

---

9th Annual Conference on Polycystic Kidney Disease, June 1998.  
Selected Talks.

Web presentation sponsored by an unrestricted educational grant from Sigma-Tau Pharmaceuticals

## The Genetics of Polycystic Kidney Disease Part One of Three

---



**Dr. Gregory Germino**

**Gregory Germino, M.D.**

Associate Professor of Medicine, Division of  
Nephrology, Johns Hopkins University School  
of Medicine, Baltimore, MD

---

### Listen to this Lecture using the Real Player



You can scroll through the talk while listening to the audio. You won't be able to listen to these files if you have not downloaded and configured the Real Player version 5.0 (earlier versions will not work!).

To download Real Player 5.0 click here.



---

**Moderator:**

I have perhaps the greatest pleasure to introduce to you Dr. Greg Germino, who if you guys don't know this name, you are very new here. He is beyond a local hero. He has done more for this Foundation and the progress of this disease than anyone on the planet so far, so far as I am concerned. He has had a lot of help with a lot of his staff, but I believe he deserves a great round of applause. Dr. Germino serves as Associate Professor of Medicine in the Division of Nephrology at Johns Hopkins University in Baltimore, Maryland. He serves on the PKR Foundation's Scientific Advisory Committee and is also a member of the American Society of Human Genetics at the American Society of Nephrology. We want

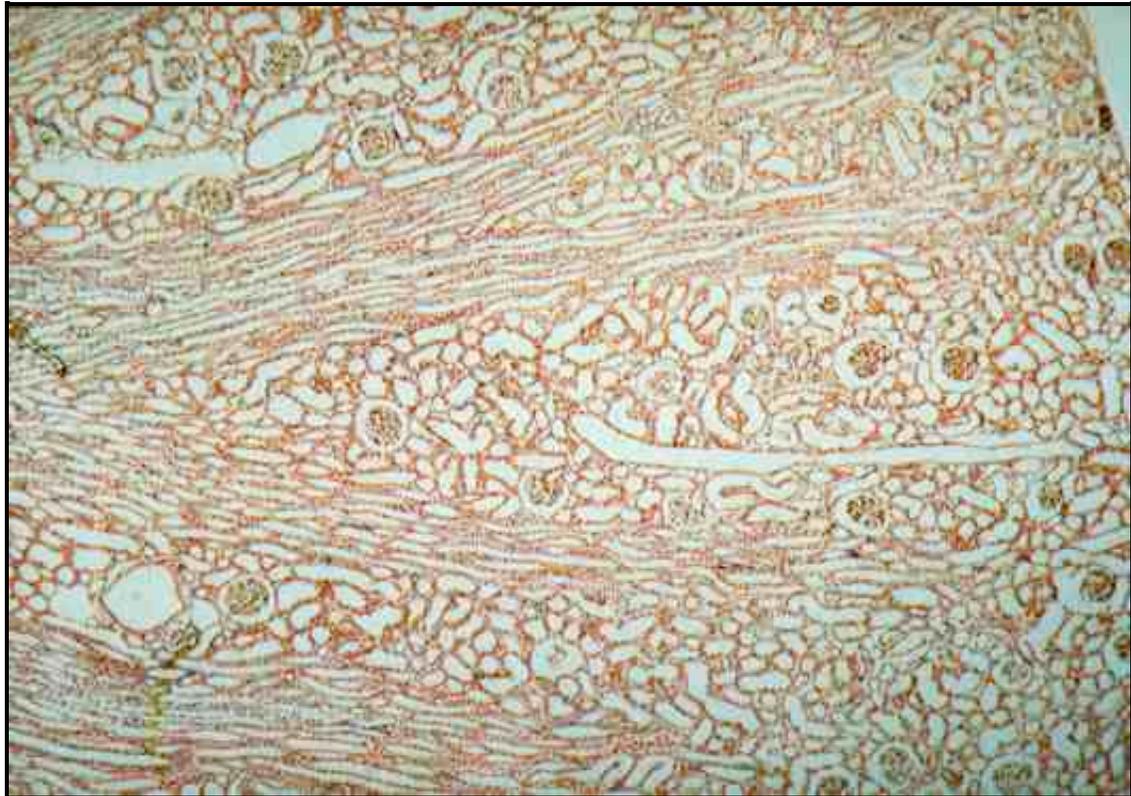
to thank him for participating in this conference today. Thanks.

**Dr. Germino**

**Introductory remarks**

It is a great pleasure to be here today and to go over with you some of the breakthroughs in the genetics of this disease over the last couple of years. The first three-quarters of the talk are going to review things that most of you or some of you may already know because these are not things that have come up over the last year. But they are important to review before we go over the very last part of the talk, which is the new breakthroughs in the animal models that have come out over this past year. Hopefully by the end of this talk, you will understand why these animals are so important, how they're so useful, how they were made and how they actually help us understand PKD.

