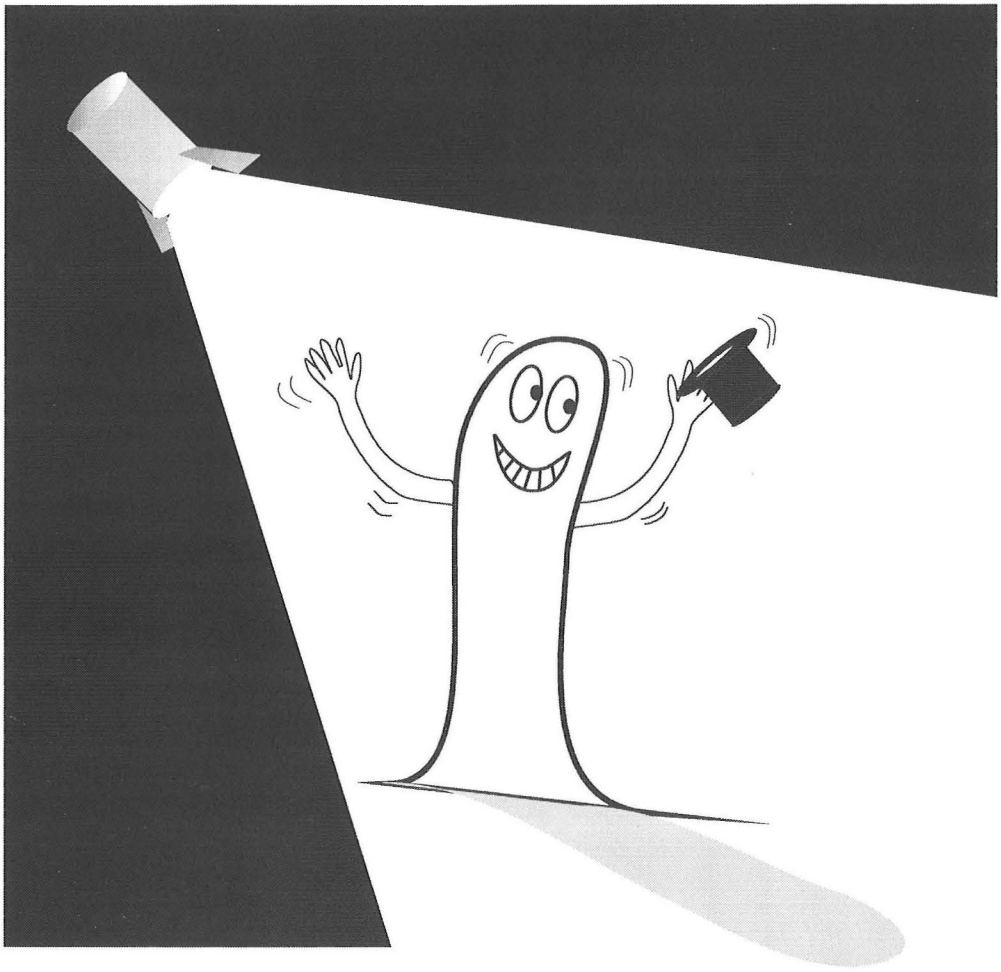
Disorders of Cilia

A Neglected Organelle in the Spotlight



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Introduction

Cilia are hair-like organelles that project from the surface of the cell. In medical school we are taught that cilia are present on certain epithelia where they beat rhythmically and have a propulsive function. For example, cilia lining the respiratory tract are involved in moving mucus from the airways to the pharynx. The beating of respiratory cilia can be inhibited in acquired conditions such as tobacco smoking and viral and bacterial infections. In this review, I will discuss **primary disorders** of cilia. First, I will review the structure and function of cilia. Second, I will discuss three primary disorders of cilia: primary ciliary dyskinesia, retinitis pigmentosa, and polycystic kidney disease. I will review the clinical manifestations and illustrate how genetic studies in humans and experimental animals, including some from our laboratory, have elucidated the mechanism of disease. These studies have revealed an unexpectedly broad role of cilia in the normal development and function of numerous organs, including the brain, eye, heart, and kidney. Because of space limitations, this review will focus on the molecular pathogenesis of ciliary disorders. For discussions of diagnosis and treatment the reader is referred to standard textbooks.

Historical Perspective

Cilia were first observed by the Dutch fabric merchant **Antoni van Leeuwenhoek** in the 17th century. Contrary to popular belief, van Leeuwenhoek did not invent the microscope, but he was particularly skilled at grinding glass lenses and produced more than 400 instruments, some capable of magnifying up to 200×. van Leeuwenhoek was also endowed with an insatiable curiosity, and he examined virtually any object that could be placed under the lens. He reported his findings in a series of letters to the Royal Society of London and is credited with the discovery of bacteria, erythrocytes, and insect parasites. On Christmas Day 1702 van Leeuwenhoek discovered an organism in pond water, called *Vorticella*, about which he wrote: "In structure these little animals were fashioned like a bell, and at the round opening they made such a stir, that the particles in the water thereabout were set in motion thereby...which sight I found mightily diverting." van Leeuwenhoek correctly concluded that this motion was a means for bringing food to the organism. We now know that the motion was created by **motile cilia** surrounding the mouth of the organism.

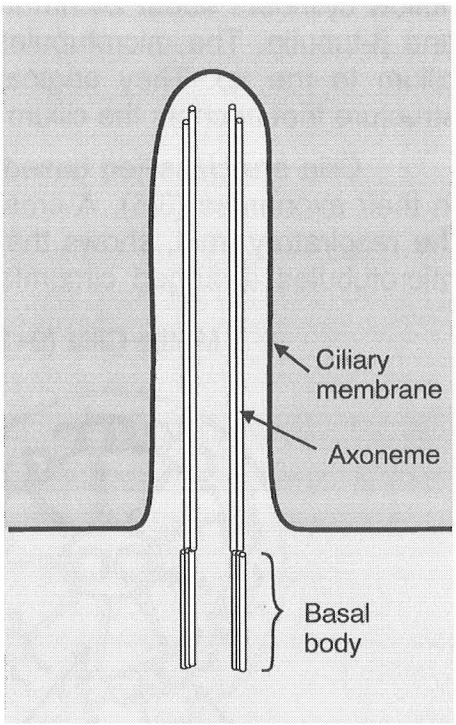

Figure 1. Structure of cilia. Cilia consist of an axoneme composed of microtubules surrounded by the ciliary membrane. Cilia are anchored in the cell body by the basal body.

van Leeuwenhoek is also credited with the discovery of flagella, which are modified cilia that are longer in length and present in only 1 or 2 copies per cell. In his own semen—which was acquired, he stressed, not by sinfully defiling himself but as a natural consequence of conjugal coitus—he observed a multitude of "animalcules," less than a millionth the size of a coarse grain of sand and with thin, undulating transparent tails. The tails that van Leeuwenhoek observed represent **flagella** that propel the sperm to the egg. van Leeuwenhoek reported his discovery to the Royal Society but requested that they not publish his letter, believing it would lead to disgust or scandal. They published it anyway, and it turned out to be one of his most important contributions. More than 300 years after van Leeuwenhoek's original discoveries of cilia and flagella, we are finally beginning to understand the normal functions of these organelles and their roles in human disease.

What are Cilia?

Cilia (sing., cilium) are thin, hair-like appendages that project from the cell surface (1, 2). They are 0.2Error! Not a valid link.0.3 μm in diameter and 1–20 μm in length. Cilia consist of a shaft called the **axoneme** that is composed of microtubules surrounded by a membrane bilayer that is continuous with the cell membrane. **Microtubules**, which are a constituent of the cell's cytoskeleton, are hollow cylinders about 24 nm in diameter that are composed of polymerized α- and β-tubulin. The microtubules in the axoneme extend from the base of the cilium to the tip. They originate from the **basal body**, a microtubule-based structure that anchors the cilium in the cell body (Fig. 1).

Cilia are classified based on their motility and the pattern of microtubules in their axonemes (3-5). A cross-section through **motile cilia**, such as those in the respiratory tract, shows that their axonemes contain nine outer doublets of microtubules arranged circumferentially around two central microtubules (**9+2**

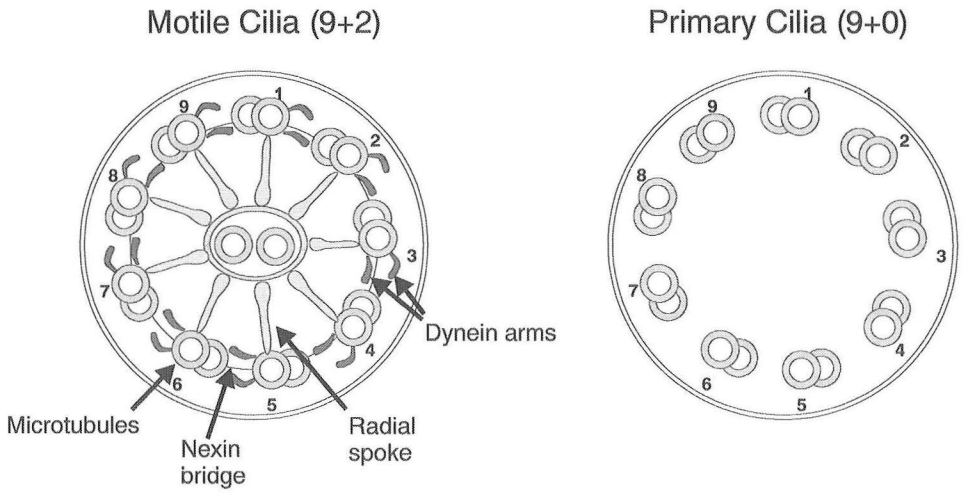

Figure 2. Cross-sections of the axonemes of motile cilia (left) and primary cilia (right). Motile cilia contain a 9+2 pattern of microtubules, inner and outer dynein arms, nexin bridges, and radial spokes. Primary cilia contain a 9+0 pattern of microtubules, lack dynein arms, and are immotile.

pattern) (Fig. 2). A motor protein, called dynein, forms inner and outer **dynein arms** between adjacent microtubule doublets. In addition, the axonemes of motile cilia contain nexin bridges and radial spokes that cross-link the microtubules. Motile cilia are found in many copies per cell and move with a whip-like motion. The movement of cilia is produced by the dynein arms, which are ATP-dependent molecular motors that are attached to one microtubule doublet and move along the adjacent microtubule doublet. The movement of the dynein arms causes the microtubule doublets to slide against each other, and this sliding motion forces the cilium to bend. The nexin bridges and radial spokes help translate the sliding motion of the microtubules into ciliary beating.

