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Cover Page Footnote
We would like to thank Dr. DebBurman for his patient instruction and his assistance with primary literature. We would also like to thank Lital Silverman and Mike Zorniac for helping us edit the paper. Josh Haas and Krista Kusinski have helped us immensely in understanding how to do science. Our minds have suckled and become mature in their cognitive powers at our gracious mother institution, Lake Forest College.

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Polycystic Kidney Disease: The Cyst-ematic Destruction of Renal Function

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Summary

Polycystic kidney disease is the most common genetic, life-threatening disease, affecting more than 12.5 million people worldwide. Fluid-filled renal cysts that eventually destroy renal tissue and renal function altogether are characteristic of polycystic kidney disease. The autosomal dominant form of the disease which is also the most common form, ADPKD, is linked to mutations in the genes PKD1 and PKD2. The complete normal function of PKD1 and PKD2 is unknown, but most research suggests that they play some role in cell signaling and controlling the cell cycle. The diseased phenotype is thought to be caused by mutations in these genes that cause misregulation of the cell cycle leading to proliferation. The recessive form of polycystic kidney disease, ARPKD, is triggered by a mutation in the gene PKHD1 and is manifested more severely than the dominant form. ARPKD has not been as widely studied as ADPKD because it affects fewer people than the dominant form of the disease. Currently, there is no treatment for any of the forms of PKD, though some studies have shown some promise in effectively attenuating renal cystic growth.

History, Basic Facts, and Epidemiology

Polycystic kidney disease (PKD) was first documented around 1540. It was known as Bright’s disease until the mid 1980’s when it acquired its current name. It is the most common genetic life-threatening disease, affecting 1 out of 1000 people, 600,000 Americans, and 12.5 million people worldwide.

There are two forms of PKD: autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD). The genes responsible for ADPKD are PKD1 and PKD2, and the mean age of onset is between 30 and 40 years of age. The disease infects men, women, and all races equally.

PKHD1 gene causes the recessive form of PKD (ARPKD). This type of the disease is the more aggressive and lethal form. The mean age of onset is during prenatal development. Similarly to ADPKD, all genders and race are equally affected. Most infected individuals of ARPKD develop renal failure by their early twenties. Patients with ADPKD or ARPKD die as a result of the disease, but medicine may be taken to relieve pain and to prolong life.

Symptoms

PKD is characterized by the formation of cysts on the kidneys. Cysts are blister-like structures that enlarge over time and fill with fluid. The proliferation of cysts on the kidneys impairs proper kidney function. The major symptoms of PKD stem from poor kidney function. High blood pressure is an early symptom of polycystic kidney disease. Other symptoms include blood in the urine, urinary tract infections, kidney stones, and dehydration. As the disease progresses, other major health problems can arise. Mitral valve prolapse, which is characterized by the inability of the mitral valve of the heart to close all the way, can lead to chest pain and heart palpitations. Intracranial aneurisms, or the bursting of blood vessels in the brain, may also occur in PKD patients. Cysts can eventually spread to the liver. These cysts do not impair the function of the liver, but can be painful for the patient.

PKD1 and 2 as causes of polycystic kidney disease

Renal cystogenesis is caused by rapidly proliferating epithelial cells in kidney ducts and tubules. Attempts to elucidate the molecular mechanism of the disease have differed on PKD1 and PKD2 mutations, which result in grossly increased cell proliferation due to an acceleration of the cell cycle. Polycystins-1 and 2 (PC1 and PC2), the proteins coded for by PKD1 and PKD2, respectively, are membrane-bound proteins. Researchers have found that the cell-regulatory functions of these proteins are due to the effects they have on other regulatory pathways. How PKD1 and PKD2 interact with these pathways and which pathways they interact with are the two central questions of most ADPKD research.