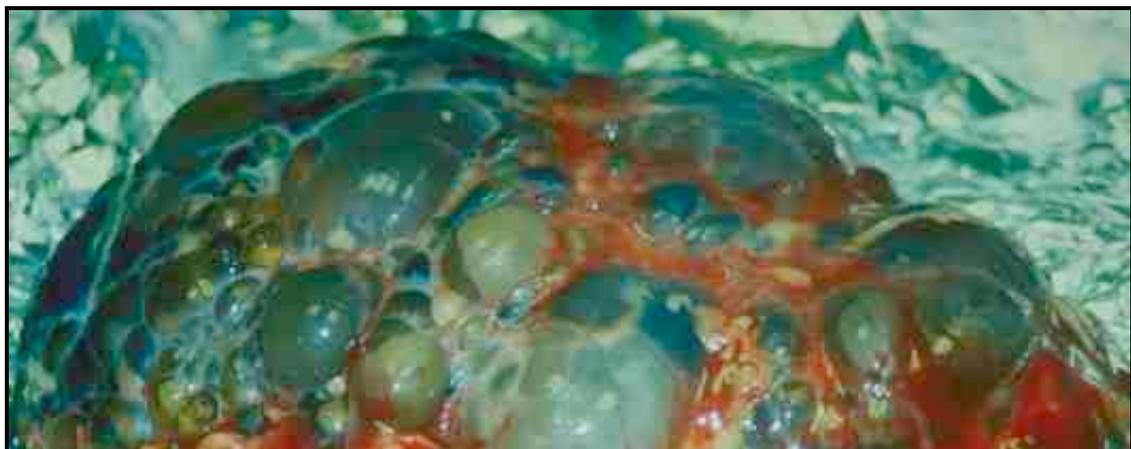
1:47.8

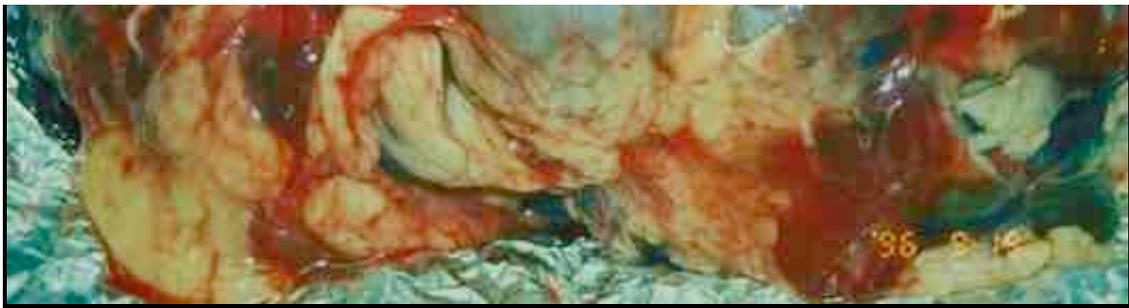


**How does a normal kidney become polycystic?**

One of the obvious questions that we would like to understand and all of you who suffer from PKD want to understand is: What makes a normal kidney, which is illustrated here, turn into the monstrous organ that we sometimes see, here?

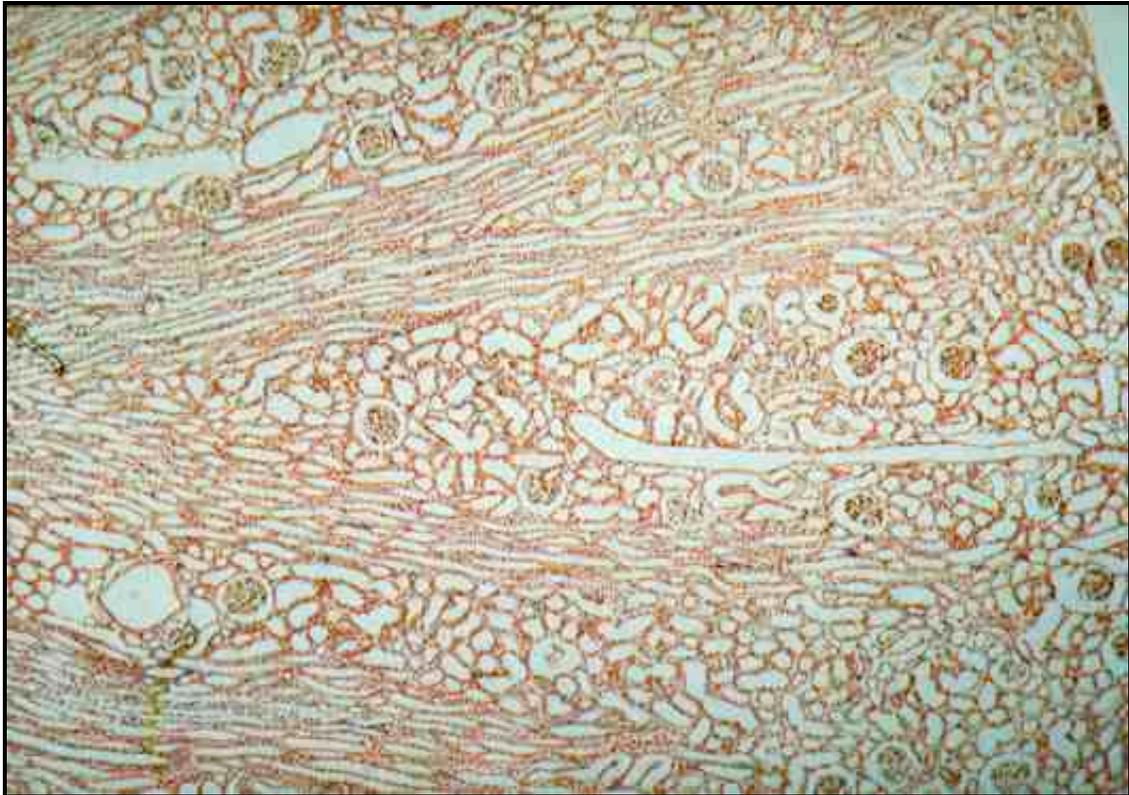
2:04





The normal kidney is about the size of an adult male's fist. As you can see, this is far larger than even Michael Jordan's fist. It is a huge, huge disrupted organ.