Motile cilia are found on the free surface of epithelia in specific places in the body. In the **respiratory tract**, motile cilia are involved in mucociliary clearance (4, 6) (Fig. 3). **Mucociliary clearance** refers to the movement of mucus and entrapped particles from the lungs to the oropharynx. The epithelial cells lining the airways contain 200–300 motile cilia that are 5 μm long and beat approximately 10–15 times per second (Fig. 4). The coordinated beating of the cilia propels an overlying carpet of mucus from the lower respiratory tract to the oropharynx. This process is essential for removing bacteria and other inhaled particles from the lungs. Motile cilia are also found in the **fallopian tubes** (oviducts), where they help to transport the egg from the ovary to the uterus. In the male genital tract, motile cilia in the **efferent ductules** transport the sperm from the testis to the epididymis. The ependymal cells lining the cerebral ventricles, cerebral aqueduct, and central canal of the spinal cord contain approximately 40 cilia that are 10 μm in length and beat continuously 30–40 times per second (7). Although their function is poorly understood, **ependymal cilia** may be involved in the directional circulation of cerebrospinal fluid. **Spermatozoa** are propelled by the undulating movement of a modified cilium, called a flagellum, which has the same 9+2 pattern of microtubules as motile cilia.

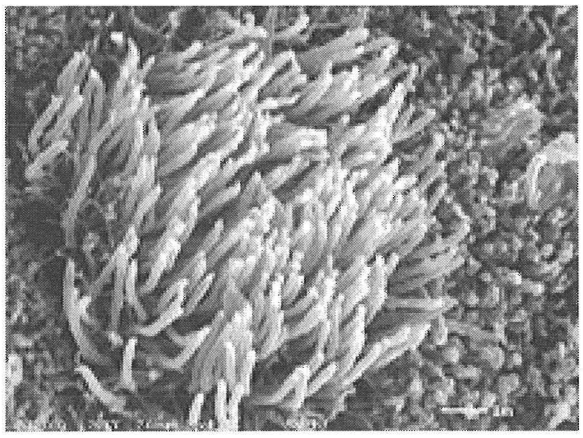

Figure 3. Scanning electron micrograph of motile cilia on the surface of a respiratory epithelial cell.

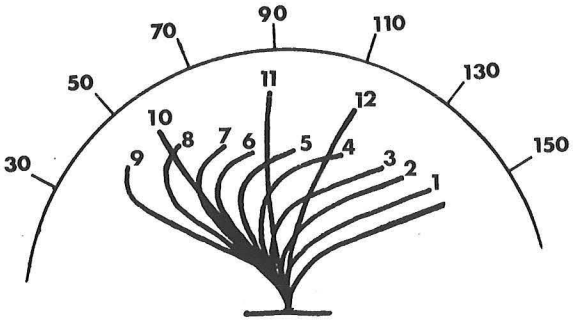

Figure 4. Diagram of ciliary beating in the respiratory tract. Steps 1-8 are the recovery stroke. Steps 9-12 are the power stroke. Direction of mucus movement is to the right.

The other major class of cilia is known as **primary cilia** (1). The axonemes of primary cilia contain nine peripheral microtubule doublets but lack the two central microtubules (**9+0 pattern**) (Fig. 5). Primary cilia originate from a basal body that is located in the cell body between the Golgi apparatus and the cell membrane. In addition to anchoring the cilium in the cell, the basal body serves as one of the centrioles in the mitotic spindle during cell division. Most primary cilia lack dynein arms, nexin bridges, and radial spokes and are **immotile**. One exception is in the embryonic node, which contains primary cilia that have a 9+0 pattern and are motile (see below). Primary cilia are found in only one or two copies per cell. Although they are not as well known as motile cilia, primary cilia have been found on almost all cells in the body, with the exception of hepatocytes and blood cells (1). They are present on smooth muscle cells, cardiac myocytes, fibroblasts, neurons, vascular endothelial cells, chondrocytes, bone cells, endocrine cells, etc. A list of cells containing primary cilia can be found at the website: http://members.global2000.net/bowser/cilialist.html. Renal tubular epithelial cells contain 1–2 primary cilia that have a typical 9+0 ultrastructure (8-10). The primary cilia in the kidney are 2–10 μm in length and project from the apical cell surface into the tubule lumen. Renal cilia have been identified in all segments of the nephron from Bowman's capsule to the collecting ducts with the exception of intercalated cells.

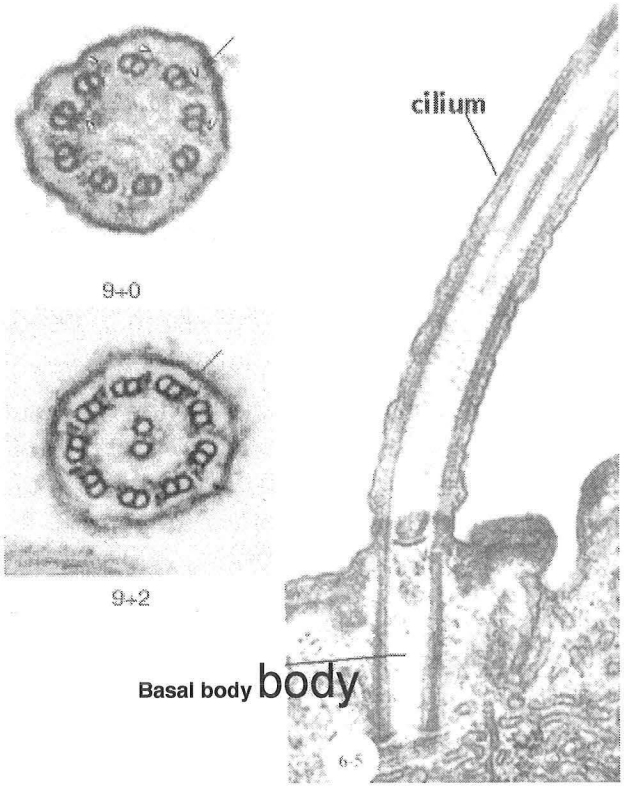

Figure 3. Transmission electron micrograph of a primary cilium (right). Left panels show cross-sections of axonemes of a primary cilium (upper) and motile cilium (lower).

Although primary cilia were recognized decades ago, their function was not known and they were often considered to be vestigial. However, recent studies suggest that primary cilia may have a **sensory function** (11). Cilia are highly evolutionarily conserved throughout the animal kingdom. In the roundworm, *C. elegans*, primary cilia have been found on chemosensory neurons that are involved in chemotaxis and mating. *Drosophila* contains neurons with 9+0 cilia that transduce mechanical and chemical sensory stimuli. Neurons in the mammalian brain contain primary cilia on which receptors for somatostatin and serotonin are concentrated (11). Olfactory neurons in the nasal cavity contain chemosensory cilia on which the odorant receptors are located.

Primary cilia in the kidney have a mechanosensory function (see below). Recent analysis of genetic diseases in humans has revealed that rather than being vestigial structures, primary cilia play essential roles in the development and function of numerous organs, including the heart, brain, eye, and kidney.

Primary Ciliary Dyskinesia

Primary ciliary dyskinesia (PCD) is a genetic disorder of motile cilia[1] (4, 12, 13). PCD is sometimes called **immotile cilia syndrome** (ICS), but this name is inaccurate since the cilia usually have abnormal motility rather than completely absent motility. PCD is characterized by abnormalities in the structure or function of motile (9+2) cilia. The most common **ultrastructural abnormality** is complete or partial absence of dynein arms (Fig. 6), but radial spoke defects and loss of central microtubules have also been observed (3, 6). Normal ciliary structure is seen in up to 30% of patients and does not exclude the diagnosis. Respiratory epithelial cells isolated from affected individuals show **decreased ciliary beat frequency** or abnormal wave patterns when visualized by videomicroscopy (6, 13). Since ciliary dyskinesia can be secondary to allergies, infections, or drugs, abnormalities should be documented in samples obtained at more than one site or time. Sperm immotility can establish the diagnosis in postpubertal males.

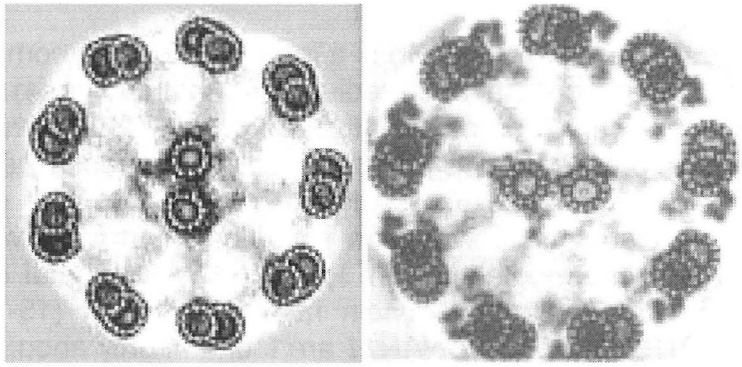

Figure 6. Ultrastructure of axonemes in primary ciliary dyskinesia (left) and normal cilia (right). Note the absence of dynein arms in PCD.

The clinical manifestations of PCD are directly related to the sites of motile cilia and flagella in the body (Fig. 7). Defects of cilia in the trachea and lower respiratory tract inhibit mucociliary clearance and cause chronic **bronchitis**, recurrent **pneumonia**, and **bronchiectasis**. Neonatal respiratory distress syndrome is common in newborns. The absence of mucociliary clearance in the upper airways, including the paranasal sinuses and auditory tubes, produces **sinusitis**, chronic **otitis media**, and conductive **hearing loss**. Sperm immotility produces **sterility** in virtually all males. Females are generally not sterile but may have reduced fertility and increased frequency of ectopic pregnancy due to abnormalities of cilia on the fallopian tubes. Some patients with PCD develop **hydrocephalus**, which is thought to be due to impaired motility of ependymal cilia in the cerebral ventricles and aqueduct. Other disease manifestations that are less easily explained include esophageal abnormalities and biliary atresia.

