Genetics and Pulmonary Hypertension

This winter, Greg Elliott, MD, Professor of Medicine at the University of Utah School of Medicine, and Emeritus Professor at Intermountain Healthcare, and Usha Krishnan, MD, Pediatric Cardiologist and Professor of Pediatrics at Columbia University Medical Center, gathered with Wendy Chung, MD, PhD, Professor of Pediatrics and Chief, Division of Clinical Genetics, Columbia University; Paul Yu, MD, Associate Professor of Medicine at Harvard Medical School and Brigham Women's Hospital in Boston; and Eric Austin, MD, Pediatric Pulmonologist at Vanderbilt University Medical Center, and Associate Professor at Vanderbilt, to discuss the genetics of pulmonary hypertension.

Dr Elliott: Let's begin by introducing the participants in today's Pulmonary Hypertension Roundtable focused on the genetics of pulmonary hypertension. I'm Greg Elliott. I'd like to introduce my comoderator, Dr Usha Krishnan.

Dr Krishnan: Hi, it's a great honor to be here. I'm a pediatric cardiologist, with my main interest being Pulmonary Hypertension and a healthy curiosity in the genetics of PH. I'm excited to learn from the experts on this panel. We're joined by Drs Wendy Chung, Paul Yu, and Eric Austin. I'm going to request each of them to introduce themselves, and we'll start with Wendy, and then Paul, and then Eric.

Dr Chung: Hi, I'm Wendy Chung. I'm very pleased to be here. I'm a pediatric geneticist, also based out of Columbia University with a long-standing interest in pulmonary hypertension, and I'm always learning because we don't know everything about genetics or causes of pulmonary hypertension.

Dr Yu: Thanks. I'm Paul Yu. I'm an adult cardiologist with an interest in pulmonary hypertension and a research effort focusing on signaling in the bone morphogenetic protein (BMP) and TGF-beta signaling pathway. I've been interested in the genetics of pulmonary hypertension as a way to get new insights into the mechanisms of disease, and, hopefully, to identify new treatment targets. I have a small academic practice and follow some patients with pulmonary hypertension, and I'm part of a pulmonary vascular disease program at our institution. Some of my research is informed from that clinical experience, but just as importantly, from clinical PAH experts in our communities such as the ones we have on the call here, with whom I have the privilege of consulting and working.

Dr Austin: Hello, I'm Eric Austin. I direct the pediatric PH program at Vanderbilt. I've had the privilege of working with each of the people on this call, and many other people in the field for many years, with an interest in pulmonary hypertension. I have a laboratory working on translational investigations of individuals with pulmonary hypertension, and also human cohort studies including clinical trials trying to translate what we can learn.

I know less about genetic science than everyone on here, but I do my best, and really feel it's important. I'm grateful that we have this topic because our pediatric PH patients and their families really embrace this, so I'm grateful for this opportunity with you as well.

Dr Elliott: Eric, I'll take my prerogative as one of the moderators to introduce the audience to some of the history of our current understanding of the genetics of pulmonary hypertension. I'd like you to start by just giving the audience some idea of the work and the discoveries that shape the current understanding of heritable pulmonary hypertension.

Dr Austin: Absolutely. Pulmonary hypertension, as we know it as a heritable disease, was originally described in the 1950s by Dr Dresdale up in New York, who described early families who had pulmonary hypertension that ran through the family. That work continued through the years, and investigators began to think about "how can we capture these families and use them to understand the biology and the genetics of the disease better over time".

In the '90s, there was a great interest as the genetic revolution was happening, and really well on its way in discovering what are the underpinnings genetically that cause PH, particularly, pulmonary arterial hypertension, what we then called primary pulmonary hypertension, in families. Familial pulmonary arterial hypertension (PAH) (a subtype of heritable PAH) is 2 or more individuals who have pulmonary hypertension in the family. Many familial PAH patients and families consented to participate in studies in which investigators in the '90s really tried to determine the shared genetic cause of PAH within and across families.

Multiple groups were instrumental in making this discovery. There were international collaborations in North America and to the UK and beyond. There was work at Columbia University that was led as well. Large investigations culminated in 2 distinct papers that both found that the primary gene at the time, that we understood associates with PAH, was the gene known as bone morphogenetic protein receptor type II, or BMPR2. Those investigators, collectively, really put in a tremendous amount of work in the discovery of BMPR2 as the predominant cause of familial, and now, what we call heritable PAH. And, those family participants were instrumental.