2:18



### **Architecture of the normal kidney**

If you go back for a second, the kidney is made up of about a million filter units called nephrons. There are about a million of them per kidney. These long structures are called tubules. These are typically the size of about a thin hair, so they are very, very small. And it is these structures that somehow misbehave and go on to form the cysts that we see in the cystic kidney. As you know, individual cysts can be up to 8 cms in size, so three to four inches in size. So something has gone wrong in a tubule which is normally the size of a human hair to make it the size of three to four inches in diameter. What we would like to know is what are the processes that govern that?

### **Signs of PKD can be present very early in life**

We know that the disease is inherited. We know the disease starts probably in utero. If you examine a woman who is known to have PKD or her husband is known to have PKD and you look in utero at the fetus and if the fetus happens to have inherited PKD, we know that you can see a small couple of cysts perhaps... not always, but you can sometimes see a couple of cysts. Even in somebody, in a child for instance, we see just a handful of cysts. And yet over the course of 40, 50, 80 years, the number of cysts increase, the size of the cysts increase.

**Understanding the reasons for polycystic transformation may well lead to therapies**

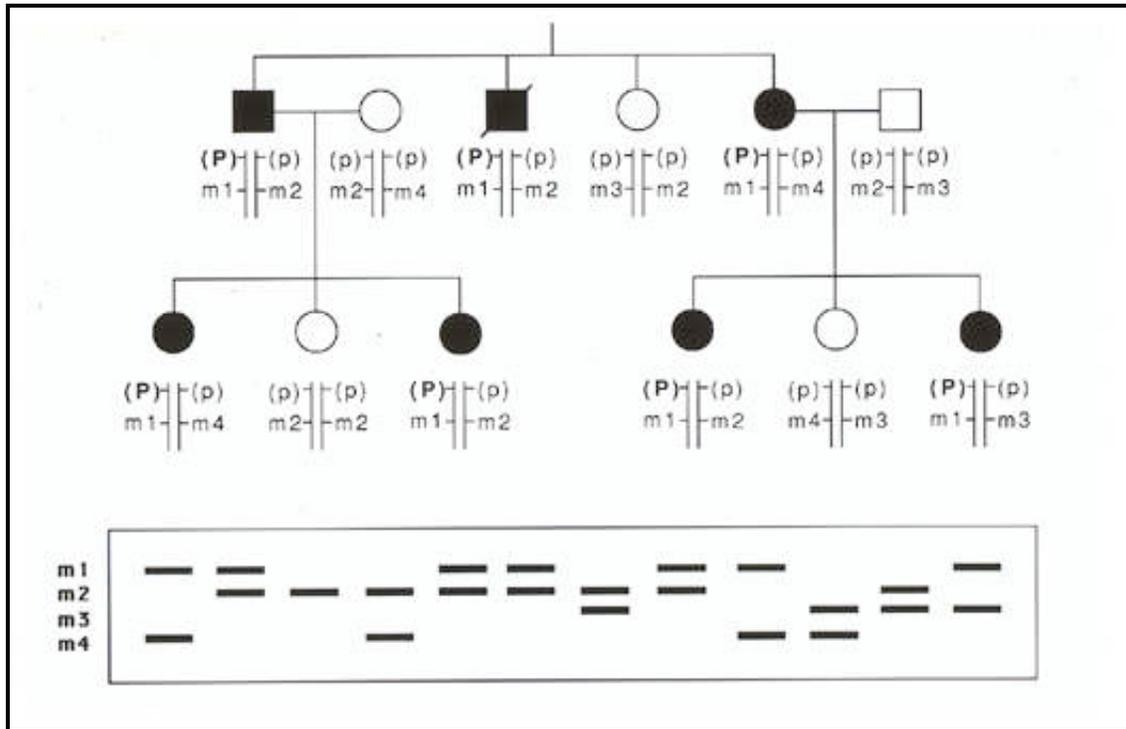
## or prevention

The question is: What is going on? If we can understand the steps that are responsible for this process, perhaps we can intervene long before someone goes on to develop that large, horrible kidney that we just saw.

## Genetic approaches to the mechanism of PKD

What I want to review with you is how we have been using genetic approaches to try to understand this. PKD, because it is a genetic disease, provides us a great tool, and that is family material.

3:59.7



**Legend:** Genetics of autosomal dominant PKD. Affected persons are shown as filled squares (men) or filled circles (women). In this family, all affected persons share the same genetic marker (m1).

## Autosomal dominant inheritance of PKD

We know that the disease is autosomal dominant. What I mean by that is that it doesn't skip a generation and it only takes one abnormal copy of the gene to cause disease. For autosomal dominant diseases, as most of you know, it takes only one abnormal copy to cause the disease. That is the reason it doesn't skip a generation. If somebody has it, they pass it on to the next generation.

As illustrated in this example, you have a parent, a father, who passes it on to approximately half of the children. This is different from autosomal recessive PKD where neither parent is typically affected and only the children, one out of four, are at risk of inheriting it. In autosomal recessive PKD it takes two abnormal copies. Because a parent has only one abnormal copy, they are fine. It takes only one to stay healthy. In autosomal dominant disease, it just takes one abnormal copy that is inherited to cause the disease process. That's why every time someone has a child, there is a 50/50 chance that their child will inherit the disease.

