, and when we re-express, this is 16 week old kidney. This is the same animal. When we re-express, you begin to see the kidneys, the cysts disappear and I'll show you a better, not a better, but a different picture of that. So here, for example, is this very large cyst in the lower pole, this kidney and when we re-express now you no longer see that there, this cyst is probably this cyst, so you no longer see it there and when you take out the kidney, though you can see that while the cyst is gone. It's still a scar, right? I mean, this is not going to go back to being a normal nephron. So you still have a scar there. Similarly up here you have a couple of cysts above this larger one. When you re-express, the larger one gets smaller, and these cysts disappear, and when you look over here, you kind of see there's some stuff going on there. When you look microscopically at that region you can see that there's some scar tissue there. But there's also normal pranccum around it. This is that cyst that remained but got smaller right there, and you know. And so so I think that there's, you know there's protection involved here. But you can't fix certain things.
- So I think timing of these things are going to be important. Last thing I mentioned to you is that liver cysts are the same mechanism. So we did re-expression of liver cysts. This is the starting point. This is untreated, and this is re-expressed, and the liver cyst, except in this one, went away. And if you look at this microscopically or histologically, this is what the 13 week livers look like these are the normal bile ducts. You can't even see them here. These are venules here. It's a portal triad. So the bile duct is these small things. You get this proliferation from the Pk. 81 knockout, and this is Pkd 2. I apologize, and 16 weeks it progresses. When we re-express. It turns out that you don't go back to the normal portal triad, but the cysts empty out, and you can see that there's still probably some proliferative bile ducts remaining, but they no longer are cystic. So this begs the question of the mechanism here. I don't have an answer for you but we can talk about it. I mean, I have ideas. And then this is sort of the natural history of what I showed you before 13 weeks. You re-express very rapidly. You get normal kidney size at 17 weeks, 16 weeks. You re-express very rapidly by. You know you get very rapid decline in kidney size. And then, interestingly, this is actually not unsignificant. I'm not sure it got significant, but it continues to get smaller over time. So you know, we wanted to know. Well, you know, you might have good kidneys in 19 weeks. What happens at 24 weeks? Does it start getting worse again? Well, no, it actually gets continues, it's sustainable thing. So if you could do gene therapy, it would work all I will say, and it's 1. 0, 8.
- So I am probably okay with stopping at this point. I have one more story to tell you. I can show you perhaps 3 slides on this one, and I'll populate the thing first, st and then I'll stop. And so the other funny thing about Pkd is that if you knock out cilia as a 1st order approximation, the kidneys are normal. If you knock out. Pkd, one same Cree same time, point very cystic. If you knock out both cilia and Pkd it actually prevents this growth. Excuse me.
- So this brought us to this idea of Cdca, which is a cilia dependent cyst activation. So what it says is normally, polycystines are on cilia. They regulate some function of physiologic function of the kidney tubule, maybe flow ligands of flow, etc. Etc. On the luminal side, and they probably adapt the kidney tubule cells to whatever

environmental stimulus they are. In the case of Pkd, where you lose these genes, this Cdca pathway, which normally is up and down now is just one directional. It's effectively a gain of function, and this grows the cyst. And if you don't have cilia I won't say this doesn't work at all, but it's markedly reduced. So really, cilia, drive this gain of function. And so we wanted to look at some. I'm going to skip a few slides now, but we wanted to look at some of the factors involved here.