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[1] The nomenclature of this disease can be confusing. The term "primary" is used to distinguish the disease from "secondary" ciliary dyskinesia, which is caused by drugs, smoking, or infections. PCD does not affect immotile primary cilia.

PCD occurs in 1 in 16,000 live births and is inherited as an autosomal recessive trait. PCD is genetically heterogeneous and can arise from mutations of more than three genes. Not surprisingly, since the disease is characterized by abnormalities of ciliary motility, all of the identified genes that cause human PCD encode subunits of axonemal dyneins in the inner or outer dynein arms (Table 1). In addition, a transcription factor called HFH4 that is involved in the transcription of the genes encoding the dynein subunits has been shown to cause PCD in experimental animals (14).

- Impaired mucociliary clearance
  - Rhinitis
  - Sinusitis
  - Otitis media
  - Recurrent pneumonia
  - Bronchiectasis
- Impaired sperm motility
  - Male infertility
- Diminished female fertility
- Hydrocephalus
- Situs inversus

Figure 7. Clinical manifestations of primary ciliary dyskinesia.

Axonemal dynein is a large multiprotein complex composed of 2–3 heavy chains and several intermediate and light chains (4). The heavy chains contain a globular motor domain that hydrolyzes ATP and moves along the microtubule, which produces ciliary beating. The intermediate and light chains are located towards the base of the dynein arms. Mutations of three genes that encode dynein subunits have been identified in patients with PCD: ***DNAI1*** (axonemal dynein intermediate chain 1), ***DNAH5*** (axonemal dynein heavy chain 5), and ***DNAH11*** (axonemal dynein heavy chain 11) (15-19). Together, mutations of *DNAI1*, *DNAH5*, or *DNAH11* are found in only about 25% of patients with PCD. It is probable that mutations of other dynein subunits or axonemal components are responsible for the remaining cases.

Gene	Chromosome	Protein
DNAH5	5p15.2	Dynein heavy chain 5
DNAI1	9p21-p13	Dynein intermediate chain 1
DNAH11	7p21	Dynein heavy chain 11

Table 1. Genetics of primary ciliary dyskinesia

Kartagener's Syndrome

Kartagener's syndrome refers to the association of chronic sinusitis and bronchiectasis with ***situs inversus*** (mirror image reversal of the internal organs) (6). 50% of patients with PCD have *situs inversus* and are therefore classified as having Kartagener's syndrome (4). *Situs inversus* is a reversal of the normal left-right asymmetry of the internal organs in which the heart is transposed to the right side of the thorax (dextrocardia). The left lung has three lobes and the right lung has two lobes, the stomach and spleen are the right side of the abdomen, and the liver is primarily on the left (20, 21) (Fig. 8). Most patients with Kartagener's syndrome have complete *situs inversus* (*situs inversus totalis*), which is generally well tolerated. However, incomplete *situs inversus* (*situs*

ambiguus) can produce congenital heart abnormalities such as double outlet left ventricle.

Kartagener's syndrome is caused by mutations of the **same genes** that cause PCD and should therefore be considered as a subclass of the broader disorder. This point is best illustrated by the existence of identical twins, both with PCD, in which one twin has *situs inversus* (Kartagener's syndrome) and the other twin has normal laterality (*situs solitus*) (22). These patients raise several interesting questions: How do abnormalities of motile cilia produce *situs inversus*, and how does the same gene mutation produce *situs inversus* in one individual and normal laterality in another individual?

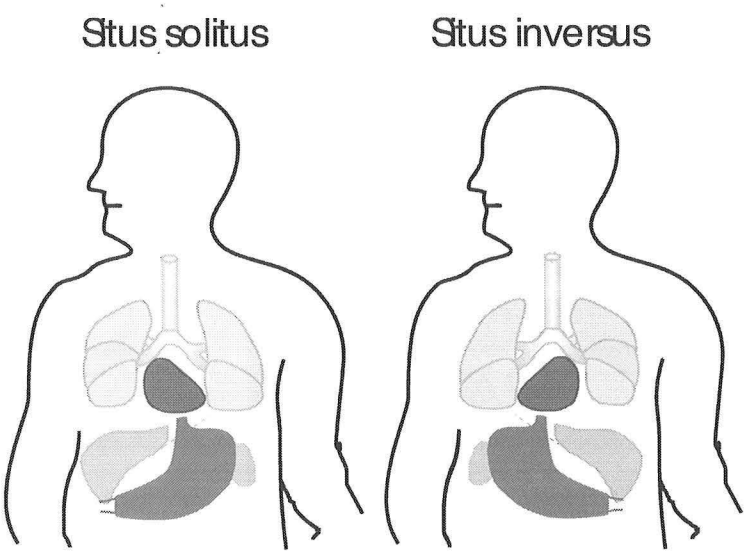


Figure 8. Normal left-right asymmetry (left) and mirror-image reversal of internal organs (right).

Left-right asymmetry is established during embryonic development by a structure known as the node (21, 23-25). The mammalian **node** is a triangular shaped structure that appears on the surface of the embryo near the site where gastrulation occurs. The cells comprising the node contain a single cilium of the 9+0 type (Fig. 9). However, unlike most 9+0 cilia, which are immotile, **nodal cilia** contain dynein arms and are motile. Nodal cilia rotate with a vortical motion in a counterclockwise direction, which generates a leftward flow of fluid across the ventral surface of the node (so-called **nodal flow**) (26-29). The leftward fluid flow initiates a signaling cascade that results in asymmetric development of the visceral organs (30, 31). The signaling pathway involves detection of nodal flow by ciliated cells on the left side of the node, increased intracellular calcium, left-sided expression of the signaling molecules nodal and lefty-2, expression of the transcription factor Pitx2 in the left lateral plate mesoderm, and asymmetric expression of downstream effector genes (20, 24) (Fig. 9).

The mechanism by which abnormalities of motile cilia produce *situs inversus* has been further elucidated by studies of ***iv/iv* mice** that have abnormalities in left-right asymmetry (32). *iv* (*inversus viscerum*) is an autosomal recessive mutation in which 50% of the affected mice exhibit complete *situs inversus*. *iv/iv* mice carry a missense mutation of **left-right dynein** (*lrd*), the mouse orthologue of the *DNAH11* gene that is mutated in some humans with Kartagener's syndrome (33). *iv/iv* mice are therefore an excellent animal model of human *situs inversus*. Videomicroscopy studies have shown that the nodal cilia in *iv/iv* mice are immotile and that the leftward fluid flow across the node is not established (34). Consequently the embryos develop with **random** left-right

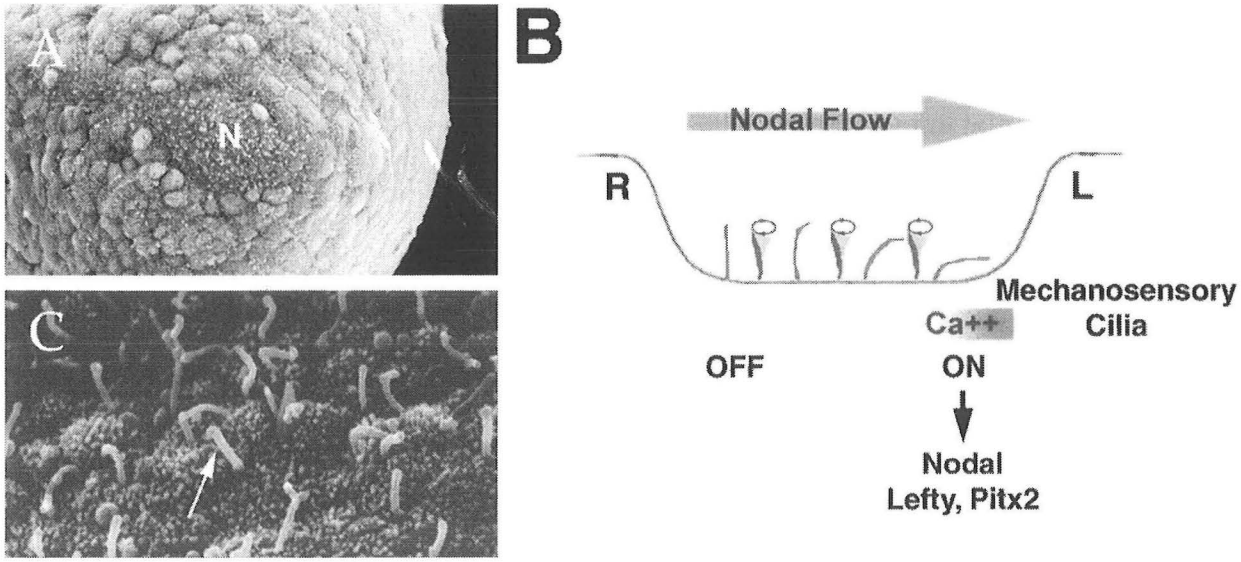


Figure 9. Establishment of left-right asymmetry in the embryonic node. Left: Low power and high power scanning electron micrographs of the embryonic node showing primary cilia on the ventral surface. Right: Nodal cilia rotate with a vortical motion producing leftward fluid flow, which is sensed by other nodal cells containing immotile mechanosensory cilia. Nodal flow induces an increase in cell calcium and expression of nodal, lefty, and Pitx2 in cells on the left side of the embryo. From references 25 and 27.

asymmetry. Nonaka *et al* have shown that the production of artificial leftward fluid flow across the node of *iv/iv* embryos restores normal laterality (35). These elegant experiments clearly demonstrate that leftward nodal flow establishes normal left-right asymmetry. In the absence of nodal flow, due to abnormalities of ciliary motility, half of the embryos will develop with normal laterality (*situs solitus*) and half will develop with *situs inversus*. This explains why only 50% of the patients with PCD have *situs inversus* (Kartagener's syndrome), even though the underlying mutation in both diseases is the same.