There are certainly other causes, and people immediately, of course, began to try and understand not only why and how does that cause disease, also, they tried to figure out—is BMPR2 also applicable to other forms of PAH? Sub-

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sequently, it was determined that somewhere in the order of 15% to 20% of idiopathic PAH may harbor mutations in BMPR2. People such as Dr Yu began to think about signaling and why does BMPR2 contribute to the pathogenesis of PAH. People such as Dr Chung and colleagues began to think, "Well, wait a minute. Not only do we need to expand on our understanding of BMPR2, but can we go beyond BMPR2 and understand other causes of genetic-associated PAH?"

An early discovery was in the disease HHT, which we already knew caused a form of primary pulmonary hypertension in a small number of HHT patients (HHT is short for hereditary hemorrhagic telangiectasia). This led to the discovery that not only BMPR2, which is a TGF-beta superfamily member, can cause PAH, but so can HHT-associated genes that are mutated, endoglin and ALK1. Those reports came from here in North America as well as in the UK. Dr Elliott was part of many of those studies. Discovering that now we had BMPR2, ALK1, and endoglin, in the early 2000s subsequent work studying more and more families led to more and more discoveries of genetic-associated underpinnings of PAH.

Dr Chung leads a lot of those right now, with many of us in both adults and children, but other people across the pond, in Europe, particularly, in the United Kingdom and in France, have investigated these, as well as individuals in Asia. It is now known that a large percentage of pediatric PAH and a decent percentage of adult PAH is actually related to genetic causes. Not only BMPR2, not only ALK1 and endoglin, but other irregularities in other genes as well.

So, we've gone beyond TGF-beta to other genes that may or may not be related to TGF-beta signaling of a great interest, including TBX4, SOX17, and EIF2AK4. EIF2AK4 is associated with a less common form of pulmonary hypertension, but still incredibly important, known as PVOD-PCH-spectrum disease. While there is much more to share, I hope that gives a decent quick overview, Dr Elliott, about where we've been and where we are now as a field. So much exciting stuff going on and a lot more to learn, but we've learned that genetics truly is majorly important to the pathogenesis of many PAH forms.

Dr Krishnan: Thank you so much, Eric.

Dr Elliott: Eric, thank you. You just covered an incredible amount of work in a short time. All the people who contributed, and there were, as you mentioned, so many people around the world who contributed to these discoveries, they would be really impressed to hear how fast you ran through it in comparison with how long it took them to assemble all the pieces of this big puzzle and begin to make sense out of it all. Just the discovery of BMPR2 took years of hard work.

Paul and Wendy, do you have anything to add to that history so well stated before we move on?

Dr Chung: No. I'll just say that, as Eric said, he rattled off a bunch of different genetic causes. They're not all created equal in terms of the proportion of PAH patients with variants in these genes or with the same associated clinical features. We're also still learning about genetics. We still don't understand the cause of PAH for most people. Even in some cases where we have clear family histories of multiple people in the family with pulmonary hypertension and we think it should be genetic, we haven't figured it out yet. We have figured out a lot but not everything.

Dr Elliott: Wendy, that's a terrific point. We're not out of a job, there's more work to be done. Paul?

Dr Yu: I agree. I think, as Eric so nicely summarized, some of these mutations are complex in that they can present as different phenotypes besides pulmonary hypertension, including the HHT syndromes. I think that's one really important unanswered question, of how the same mutations in 1 gene can manifest differently as HHT or as PAH, or in some cases, as PVOD, as your group, Greg, recently reported. These different presentations can sometimes be found among different members of a family carrying the identical mutation. What are the additional genetic or nongenetic factors that cause these various mutations to manifest as any disease, and when they do, what causes them to manifest as a particular disease on this spectrum of vascular disorders?

Dr Krishnan: Right now, what we know is really the tip of the iceberg. As far as the genetics of pulmonary hypertension, there's so much more to learn. With that background, let's pivot the discussion to the current knowledge of genetics and how can we use it to help our patients. Wendy, very often, cardiologists and pulmonologists and other practitioners are not comfortable discussing the genetics with their PAH patients. What should doctors tell their patients about heritable PAH? When should genetic counselors be involved and what kind of panels and what is available everywhere? Could you please start the discussion and then we can have Paul and Eric to comment after you?