## Genetic disease mean an abnormal gene which means an abnormal protein

We know in a genetic disease there is a gene that is abnormal. Genes are coded in DNA. The DNA code itself does nothing. It is like a hard disk of a computer. It stores information. That DNA provides the template for making all of the really useful and working molecules in the body. Those are the proteins. That is what we want to understand in PKD. We want to know what the protein is and we want to know why it is abnormal and how the abnormality causes a disease to form.

### **Difficulties in identifying the abnormal protein**

To get at that, you could just grind up a cystic kidney and try to look at the protein and see if you can identify the abnormal protein, but that is a very hard way to go. People have tried to do that for 10 to 15 years and couldn't find it because there are thousands and thousands of different proteins, and you can't really know which one is specifically the principal problem. There are a lot of other problems that develop as a result of the principal problem.

### **Advantages of molecular genetic techniques: looking for the abnormal gene directly instead of looking for the abnormal protein**

But with genetics, you can identify the chromosome segment where a gene lies by looking at the inheritance of markers on a chromosome with the disease phenotype. And that is illustrated on this slide. With a very large family, you know there are individuals who have the disease and you know there are individuals who don't have the disease. You basically test for the inheritance of markers within a family.

### **Looking for genetic markers of PKD in affected family members**

So these are markers. This is a marker the position of which on a chromosome is already known. We don't have to go into how we know that, we already know it. So we have a marker that tags a specific part of the chromosome. If we are close to where a diseased gene lies, we should find that everybody who has the disease has the same tag, shares the same tag, in a given family.

Indeed, if you look at this example, everybody who has the filled circle or the filled square all have the same top band that they share.

7:24.3

<b>Genetics of Polycystic Kidney Disease in Humans</b>		
<b>Autosomal Dominant:</b>		
PKD1	16p13.3	gene cloned (1994, 1995)
PKD2	4q21-23	gene cloned (1996)
<b>Autosomal Recessive:</b>		
ARPKD	6p21-p12	interval cloned

### **Two major types of autosomal dominant PKD (PKD-1 on chromosome 16 and PKD-2 on chromosome 4)**

For PKD that was done actually almost 10 years ago. We were able to discover that PKD-1, which is the most common form of autosomal dominant polycystic kidney disease is on chromosome 16. There is a second form, however, (PKD-2) that is on chromosome 4, and that gene was just discovered in 1996.

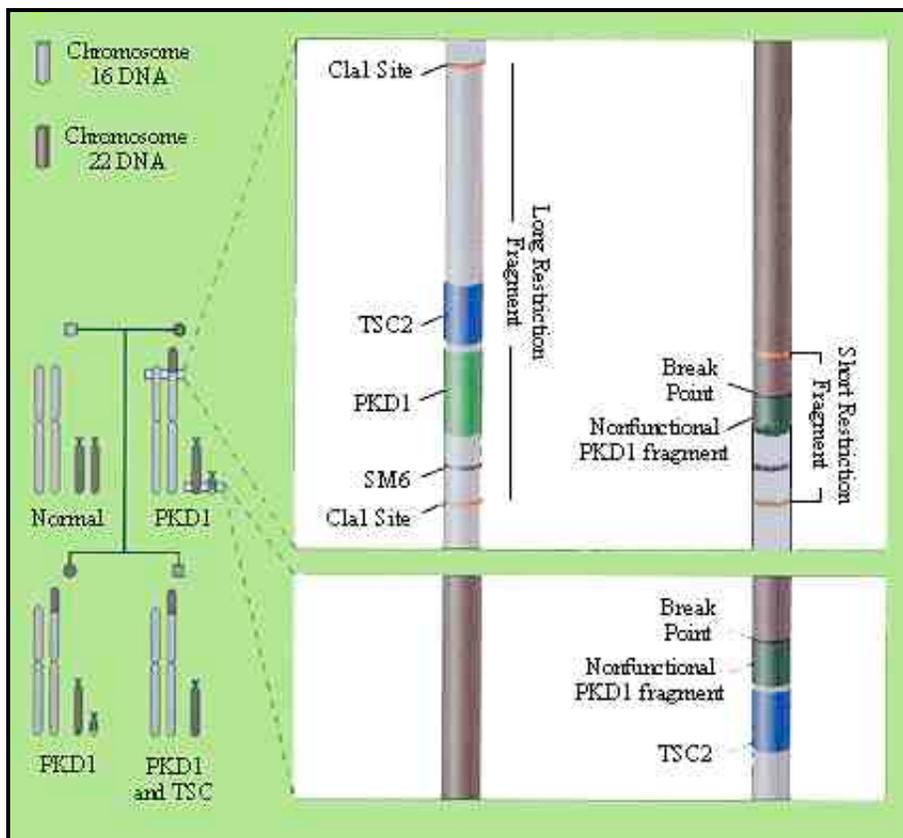
### A third type of autosomal dominant PKD also exists, the location of which remains to be discovered

There is at least one other form of PKD that is yet to be discovered. The reason we know that there is another form is, because there have been a number of families, including a family described by Dr. Turco here who is visiting from Italy, where there are many members of the family who have the disease but don't share the same chromosome tags (markers) for chromosome 16 or chromosome 4. Now we don't know what chromosome this new form of PDK is on yet. Maybe Dr. Turco knows. I am sure he is looking, as well as a number of other groups around the world. So there are more PKD genes yet to be defined, but PKD-1 and PKD-2 have given us plenty of information about the disease process.

