The Genetics of Polycystic Kidney Disease
Part Two of Three

Gregory Germino, M.D.
Associate Professor of Medicine, Division of Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD

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Phenotypic Variation
How one abnormal PKD-1 gene might cause disease: looking at the patients for an answer
We were interested in trying to sort this problem out. How can we define how a mutation causes disease? As we thought about the question, it dawned on us that perhaps we could just look at the patients and they would give us the answer. Indeed that is what has happened.

Inter-family variation in the severity of PKD
What we all know about PKD is that the disease, one of its sort of classic features is that it doesn't follow the same course necessarily in everybody. We know that there are considerable differences among families. That is what I call inter-familial variation. There are a couple of different reasons for that that we have defined. One is that we know that you can have different forms of PKD. There is PKD-1, there's PKD-2, and PKD-3.

Structure and function of the PKD-2 gene
The PKD-2 gene here... this is my picture of what the PKD-2 protein would look like. Again,
it sits on the cell surface. It has parts on the inside and parts on the outside. These are the parts on the inside; those are the parts on the outside. It has a different function than PKD-1.

Although it looks a little like PKD-1 and that is how Steve Somlo's group up in Einstein were able to find it (Mochizuki et al, Science 1996 May 31;272(5266):1339-1342). It has a very different function: We think it makes a little hole, a little pore, in the lining of the cell to let things come in and out of the cell. Perhaps PKD-1 regulates that. But it lets things come in and out of the cell in a regulated way. That is very important in kidney cells.
Differences in how patients with PKD-1 and PKD-2 mutations present
What we do know about PKD-2 besides what the protein looks like is that there are some differences in how PKD-2 patients present. We know that PKD-2 tends to present a little bit later in life. So the average age of end-stage renal disease in patients with PKD-1 is in their early to mid-50s. Whereas with PKD-2, it tends to be later, around 70 or 71. We know that patients with PKD-2 tend to live a little bit longer because they have renal failure typically later in life. So they don't have as many cysts early on. They get diagnosed later and they have less hypertension.

So there are some genetic differences just simply due to the fact that if you have PKD-1 versus PKD-2, you may tend to have a more less severe disease. That gives us some clues about PKD-1 and PKD-2. It does give us some information, but this isn't the only reason that there is a difference.

Phenotypic Variation

Intra-familial
- modifying genes
- dynamic mutations
- environmental factors

Reasons for intrafamilial variation within the same PKD gene mutation
Let's just take out all of the PKD-2 families and all the PKD-1 families and we separate them. We are going to focus right now just on PKD-1. If you look at PKD-1, as many of you know from your own families, there is considerable variation even within a given family. We call that intra-familial variation.

Modifying genes
There are a number of different reasons or different factors that can play a role in that. One is called modifying genes. What do I mean by that? Throughout our DNA there is plenty of variation. I suspect if I took every single one of you and analyzed each of your DNA samples, we would find little subtle differences up and down your different chromosomes.
That's why we look differently; that's partly why we act differently. There is a lot of variation. Now some of these variants are quiet--we never see them; and some of them subtly affect some of the other proteins in the body. Some of those other proteins in the body can complement, i.e., they can either protect or worsen, a primary mutation or a primary factor.

Imagine that you have two people making a handshake or somebody trying to grab somebody. It is the strength of this individual and the strength of the other individual that is going to make the link. If one individual is weak and the other individual is strong, the link still may work. On the other hand, if they are both weak, you may have a break in the link--the person falls or whatever else. So you can imagine that in many systems, it is a combination of things, the net effect of multiple parts, that determines how something works. We know that is true in PKD.

Lisa Guay-Woodford ([Kidney Int 1996 Oct;50(4):1158-1165]) has looked at animal models, for instance, of PKD and has shown that if you take what is known to be a pure mutation of one of the forms of PKD and you put it into a different animal background, so you mix it up a little bit, the disease gets either worse or better, which is due to the action of other (modifying) genetic factors.

**Environmental issues**
The other important factor, of course, is environmental issues. People live different life styles. They may have different exposures or whatever else, different diets. Those things theoretically may play a role in influencing how a disease is expressed. I just want to present a couple of examples of some of the variation that we see within a given family.

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**An example of intrafamilial variation: susceptibility to vascular damage**
This is an aortogram. An aortogram is where you take dye and you inject it into the aorta or the large blood vessel that feeds all the organs in an individual. This individual was a 27-year old African-American fellow who came from a family that was known to have PKD. Everybody else in the family had just typical PKD. This guy at age 25 presented with a severe headache and was discovered to have two aneurysms, two of them, that were
successfully clipped. He did well until age 27 when he came back with a large aortic dissection. What that is is a tear in the aorta, so a tear in the major blood vessel that feeds all your important organs. He had a really bad back pain, as you can imagine. What is illustrated here is the dye going through the tear, going through the torn wall. He did very well and has survived, but he was the only person in his family who had this complication. This is a recognized, rare fortunately, but recognized complication of PKD.