Dr Chung: Sure. I would say there are different scenarios that I see clinically; I'm going to start with the adults because that's where we see more individuals with pulmonary hypertension. There are some individuals with a family history of pulmonary hypertension. Historically, we haven't recognized everyone with a family history, because a generation ago, people may not have been diagnosed with pulmonary hypertension. In some cases, people didn't necessarily communicate the information within their family. My point being that you don't always get a positive family history, even when you go back afterwards and look carefully at the family.

Then in those cases, hopefully, the physician caring for the patient has at least gotten a cursory family history to know there might be something. Oftentimes, that discussion naturally comes up because people say, "Oh, my uncle Joe had this and is this the same thing in me? Should I be worried about my kids?" That conversation naturally comes up, and depending on how people feel about it, it's driven by the patient who is worried about their children or their nieces and nephews or

other relatives. The genetics of pulmonary hypertension are tricky because even if you have the genetic variant, it does not mean 100% that you'll develop the disease.

In fact, there are differences between men and women. Females are at about 40% lifetime risk. Males are at about 20% lifetime risk. We can't necessarily say exactly if or when any one person will develop pulmonary hypertension. At this point, I don't have a cure that I can give someone to prevent the pulmonary hypertension. That level of uncertainty for many people doesn't sit well. They'd rather not know unless there's news you can use, something that you could clearly do to prevent this condition. Otherwise, it causes some people angst or concern. They worry about discrimination, they worry about a lot of things, but that causes a lot of people to just be ostriches, bury their head in the sand and not have to deal with it.

There's also an interesting chicken and egg problem. We don't know about a large number of people who are at risk from whom we could actually learn quite a bit in terms of primary prevention and getting to the point where we could potentially prevent this disease.

There's the bigger group of folks which Eric was mentioning. The people who don't have a family history of pulmonary hypertension. Of those IPAH adults, 20% have one of these BMPR2 mutations, and a few more have another gene. For whatever reason, they don't recognize this because they really truly don't have a family history of pulmonary hypertension, and other people who might have the genetic susceptibility just simply aren't demonstrating any evidence of disease.

Again, when they think about their kids for instance, even though they don't realize they have the gene, they still potentially have passed on that genetic susceptibility to the next generation. Even though they're ignorant to it, the gene's still there. A lot of the doctors don't feel comfortable bringing it up in those cases because it's just pointing a finger at something that people don't want to even think about. They don't want to think their kids might be at risk, they don't want to think they might themselves have passed on a genetic predisposition. They don't want to feel the guilt associated with that because, again, they don't feel like there's news you can use. They're just like an ostrich—bury their head in the sand and go on from there. That's, I would say, the culture, sociologically, that we often see in adults.

I think it's going to be a completely different story if we get to the point where we can actually do something for disease prevention. If we had that magic bullet in terms of early diagnosis, early treatment, and early intervention to slow down or halt the disease, it would be a completely different discussion. But this is the chicken and the egg. If we don't do those studies, if we don't identify people early, if we don't start treatment early, we'll never know, we'll never know if those interventions work, and so we're stuck.

Eric and I are pediatricians. Parents of young children, they have a very different perspective on this. They're watching their little children, oftentimes, they're really little, and their children are sometimes dying of this disease. For them, they are oftentimes not finished having their children. They think of having other children, they think about their other children who are still at risk, and they're panicked. It's a terrible thing to lose a child. To go through that and the heartache that goes with it is just unbearable, and they can't imagine that, so they will do anything and everything to make sure that they can either save a child who's already with them and do anything to help a child who's already sick. If they're thinking about having more children, they want to avoid being in this situation again in the future.

I find parents of children are very aggressive, information-seeking, and want to have answers. They'll do whatever they can to keep their families safe and healthy.

Dr Krishnan: What about tests though? When do you order whole exome sequencing vs whole genome sequencing, and when do you just order a PH panel? When are genetic counselors involved in this process? Is it better to involve a genetic counselor to talk about the genetic background of PAH to families?

Dr Chung: There's no one way to skin a cat, so to speak. I think your strategy is based on your institution, based on the expertise that's there. I'll also be radical and say we're doing much of this now by video conferencing. There are ways of being able to get expertise that may not be in your clinic, so to speak, but you can teleport someone to be able to come into your clinic or to be able to deliver these services. Genetic counselors have a masters in genetic counseling and human genetics. They're very nice people, very knowledgeable, and patient.

They can sit for sometimes hours, talking to families to educate them about what this is, be able to understand their family, understand social dynamics, and do all the paperwork to get the job done. They explain things in a way that the family understands, and help with preauthorization to ensure the family doesn't get stuck with big bills, and communicate information to other family members. The problem is we don't have enough genetic counselors.