Nephronophthisis

Next, we turn to abnormalities of primary cilia. The first disorder of primary cilia that I will discuss is nephronophthisis. Nephronophthisis is an autosomal recessive disorder that produces **chronic renal failure** in children and adolescents (36, 37). In some populations, nephronophthisis is the most common genetic cause of chronic renal failure in the first two decades of life. The renal histology is characterized by disruption of the tubular basement membrane, interstitial infiltrates, renal fibrosis, tubular atrophy, and **renal cysts** at the corticomedullary junction. The pathologic features of nephronophthisis are similar to adult medullary cystic disease. Clinically, children with nephronophthisis present with progressive renal insufficiency, urinary concentrating defects, salt wasting, anemia, and growth retardation (Fig. 10). Extrarenal manifestations include retinitis pigmentosa, Cogan syndrome (ocular motor apraxia), liver fibrosis, and cone shaped epiphyses.

Nephronophthisis is genetically heterogeneous and can be caused by mutations of at least four genes, named *NPHP1–NPHP4* (38-43) (Table 2). Clinically, the diseases caused by mutations of the different genes are similar, although the age of onset varies. The median age at onset of ESRD in patients with mutations of *NPHP1*, *NPHP2*, *NPHP3*, and *NPHP4* is 13 years, 1–3 years, 19 years, and ~20 years, respectively. Retinal abnormalities have been described in patients with all four forms of nephronophthisis. The association of retinitis pigmentosa with NPH is known as the **Senior-Løken syndrome**.

- Urinary concentrating defect
  - –Uosm < 800 mOsm
  - –Polyuria, polydipsia, enuresis, dehydration
- Salt wasting
- Failure to thrive
- Anemia
- Progression to renal failure
- Extrarenal manifestations
  - –Retinitis pigmentosa (Senior-Løken syndrome)
  - –Ocular motor apraxia (Cogan syndrome)

Figure 10. Clinical manifestations of nephronophthisis.

NPHP1 encodes a 732-amino acid protein called **nephrocystin**, which contains a coiled-coil domain involved in protein-protein interactions and an SH3 domain that binds to proteins containing a P-X-X-P motif. Nephrocystin is a docking protein that interacts with signaling proteins, such as the proline-rich tyrosine kinase Pyk2 and crk-associated substrate $p130^{Cas}$, and cytoskeletal proteins, such as tensin and filamin (44-46). *NPHP2* encodes **inversin**, a 1,062-amino acid protein that contains ankyrin repeats, IQ domains, D boxes, and a bipartite nuclear localization signal. Inversin interacts with calmodulin in a calcium-dependent manner and interacts with the anaphase promoting complex Apc2 and β-catenin in the nucleus (47-50). *NPHP3* encodes **nephrocystin-3**, a protein of 1,324 amino acids that contains a coiled-coil domain, a tubulin tyrosine ligase domain, and eight tetratricopeptide repeats that may be involved in protein-protein interactions. The protein encoded by *NPHP4*, called **nephrocystin-4** or **nephroretinin**, consists of 1,250 amino acids and contains a proline-rich domain.

Gene	Protein	Age@ESRD	Cilia	RP
NPHP1	Nephrocystin	13 yr	Yes	Yes
NPHP2	Inversin	1-3 yr	Yes	Yes
NPHP3	Nephrocystin-3	19 yr	Unknown	Yes
NPHP4	Nephrocystin-4	~20 yr	Yes	Yes

Table 2. Genetics of nephronophthisis. Nephrocystin, inversin, and nephrocystin-4 have been localized to primary cilia. Mutations of all genes are associated with retinitis pigmentosa (RP).

Many of the NPHP proteins contain domains that could be involved in protein-protein interactions. Indeed, nephrocystin has been shown to directly interact with inversin, nephrocystin-3, and nephrocystin-4 (39-41). Nephrocystin, inversin, and nephrocystin-4 have been localized to **primary cilia** in cells in the kidney and embryonic node (39, 51, 52). Moreover, the carboxyl-terminus of nephrocystin binds to β-tubulin, a component of the ciliary axoneme (39). These findings suggest that the NPHP proteins form a large multiprotein complex that is located in the ciliary axoneme. Mutations that disrupt components of the complex may impair ciliary function and lead to renal cysts, renal fibrosis, and retinitis pigmentosa. How mutations of ciliary proteins produce renal cysts will be discussed later in this review. In the next section, I will discuss the association of cilia with retinitis pigmentosa.

Retinitis Pigmentosa

Retinitis pigmentosa (RP) is an inherited form of retinal degeneration that leads to progressive blindness, usually by age 60 (53). Patients with RP present with gradual loss of visual acuity, loss of night vision, and tunnel vision (Fig. 11). RP is the most common inherited form of blindness, affecting more than 100,000 individuals in the U.S. A severe form of RP that presents with nystagmus and blindness in children is called **Leber congenital amaurosis**. Funduscopic examination reveals waxy pallor of the optic disc, constricted retinal arteries, retinal pigment epithelial atrophy, and characteristic "bone spicule" hyperpigmentation of the retina. Retinal pathology shows increased photoreceptor cell death (apoptosis), which leads to a thinning of the photoreceptor cell layer (Fig. 12). The genetics of RP are very complex, and autosomal dominant, autosomal recessive, and X-linked forms of the disease have been described. Mutations of over 30 different genes have been shown to cause RP, including genes encoding visual pigments, components of photoreceptor disk membranes, proteins of the retinoid cycle, transcription factors, and pre-mRNA splicing factors (53). Some forms of RP are caused by mutations of ciliary proteins. The **RP1 protein** that is mutated in autosomal dominant RP is localized in the connecting cilium of photoreceptor cells (54). The **retinitis pigmentosa GTPase regulator** (RPGR) and an **RPGR-interacting protein** (RPGRIP), which cause RP and Leger congenital amaurosis, respectively, are also localized in the connecting cilium (55). As discussed above, mutations of the ciliary proteins nephrocystin, inversin, and nephroretinin (nephrocystin-4) cause RP.

- Progressive blindness
  - –Decreased visual acuity
  - –Tunnel vision
  - –Decreased night vision
- Retinal abnormalities
  - –Bone-spicule pigmentation
  - –Constricted retinal arteries
  - –Waxy pallor of the optic disc
  - –Atrophy of RPE

Figure 11. Clinical manifestations of retinitis pigmentosa.

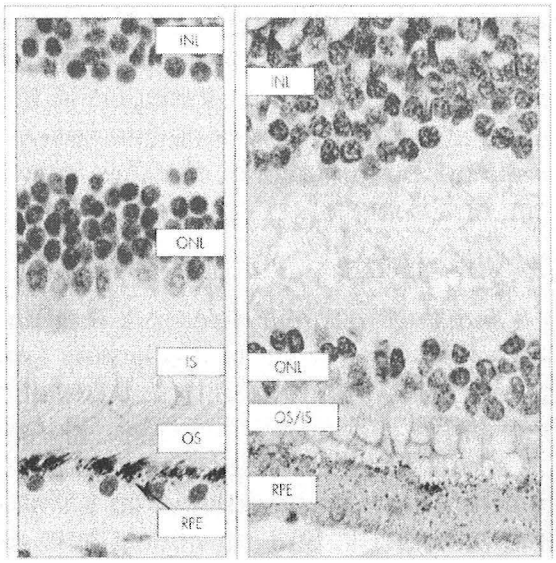


Figure 12. Histology of normal retina (left) and retinitis pigmentosa (right). Note decreased thickness of the inner segment (IS) and outer segment (OS) photoreceptor cell layer in retinitis pigmentosa.

To understand how abnormalities of cilia might produce RP, we must first review the structure of **photoreceptor cells**. The rods and cones of the mammalian retina contain an inner and outer segment. The outer segment is filled with stacks of photosensitive disk membranes, which contain the light-sensitive visual pigments (rhodopsin in rods and opsins in cones). The inner segment contains the mitochondria, endoplasmic reticulum, and other cell organelles. The outer segment is connected to the inner segment by a thin structure called the **connecting cilium** (Fig. 13). A cross-section of the connecting cilium reveals that it has a 9+0 axonemal structure typical of a primary cilium (2). Therefore, the entire outer segment can be viewed as a modified sensory cilium. Moreover, the outer segment contains no protein synthetic machinery. Therefore, the opsins and other proteins are synthesized in the inner segment and transported through the connecting cilium into the outer segment. Since the outer segment is continuously turning over, proteins are constantly trafficking through the connecting cilium. A common feature of several forms of RP is **abnormal trafficking** of visual pigments from the inner segment to the outer segment. The accumulation of proteins in the inner segment produces apoptotic cell death. Therefore, gene mutations that interfere with protein transport in the connecting cilium will lead to degeneration of the outer segment and RP.

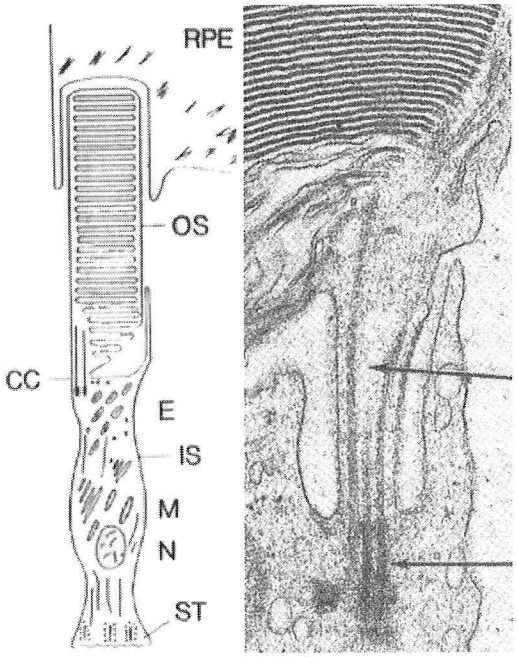


Figure 13. Left panel: Diagram of a photoreceptor cell showing the connecting cilium (CC). RPE, retinal pigment epithelium; OS, outer segment; IS, inner segment; M, mitochondria; N, nucleus; ST, synaptic terminus. Right panel: Electron micrograph of the connecting cilium.