**Intrafamilial variation of severity of onset**

As many of you know, the University of Colorado has been following families with PKD for many, many years, and they have identified a large number of families who have what they describe as anticipation. What that means is that in a given family, the disease appears to get progressively worse from generation to generation. Whether or not PKD really has anticipation can be debated by many, many geneticists. I won't get into that today. But certainly it is true that there are plenty of families, as illustrated here, where in the grandparent... the grandmother is 77 (panel A, left, row 2 in figure) and has pretty reasonable renal function. Her daughter at age 53 (panel A, row 3) was in renal failure and on dialysis. Their child at age 20 (panel A, bottom row) already had significant hypertension, significant cystic disease and actually had already had some decline in their GFR. Progressive worsening within a family. So this is example of, again, intrafamilial variation.

A lot of different models have been proposed to explain it. I won't go into what those models were genetically because they are not important for today. Let me just say that there has been one report published that has actually looked at this issue to try to see if they can explain why the disease is worse in some people within a family and not in others.
Another example of variability of onset in the same family

This is a study from England. In this particular family, the father had typical PKD and moderate cystic disease. He had two children. They were fraternal twins. The boy had severe disease as a child. This child was only age 5 years old and had hypertension and big, cystic kidneys. His sister had virtually nothing clinically. So these two children both had very different phenotypes, very different presentations clinically.

One was affected severely; one, barely affected at all. And yet genetically they were found to have exactly the same mutation. And so why did these two kids have such different presentations? As a matter of fact, they were even in the same pregnancy. So whatever pregnancy factors you might imagine that could potentially influence this were well controlled as you can possibly control these things.
One possible explanation for intrafamilial variation: variation of expression of PKD in the tissues of the same individual

So we thought about this. We said, "This is odd. There must be some reason for this." As we thought about it some more, we concluded that the individual himself or herself has intra-individual variability. If you look at an individual's kidney, particularly in the earliest stages when they have the very first cysts, it is not homogeneous. Cysts are focal. Only some of the tubules actually develop cystic change. We call this intra-individual variation.

Not all tubular cells in the kidney are involved in cystic change, yet they all carry the same mutation. Why is this?

Here is an example of that from an actual photomicrograph from micro-dissected early PKD kidney. Here is the normal tubule that I showed you before. As you can see, there are these little balloons. This is a close up of that balloon. There is a little out-pocket, like a little water balloon, coming off of the normal tubule. Every single cell in this tubule has inherited this same mutation presumably because they all are derived from the same egg and the same sperm. So every one has the same mutation. Yet if you think about it, why did only these cells here go wrong? What's different about these cells from these cells?

We figured this must hold the clue to understanding. Even in a given individual, where all the genetic factors in the world as far as you can tell have been controlled because every cell has the same genes as every other cell, why do some cells go bad (and succumb to cystic dilatation) and some don't? That's the question.
Focal tubular dilatation in only one part of the nephron. Why does it start in one place and not another?
Schematically again, this is my attempt at a drawing. You can see that this is my model of what a normal filter unit and tubule would look like in a kidney if you took it out. Something is going on right here. So we targeted these structures for study. We had a hypothesis. We had a model that we wanted to test. It is well known in other disease, like tumors, where a disease starts out as a focal process, there is a genetic reason for that. The genetic reason for this is, that the defect really isn't dominant, but recessive.

The two-hit hypothesis
What I mean by that is illustrated on this slide. Every cell inherits the mutation. That is marked by an M in this schematic, in this cartoon. So every cell has the same mutation. That would be what you would imagine by a dominantly inherited mutation. Just one copy is inherited which is mutated.
However, over time and in tissue, as you are building your kidneys or whatever, some cells have an accident. Now it is well known that this happens all the time in all of us. But there are a couple of cells that have had a second mutation occur in the other (previously normal) copy of the gene. So the copy that before was normal, that was protective, now has acquired another change, a mutation. That is called a somatic mutation or an acquired mutation.

So now suddenly, the PKD-1 gene, in our example, could potentially be mutated in both copies. If that were the case, then this cell now suddenly has lost its break, its control, and continues to grow. So this is the model that we wanted to test. Is PKD really a recessive disease? Even though we inherit it in a dominant fashion, in terms of how disease develops in people on a tissue level, it may actually be recessive in that it may take two abnormal copies to cause the disease. That was the question we asked.

Testing the two-hit hypothesis: obtaining tissue directly from cysts
This just illustrates how we went about doing it. We had to get a way of looking at the cells that line individual cysts. I won't bore you with the details except to show you a picture of what it looks like. We would get cystic kidneys sent to us from patients who have had them taken out for medical reasons and we were able to puncture and isolate from an individual cyst, as illustrated here, all the cyst contents.