### Autosomal recessive PKD (ARPKD) linked to chromosome 6

Because of genetic testing, we also know that the autosomal recessive form of PKD is completely separate. It is a very different disease process. Not only is it differently inherited, but we know genetically that it maps to a different chromosome, to chromosome 6. It is therefore the result of a different gene. It has a different protein; the protein has a different function. I am not going to talk about this much more today except to say that we are narrowing in on the autosomal recessive PKD (ARPKD) gene. We have a number of candidate genes that are being looked at. Hopefully within the next ...I don't know how long--one to five years, I guess, is a reasonable estimate... but at some point soon, we hopefully will be able to do for ARPKD what we have done for PKD-1 and PKD-2.

9:13.6



Source: *Germino GG. Hosp Pract (Off Ed) 1997 Mar 15;32(3):81-82.*

### Identification of the genetic problem associated with PKD-1 in 1994

For PKD-1, we were able to identify a piece of a chromosome to where the gene was. The gene was discovered in 1994 by a group of investigators in Europe [Cell 1994 Jun 17;77\(6\):881-894](#) They had found a family that had a very interesting property. This family actually had two different diseases, two different forms of cystic disease.

### A genetic clue from a coexisting condition: tuberous sclerosis

The mother had polycystic kidney disease. Her daughter had polycystic kidney disease, but her son had polycystic kidney disease and tuberous sclerosis. Now you don't need to know for today's discussion what tuberous sclerosis is, but investigators independently had shown that tuberous sclerosis mapped to the same chromosome region that polycystic kidney disease mapped to.

When these investigators looked carefully at the family, they discovered that this family had a break in their chromosome 16. And this was the clue that told them where the PKD gene was. The PKD gene was positioned at the point of the chromosome break. In any case, there was a break in the chromosome. And we know in genetics that typically, when you have a chromosome break that is associated with a disease, and it is inherited with the disease, that where the break occurs in the chromosome, is where your gene of interest lies.

And so these investigators then were able to use molecular tools to clone out, and what I mean by that is to isolate, a single piece of DNA of about 10,000 base pairs out of the three billion that we all have. They took a small piece of DNA and were able to characterize this and define the gene sequence.

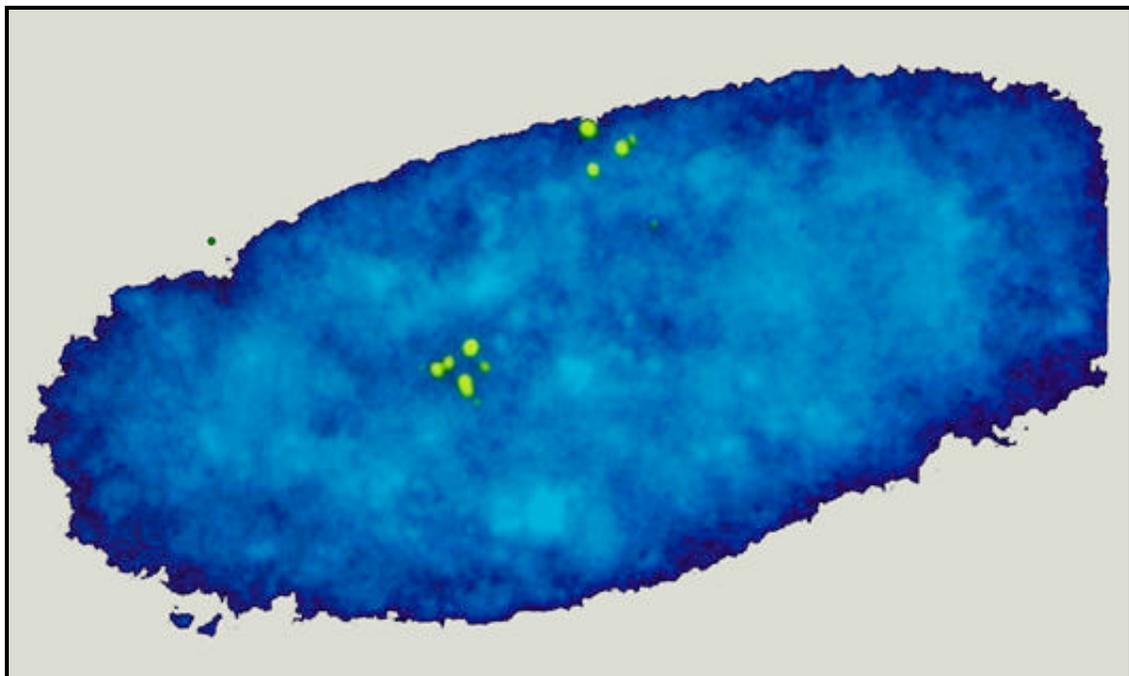
### **Gene duplication in PKD-1: a source of problems**

They discovered, however, as they were characterizing this piece of DNA that it had some very funny properties that made it very unique among genes. One of those properties is that about 70 percent of its length was present in multiple copies. Now for most genes we have just one copy, and one is more than enough. But with PKD, three-quarters of this particular gene was duplicated somewhere else.

Now why that is important is because it makes it difficult to evaluate PKD. When we look at sequence, when we take a piece of DNA and we clone it, we determine its DNA sequence. DNA is comprised of four base pairs and we can decode it by looking at how these four units are put together. The problem is when you pull it out a specific piece, you have to know that you're looking at the specific piece that goes with PKD, and not its "cousins". The "cousins" may have mistakes or may have other artifacts that can mislead us in decoding the PKD-1 sequence.