We also don't have enough genetic counselors who understand the genetics of pulmonary hypertension. Even though you might have a fantastic genetic counselor upstairs or down the hall from you, if you don't train them about pulmonary hypertension, and if they don't listen to this podcast and learn about it, then they're not as strong an asset for your program. We can educate them in terms of integrating them, and I would go for a model of integration, pushing in and bringing the genetic counselors into your program to be able to help you. If that's not possible, you can reach out to your genetics team and refer them over. My advice is, get 1 go-to person at your institution who really knows what they're doing and works well with you, and works with the laboratories since there are certain laboratories that are good in terms of good, reputable, and patient-friendly. They have billing policies that don't leave people stuck with big bills.

Your other question about what tests to order, I'll just make it very simple to say that you can do targeted testing with just pulmonary hypertension genes. The number of those genes, as Eric describes, keeps growing over time as we

find more genes. Sometimes, a test that you ordered 5 years ago is out of date because we've identified more genes over time and you miss something. That's a problem in terms of genetic testing. You may have to go back and order that test again if you miss something, and a family clearly has a strong family history, and you don't give up.

The other thing that we've developed as geneticists, is THE genetic test, T-H-E genetic test. For some people that's overwhelming, either in terms of cost or information, but for those people who get a negative targeted test and are really information-seeking want to go for the gusto, and go for what we call an exome. In particular, if it's a child, I include both the parents or include multiple family members who have pulmonary hypertension so we can do a comparison within the family genetically and compare what is genetically the same to narrow down that search space and find that pulmonary hypertension gene.

When I do that in kids, I very often find a gene I have never, ever seen before in pulmonary hypertension. We are still at that cutting edge of still searching and finding new information. The good news is that many times that mutation is de novo, or new in the child with pulmonary hypertension, and won't happen again in other kids. So parents can breathe a sigh of relief that they're not going to have to worry about that for their other kids or for future kids.

Dr Krishnan: Is that why mutations found in children are different from mutations in adults?

Dr Chung: Yes. For mutations in kids, if you think about it from a population genetics point of view, this is true of isolated pulmonary hypertension, but it's also true for pulmonary hypertension associated with other conditions we see in children. For instance, we see more diaphragmatic hernias and congenital heart disease in children associated with pulmonary hypertension. If you think about it, 2 generations ago, or even 1 generation ago, before we had fantastic surgeons, before we had fantastic pulmonologists or cardiologists, those children were born, and they didn't make it very long. They died of their disease, and they didn't live to have children of their own.

They couldn't pass those genes on because they didn't make it into their 20s to be able to have kids. From a population point of view, those mutations were born and died and born and died every generation. It's really only now that some of my patients are actually living long enough that they can be able to have children of their own, if they can survive. We are certainly now getting some kids up until the age where they might be able to have their own kids.

Dr Krishnan: Wow! Paul and Eric, any comments before we go on to the next question?

Dr Yu: I think that what Wendy said was absolutely helpful for understanding the role of genetic counseling and family planning for people who are currently unaffected. I wanted to ask our panel members how each of you feels that the knowledge of having a particular mutation affects your treatment plan and your approach to the affected individuals, also known as the probands? Given that we know from Dr Elliot's work and Dr Marc Humbert's work, that people with BMPR2 mutations are more severe clinically at the time that they present, and may have a more challenging disease, do positive genetic testing results affect how aggressively we should approach those patients, above and beyond their clinical status and hemodynamic parameters that you're already factoring into those decisions?

Dr Austin: I'll take that to start. I think that's a great question. What you're alluding to Paul, is true, that the metrics that we use to determine severity of PAH in the current era, including features that we learned from work by Drs Elliott, Humbert, and others, is that mutation carriers with PAH have metrics suggestive of a more severe disease condition than people who have nonheritable PAH. For me, as a practicing pediatric PH physician, this knowledge does influence the way I think about things.

The truth is, as you know, there's a lot of data now that upfront, early aggressive therapy, in many PAH patients, may be the right choice. But, I'm particularly aggressive in our practice and I think others are (but I can't speak for others of course) with PAH patients with a known PAH-associated gene mutation. I suspect there is a common scenario in which we think, "Well, we've got a person, they found a BMPR2 mutation in this person, so I'm going to be even more aggressive to move to a three-drug therapy option including some form of prostacyclin or prostacyclin-derivative fairly quickly." I am biased in that direction, and I do talk, frankly, to families about this potential treatment approach.