Additional evidence that ciliary abnormalities can produce RP has been provided by studies of mice with mutations in ciliary transport. Proteins are transported in cilia by a motor protein called kinesin-II (see below). A **retina-specific knockout** of kinesin-II produces

retinal abnormalities that are strikingly similar to those found in humans with RP (56). Because of the absence of ciliary transport, the opsin visual pigment accumulates in the inner segment, which leads to accelerated photoreceptor cell death. These findings demonstrate that the inhibition of protein transport in the connecting cilium produces the histological features of RP. Additional studies will be required to determine how RP is produced by mutations of other ciliary proteins, such as nephrocystin, nephroretinin, and inversin.

Polycystic Kidney Disease

Polycystic kidney disease (PKD) is a family of genetic disorders that are characterized by the accumulation of multiple fluid-filled cysts in the kidney and other epithelial organs (57). The renal cysts originate from the renal tubules and progressively enlarge and multiply over time producing massive kidney enlargement and renal failure. PKD can be inherited as an autosomal dominant trait or an autosomal recessive trait. **Autosomal dominant polycystic kidney disease (ADPKD)** is a common disease that affects 1 in 500 individuals from all ethnic groups worldwide (Table 3). ADPKD is caused by mutations of ***PKD1*** on chromosome 16 (85% of cases) or ***PKD2*** on chromosome 4 (15% of cases) (Table 4). Individuals with ADPKD typically present with massively enlarged kidneys and renal insufficiency in the third and fourth decade but can also present in childhood or *in utero*. ADPKD is a systemic disorder, and extrarenal manifestations include hepatic and pancreatic cysts, cerebral and aortic aneurysms, colonic diverticuli, cardiac valvular abnormalities, and left ventricular hypertrophy. Mutations of *PKD1* and *PKD2* produce identical clinical manifestations, but *PKD2* patients present later in life and have longer renal survival. Based on the findings of loss of heterozygosity and somatic mutations in cysts from ADPKD patients, a two-hit model of cystogenesis has been proposed (58, 59). Consistent with this model, knockout mice that lack both copies of either *Pkd1* or *Pkd2* develop severely cystic kidneys *in utero* (60-65).

Autosomal recessive polycystic kidney disease (ARPKD) is less common than ADPKD (incidence: 1 in 20,000) and primarily affects infants and children, but survival up to age 55 has been reported (66, 67) (Table 3). The

	ADPKD	ARPKD
Incidence	1/500	1/20,000
Age of onset	Adult	Infancy/childhood
Location of cysts	All nephron segments	Collecting ducts
Liver involvement	Liver cysts	Biliary dysgenesis
Extrarenal manifestations	Cerebral aneurysms Pancreatic cysts Cardiac valvular Hypertension	Portal hypertension Systemic hypertension

Table 3. Clinical manifestations of autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD)

kidney cysts in ARPKD originate from the renal collecting ducts. ARPKD is invariably associated with **biliary dysgenesis**, a congenital abnormality of the bile ducts that causes portal fibrosis and portal hypertension. ARPKD can be classified based on the age of onset into neonatal, infantile, childhood, and adolescent forms. Neonates with the severe perinatal form of ARPKD typically present with massively enlarged kidneys and intrauterine renal failure. Since the kidneys are required for production of amniotic fluid *in utero*, affected individuals develop Potter's facies, limb deformities, and pulmonary hypoplasia due to **oligohydramnios**. Up to 30–50% of newborns with the severe perinatal form die shortly after birth from respiratory failure or sepsis. Children with late-onset disease present with enlarged kidneys and progressive renal failure as well as esophageal varices, hepatosplenomegaly, and hyersplenism due to **portal hypertension** (67). Other clinical manifestations include systemic hypertension, growth retardation, urinary tract infection, and hyponatremia. All forms of ARPKD are caused by mutations of ***PKHD1*** (Polycystic Kidney and Hepatic Disease 1) on chromosome 6 (68, 69) (Table 4). Missense mutations of *PKHD1* are more commonly associated with a nonlethal presentation of ARPKD, whereas truncating mutations are more commonly associated with the severe perinatal form of the disease (70-72).

	ADPKD	ARPKD
Inheritance	Autosomal dominant	Autosomal recessive
Gene (Chr)	*PKD1* (Chr 16) *PKD2* (Chr 4)	*PKHD1* (Chr 6)
Protein product	Polycystin-1 Polycystin-2	Fibrocystin (polyductin)
Protein size	PKD1: 4,302 aa PKD2: 968 aa	4,074 aa
Tissue distribution	Widespread	Kidney, liver, pancreas
Subcellular localization	PKD1: Cilia, PM PKD2: Cilia, ER	Cilia

Table 4. Genetics of autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD). PM, plasma membrane; ER, endoplasmic reticulum.

Polycystin-1, the product of the *PKD1* gene, is an integral membrane protein that is predicted to contain a large extracellular domain, 11 transmembrane segments, and a C-terminus that is located in the cytoplasm (73-75) (Fig. 14). The extracellular domain contains an array of motifs that are involved in protein-protein or protein-carbohydrate interactions. Polycystin-1 has been shown to activate signaling pathways involving Wnt/β-catenin, heterotrimeric G proteins, MAP kinase, and Jak/STAT (57). The *PKD2* gene product, **polycystin-2**, is composed of 968 amino acids and is also predicted to be an integral membrane protein (76) (Fig. 14). Polycystin-2 shares structural features with transient receptor potential (TRP) channels as well as voltage-

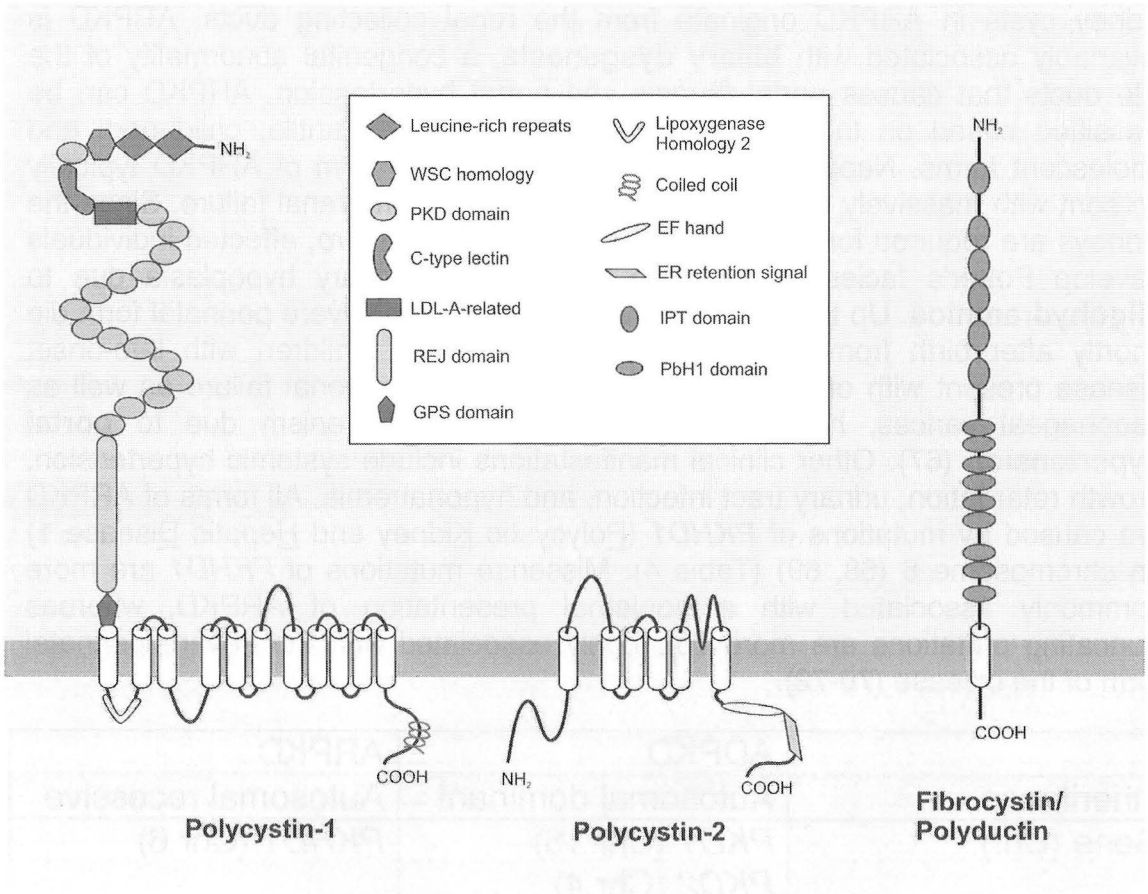


Figure 14. Structure of polycystin-1, polycystin-2, and fibrocystin

activated calcium and sodium channels. Single channel recordings and patch clamp analyses have shown that polycystin-2 is a non-selective cation channel that can conduct calcium ions (77-80). Polycystin-2 physically interacts with polycystin-1 both *in vitro* and *in vivo*.

The ARPKD gene, *PKHD1*, encodes a protein called **fibrocystin** or **polyductin** that is composed of 4,074 amino acids (68, 69). Fibrocystin is an integral membrane protein containing a large extracellular domain, a single transmembrane segment, and a short carboxyl-terminal domain (Fig. 14). A splice variant that may encode a secreted form of the protein has also been identified. The extracellular domain contains multiple IPT domains (Ig-like, plexin, transcription factor) and PbH1 repeats (parallel beta-helix), and the carboxyl-terminal domain contains potential phosphorylation sites. Although its function is not known, the structure of fibrocystin suggests that it may be a membrane receptor or ligand.

Is PKD a Ciliary Disorder?