It took another year after the original gene was discovered to completely analyze the full gene sequence for PKD-1. At some point we will be able to show you what that looks like.

12:51.7

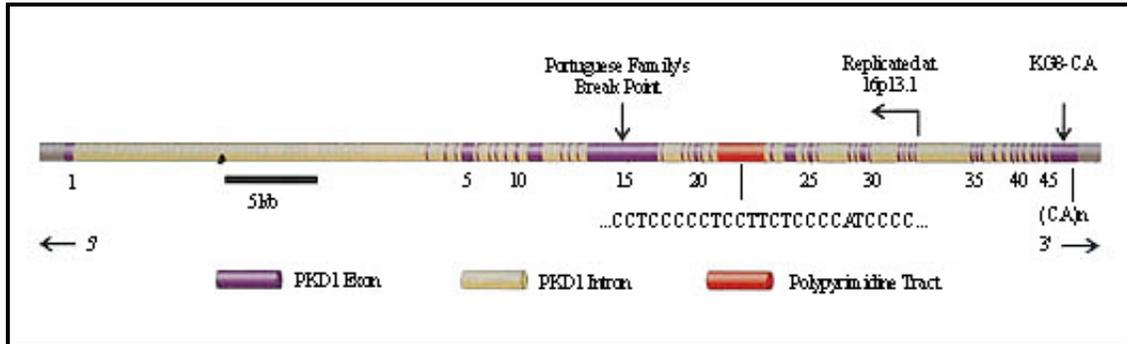


*Source: Courtesy of Antonio Baldini, Baylor College of Medicine*

### Gene duplication in PKD-1 illustrated

This is an example of what I mean by having more than one copy. This is an example of a cell nucleus. Now typically with a cell nucleus if you were to hybridize, that is to take a piece of the DNA and put it on it and find its match... if you were to normally do that in any one of our cells, with a normal gene you would find just two yellow dots. What you can see for PKD--this is a piece of PKD on a cell--you see a cluster of dots. There are four or five dots over here and four or five dots over here. That is just an example illustrating that we have more than one copy of the gene, and the copies are very, very similar. That made it very, very difficult to decode the PKD-1 gene.

13:56



Source: Germino GG, Hosp Pract (Off Ed) 1997 Mar 15;32(3):81-82

### The structure of the human PKD-1 gene

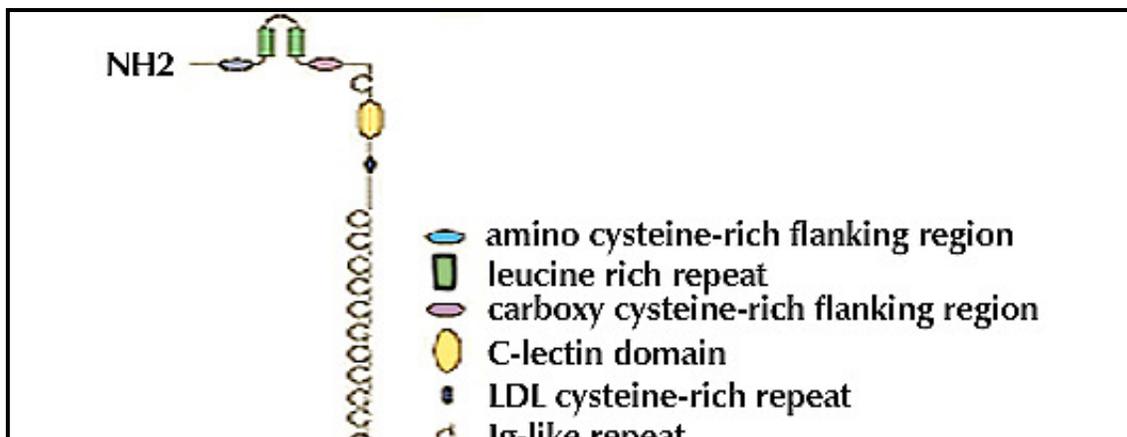
In 1995, we were able to do that by taking out a piece of DNA that had unique pieces on either side and then determining the entire sequence, just brute forcing it, as you have probably heard Craig Ventner doing for other chromosomes. It's in the newspaper all the time about the sequence being determined for different bacteria for instance.

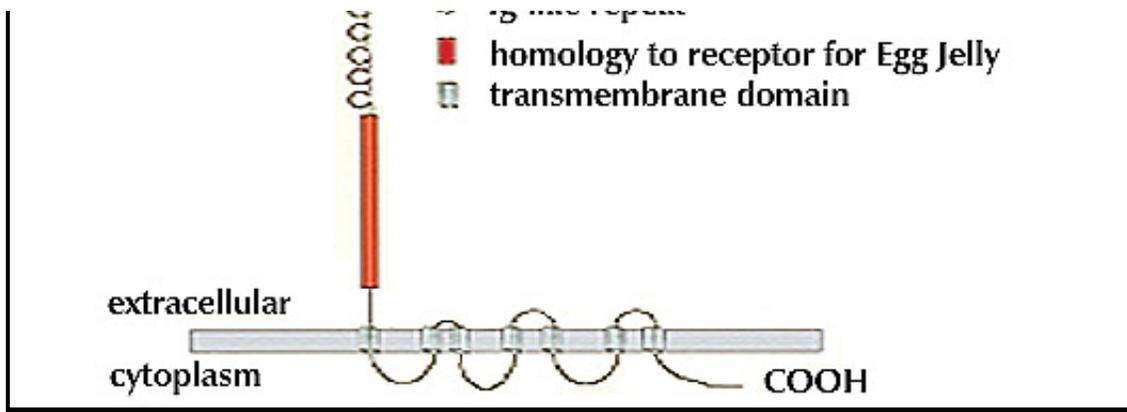
We used the same strategy for the human PKD-1 gene. This is the structure. What the structure tells us...it is just As, Cs, Gs, and Ts; that gives us no information. It is only when we decode it that we can determine what the likely protein is, and there are programs for doing that.