But, I try not to be overly influential about it, because we just don't have a robust amount of data. Wendy, I hope I didn't interrupt your response.

Dr Chung: No, no. Full disclosure, I'm not the one who's actually prescribing the medications here. All of my other colleagues are the ones who are actually doing the management, but I think about the comparisons between other genetic conditions and pulmonary hypertension I would argue more aggressive treatment and even earlier before vascular remodeling, could cut this off at the beginning early on in this process.

Realizing that not everyone at genetic risk is going to get sick, we have to have real time markers to see who's progressing to develop pulmonary hypertension. But let's not wait for symptoms, let's think about how we can intervene earlier. Mutations in BMPR2 are dominantly inherited, and if you have 1 of these genes, there's 50/50 chance that your brother, your sister, your kids have this as well, so identifying 1 person in the family, you can potentially save lives other members of your family by giving them a heads up with this information.

Dr Elliott: Yes. As a moderator, I want to jump in for a couple of points. One, Wendy, to your point, we in Utah, and, Eric, some of your senior colleagues at Vanderbilt have seen an occasional family member who lives in a rural community where they were thought to have asthma until they presented with very advanced PAH. Had the BMPR2 mutation been uncovered early and

treatment started early, we would expect that they would have had a better outcome. Discovery of pathogenic BMPR2 mutations creates an opportunity for earlier recognition and treatment of this progressive, often fatal, disorder.

One other gene marker that's important on the treatment side, is EIF2AK4, which causes pulmonary veno-occlusive disease (PVOD) / pulmonary capillary hemangiomatosis (PCH). PVOD/PCH doesn't respond well to our current PAH therapies, probably because it's a very different pathway, isn't it? It's not a TGF-β pathway like BMPR2 or ALK1. In fact, some of our PAH therapies can precipitate pulmonary edema in patients with PVOD/PCH. There's a great example of the value of genetic testing. Recognizing EIF2AK4 mutations when it looks like idiopathic or familial PAH can very definitely influence our sense of prognosis, disease progression, and response to therapy, so I would always put that in there.

Fortunately, the data that we've seen so far tells us that PVOD/PCH caused by EIF2AK4 mutations is rarely found in patients diagnosed with Group 1 PAH, at least in adults. Two studies, 1 study in the United States and a larger study in Great Britain and Europe, reported that only about 1% of patients diagnosed with IPAH had occult PVOD caused by biallelic EIF2AK4 mutations. The percent may be higher in families thought to have familial PAH.

Dr Krishnan: The recognition of a gene coding for PVOD really triggers a lung transplant referral as there is no treatment, and the condition is one of rapid progression to death.

Dr Elliott: Yes, I think that's a good point. Here in Utah, our approach is to give the patient with PVOD/PCH an early referral to the lung transplant program. We know that these patients are unlikely to benefit from PAH-specific therapies, and their prognosis for survival is generally poor by the time they seek medical attention and are diagnosed.

Dr Elliott: Maybe we should move ahead, Usha, to your question about penetrance.

Dr Krishnan: Okay. I was going to address this question to Paul initially, and then to Eric. Paul, most of us who are not geneticists sometimes find it difficult to understand what penetrance means. What can you tell us about the penetrance of PAH with mutations in genes like BMPR2? Can you help our audience understand incomplete penetrance? Are there epigenetic or environmental factors that can influence penetrance?

Dr Yu: Thanks Usha. Wendy nicely addressed part of this question with her earlier comments. As she noted, there was a great study from Dr Austin and his colleagues at Vanderbilt several years ago that looked at 53 different families with heritable pulmonary hypertension. From that large set of families, there was a unique opportunity to observe multiple generations for long periods of time to get us better estimates than we had been previously able to obtain from cross-sectional studies, meaning at one static point in time, to answer this question of penetrance.

In that study, the estimates of penetrance were a little bit higher overall than previous estimates, maybe 25% over the lifetime of patients with BMPR2 mutations, for example, where previous estimates were somewhat lower, in the range of 15% to 20%. This study shows that the lifetime penetrance of mutations in BMPR2 and other genes is a concept that is evolving as we have more numbers of unaffected and affected mutation carriers identified, and more patient-years of observation. As Wendy pointed out, there might be a difference between men and women, as in this Vanderbilt study men were found to have 14% while women were found to have 42% penetrance. This factor, gender itself, is obviously a modifier of penetrance. We don't know exactly what the mechanisms are, if they are related to the influence of sex hormones, or the influence of other factors.