We put in a special solution that makes the cells that line this cyst to fall off. So we can get a very pure population of just the epithelial cells that line the cyst wall. It's a very pure population, which is key. We then look at them in terms of their DNA and ask the question: Have these cells inherited or developed a second mutation of PKD?
Testing the other previously "healthy" chromosome for an acquired PKD gene defect in cells from the cyst lining

The way we look at that is shown here in this cartoon. We know where PKD-1 is, and we want to know whether the chunk of chromosome on the other side, the other copy that is normally protective, is missing or somehow altered in the kidney.

We can look at that using this technique. We have tags that line up and down a chromosome, the same tags that we use doing genetic studies on families. We can use those tags to look at the cells from an individual cyst. If there is a hole, if there is a deletion or some sort of mutation, instead of having two bands here, we get one band. So we are testing in the individual cysts from a given person whether or not we go from two bands to one band when we know they should have two bands.

Evidence of acquired mutation of PKD-1 in DNA from cysts of a patient with PKD.
For PKD-1 there are plenty of little tags inside the gene that we can use to actually look specifically and directly just at PKD-1 and see if PKD-1 suffers another loss or deletion. This is what we found.

In this picture we have taken DNA from individual cysts. We took 16 different cysts from one person's kidney. We puncture the cysts, drain all the contents, clean it up, make DNA and then do that test that I just showed you. Now we know in this given person that in their blood and in most of their cysts, you can see there are two bands, one band from each chromosome. So it's tagging each chromosome for PKD-1. What you can clearly see is that in this lane here and this lane here, Number 14 and Number 8, instead of seeing two bands, you only see one band. And indeed you see it is the same band in both cases that is missing. We know that these two cysts have acquired a deletion. They have lost a piece of their PKD-1 gene, and they lost that while their kidney was forming or afterwards. They didn't inherit that. These other lanes are all from the same individual. So they have an acquired mutation of the normal copy.
Results confirmed in other patients: Acquired mutations in the second, healthy PKD-1 gene in DNA taken from renal cysts
This just summarizes our results looking at this in a lot of different people, looking at a lot of different cysts, and we found a lot of different times mutations that were acquired--they were not inherited mutations.

Proving that the normal copy of the PKD-1 gene was mutated in DNA from renal cysts
This is how we show that it was indeed the normal copy that got lost. This is really critical. You have to be able to know that it's not just the mutated copy because we know that they already have one mutated copy because they have PKD. So we wanted to know whether or not it was the normal copy that got lost in the tissue.

This is just a picture of a family analysis. We've coded it so you can't tell who is male and female in this cell. What we found in our tissue is that it was this particular tag, this 106 tag, that was lost. We know it is the 110 tag in this family that associates with disease. You see everybody who has disease--they all have 110. This person's kidney lost the 106 tag, so we know it was the normal copy that got mutated, that got lost.
So this is the model for what goes on in cystic tissue. This is how we believe people develop cysts in ADPKD. Everybody who has ADPKD inherits a mutation in one copy of their PKD gene, whether that is PKD-1 or PKD-2 or PKD-3. It is autosomal dominant, one copy. However, in their tubes at some point in time, whether it is very early or even perhaps later in life, they acquire another mutation. That mutation allows this particular cell that is now lavender to grow. And it bubbles out and just continues to grow. It has lost that protein that is sitting on the outside telling it to stop growing. It keeps looking for the signal, never gets the signal; it just keeps going and going and going.

Does the two-hit phenomenon in PKD also hold for cysts in non-renal tissues
We wanted to know if this is the mechanism responsible for other complications of PKD. As most of you know, people with PKD just don't get kidney cysts, they also get liver cysts. About 40 percent get liver cysts. We reasoned that it probably is the same process, and we actually wanted to test that. This is just a CT scan illustrating one of the individuals who we have seen who has had really massive cystic disease of the liver. Here is the kidneys. You can see that they are pretty big and they're pretty full of holes. Here is the liver, which is massively filled with holes, filled with cysts. This person actually had to have part of his liver taken out because it was so big, just distended.

**Source: Watnick TJ et al. Molecular Cell 2:247-251**

**Acquired mutations in PKD-1 in cysts from patients with both renal and liver cysts:**

**Results in renal cysts**

So we got samples from people like this, and we did the same study. In this particular example it is a different tag, but the point is the same. In Lane 8 and in Lane 12, you see a single band when there should be two. And it is the same band that was missing. Moreover, in Lane 11, you see they have a funny new pattern. These are all from the same individual. These are individual cysts from the same person. They should all look the same in a normal case. What you can see is 3 of these 12 look different. And in all cases, it was another acquired mutation of the previously normal PKD-1 copy.
We found in one of the cysts there is actually a small chunk actually missing, just missing. And that has important implications for what happens to the gene product or the protein that it makes.

Acquired mutations in PKD-1 in cysts from patients with both renal and liver cysts:

Similar finding in liver cysts

We found the exact same pattern on liver cysts. I won't go over the data to bore you, but basically it is exactly the same thing. Indeed it is acquired mutations of just PKD-1 that are sufficient to cause this.

References at the end of part 3