The Normal PKD1 gene and polycystin-1

The majority of PKD research has focused on the PKD1 gene, because mutations involving it account for 85% of all cases of ADPKD. Located on chromosome 16, this gene encodes polycystin-1 (PC1). The complete function of the gene and its protein product is unknown; but an understanding of PKD1 has begun to take shape as researchers discover more about it.

Using computer analysis, Martijn, et al. (2000) found that PC1 is a large transmembrane protein containing an extracellular N-terminus and an intracellular C-terminus. The C-terminal region has multiple hydrophobic domains (Bhunia, et al., 2002). Polycystin-1 anchorage to adhesion complexes is needed for signal transduction in order to maintain cell adhesion protein sorting and cell polarity (Martijn, et al. 2000).

The PKD1 gene is thought to be a member of the tumor suppressor family of genes. This is supported by Boletta, et al. (2000), who found that polycystin-1 expression resulted in reduced growth rates and spontaneous tubulogenesis in epithelial kidney cells. One downstream effect of PC1 activity might be the inhibition of apoptosis. Polycystin-1 decreases the proliferation of epithelial cells in developing tissues, allowing them to proceed down terminal differentiation pathways without undergoing apoptosis. (Boletta, et al. 2006).

The diseased function of PKD1: two hypotheses

Although a few of the pathways by which PKD1 regulates the cell cycle have been discovered, the exact nature of this regulation is still unknown. There are two conflicting hypotheses about how PC1 interacts with the regulatory pathways. The first suggests that PC1 controls many different regulatory pathways. Bhunia, et al. (2002) argue that mutations in PKD1 give the diseased phenotype because all of these pathways are disrupted as a result. A disruption to only one or two tumor suppressors, for...
Figure 1. Overview of the molecular causes of polycystic kidney disease. PKD1 resides on chromosome 16 and encodes polycystin-1 (PC1). PKD2 encodes PC2 and PKHD encodes fibrocystin. These three proteins are thought to interact with other regulatory pathways in the cell. Mutations would then cause dis-regulation of the cell cycle and proliferation of kidney epithelial cells, resulting in cysts. A few specific pathways have been discovered, such as the JAK-STAT pathway and PC1's interaction with p53, but there are thought to be other pathways that are affected by PC1 and PC2 (Low, et al., 2006). Fibrocystin is known to be cleaved (as is PC1) and translocated into the nucleus, but its precise function in the nucleus remains obscure. p53 and p21 are both tumor suppressors whose expression is partially regulated by PC1 or PC2. Question marks and dotted line indicate hypothesized pathways.

either, does not cause cystogenesis, because there are many other regulatory pathways to compensate. These “back up” pathways remain properly regulated by PKD1 (Bhunia, et al., 2002). This may explain why PKD is a genetic disease—there needs to be a basic problem that affects all of these regulatory pathways.

The second hypothesis is that there must be a master pathway that is commonly affected by PKD1, PKD2, and all of the numerous other genes that give a PKD-like phenotype when mutated (Shillingford, et al., 2006). In support of this hypothesis, it is argued that all the nearly identical polycystic phenotypes must result from the disruption of a single, common pathway, and that all the genes that can cause this phenotype must necessarily be affecting this same pathway. For example, the ADPKD phenotype caused by PKD1 is virtually indistinguishable from that caused by PKD2. This strongly argues for a common target pathway for the protein products of both genes. Although they seem quite different, the “multiple-pathways” hypothesis and the “common pathway” hypothesis do not necessarily contradict each other.

Feedback Inhibition in the PKD1 gene, polycystin-1 and the tumor suppressor p53

In seeming support of the multiple pathways hypothesis, PC1 has been implicated in many cell cycle control pathways. Van Bodegom, et al. (2006) found that the tumor suppressor protein p53 is a transcriptional repressor of PKD1. Kidneys of p53 null mice exhibited up regulated PKD1 mRNA levels compared to wild type mice, indicating that more polycystin-1 was being transcribed. Polycystin-1 activates p53, which in turn controls PKD1 gene expression. This finding illustrates a type of feedback inhibition among PKD1, the protein it encodes, and p53, which represses PKD1.