In addition to sex hormones, there are genetic differences between men and women, as well as epigenetic and environmental differences. There could be as-of-yet unidentified factors, and there's a ton of research activity focused on addressing this question, where the factors that modify penetrance are an important part of the puzzle. Several large studies that came from Wendy's group, and Bill Nichols' group, and Nick Morrell's group sequencing the genomes of thousands of individuals with Group 1 PAH found that the mutations that cause heritable PAH, now totaling over a dozen and a half mutations identified over the course of the past 20 years, are also found at substantial rates in patients who do not necessarily present phenotypically as heritable PAH but rather as other etiologies of PAH. While we have generally been more suspicious of these mutations and thus more likely to pursue genetic testing in our patients who have a strong family history, or who appear to fit within a syndromic pattern, especially when they're pediatric patients, that strategy may be too limiting, it turns out. One of the first such large-scale sequencing studies from Bill Nichols and Wendy looked at the exomes sequenced from almost 2600 patients and found that some of these mutations are enriched in patients thought to have PAH from exposure to stimulants, or from congenital heart disease, liver disease, or even HIV. In answering to your question about penetrance, these sequencing studies suggest that some of the clinical factors that we already know predispose to PAH can probably interact with genetic changes to promote penetrance.

A possible corollary to this idea is that we might start looking for mutations more broadly in our patients, to encompass more Group 1 PAH patients, some of whom have less obvious syndromic findings, or who may have de novo mutations or unclear or unavailable family histories, but I'd welcome your thoughts on that.

Dr Austin: That's an interesting question. Some people are very aggressive about Group 1 PAH mutation screening because of what you said, that we have probably a number of individuals with other PAH forms with PAH-associated gene mutations. For example, early work showed that about 6% of congenital heart defects, actually, with PAH were associated with BMPR2 mutations. Wendy could comment on what the updated percent-

ages are in that scenario, however. So, I think you're absolutely right, Paul.

It's fascinating to think that the genetic-associated PAH was once thought of as a niche group. I think it was 6% of the 1980s NIH Registry had a family history. It turns out that 6% really, is probably much lower than in actuality; but, even if it was just that 6%, genetics has dramatically informed what we know about other forms of PAH that had no known family history. Anyway, we are now screening in our clinic, congenital heart, idiopathic, and familial forms routinely.

But, we do not go beyond that routinely, unless we have some other syndromic-associated target. Wendy, you could probably comment better about the distribution in congenital heart disease and other forms. I'd be fascinated to actually be reminded of that data.

Dr Chung: We've looked at things that go from both directions. With Erika Berman Rosenzweig, and Usha Krishnan, we've taken individuals who came to them because they had pulmonary hypertension, and we see who has a history of congenital heart disease, or diaphragmatic hernia, or family history of PH, or none of the above. The numbers are still modest. We're talking about 200-ish families that we've studied that way, but we've seen some of the genes. Some of the genes, for instance, a gene associated with Noonan syndrome, was in a person we didn't recognize as having Noonan syndrome.

We did afterwards, looking back and saying, "Oh, well, yes, maybe it could be," but didn't recognize this gentleman had Noonan syndrome and went through his whole childhood without being diagnosed. We've also done it in the other direction, which is interesting because in many cases, I'll see the newborns with a diaphragmatic hernia or congenital heart disease. I'll ask myself, is this a baby who's going to have trouble with pulmonary hypertension in the future? Can I make any prediction based on what I'm seeing with the genetics that'll help our pulmonary hypertension team in terms of monitoring or early intervention, early recognition, and early treatment?

Kids are different. Kids are not just little people in little packages. There really are developmental differences. There's a difference in terms of the way the pulmonary vasculature develops. It's in part a plumbing issue, with the heart and plumbing into the pulmonary vasculature as well.

Sometimes it's a problem in the anatomical neighborhood or field effect, so with diaphragmatic hernia, sometimes we see, developmental lung problems, and it probably isn't just the vasculature but could be alveologenesis in general. Then in some of these cases it's broader. I have to admit, if you'd asked 10 years ago, how many pulmonologists were looking down at kids' toes or feet or knees, or asking about hip problems, they would say, "No, we never take off the shoes." The thing that I'm alluding to is we know about a mutation gene called TBX4, that we used to call small patella syndrome. We were focused on their small knees or kneecaps.