- And I will not go through. This takes a little while for this thing to move forward. Sorry and I will show you the punchline, and then we'll stop.
- So we found this factor
- called Gls-two. Amongst these things, and double knockouts of Glyc, 2 prevent cyst formation. So it's a factor that we found related to the Cdca. It works in liver as well. And importantly, we used asos to actually knock it down as a therapy in the mouse, and it actually reduced kidney size from the cystic to the non-cystic. So what we now have a variation on the cartoon I showed you before is that this is the normal Cdc function. When you do this, then you have it.
- This continued effect. But now we have a downstream target. Here I have gls 2 going up, and so glist 2, it turns out, is required to grow the cysts. So if you get rid of glist 2 by the aso or the knockout I showed you, even though you have intact cilia and no polycystines in them. You don't grow the cyst. So now we have 2 points on the map here, which is better than 1, 1 being polycystines and cilia. Now we have a nuclear transcription factor, which is what glse 2 is. And now we just want to answer the questions of what's between the cilia and the polycystines. And what's
- Gls-two? What are the transcriptional targets of gls? 2.
- So I will stop with that and show you a picture of us in Orlando a few years. This was like a reunion from former lab members present lab members, my wife and I. You know I showed you pictures of folks. I didn't get a chance to talk about specifically, and with that I'll stop and answer any questions.
- We'll open it up to the, to the room for any questions.
- Go over here.
- Thank you so much was fascinating. Could you talk a little bit about what their effects of lack of cilia on the cell? And can the cell survive without cilia.
- Yeah. So it depends on the cell.
- So you know, if if you have no cells in your olfactory epithelium, you don't smell if you don't have them in your eye, in your retina you can't see. And neurons probably is real important, too. It turns out in the kidney. If you take adult mouse and you knock out the cilia. The kidneys are pretty much okay from what we know, but of course we don't know what functions, you know, because other sensory functions in cilia probably are lost. Mice have pretty low metabolic demands, but the cells definitely survive, and they're not that proliferative. Now the trick with cilia is if you knock them out and you wait long enough. They actually grow cysts separately. So this is work that Peter Garashi did many years ago. So they actually do form cysts. But it's a different time scale. That's actually why we did the experiment. So Pkd knockouts with intact cilia is rapid cilia knockouts, slow cyst, and the slow cysts are going to be not relevant to, you know, if you get cysts at 150 years of age. The problem is that you know, how do you target cilia specifically in the kidney without touching cilia anywhere else?
- And that's, you know, a fraught question. But but yeah sorry.
- Do your mice get brain aneurysms as well? And if so, do you see those disappear? Yeah. So one of the limitations of the brain aneurysm issue is that there's no animal

models for it. People have tried to induce hypertension mice with Pkd, and you know I don't think the aneurysms are too hit. So I think the genetic mechanism is different. I think that having one copy of Pkd is a sensitizing event.

- You know, there's 3 candidate or 2 candidate cell types, endothelia, or smooth muscles around these larger vessels. But it's hard to know. There were a couple of mutations, one particular mutation in human pkd, that seems to be more common in patients with aneurysm. But it doesn't have statistical significance. We're doing some stuff. We made those animal models, actually. And they're being investigated by my colleague, Terry Watnick.
- But the short answer is, we don't have a model, and we don't understand the genetics. So we've actually been recruiting patients about 500 patients now with Pkd and aneurysm, and we're doing Gwas. And whole exome in the hopes that we get some clues to what the risk factors are small population. But again, if we do it right, we might get something. But so far nothing so very nice. Talk, amazing overexpression of Pke. One causes stops nuisance from occurring, and it induces the currencies to start go down, so the process must be must involve some sort of autophagy or other genes. So if one can trigger those autophagy genes, can you slow down the cystogenesis?
- All right. So couple of things. One is we tried. Not so the back transgenics are actually low copy number expression. They're not really overexpression the way conventional transgenics are very high copy numbers of Pkd one. And people ask this all the time, actually cause cyst, okay. But we're talking 30 to 90 copies. What we've done here is just one to 5 copies which has no phenotype.
- The second thing is, actually, we looked at a few of these things, not everything, though there's no apoptosis in that reversal. So it's not like. There's a slew of cell death when you re-express it. That gets rid of all the cyst cells and autophagy is interesting. It turns out that Pkd knockouts actually have reduced autophagy, and when you re-express it you have increased autophagy. But I take that as autophagy without apoptosis. So really what the cell is doing is rebuilding itself in a different form back to the columnar epithelia. So that's what we've looked at. There may be some pyoptosis in these things, but we haven't explored that a huge amount. I don't think cell. I think it's a change in cell state more than a change in the population of cells. What we're doing now is lineage tracing these cells. So knowing which cells, we're getting the expression on and seeing what their fate is by using the fluorescent reporter. But we haven't completed those studies yet.
- Yeah, I'm I really enjoyed your talk.
- And and I've got a lot of questions. But I'll just ask just a couple 1, st one is only one to 3% of nephrons develop cysts. And so we're just wondering those genes for Pkd, one or 2.
- And those abnormal genes are then not present in the other nephrons, or or or what is going on to keeps them from getting cyst. So it turns out. It's it's the second hits that aren't present. The nephrons. These genes are ubiquitous, and they're actually required for the nephrons. They're required for other stuff, too. But it's that second mutation, the somatic mutation that only occurs in a minority of locations in a minority of nephrons. So that's what's driving it. So you know it turns out that the reason so why is Pkd, one more severe than Pkd, 2 rhetorical question. So the reason for that is nothing to do with the function of genes. If you knock out Pkd, one germline in the mouse, so you make it null. It's actually a more severe phenotype, not a cystic phenotype, but has lots of other phenotypes. But if you just compare