PKD1 expression arrests the cell cycle

Another important discovery is that PKD1 expression induces accumulation of cells in the G0/G1 phase of the cell cycle. It seemed likely that this cell-cycle arrest was due to interaction with a tumor suppressor, but research was needed to establish this. In 2002, Bhunia, et al. discovered that over expression of PC1 resulting in increased downstream activity of p21, a Cdk inhibitor. They found that PC1 physically attaches, along with PC2, to JAK2, allowing JAK2 to phosphorylate STAT1, a transcription factor. STAT1, when phosphorylated, induces expression of p21, a tumor suppressor. So in this pathway, PKD1, along with PKD2, functions as a tumor suppressor, since it acts as the initiator of a signaling pathway that eventually leads to the expression of p21.

This suggests a mechanism for the development of polycystic kidney disease. If a mutation in PKD1 reduced its ability to interact with JAK2, then an important tumor suppressor would be down-regulated in the cell, resulting in cell proliferation. In support of this, Bhunia, et al. (2002) found that the levels of phosphorylated STAT1 and p21 were much lower than in mice that had the PKD1 gene than in mice that did not. Mice lacking the PKD1 gene also developed cysts, while those having the gene did not. This study argues strongly for the common-pathway hypothesis. Both PC1 and PC2 are required to activate JAK2, so a mutation in either of them would disrupt the same pathway in nearly the same way.

The above study may suggest that there are other nuances of the function of PC1. The implication is that PC1 interacts with JAK2 at the membrane, and that STAT1 is
then phosphorylated before going to the nucleus to act as a transcription factor. There may be other ways PC1 can affect transcription in the nucleus.

Qian, et al. (2002) showed that PC1 can be cleaved at the GPS domain on its extracellular N-terminus. They deleted various segments of the extracellular N-terminus of PC1, expressed these in mammalian cell lines, and tested for cleavage. Cleavage only occurred if a specific domain, called the receptor for egg jelly (REJ) domain, was present. If this domain was missing in whole or in part then cleavage would not occur.

Several mutations to the REJ domain, that occur in nature, were identified and expressed in mammalian cell lines. These engineered cells were then cultured in an in vitro tubulogenesis model, where they were found to develop cyst-like structures. Cells with wild-type PC1 developed tubules as normal (Qian, et al., 2002). Apparently, cells need PC1 to be cleaved at the GPS domain in order to function properly.

Cleavage of PC1 is important for signaling

The finding that PC1’s extracellular N-terminus could be cleaved raised the question of whether the cytoplasmic terminus could also be cleaved. Could this be the mechanism by which PC1 regulates the cell cycle? In 2006, Low et al. showed PC1’s cytoplasmic C-terminus tail undergoes cleavage and translocation to the nucleus. Interestingly, this study also found that PC1 interacts with a STAT protein. The C-terminal tail coprecipitated with STAT6 and P100. STAT6 is a transcription factor that acts in concert with P100 to induce gene transcription. P100 and STAT6 were also found to colocalize on the cilia of cells in culture, which further supports that they are closely associated with one another. Also, STAT6 dependent transcription was increased when the C-terminal tail of PC1 was present in the cell, but not when PC1 remained fully attached to the membrane. Additionally, the expression of the PC1 C-terminal tail alone was found to cause cystogenesis in Zebra fish embryos (Low, et al., 2006).

Low, et al (2006) also outlined a possible mechanism by which disruption or malfunction of this pathway could contribute to cystogenesis. Because PC1 is located primarily on the cilia of the epithelial cells, it has been widely hypothesized that the polycystins may play a role in mechanosensation (Deldas, 2004). Low, et al. (2006) tested the effect of flowing versus stagnant fluid on a culture of cells that expressed the full-length PC1 protein. They found that under flow conditions, STAT6 localized to the nucleus, but under no flow conditions, STAT6 remained localized on the cilia. If the PC1 C-terminus remained associated with STAT6, the no flow condition must have resulted in cleavage of this tail and subsequent translocation to the nucleus, where the complex would presumably increase gene transcription. Low, et al. (2006) suggest that cyst formation in kidney tubules may cause fluid flow to stop, cause cleavage of PC1 and up-regulation of STAT6-dependent gene transcription. This could cause further cell proliferation, worsening the condition of the already diseased kidney.

