John: This podcast is for informational and educational purposes only, and is not to be considered medical advice for any particular patient. Clinicians must rely on their own informed clinical judgements when making recommendations for their patients. Patients in need of medical advice should consult their personal healthcare provider.

Stephanie: From UPMC Children's Hospital Pittsburgh, welcome to That's Pediatrics. I'm Steph Dewar, Director of The Pediatric Residency Training Program and member of the Hospitalist Division. Happy that you're back with us today.

John: And I'm John Williams, Chief of Pediatric Infectious Diseases.

Stephanie: We're here today with Dr. Carlton Bates who is the Chief of the Division of Pediatric Nephrology, and Vice Chair of Basic Research in the Department of Pediatrics. In addition to caring for children with pediatric kidney disease for many years, Dr. Bates has a well-recognized research program focused on genetic regulation of kidney and bladder development, with nearly 20 years of continuous funding from the NIH. Welcome Dr. Bates.

Carlton: Thank you, glad to be here.

Stephanie: Well, we're so curious to hear about what are the types of patients that you're here at Children's taking care of.

Carlton: We have quite a wide range of children. Sometimes people might ask, "What does a pediatric kidney doctor do?" It is somewhat a specialty of rare diseases, but some of the more common things that we see patients we take care of, are those that have problems with hypertension and kidney stones or crystals in the urine. Those are probably the two major populations. And then one might ask, "Well, why would we care for patients with hypertension?" And that's because a significant portion of children that have hypertension have something wrong with their kidneys. Either compromised blood flow, a scar in their kidneys. Now, unfortunately these days, an increasing cause of hypertension is primarily related to obesity, as well as genetics. That is our most common diagnosis.

I'd say those are the two primary conditions we see. Certainly we have many other patients that have proteinuria, hematuria, more rare genetic types of diseases. It is an interesting specialty. We do have quite a spectrum of patients that we see. We never quite know when we're going to clinic exactly what we're going to see.

John: So, well get into this, Carl, when we talk about your research in a couple of minutes. But, it sounds like from what you're saying, that there's also a lot of ways that things can go wrong in the kidney; either in the way it forms, the way the plumbing is connected, or the way it functions.

Carlton: Yes.

John: Is that these rare disease you're talking about?

Carlton: Right. It can range from, as you said, there are genetic diseases leading to malformed kidneys. More often than not we don't understand the genetics, we know there's something wrong with the genes, but it doesn't follow a typical Mendelian inheritance pattern. So many of the causes, like posterior urethral valves, which is the number one cause of structural disease leading to end-stage kidney disease. We don't even understand what valves are. At an embryologic level you're not supposed to have them, and somehow, these things appear. No one is even sure exactly what they are, why they appear.

Whereas, there are other clearly defined genetic disorders. Some of that has to do with our knowledge base increasing. For instance, there are nephrotic syndrome, inherited forms of nephrotic syndrome, that we've known about for some time, but then there've been others that we thought were more sporadic that we've identified, that no, in fact, they are monogenic forms of nephrotic syndrome. Probably well over 20% of patients in an early stage that have nephrotic syndrome have a genetic cause.

So, the mutations that you see can lead to problems with glomerular formation, tubular formation, tubular function, overall kidney architecture that doesn't form right, vascular problems within the kidneys. So, yes, there're quite a wide range of things that can go wrong.

Stephanie: So, most of the patients then that you see on an ongoing practice in your clinic, are these patients who have underlying disorders of structure or function, or are these new referrals from out in the community?

Carlton: Well, probably about a third of the patients we see in clinic are new. And the others are patients that we continue to follow. The frequency with which we follow up patients depends on their underlying condition. There's some that have relatively minor conditions, like persistent microscopic hematuria that we'll see, probably, on a yearly to every other year basis. To those that have advancing chronic kidney disease, that will see us frequently as every other month.

So, it really varies. Most of our patients we see at about every six months or so. The average patient with kidney stones that has hypercalciuria, we'll follow on about a six month basis. Or patients with hypertension, every six months to a year. The rest of them, as I say, it really varies on what their underlying disease state is, as well as how progressive it is.

Stephanie: I'm wondering what your current research interests are and what exciting new you have to share about that front?

Carlton: So, most of my personal research has focused on the molecular control of kidney development. Kidney and lower-urinary tract development. And one might ask, "Why do we care?" Well, if you look at the leading causes of chronic kidney disease, about half are structural. And among that half its split fairly evenly into aplasia, dysplasia, hypoplasia. Meaning that it just didn't form right. The kidney didn't form right.