### Two properties of the PKD-1 gene: multiple copies, and an unusual element in the middle of the gene

There are two principles or two properties of the gene that I just want to highlight for you that are important. The first is, that this portion of it is present multiple times elsewhere. The second feature is an unusual element in the middle of the gene. As I said, DNA typically has four base pairs of sequence: A, C, G, and T; sort of like the 1s and 0s in a computer code. In this particular part of the gene for PKD-1, it is just Cs and Ts. Very, very boring... Cs and Ts. But we think it has a very important role to play in causing the disease, as I hope to make clear a little later in the talk.

15:19.5

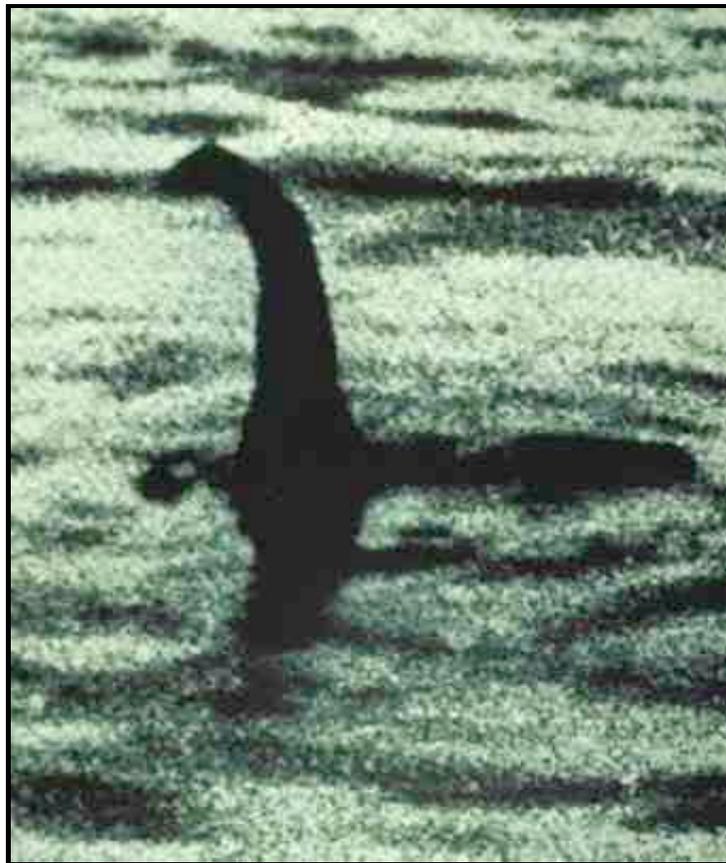




### The protein corresponding to the abnormal gene in PKD-1

This is what the decoded DNA sequence tells us about the protein. The protein is a very, very large protein. Again, it is a very unusual protein among the family of proteins that have been defined.

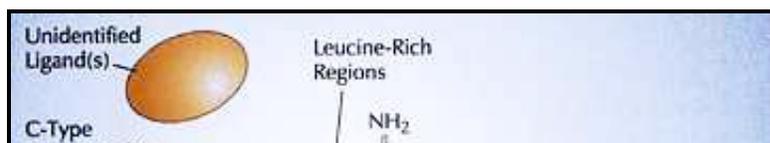
15:38

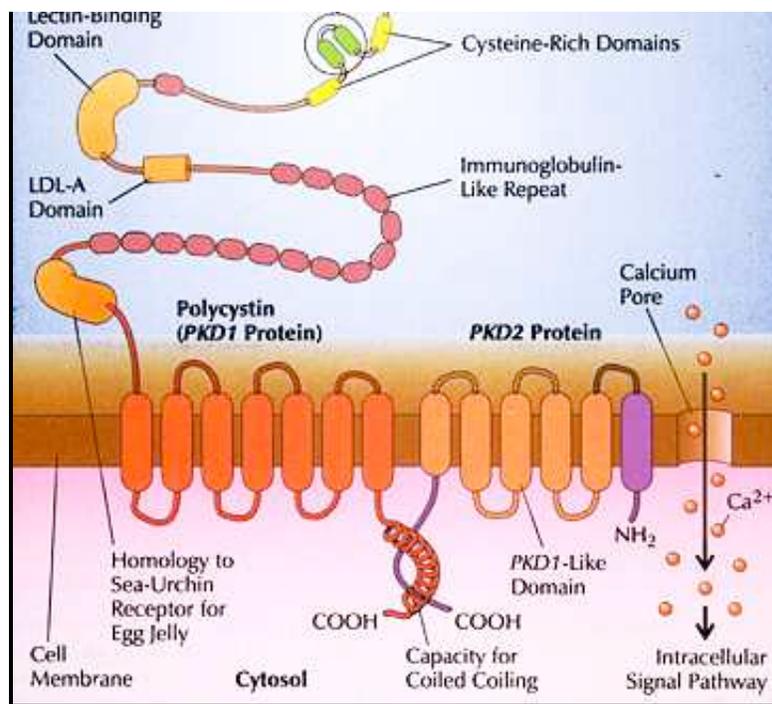


### Structure-function relationships

It looks a little bit like the following. Indeed it is just about as mysterious because it has been very, very difficult to really find out what it does. What we think this protein does is it sits on the cell surface. And the cells are surrounded by a matrix, like a jelly, and again contact other cells. We think this protein, the top part of this, sits on the outside of the cell, either going into the jelly or talking to other cells.