PKD2 gene and polycystin-1

Although most cases of ADPKD are due to PKD1, about 15% of all cases of ADPKD are caused by mutations in PKD2 (Wu, et al., 1998). The gene PKD2 is located on chromosome 4 in the region 4q21–q23. The complete functional role of the protein product of PKD2, polycystin-2 (PC2), is still unknown. Recent studies have begun to uncover the properties of PC2. This will provide insight into the mechanism of cyst formation that is caused by lack of PC2. How PKD2 is involved in the cell cycle is at the focus of research, since cyst formation is clearly caused by abnormal cell proliferation. Insight into the normal function of PC2 will allow the formation of possible hypotheses as to how cysts form on the kidneys as a result of PKD2 mutation.

How PKD2 Mutations Cause Cyst Growth

According to some studies, polycystin-2 has channel-like properties (Gonzalez-Perrett, et al., 2001). It acts as a nonselective cation channel across an epithelial membrane. It is also a Ca$^{2+}$ permeable membrane. PKD2 in some cases specifically regulates Ca$^{2+}$ transport in response to store depletion through interactions with specific TRPC proteins (Tsikas, et al). Abnormal function of this channel could cause excessive fluid buildup in renal cells, which results in renal cyst formation and eventual destruction of renal tissue. This presents a possible mechanism of cyst formation in ADPKD patients.

Gallagher, et al. (1999) show that polycystin-2 may also have a link with the actin cytoskeleton through its interaction with a protein Hax-1. Hax-1 interacts with cortactin, an actin-binding protein. The relationship between the three proteins suggests that PKD2 is involved in the formation of cell-matrix contacts. In this case, cyst formation in PKD patients could be explained because of dysfunctional formation of cell-matrix contacts leading to cystic enlargement in the tubular structures in the kidney. This proposes another mechanism for cyst formation in PKD patients with a mutation in PKD2. However, the specific pathway in this protein complex is not clear. There are a number of possible ways that the three proteins affect cell-matrix interactions. The correct pathway has yet to be found and may be the target of future studies.

In order to suggest reasonable treatments for PKD, we must understand the disease completely. Studies on PKD2 and its protein product, PC2, continue so that we may fill in this gap in knowledge in the hopes of finding a cure for this disease. Studies that delve deeper into the findings of others to further the research will bring us closer to finding a cure for PKD. For example, targeting the molecular pathway for the interaction between polycystin-2, Hax-1, and cortactin will allow a better understanding of the normal function of polycystin-2 that is disrupted by mutation, causing cyst proliferation.

Relationship between PKD1 and PKD2

The fact that ADPKD is manifested the same way in patients with mutations in PKD1 and mutations in PKD2 supports the common pathway hypothesis, although this hypothesis remained unverified for several years. It is important to note that PC1 was not able to phosphorylate JAK2 without PC2. When PC2 was present with PC1, JAK2 was phosphorylated and p21 levels increased. Therefore, the activity of PC1 depends on the presence or absence of PC2. This finding helps uncover the relationship between PKD1 and PKD2. Neither polycystin can activate the JAK-STAT pathway alone, which explains why a mutation in either gene causes abnormal cell proliferation of cystic epithelia.