Other people were looking at their lungs, but people weren't putting everything together. When you do, we don't understand why within the same family with the same TBX4 mutation, someone has a hip issue when they're 30, but they don't have any pulmonary problems. I've got families where we find pulmonary hypertension, but not until the 70s, and then I'll have in that same family, a little one who's got pulmonary hypertension at the age of 10, and we don't know why. There may be a second or third contributing factor, be it genetic, be it infectious, be it something else.

There are probably other things that go into this equation and help us determine risks, but it's a numbers game. In terms of being able to get to the to truly see what is influencing risk, we need to study many people.

Dr Austin: There is an interesting study that was recently published out of France from David Montani and Marc Humbert and colleagues, in which I believe they followed 55 individuals who had BMPR2 mutations who were otherwise well, without PAH. They showed over a 2 to 3 year period about 1% of males and 3.5% of females actually were diagnosed with PAH. I think this gets at the notion that Wendy alluded to that if we could capture people who are genetically at risk, but not with overt disease early, could we somehow alter that trajectory. This reservoir of people who actually have a genetic risk in these families but don't have disease is incredibly high. I hope that some day we can provide them some disease-modifying therapeutic approach.

Dr Elliott: In the short time that we have left, we have maybe 3 questions that remain for discussion. First, does anyone have experiences to share over insurance coverage in the United States and limitations of genetic counseling or testing, or do you feel like that's not a barrier so much?

Dr Chung: I'll just say there are a couple of different insurance questions. Number 1, testing is largely covered by insurance. I don't want people to be reluctant because they're afraid they're going to get bills for thousands of dollars; that doesn't happen anymore. That's number 1. Number 2 is some people worry about what I'll call discrimination. Whether it's insurance discrimination or something else, for those individuals who are not showing any signs or symptoms of pulmonary hypertension, they're worried. "If I've got the genetic predisposition, am I going to have trouble getting health insurance, life insurance, long-term care, disability insurance? Is someone going to hire me? Am I going to get into the right college?" Whatever it is, but people are worried.

With this, what I will say is, there's a federal law in place to protect individuals from having your health insurance rates raised or being denied health insurance. It's a very good law. I'm not worried about health insurance, but here's the rub. It doesn't protect in terms of life insurance, long-term disability, insurance long-term care, so people worry about that. Again, I haven't personally seen it happen, but people worry about that. What I have seen is that some people will get their life insurance, and then they'll get their genetic testing. As long as you've got your insurance policy in place and you pay your premiums, you

should be okay in terms of having a policy. For people who are really concerned, that's one way of approaching it.

Dr Elliott: Wendy, that's very helpful. Eric, any comment about using genetic tests to facilitate a diagnosis in a PH patient?

Dr Austin: Yes. You did specifically discuss wisely earlier, EIF2AK4 with regard to PVOD and PCH, and whether that could spare us a lung biopsy and really inform. That is really a very heavy hitter and a major player for us. If we're concerned at all about a spectrum of disease that may not respond well to vasodilator therapy such as PVOD/PCH-spectrum disease, that would be a large contributor to the way genetics really impacts a person's diagnosis.

If we found a BMPR2 mutation in a person with PAH for whom we thought it was idiopathic but we weren't sure, that would make us probably feel better that we were dealing with PPH in the traditional form, primary pulmonary hypertension, and not as worried about connective tissue disease or other forms. Although, as Paul said earlier, there are some concerns that there are individuals out there who have maybe two hits in that. I think for the sake of brevity, I'll say that in my experience the most is that the PVOD/PCH is where genetics with diagnosis is most key, but it's also true that genetics is incredibly important for informing familial understanding as we just have been talking about for a while.

Dr Krishnan: Paul, a question just leading from that would be, if you have a genetic diagnosis, is there any approach, any advances, or anything in the pipeline regarding treating patients with certain mutations?

Dr Yu: There may be 2 ways to answer that. I think that genetics have been really helpful in highlighting potential therapeutic strategies. I'll circle back to your question about whether we have specific treatments for those genetic causes, but as I mentioned earlier, our lab is interested in regulation of the bone morphogenetic protein or BMP and TGF-beta signaling pathway. We know that there are a whole variety of vascular syndromes that can be caused from genetic mutations in the pathway, of which PAH is one, as are HHT, and occasionally PVOD. What we've been excited about are a couple of different approaches that try to reorder or redistribute the signaling in this pathway.