the 2, the progression is the same. Pkd, one and 2. The difference is that. Pkd, one is a 12,000 nucleotide coding sequence. Pk, 82 is a 4,000 3,000 nucleotide coding sequence. So the frequency of second hits is going to be your 1st order. Approximation be 4 times as great in Pkd one, and what people have done is they've measured the rate of cyst growth. Pkd, one and 2 cysts individually grow at the same rate. But the number of cysts in Pkd 2 is lower. So it's all related. Again, if you have Peter Harris here then he's going to give you a different story. But let's let's say this is an area of conflict. It's all related to the number of second hits, and that that results. That explains the stochastic nature, the variation within families to some degree. It explains why only some nephrons are involved, and explains the differences to me between Pkd. One and 2, and and then you also showed one other thing. You showed some a fair amount of inflammation going on as well as indicating that Mcp. One is not a good prognostic factor. Yes, so I'm just wondering if by accident someone transplanted a very early polycystic kidney into, you know, transplanted it, you know, like how it happened with diabetes, but whether something like that happened with polycystic, and you put on immunosuppression. Whether it prevented the cyst from coming from developing so 1st part of the question is, you know, so if a young kidney donor, especially without a known family history. Certainly there have been instances of polycystic kidneys that were not known to be polycystic transplanted in the patients. So answer number one is those kidneys develop cysts over time. Anecdotally.

- And this is anecdotal. So you know, like there may be 2 different recipients for that kidney from that one donor the rate of growth in the system, the recipients for the 2 kidneys where you saw in the images, they typically in one patient, they're typically similar. They actually can be discordant. That's anecdotally so you know, it's hard to assess the immunosuppressive effects on that. But things like, for example, I didn't mention this but acute kidney injury can make Pkd cysts grow worse. So if you take a mouse and you knock out Pkd gene, and then you do ischemia reperfusion. The cysts grow much faster. I don't know how much, how clinically important that is in humans, but certainly, if you had an atn and you have Pkd. It cannot be good for you and maybe bad for you.
- But in the transplant maybe the cold ischemia, time or differences in the transplant procedure might result in the discordance between the same genetic kidney being transplanted in 2 different patients don't know about the immunosuppressors. There was a time when Mtor inhibitors were considered as therapy for Pkd, I think the data for that is not good. The studies were negative, and I wouldn't go there in the future. But that's just me.
- I'll try to be the last sorry fast. Thank you. Excellent talk, I totally understand, based on your proposed mechanism, why you could reverse the progression disease. But why do the cysts get better? Why do you? Why does Restoration suck back the fluid? That was less clear? Yeah, so the short answer is, I don't know. The longer answer is, Well, I can think of. And you guys, if you guys have other suggestions, I'm open to it. I can think of 2 mechanisms. One is, it just changes the transfer properties of cell. And then you get basically transcellular resorption rather than secretion going on.
- The other possibility is that there's a step in there where there's loss of integrity of the you know the tight junctions in essence. And so you get interparescellular fluid going in fluid, going paracellularly into your system, and then getting picked up by the lymphatics and getting emptied out. So I don't know which of those 2. It is in

some ways the rapidity suggests. Maybe the latter like there's, you know, but I don't even know how. I don't know how to get at that in the mouse, which is pretty small kidney in the mouse. It's really hard. The mice are. The kidneys are small, and you know the Ws 25, that one with the MRI. Maybe we could do. We've talked about this. I don't think we have the skill set to do it. I think it's technically challenging, and I can imagine that the injection itself will empty the cyst, and people will get nervous in a pig model. You could do that

- I can't afford a pig model. I don't want to work with a pig model, but you know, but that's that's they do exist.
- So when it regenerate and return back. Is there that regeneration, for example?
- Right? So so. And you know, when you talked about transport, I'm the wrong person to talk to. But Michael Kaplan is the right person to talk to. But there was a thought that the secretion into cyst is chloride, driven chloride dependent, and Jared Grantham and Michael, many years ago, actually looked at Cftr, and there was some thought that it was Cftr. Alan Verkman did some Cftr inhibitors in these models, and got kind of a middling result. I'm pretty sure Vertex has tried this kind of stuff. I don't think Cftr is the answer here, but I'm not a transport person. But it was definitely. It's definitely one of the possibilities for what drives.
- What is driving secretion is a chloride, dependent secretion.
- All right. We are at time. So thank you, Dr. Samo, for your time.
- Thank you for coming here and being our distinguished lecture, and on behalf of the division and department.
- So thank you very much.