Location, Function, and Localization of PKHD1

As more evidence is being found to support this interaction between PKD1 and PKD2, scientists are beginning to better understand the causes of ADPKD. However, as knowledge for ADPKD is advancing, research on ARPKD has only begun. Ward, et al. (2002), Onuchic, et al. (2002), and Zhang, et al. (2004) have just discovered the gene
responsible for PKHD1 in the 6p21cen-region on chromosome 6. Accordingly, this has opened research possibilities for researchers to discover the gene responsible for ARPKD, what protein PKHD1 encodes, and to ascertain what mutations cause ARPKD. Furthermore, researchers are postulating possible kidney functions.

PKHD1, thus far, is known to encode a 4,074 amino acid chain protein and is composed of 67 exons. The protein is known as fibrocystin and/or polyductin (Zhang, et al., 2004). It contains multiple TIG and TIG-like domains, TMEM2 and DKFZ homology, and a transmembrane domain. Fibrocystin colocalizes to the basal bodies of the primary cilia in the renal epithelia of the kidney and is an integral trans-membrane protein (Zhang, et al., 2004). Its current function is unknown, but Zhang, et al. (2004) and Hiseberger, et al. (2006) hypothesize that polyductin may initiate epithelial differentiation, alter ciliary function, or act as a receptor protein. Zhang, et al. (2004) have found that the expression of fibrocystin, in the mice PKHD1 ortholog, is essential during embryological organogenesis (Zhang, et al., 2004). Fibrocystin lines the fetal epithelial cells of neural and mesonephric tubes, which eventually differentiate into collecting ducts. The protein is also expressed on human adult kidney ducts and tubules. Thus, it seems that the expression of PKHD1 is essential in development and for normal function of the kidney (Zhang, et al., 2004).

Mutations in ARPKD

To begin research on the particularities of ARPKD, researchers are establishing studies on known mutations of PKHD1. However, one difficulty hindering research on ARPKD is that mutations randomly span the chromosome. In addition, all mutations are “private mutations” unique to an individual (Bergmann, et. al., 2005). Hence, there can be an infinite amount of mutations with an infinite amount of causes. This also causes different phenotypes in individuals depending on the severity of the case. The symptoms can range from mild kidney dysfunctions to death. Moderate symptoms are generally associated with missense mutations while death correlates with termination-type mutations. Other mutations that have been discovered include insertion, deletion, frame-shift, and truncation (Rossetti, et al., 2003). Specific mutation cases and up-to-date information on ARPKD can be found in the ARPKD mutation database (URL: http://www.humgen.rwth-aachen.de).

Fibrocystin Cleavage is Regulated by Ca\(^{2+}\)

Even with the difficulty of research by the numerous mutations of PKHD1, studies are still performed. Hiseberger, et al. (2006) elucidated a possible pathway for fibrocystin production through the ADPKD pathway including polycystin-1 and 2. One known mechanism of polycystin-1 is that it undergoes regulated proteolytic cleavage at the C-terminus with the mediated release of Ca\(^{2+}\). Therefore, Hiseberger, et al. (2006) hypothesize that fibrocystin undergoes similar regulation by Ca\(^{2+}\), which, in turn, causes proteolytic cleavage at the C-terminus. It is nevertheless postulated that a mutation or an interference with this pathway may be a factor for PKD, but this theory has yet to be supported (Hiesberger, et al., 2006).

Although PKHD1 was only recently discovered, researchers are already mapping the abundant mutations of PKHD1. This, however, is only the start, as scientists are beginning to elucidate fibrocystin function and its importance in the kidney. As research continues to enhance knowledge about ARPKD, as well as for ADPKD, the chances of finding a treatment also increase. For now, there are short-term treatments and clinical trials for potential drugs.