The other half are plumbing problems, that something is obstructing urine flow. Posterior urethral valves, ureteropelvic junction obstruction, UVJ obstruction. So, those two categories make up over half of the cases of patients with chronic kidney disease leading to end-stage kidney disease. So to make an impact on that... So, you might say, "Well, we have treatments. We have dialysis and transplant. That's effective, right?" Not really. There are no cures for end-stage kidney disease. If you are unfortunate enough to be born, or to be a child... we won't even go birth, birth is worse. A child, and you develop end-stage kidney disease, you lose. You lose, on average, 50, 5-0, 50 years of life. 50 years of life on dialysis.

Well, we can transplant them, right? We can do a lot better. Well, you only lose 25 years of life if you get a kidney transplant, your quality of life is much better. When you provide dialysis for patients, you give them basically enough kidney function back with dialysis so that they don't die, is really the truth of the matter. If your GFR is below 10 mL/min from a .73 meters squared, you're going to die. We give them enough dialysis that we're barely above 10.

So, imagine that you're a child, you're trying to grow, thrive, learn, go to school when you have almost the level of kidney function provided that you don't die. And it actually speaks to one thing about our program that I'm very happy about, is we are a very aggressive kidney transplant program. Despite the size of Pittsburgh, we are in the top 10, if not the top 5, for the number of patients that we take care of that have end-stage kidney disease. A large portion of those come from outside of Pittsburgh. Buffalo, Morgantown, they send all of their kidney transplants to us. In fact, we have patients that have come from 18 states outside of Pennsylvania for kidney transplants. Including programs that have transplant programs, but they just feel the patients are too complicated, or too small, medically complex to transplant.

We have a very small chronic dialysis program. And I'm proud of that, because we don't let the kids languish on dialysis. We transplant them. But even that said, transplants not a cure either, it's better, you feel better, you now have 50% to 70% GFR. But you still have problems, and you still die young. Unlike cancer with our 80% cure rates, we have 0% cure rates for end-stage kidney disease. That's why I think looking at... My point in my research, what I always put in from my significant sections is these very issues, that we have palliative care for end-stage kidney disease. If we want to make an impact we need to figure out what goes wrong with kidney development, and what can we do to make it go right.

As an extension of my work related to understanding the molecular control of kidney development, and this is really in the mouse. We make lots of mouse models where we manipulate gene expression, and we reproduce the kinds of structural kidney disease that you see in children. Absent kidneys, duplex kidneys, hypoplastic kidneys, dysplastic kidneys, cystic kidneys. Well, that's all well and good, but how do you take that and do something that's translatable?

I've been working recently with a team including members of the McGowan Regenerative Institute at Pitt on looking at, "Can you regrow a kidney in a dish?" And there are lots of people looking at this now, but my collaborate Eric LaGasse floored me

when he showed that he could take an embryonic mouse kidney, mince it up, inject it into a host lymph node, it reconstituted kidney tissues that were profused, that entrapped a fluid that concentrated urea. The only fluid that concentrates that urea is urine, he made functioning kidney tissues within a lymph node. This is stunning to me.

So we're working with him now on, "Well that's great. You take minced embryonic kidneys, we can't really do that as a source. Can you get progenitors?" So there's a large movement in the research community looking at induced pluripotent stem cells and converting them, driving to a renal state. The kidney's complex though, it's not like... Not to denigrate other organs, but a liver cell, where you take hematoblast, and you make that at an hematocyte. Well, the kidney has, really, four different progenitor cell types that all need to be present. The nephron progenitors that make nephron epithelial cells, at which there are 21 different epithelial cell types, that's complicated enough. A ureteric epithelium, which is the progenitor for the collecting system. And those two need to be cross-talking and signaling to each other. Then around that is a renal cortical stroma that acts as an organizer for all this to work. And then, of course, you need blood vessels. Many which are coming within the kidney.

So, there are people out there that can make nephron progenitors grow into these blobs that kind of have epithelial structures. But we're looking at the lymph node as a bio reactor that has these amazing organizing properties. The lymph node can organize liver cells, beta cells in the pancreas to make functioning tissues. If you think about it, cancer's teaching us that. How does cancer spread through the body? Through the lymphatic system. They've known that the lymph node's a potent bioreactor for some time.

We're trying to harness that capability to say, "Let's take your favorite IPS cells, make them into nephron progenitors, make them into ureteric cells, combine them together, put them in the lymph node." In fact, we're actually now talking to a group from Japan that has done some really successful research doing exactly that in a dish, but they can only go so far. These tissues that they're combining in a dish, while they kind of look like early kidneys, they're really fetal kidneys. They don't mature. If they grow them longer in vitro they just turn into fibrotic masses and die. They're not profused, so how do you really test for function? You need profusion to then look at urine production, glomerular filtration, and then tubular function. So we are working with groups like this now in the lymph node. That to me is really exciting work, and of course, the challenge is always getting external funding agencies to be as excited. That's one piece that I'm working on.