The first hint that cilia might be involved in the pathogenesis of PKD came from studies of the *orpk* mouse model of ARPKD (81). ***orpk/orpk* mice** develop renal collecting duct cysts, biliary dysplasia, and portal fibrosis and usually die

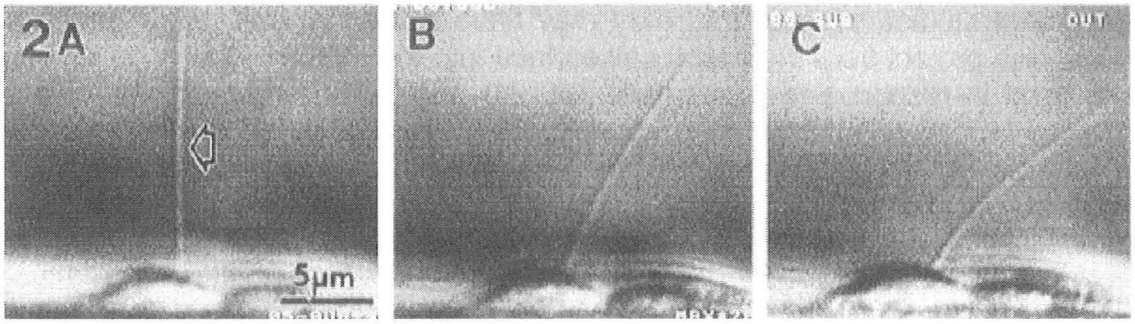


Figure 15. Bending of a renal cilium (arrow) in response to increasing rates of fluid flow. From reference 94.

within the first week of life. The gene that is mutated in *orpk* mutant mice (*Tg737*) encodes **polaris**, a protein that localizes to the axoneme and basal body of both motile and primary cilia (82, 83). Polaris is expressed in the kidney and has been localized to the primary cilia of cultured renal epithelial cells. Recent studies have shown that polaris is involved in ciliary assembly, and *orpk/orpk* mice have stunted primary cilia. Two additional mouse models of recessive PKD involve ciliary proteins: 1) ***cpk/cpk* mice** have mutations of cystin, a 145-amino acid protein that localizes to the primary apical cilium when expressed in cultured collecting duct cells (84). 2) ***inv/inv* mice** develop cysts in the renal collecting ducts and pancreas and have mutations of inversin (47, 85). As discussed earlier, inversin is a ciliary protein that is mutated in humans with nephronophthisis type 2. In addition to renal cysts, *inv/inv* mice exhibit complete *situs inversus* due to abnormalities of nodal cilia (34, 85). Dextrocardia and *situs inversus* are also observed in mice that are deficient in polaris or polycystin-2 (82, 86). The coupling of defects of left-right axis determination with kidney cysts in several mouse models lends indirect support to the notion that cilia may play a role in renal cystic disease.

A second hint that cilia were involved in PKD came from the **subcellular localization** of polycystin-1, polycystin-2, and fibrocystin. Homologues of polycystin-1 and polycystin-2, the proteins that are mutated in humans with ADPKD, have been identified in the roundworm *C. elegans*. These proteins, named LOV-1 (location of vulva) and PKD-2, respectively, are localized to the primary cilia of a specialized group of sensory neurons that are involved in mating (87). Worms with mutations of LOV-1 and PKD-2 exhibit abnormal mating behavior due to sensory defects. Polycystin-1 and polycystin-2 have also been identified in the primary cilia of mammalian kidney cells. Polycystin-2 co-localizes with tubulin in the cilia of cultured kidney cells and native renal tubules (88, 89). Polycystin-1 is also expressed in renal cilia where it co-localizes with cystin and polycystin-2 (90, 91). Fibrocystin, the protein that is mutated in human ARPKD, has recently been localized in the primary cilia of Madin-Darby canine kidney cells (MDCK cells) (92). Although polycystin-1, polycystin-2, and fibrocystin are also found in other sites in the cell (Table 4), their common localization in renal cilia strongly suggests that the latter is involved in PKD.

The function of polycystin-1 and polycystin-2 in renal cilia is not known, but recent studies suggest that they may have a **mechanosensory function**. Renal cilia project from the apical cell surface into the lumen of the renal tubules and bend in response to **urine flow** (93, 94) (Fig. 15). Bending of the cilia of cultured MDCK cells, either mechanically or with fluid flow, induces an increase in intracellular calcium concentration (95). Nauli *et al* recently showed that polycystin-1 and polycystin-2 are required for the flow-induced increase in intracellular calcium (91). *Pkd1* mutant cells contain primary cilia but do not increase intracellular calcium in response to fluid flow. Incubation of wild-type renal epithelial cells with blocking antibodies to polycystin-1 and polycystin-2 also inhibits the response to fluid flow. Taken together, these findins suggest that polycystin-1 and polycystin-2 have a mechanosensory function in renal cilia that is coupled to intracellular calcium (96, 97).

PKD in Mice Lacking Renal Cilia

To directly test the role of primary cilia in the pathogenesis of PKD, our laboratory has produced mutant mice that lack renal cilia (98). We reasoned that if ADPKD is caused by mutations that inhibit the function of polycystin-1 and polycystin-2 in renal cilia, then mutant mice that completely lack renal cilia should develop polycystic kidneys. Cilia are synthesized by **intraflagellar transport (IFT)**, a process that was first described in the green alga *Chlamydomonas* (2, 5). Since cilia do not contain endoplasmic reticulum or ribosomes, their protein constituents must be imported from the cell body then transported to their proper locations along the ciliary axoneme. IFT refers to the transport of large multiprotein complexes (IFT particles) that carry cargo from the base of the cilium to the tip (Fig. 16). IFT particles are transported by molecular motors that move along the microtubules of the ciliary axoneme. The outward (anterograde) movement of IFT particles is mediated by the motor protein, kinesin-II, whereas the retrograde movement is mediated by a cytoplasmic dynein[2].

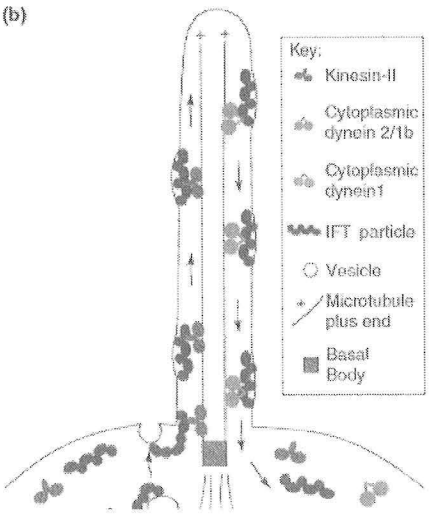


Figure 16. Diagram of intraflagellar transport (IFT). Kinesin-II mediates outward movement of IFT particles along the ciliary axoneme. Retrograde transport is mediated by cytopiasmic dynein 1. From reference 5.

Kinesin-II is a heterotrimeric protein composed of two motor subunits, KIF3A and KIF3B (Kinesin family 3A and 3B), and one non-motor subunit, KAP3 (Kinesin associated protein 3) (99). KIF3A and KIF3B contain a globular domain that moves processively along the microtubules in an ATP-dependent manner. An extended coiled-coil mediates heterodimerization and the interaction with

[2] These molecular motors are different from the axonemal dyneins that are responsible for ciliary beating. Kinesin-II and cytoplasmic dyneins mediate protein transport within the cilium and are found in both motile and primary cilia. They are not involved in ciliary motility.

KAP. Conventional knockout mice that completely lack KIF3A or KIF3B are unable to synthesize cilia. The absence of cilia in the embryonic node produces randomization of left-right asymmetry, further verifying the involvement of nodal cilia in left-right axis determination (26). In addition, the conventional kinesin-II knockout mice develop abnormalities of the neural tube, pericardium, branchial arches, and somites and are embryonic lethal (28, 100). Although these studies demonstrated that kinesin-II was essential for the synthesis of cilia, the early embryonic lethality prevented study of the kidney phenotype.

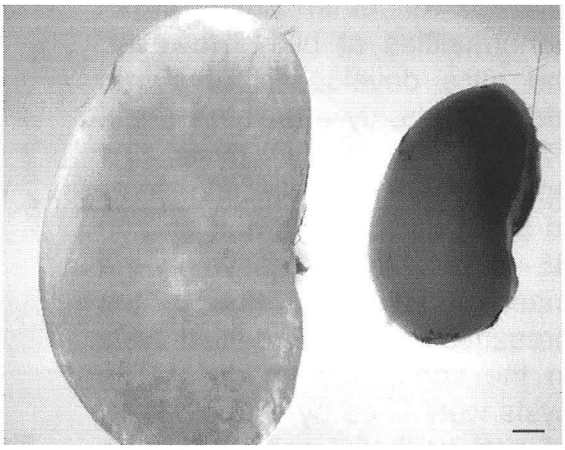


Figure 17. Gross appearance of kidneys from kidney-specific kinesin-II knockout mice (left) and normal mice (right).

To circumvent embryonic lethality, we created a kidney-specific knockout of the KIF3A subunit of kinesin-II in transgenic mice. We used a genetic technique, called **Cre/loxP recombination**, to inactivate the kinesin-II gene (*KIF3A*) specifically in renal tubules (98). In all other tissues besides the kidney, the kinesin-II gene was not affected. Analysis of **kidney-specific kinesin-II knockout mice** showed that the expression of kinesin-II (KIF3A) mRNA and protein was inhibited in the kidney but was unchanged in other tissues. The inactivation of kinesin-II resulted in the absence of primary cilia in the renal tubules. Kidney-specific kinesin-II mutant mice were viable and did not have *situs*