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Figure 2. Proteins involved in polycystic kidney disease. This diagram shows the autosomal dominant polycystic kidney disease (ADPKD) proteins, polycystin-1 and 2 and the autosomal recessive polycystic kidney disease (ARPKD) protein, fibrocystin. These models of the proteins structure demonstrate the cleavage sites, conserved domains and regions of homology with other proteins. The locations of N-terminus – in the extracellular space – and the C-terminus – in the cytosol - are shown for each protein.
Current treatment research

Currently there is no effective therapy for ADPKD or ARPKD. Kidney transplantation and dialysis are the only methods that can be performed to prolong life. Murine models have been successfully employed to increase the understanding of the molecular mechanisms of polycystic kidney disease. These tests have been used to explore possible therapeutic techniques for polycystic kidney disease, mostly ADPKD, the more common form. Current treatments of polycystic kidney disease involve caspase inhibitors, CDK inhibitors, Triptolide, and mTOR inhibitors.

Caspase inhibitors

The caspase’s role in apoptosis makes it a suitable possible target for treatment of PKD. Caspase inhibition in other animal models has been successfully used on non-renal organs; heart, liver and pancreas, that have been injured due to apoptosis (Tao, et. al., 2005). IDN-8050, a caspase inhibitor, was used to treat Han:SPRD rats who had PKD. IDN-8050 significantly reduced apoptosis and cell proliferation in cystic and non-cystic tubules. Triptolide, a potent and selective inhibitor, was used to treat Han:SPRD rats who had PKD. IDN-8050 significantly reduced apoptosis and cell proliferation in cystic and non-cystic tubules. Triptolide arrests epithelial cell growth and attenuates cyst formation by restoring Ca²⁺ release (Leuenroth, et. al., 2007). Thus, Triptolide offers a therapeutic solution of PKD through the restoration of calcium signaling in the diseased kidney. Future studies in other animal models and clinical trials should be possible since there is an existing common use of the drug by humans with no knowledge of harmful side effects.

mTOR inhibitors

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription (Walz, 2006). mTOR has previously been identified for its role in proliferation of activated T-lymphocytes (Walz, 2006). As a result, Rapamycin has been approved by the Food and Drug Administration (FDA) as an immunosuppressive drug used after organ transplantation to prevent transplant rejection (Shillingford, et. al., 2006). The mTOR inhibitor, Rapamycin effectively blocks cyst progression, inhibits cyst and kidney growth and prevents renal failure (Shillingford, et. al., 2006). These findings nurture the hope that therapeutics for ADPKD, since the drug has been safely used over extended periods of time in humans and inhibits renal failure. Future studies in other animal models of PKD and even clinical studies in humans should prove vital in the search for a cure.

Conclusion

Polycystic kidney disease is characterized by the abnormal growth of fluid filled cysts on the kidneys. This cystic growth eventually impairs the normal function of the kidney and the urinary system. There are many gaps in our understanding of this disease such as the mechanism of cyst formation and how this process can be regulated. By uncovering the normal protein function(s) of PKD1, PKD2, and PKHD1, we will be able to determine how the mutated forms of these proteins alter the cell cycle. PKD1 and PKD2 play important roles in cell proliferation. PKD1 acts as a tumor suppressor, induces cdk inhibition, decreases cdk 2 activity, and arrests the cell cycle. PKD2 is a channel like transmembrane protein that regulates ion transport. PKD2 interacts with Hax-1 and cortactin which suggests a possible link between PKD2 and the actin cytoskeleton. These findings provide insight as to how the disease is initiated and how it progresses throughout the kidneys in ADPKD.

The recessive form of the disease has not been as widely studied because the PKHD1 gene was only recently discovered. PKHD1 codes for fibrocystin, a transmembrane protein, whose function is not completely known. One difficulty inhibiting the discovery of ARPKD is that each case of the disease has private mutations.

Without a real cure for PKD, kidney transplant and dialysis are the most effective forms of treatment. Other therapies are being considered, such as the CDK inhibitor Roscovitine. However, these therapies have only been tested on animal models and their effects on human patients with PKD can only be hypothesized. The focus of future
therapy research should be on further testing of potential drugs, like roscovitine, on PKD in different animal models to test whether they are equally effective. This, however, cannot be accomplished without first discovering the normal function of PKD1, PKD2, and PKHD1 and how these gene mutations cause ADPKD and ARPKD.

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