The other piece is I've fallen in love with the bladder. I never knew I would fall in love with the bladder, but I have. And it's a very important organ if you're a nephrologist. If you don't have a functioning bladder your kidneys aren't going to be very happy. And, actually, there's a real lack of intensive research at a basic science level looking at bladder disease. Urologists are very talented people, but they're mostly clinically focused. Whether it's they're straight up clinicians, or focused on clinical research. Not really interested in laboratory research as much.

So, there's a real big problem out there, which is that bladder injury happens a lot. One example is cyclophosphamide. A great drug, helps cure patients that have lymphomas,

or that have bad types of glomerular disease, or rheumatic disease. But, there are problems with it. And one is that a toxin called acrolein accumulates in the urine, and it leads to direct bladder toxicity. Leading to hemorrhagic cystitis, and more importantly, bladder cancer.

There was a large survivor study, patients with lymphoma, 6000 of them that overall had a 4.5 fold increased risk of bladder cancer. If you stratify them by dose, those that get the highest dose, which is 50 grams of more, had a 15 fold increased risk of bladder cancer. This is despite all the stuff we're currently doing. Mesna, which binds acrolein, hydration. What we're doing isn't working. And no one's really looked at this. How does acrolein cause bladder toxicity? What does it do, and is there anything we can do about it?

We have some really interesting, I think, interesting research looking at exactly that topic. We are specifically interested in how fibroblast growth factor receptors might play a role in disease, or even in therapy for patients that have this bladder disease. Well, why would that be the case? It's been known for some time that keratinocyte growth factor, FGF-7, is a potent epithelial mitogen; and if you give too much, that actually can cause hypoplasia and even cancer. But it's also been shown in many wound healing studies, oral mucositis, lung alveoli injury, gut injury models. Both preclinical and some clinical models, that if you give patients keratinocyte growth factor, patients or animals, that it largely ameliorates injury.

So we started looking at that in the context of the bladder. There was actually a paper, the last one in 1997, that's the last one. That somebody looked at this, and there was a paper, a preclinical model, that suggested you give a patient Cytoxan, give a mouse, give a rat Cytoxan, they get horrible hemorrhagic cystitis. You give them a single dose of keratinocyte growth factor before Cytoxan, and they said you largely regenerate the urothelium. You accelerate the regeneration.

We started looking at this and they're not quite right. What actually happens is, and we've shown this same thing, it's incredible. If you give a mouse a single intraparenchymal dose of cyclophosphamide, a dose that's equivalent or similar to what you'd give a person, they get horrible cystitis. And we actually have to look at the females, because the males do, as is most often the case, much worse than the females. We weren't even looking at the males, we're looking at the females. And even they have terrible hemorrhagic cystitis.

If we give a single, one, single, one, subcutaneous, which is like giving an IV dose of keratinocyte growth factor, we almost completely block the injury. Block the injury. That's the key. It's not accelerated regeneration. We block the injury. So, what we find is that actually the acrolein, which hits the superficial cells in the bladder, the urothelium... The outermost layer, there's a necrotic cell death they slough off, and we don't block that. That happens within the first 0 to 6 hours. But from 6 to 24 hours, there's a second wave of death called apoptosis. That is almost completely blocked by keratinocyte growth factor. In those that don't have KGF... The urothelium is a multilayered epithelial, you go down to just your basal layer, and sometimes completely denude everything, and in our KGF-treated it's amazing. There's almost no apoptosis.

We've been carrying these studies forward to look at... Well, you might say, "Are you protecting cells that you should let die?" The answer seems to be no, so far. We've carried the studies out to 3 days, and now 28 days we're looking to see what the architecture is. And so far it looks pretty good, but stay tuned with that. The backside of this, is what happens if you knockout the receptor that binds to KGF? What happens to the urothelium then? Well, so as it turns out, if you do that, if you knockout the receptor, specifically, we can use fancy mouse targeting techniques to knock it out just in the urothelium. And we can do it in indusial fashion.

So we let these mice... they're born, they're fine. We give them a hit of Tamoxifen, which induces the deletion of this receptor. And in the quiescent state they don't care. It's fine. But we hit those with Cytoxan and they don't do very well, they're not very happy. What happens is we have a maladaptive regenerative response. What happens is, the basal layers, which is where the progenitors are... Normally in a quiescent state there are very few progenitors. After injury, they spread across the entire basal layer, and in the controls, the proliferate upwards and replace the damaged tissue. But it's not a simple replacement. There's a lot of DNA damage that happens, so they're sloughing the damaged cells, making new ones. Sloughing, making, sloughing, making, and this happens all the way out to... Even at 28 days it's not finished.