16:17.6





Source: *Germino GG, Hosp Pract (Off Ed) 1997 Mar 15;32(3):81-82*

### The protein associated with PKD-1: possible functions

We believe the inside sits in the cell talking to other important partners within the cell and sending information back to the control center in the cell called the nucleus. We think this protein sits on the tubule cell and helps tell the tubule how big it should be.

### Speculation on how renal tubules adjust and maintain their proper size

If you go back to the very beginning of the talk, I showed you that a tubule is very, very precisely defined. It is only the size of a hair and no bigger. Yet it does this a million times over just perfectly. How does a cell, how does a tubule know how to do this? Think about it. How does it do it every time, all the time, absolutely correctly except in PKD? There has to be information that is encoded to tell the tubes to make them just so and not any bigger. One of the ways we think it does that is by making the PKD protein. The PKD protein perhaps goes to the outside of the cell and talks to other cells and talks to the jelly and says, "You're just big enough and don't get any bigger." You can imagine if you take that away, if you make it abnormal, the cell keeps looking for the signal to stop and it doesn't and it keeps blowing up bigger and bigger and bigger. So that is what we think right now is the model for what this protein is doing.

00:00

## How autosomal dominant mutations can cause disease

### ■ Dominant negative mutation

One abnormal gene "gums up the works" by interfering with function of the normal copy.

### ■ True Dominant

Mutated gene has a new deleterious function, per se, or loss of normal regulation, but does not interfere with the function of the normal copy.

## ■ Haplo-insufficiency

Both copies are required to produce sufficient product. When one has loss of function, insufficient amounts of products are produced.

### **With PKD-1, why is disease caused by just one abnormal copy of the affected gene?**

We would like to find some other clues though that can give us some insight into how the disease develops. With an autosomal dominant disease, as I said, it takes just one abnormal copy to cause the disease process. The obvious question is: Why can't the other copy be protective? If you have one abnormal copy and one normal copy, why can't the normal copy do the job to make up for the defect of the abnormal copy?

### **Three potential mechanisms**

In diseases like PKD, in autosomal dominant diseases, there are three different ways that a diseased gene can cause disease.

#### **a) Mutated gene gums up the works: dominant negative mutation**

The first is that this abnormal copy really gums up the works. Imagine that you're trying to put together, for instance, a puzzle: If you have one piece which is necessary for the other pieces to come into the puzzle, if you cut this piece here or if you break off one of its adjoining little projections ...I don't know whatever they call it that stick out... if you break that off, all of the pieces that would have tied into it can no longer work. So the whole puzzle comes to a halt even though you have other normal pieces. That is called a dominant negative mutation. In such a case the abnormal copy is able to interfere with the function of the normal copy. That is a pretty common mechanism for causing disease with dominant diseases.

#### **b) Mutated gene functions in a deleterious fashion: true dominant**

The second way that a mutation in a dominant disease can cause disease is if the mutated product has a new function. Let's say, for instance, it's acting like a gas pedal and it is supposed to go up and down, it's supposed to turn up and down. And let's say that the gas pedal gets stuck in a locked position on the "on" position, so it is going too fast. Of course, it's going to have a bad outcome. Likewise, the same thing can happen in a diseased gene. If you have one normal copy which turns up and down properly but you have one abnormal copy that loses its ability to be turned off when it should be turned off, it can overcome the braking effect of the normal copy. That is called a true dominant. So one abnormal copy is sufficient to create a problem. It is not by interfering with the other copy, it is just because it has a new function or an overactivity.

#### **c) Mutated gene causes a decrease in activity**

The third way that a dominantly inherited mutation can cause disease is if the mutation results in a decrease in activity. So you have two copies, you take away one, and sometimes one is just not enough. If you're trying to lift something up, you can imagine you may not be able to lift it up with just one hand. Trying to do push-ups, for instance, if you try to do one-handed push-ups, you can't do it unless you're like one of those big guys. Certainly I can't do it. You need two hands to do it. This other model, this other form, is called haplo insufficiency. There again one copy is made normally, one copy is not made at all, but the total amount made is not enough and you need more product.

### **How a mutated gene causes disease: implications for therapy**

It is very important knowing how a mutation causes the disease because it will define partly how you make your therapies.

### **Therapeutic approach for insufficient production of normal protein**

For instance, if the problem is that you don't have enough normal protein, you don't have enough normal product there, just by producing more normal product will take care of the problem. So things like gene therapy make sense, or one can figure out how to bypass the

block in the cells. So if we can get into the cell and turn on whatever it is supposed to be doing, thereby skipping or bypassing the block, we can perhaps stop the cysts from forming.

**Therapeutic approach for a dominant negative protein**

If, however, it is a dominant negative, it doesn't matter how much normal you put in there. The mutated copy is always going to interfere. So it has very important implications for investigators trying to develop therapies or make models to test therapies.

**References at the end of part 3**

**[To Go To PART TWO Click Here](#)**

---

**[Back to list of talks for this symposium](#)**

**[Back to symposia list by topic](#)**

**[Back to symposia list by meeting](#)**

**[Back to HDCN home page](#)**