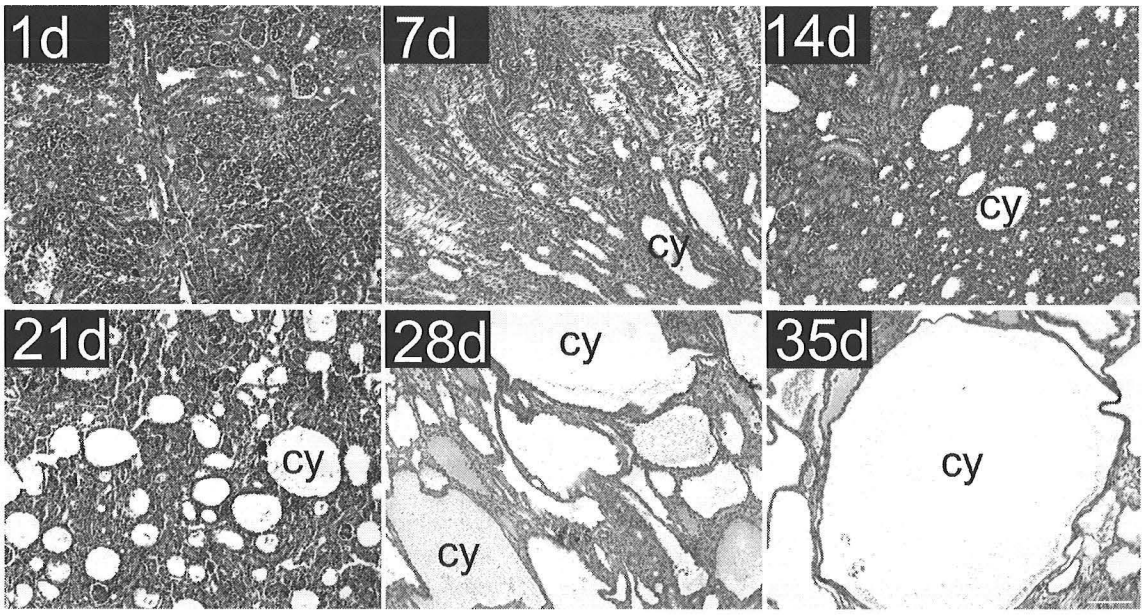


Figure 18. Renal pathology of kidney-specific kinesin-II knockout mice at the indicated number of days after birth. cy, cysts.

inversus or other gross organ abnormalities at birth. However, the mice developed **polycystic kidneys** shortly after birth (Figs. 17 and 18). Renal cysts first appeared in the collecting ducts at 5 days after birth, and by age 35 days the kidneys were massively enlarged due to the presence of large fluid-filled cysts in the cortex and medulla. The cysts were lined by a single layer of cyst epithelial cells and were surrounded by atrophic tubules and areas of interstitial fibrosis. Measurements of BUN showed that the kidney-specific kinesin-II knockout mice had normal renal function up to age 14 days. Thereafter, the knockout mice rapidly developed azotemia indicating **renal failure** (Fig. 19). Further analysis of the cyst epithelial cells revealed increased cell proliferation, increased apoptosis, apical mislocalization of the epidermal growth factor receptor, and activation of β-catenin signaling. Similar cell biological abnormalities have been observed in human cysts. These studies demonstrate that kidney-specific inactivation of kinesin-II inhibits renal ciliogenesis and produces renal cysts, thereby providing direct evidence for the role of cilia in the pathogenesis of PKD.

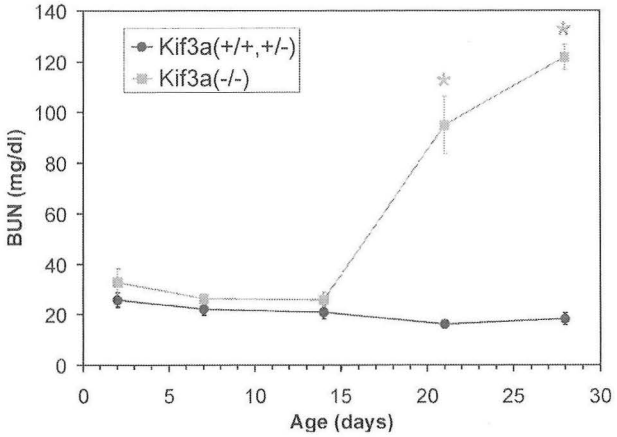
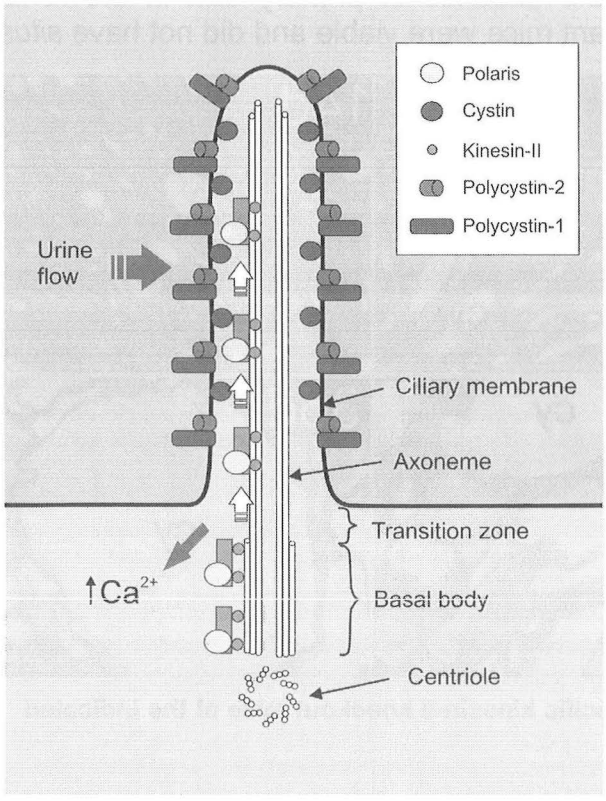


Figure 19. Renal function in kidney-specific kinesin-II knockout mice (squares) and wild-type mice (circles).



Taken together, these findings suggest a model of renal cilia that is shown in Fig. 20. Renal cilia contain polycystin-1, polycystin-2, polaris, cystin, and other proteins that are involved in PKD. Renal cilia are immotile but bend in response to urine flow. Bending of the cilium produces an increase in intracellular calcium that is dependent on polycystin-1 and polycystin-2. The increase in cell calcium initiates a signaling pathway that regulates cell

Figure 20. Model of the renal cilium. White arrows indicate anterograde intraflagellar transport (IFT) mediated by kinesin-II. Polaris, cystin, polycystin-1, and polycystin-2 are localized in renal cilia. Dark arrows indicate that renal cilia bend in response to urine flow and induce an increase in intracellular calcium, $[Ca^{2+}]_i$.

growth and differentiation. Disruption of ciliary function, either by mutations of polycystin-1 or polycystin-2 or by loss of renal cilia, inhibits this signaling pathway, which leads to uncontrolled cell growth and cyst formation.

Perspectives

Although overlooked for many years, primary cilia are returning to the spotlight. In this review, we have seen how abnormalities of primary cilia can produce *situs inversus*, retinal degeneration, and polycystic kidney disease. However, primary cilia are widespread and are present on most cells in the body. Primary cilia may be thought of as the cell's antenna permitting it to sense its surroundings and respond appropriately. It seems likely that abnormalities of primary cilia will be found to produce disease in many organs. A case in point is the recent discovery that Bardet-Biedl syndrome is caused by mutations of a protein that is expressed in the basal bodies of the cilia (101). This discovery suggests that abnormalities of cilia may produce obesity, learning disabilities, polydactyly, and diabetes in addition to eye and kidney disorders.

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References

1. Wheatley DN (1995) Primary cilia in normal and pathological tissues. Pathobiology 63:222-238
2. Rosenbaum JL, Witman GB (2002) Intraflagellar transport. Nat Rev Mol Cell Biol 3:813-825
3. El Zein L, Omran H, Bouvagnet P (2003) Lateralization defects and ciliary dyskinesia: lessons from algae. Trends Genet 19:162-167
4. Ibanez-Tallon I, Heintz N, Omran H (2003) To beat or not to beat: roles of cilia in development and disease. Hum Mol Genet 12:R27-R35
5. Pazour GJ, Rosenbaum JL (2002) Intraflagellar transport and cilia-dependent diseases. Trends Cell Biol 12:551-555.
6. Felix H, Holzmann D (2000) Function and ultrastructure of cilia in primary ciliary dyskinesia. Schweiz Med Wochenschr 130:699-704
7. Hirst RA, Rutman A, Sikand K, Andrew PW, Mitchell TJ, O'Callaghan C (2000) Effect of pneumolysin on rat brain ciliary function: comparison of brain slices with cultured ependymal cells. Pediatr Res 47:381-384
8. Webber WA, Lee J (1975) Fine structure of mammalian renal cilia. Anat Rec 182:339-344
9. Andrews PM, Porter KR (1974) A scanning electron microscopic study of the nephron. Am J Anat 140:81-116
10. Bulger RE, Siegel FL, Pendergrass R (1974) Scanning and transmission electron microscopy of the rat kidney. Am J Anat 139:483-502

11. Pazour GJ, Witman GB (2003) The vertebrate primary cilium is a sensory organelle. Curr Opin Cell Biol 15:105-110.
12. Eliasson R, Mossberg B, Camner P, Afzellus B (1977) The immotile-cilia syndrome. A congenital ciliary abnormality as an etiologic factor in chronic airway infections and male sterility. N Engl J Med 297:1-6
13. Bush A, O'Callaghan C (2002) Primary ciliary dyskinesia. Arch Dis Child 87:363-365
14. Brody SL, Yan XH, Wuerffel MK, Song SK, Shapiro SD (2000) Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. Am J Respir Cell Mol Biol 23:45-51
15. Bartoloni L, Blouin JL, Pan Y, Gehrig C, Malti A, Scamuffa N, Rossier C, Jorissen M, Armengot M, Meeks M, Mitchison H, Chung E, Delozier-Blanchet C, Craigen W, Antonarakis S (2002) Mutations in the *DNAH11* (axonemal heavy chain dynein type 11) gene cause one form of situs inversus totalis and most likely primary ciliary dyskinesia. Proc Natl Acad Sci USA 99:10282-10286
16. Ibanez-Tallon I, Gorokhova S, Heintz N (2002) Loss of function of axonemal dynein Mdnah5 causes primary ciliary dyskinesia and hydrocephalus. Hum Mol Genet 11:715-721
17. Olbrich H, Haffner K, Kispert A, Volkel A, Volz A, Sasmaz G, Reinhardt R, Hennig S, Lehrach H, Konietzko N, Zariwala M, Noone PG, Knowles M, Mitchison HM, Meeks M, Chung EM, Hildebrandt F, Sudbrak R, Omran H (2002) Mutations in DNAH5 cause primary ciliary dyskinesia and randomization of left-right asymmetry. Nat Genet 30:143-144
18. Pennarun G, Escudier E, Chapelin C, Bridoux AM, Cacheux V, Roger G, Clement A, Goossens M, Amselem S, Duriez B (1999) Loss-of-function mutations in a human gene related to Chlamydomonas reinhardtii dynein IC78 result in primary ciliary dyskinesia. Am J Hum Genet 65:1508-1519
19. Guichard C, Harricane MC, Lafitte JJ, Godard P, Zaegel M, Tack V, Lalau G, Bouvagnet P (2001) Axonemal dynein intermediate-chain gene (DNAI1) mutations result in situs inversus and primary ciliary dyskinesia (Kartagener syndrome). Am J Hum Genet 68:1030-1035
20. Bisgrove vW, Morelli SH, Yost HJ (2003) Genetics of human laterality disorders: Insights from vertebrate model systems. Annu Rev Genomics Hum Genet 4:1-32
21. Capdevila J, Vogan KJ, Tabin CJ, Belmonte J (2000) Mechanisms of left-right determination in vertebrates. Cell 101:9-21
22. Noone PG, Bali D, Carson JL, Sannuti A, Gipson CL, Ostrowski LE, Bromberg PA, Boucher RC, Knowles MR (1999) Discordant organ laterality in monozygotic twins with primary ciliary dyskinesia. Am J Med Genet 82:155-160
23. Kathiriya IS, Srivastava D (2000) Life-right asymmetry and cardiac looping:implications for cardiac development and congenital heart disease. Am J Med Genet 97:271-279
24. Mercola M (2003) Life-right asymmetry: nodal points. J Cell Sci 116:3251-3257