The pathway is complicated by the fact that there are 33 different signaling proteins in this pathway that impart signals to different cells in the body at specific times to coordinate the growth and remodeling of all of our tissues, and these include BMP proteins, as well as activin and TGF-beta proteins. There are 12 different receptors for these proteins, and a number of co-receptors. It has become clear to researchers in this field that this is a very modular system that's designed to help organisms establish important patterns required for embryonic development—in other words the blueprint of life—how do we form our limbs, how we make our digits, how we establish left and right sides of our bodies? The same signals that occur at precisely timed intervals and in precise locations during our development are also part of the carefully orchestrated sequence events that give us our pulmonary vessels, or our pulmonary vascular tree. It is likely these same signals maintain the stability of these structures in adulthood or govern the way they are repaired from injury.

In adults, we found that we can use several types of novel drug agents to try to bias the signaling in this pathway to treat diseases, based on the concept that some of the proteins are responsible for bad or disease-promoting remodeling of tissues, and others may be more protective. The genetics of PAH have suggested that the BMP signaling proteins, including BMPR2 itself, are protective factors that when lost can predispose to PAH, and that the signaling of other factors in this pathway, such as TGF-beta and activin proteins, might contribute to disease. Given the complexity of the system, most of these new types of drugs bias BMP, activin, and TGF-beta signaling only by broad strokes.

Recently we were involved in a translational effort for a molecule called sotatercept, which is basically an activin receptor that was reengineered to become an activin-blocking protein. The thinking was that activin signals generally seem to oppose BMP signaling, and that activin signals might become maladaptive in PAH. Blocking activin signals might restore the balance toward BMP signals that are insufficient in PAH disease. The genetics of PAH were the direct inspiration for this approach. The activin and TGF-beta signaling molecules are not found to be deficient or mutated in PAH, but rather increased—which led us to think that their imbalanced or unopposed signaling could be a driver of PAH. Both activin and TGF-beta turn out to be pretty good drivers of fibrosis, and thickening of vascular smooth muscle, which are 2 processes that describe changes in the lungs of people with PAH. Sotatercept is a potent blocking agent for activin signals, while appearing to leave most BMP signals more or less intact, and was special in that it had already been tested for safety in about 400 other volunteers and patients in previous clinical studies. Namely, it had been used in clinical trials for anemia, especially anemia due to beta thalassemia, and so we had many years of patient experience to learn about the effective and safe doses of this drug. When we used a rodent version of sotatercept, ACTRIIA-Fc, in animal models of pulmonary hypertension, it appeared to be very effective in not only in lessening the impact of disease as it was developing, but also appeared to be effective in reversing established disease.

The findings in rodent models of pulmonary hypertension were so conclusive that they led to a clinical effort called the PULSAR trial that was recently published in New England Journal of Medicine. In this study, sotatercept was seen to improve pulmonary vascular resistance in patients with moderate to severe pulmonary hypertension following 6 months of treatment. There are now follow-up studies, including STELLAR, a large Phase 3 study looking at this concept in more patients with PAH. We're hopeful about the results of STELLAR, and optimistic that it will pave the way for other follow-up strat-

egies that will augment BMP signaling or modify activin or TGF-beta signals in different ways to either enhance the therapeutic effect, safety, or both.

The human genetics of PAH really paved the path toward understanding which of the proteins are potentially helpful versus those that might be promoting the disease. I don't think that we have enough information yet to know if these treatments will end up being more or less effective for specific types of mutations, or without any mutations at all. For example, in the PULSAR Trial, the majority of patients were not known mutation carriers, and there were not enough patients enrolled with any of the known mutations to make conclusions about whether or not any of these mutations influenced the success. Hopefully, in the follow-up studies, we'll get more information on these subtle but important details.

Dr Krishnan: That's an exciting note to end this conversation with some hope in the air. That, I think is really, really important. Thoughts from everyone?

Dr Elliott: Actually, Usha, I just want to thank the participants and organizers of this roundtable discussion. This was a terrific conversation. It really is exciting to end our conversation with the hope that future generations of PAH patients will benefit from novel therapies aimed at the molecular causes of their disease which were discovered through genetic studies.

This whole concept of precision medicine, using molecular pathways and knowledge of pathogenesis, is incredibly exciting to someone like me who has lived through 40 plus years of advances in the treatment of PAH. I'm old enough to have given hydralazine when we were first trying to vasodilate the pulmonary vascular bed. I'm here to tell you, that was primitive therapy. We have come a long way. We'll close it there.