Our poor mutants on the other hand, they make the progenitors spread across the basal layer, but they are ineffective at producing progeny to replace the damaged urothelium. What they do is they get trapped in a process called endoreplication. Well, what is that? They go through the cell cycle, but they bypass mitosis. So they make big, fat nuclei, they make more DNA, but they don't divide. So they get these giant cells, with these giant nuclei, that don't divide.

We find this very fascinating and, actually, we are starting to even understand the mechanisms by which this occurs. We're understanding now that they're different, of course, these receptors signal through different downstream pathways. The KGF overdrive anti injury protective effect seems to be on one pathway called AKT. The problem with regeneration in the mutants seems to be irk mediated. In other words, irk represses endoreplication.

So, anyway. This is where we are with that. Probably more detail and depth than you wanted to hear.

Stephanie: I am so happy that you're in love with the bladder. I have merely made peace with my bladder, and learned to live with it every day. But that was more thinking about bladder cellular activity than I think I've ever had in my entire career.

John: It is clear that you are passionate about your research, and I think it's really cool that you highlighted the different areas from really basic, how are things forming and developing in the kidney which lead to these kidney diseases in kids, and how are medicines that we use injuring the kidney or bladder, and what are the mechanisms, and how could that be intervened in. And then how we can maybe make new kidneys instead of the treatments we have now. Just a final point, your division, I think, really

focuses a lot on research and education, right? That's a little unusual for a pediatric nephrology division?

Carlton: Yeah. So, first of all we have 10 MD nephrologists, and 2 PhDs in our faculty; which puts us at one of the largest in the country. I can't say we're the largest. But it isn't just the numbers that I think is impressive. We have four in our division that are really dedicated to patient care. That really come to work to see patients and stamp out kidney disease. And they do a wonderful job. And then the rest have other activities, mostly focused on discovery and research. And what I love is it's really diverse.

We have my basic science program, but I'm not the only one. We have junior faculty doing really exciting things, our one funded research focused on the role of Micronase in patterning the kidney, and podocytes in the glomerulus. We have people working on how the microvasculature is important in response to acute kidney injury, and how alterations in metabolism related to the microvasculature is important in acute kidney injury. We have people working on understanding the mechanisms of nephronophthisis, which is a degenerative cystic kidney disease. Looking at how aberrant DNA damage response plays a role. Which, again, has potentially high clinical relevance. We have people looking at altered membrane traffic of proteins in diseases such as nephrotic syndrome.

There are lots of kinds of nephrotic syndrome we don't know how to treat, we don't understand that pathophysiology, but this is a novel approach, an outside the box thought that aberrant trafficking of proteins contributes to this. And, while we have this fairly solidly established basic science program, we have this growing patient oriented research program that I'm very excited about. We have a faculty member whose a dual critical care pediatric nephrologist, who submitted what's called a K Award, which is a career development award. She's likely to be funded, she got a very good score. Looking at novel biomarkers that would predict poor outcomes in patients with congenital heart disease that go for heart surgery, some of these adult general heart patients get their surgery. About half have full kidney failure requiring intervention. Half don't. We have no idea to predict whose going to do what, which would then help us determine how close we would follow up on care we would give. She has some very innovative data, looking like this thing's likely to be funded.

We have a faculty member that created a high density patient database from the intensive care unit from a five year period. Granular level detail, and has identified novel associations between antibiotics and acute kidney injury that really weren't very appreciated. Pip-Tazo, Zosyn, actually fell out as very highly associated with acute kidney injury. Vancomycin did not, which was surprising. So, this is a current K-application that's in review. We have other fellows, I'm very proud to say we're 1 of only 5 pediatric nephrology programs that has a dedicated T32 to training pediatric nephrology fellows. Only 5 in the country, and of the 13 fellows that have been on the T32, 6 are committed to becoming clinician scientists. And I actually heard rumors that one that's a junior person, the 7th, is very interested. And the topics they're looking at range from hardcore basic science work, one doing induced pluripotent and stem cell work, all the way to patient-oriented research related to databases and other things.

I'm very proud of the research focus in our program.

Stephanie: So it sounds like there's a lot going on with kidneys, ureters, bladders, and urethras here in Pittsburgh. We're so happy that you were able to take the time to join us on the podcast today and share this knowledge with us.

John: It's really been a pleasure to have Carl Bates, chief of pediatric nephrology to tell us about the exciting research and clinical care in his division. I'm John Williams, we'll see you next time on That's Pediatrics.

Stephanie: And I'm Steph Dewar. Thanks for joining us.