25. Tabin CJ, Vogan KJ (2003) A two-cilia model for vertebrate left-right axis specification. Genes Dev 17:1-6
26. Hirokawa N (2000) Stirring up development with the heterotrimeric kinesin KIF3. Traffic 1:29-34
27. Nonaka S, Tanaka Y, Okada Y, Takeda S, Harada A, Kanai Y, Kido M, Hirokawa N (1998) Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell 95:829-837
28. Takeda S, Yonekawa Y, Tanaka Y, Okada Y, Nonaka S, Hirokawa N (1999) Left-right asymmetry and kinesin superfamily protein KIF3A: new insights in determination of laterality and mesoderm induction by *kif3A*$^{-/-}$ mice analysis. J Cell Biol 145:825-836
29. Hamada H, Meno C, Watanabe D, Saijoh Y (2002) Establishment of vertebrate left-right asymmetry. Nat Rev Genet 3:103-113
30. McGrath J, Makova S, Tian X, Brueckner M (2003) Two populations of node monocilia initiate left-right asymmetry in the mouse. Cell 114:61-73
31. Brueckner M (2001) Cilia propel the embryo in the right direction. Am J Med Genet 101:339-344
32. Supp D, Potter S, Brueckner M (2000) Molecular motors: the driving force behind mammalian left-right development. Cell Biology 10:41-45
33. Supp DM, Witte DP, Potter SS, Brueckner M (1997) Mutation of an axonemal dynein affects left-right asymmetry in inversus viscerum mice. Nature 389:963-966
34. Okada Y, Nonaka S, Tanaka Y, Saijoh Y, Hamada H, Hirokawa N (1999) Abnormal nodal flow precedes situs inversus in *iv* and *inv* mice. Mol Cell 4:459-468
35. Nonaka S, Shiratori H, Saijoh Y, Hamada H (2002) Determination of left-right patterning of the mouse embryo by artificial nodal flow. Nature 418:96-99
36. Hildebrandt F, Otto E (2000) Molecular genetics of nephronophthisis and medullary cystic kidney disease. J Am Soc Nephrol 11:1753-1761
37. Hildebrandt F, Omram H (2001) New insights: nephronophthisis-medullary cystic kidney disease. Pediatr Nephrol 16:168-176
38. Otto E, Hoefele J, Ruf R, Mueller AM, Hiller KS, Wolf MT, Schuermann MJ, Becker A, Birkenhager R, Sudbrak R, Hennies HC, Nurnberg P, Hildebrandt F (2002) A gene mutated in nephronophthisis and retinitis pigmentosa encodes a novel protein, nephroretinin, conserved in evolution. Am J Hum Genet 71:1161-1167
39. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beekmann F, Landau D, Foreman JW, Goodship JA, Strachan T, Kispert A, Wolf MT, Gagnadoux MF, Nivet H, Antignac C, Walz G, Drummond IA, Benzing T, Hildebrandt F (2003) Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nat Genet 34:413-420

40. Mollet G, Salomon R, Gribouval O, Silbermann F, Bacq D, Landthaler G, Milford D, Nayir A, Rizzoni G, Antignac C, Saunier S (2002) The gene mutated in juvenile nephronophthisis type 4 encodes a novel protein that interacts with nephrocystin. Nat Genet 32:300-305
41. Olbrich H, Fliegauf M, Hoefele J, Kispert A, Otto E, Volz A, Wolf MT, Sasmaz G, Trauer U, Reinhardt R, Sudbrak R, Antignac C, Gretz N, Walz G, Schermer B, Benzing T, Hildebrandt F, Omran H (2003) Mutations in a novel gene, NPHP3, cause adolescent nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis. Nat Genet 34:455-459
42. Hildebrandt F, Otto E, Rensing C, Nothwang HG, Vollmer M, Adolphs J, Hanusch H, Brandis M (1997) A novel gene encoding an SH3 domain protein is mutated in nephronophthisis type 1. Nat Genet 17:149-153
43. Saunier S, Calado J, Heilig R, Silbermann F, Benessy F, Morin G, Konrad M, Broyer M, Gubler MC, Weissenbach J, Antignac C (1997) A novel gene that encodes a protein with a putative src homology 3 domain is a candidate gene for familial juvenile nephronophthisis. Hum Mol Genet 6:2317-2323
44. Benzing T, Gerke P, Hopker K, Hildebrandt F, Kim E, Walz G (2001) Nephrocystin interacts with Pyk2, p130(Cas), and tensin and triggers phosphorylation of Pyk2. Proc Natl Acad Sci USA 98:9784-9789
45. Donaldson JC, Dempsey PJ, Reddy S, Bouton AH, Coffey RJ, Hanks SK (2000) Crk-associated substrate p130(Cas) interacts with nephrocystin and both proteins localize to cell-cell contacts of polarized epithelial cells. Exp Cell Res 256:168-178
46. Donaldson JC, Dise RS, Ritchie MD, Hanks SK (2002) Nephrocystin-conserved domains involved in targeting to epithelial cell-cell junctions, interaction with filamins, and establishing cell polarity. J Biol Chem 277:29028-29035
47. Morgan D, Eley L, Sayer J, Strachan T, Yates LM, Craighead AS, Goodship JA (2002) Expression analyses and interaction with the anaphase promoting complex protein Apc2 suggest a role for inversin in primary cilia and involvement in the cell cycle. Hum Mol Genet 11:3345-3350
48. Nurnberger J, Bacallao R, Phillips C (2002) Inversin forms a complex with catenins and N-caderin in polarized epithelial cells. Mol Biol Cell 13:3096-3106
49. Morgan D, Goodship J, Essner JJ, Vogan KJ, Turnpenny L, Yost HJ, Tabin CJ, Strachan T (2002) The left-right determinant inversin has highly conserved ankyrin repeat and IQ domains and interacts with calmodulin. Hum Genet 110:377-384
50. Yasuhiko Y, Imai F, Ookubo K, Takakuwa Y, Shiokawa K, Yokoyama T (2001) Calmodulin binds to inv protein: Implication for the regulatio of inv function. Develop Growth Differ 43:671-681
51. Watanabe D, Saijoh Y, Nonaka S, Sasaki G, Ikawa Y, Yokoyama T, Hamada H (2003) The left-right determinant Inversin is a component of node monocilia and other 9+0 cilia. Development 130:1725-1734

52. Otto EA, Wanner N, Schermer B, Benzing T, Kispert A, Walz G, Hildebrandt F (2003) Nephrocystin (NPHP1), inversin (NPHP2), and nephroretinin (NPHP4) localize to the node and to primary cilia of MDCK cells. J Am Soc Nephrol, in press (abstract)
53. Farrar GJ, Kenna PF, Humphries P (2002) On the genetics of retinitis pigmentosa and on mutation-independent approaches to therapeutic intervention. EMBO J 21:857-864
54. Liu Q, Zhou J, Daiger SP, Farber DB, Heckenlively JR, Smith JE, Sullivan LS, Zuo J, Milam AH, Pierce EA (2002) Identification and subcellular localization of the RP1 protein in human and mouse photoreceptors. Invest Ophthal Vis Sci 43:22-32
55. Zhao Y, Hong D, Pawlyk B, Yue G, Adamian M, Grynberg M, Godzik A, Li T (2003) The retinitis pigmentosa GTPase regulator (RPGR)-interacting protein: Subserving RPGR function and participating in disk morphogenesis. Proc Natl Acad Sci USA 100:3965-3970
56. Marszalek JR, Liu X, Roberts EA, Chui D, Marth JD, Williams DS, Goldstein LSB (2000) Genetic evidence for selective transport of opsin and arrestin by kinesin-II in mammalian photoreceptors. Cell 102:175-187
57. Igarashi P, Somlo S (2002) Genetics and pathogenesis of polycystic kidney disease. J Am Soc Nephrol 13:2384-2398
58. Qian F, Watnick TJ, Onuchic LF, Germino GG (1996) The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. Cell 87:979-987
59. Brasier JL, Henske EP (1997) Loss of the polycystic kidney disease (PKD1) region of chromosome 16p13 in renal cyst cells supports a loss-of-function model for cyst pathogenesis. J Clin Invest 99:194-199
60. Lu W, Peissel B, Babakhanlou H, Pavlova A, Geng L, Fan X, Larson C, Brent G, Zhou J (1997) Perinatal lethality with kidney and pancreas defects in mice with a targeted Pkd1 mutation. Nat Genet 17:179-181
61. Wu G, Markowitz GS, Li L, D'Agati VD, Factor SM, Geng L, Tibara S, Tuchman J, Cai Y, Park JH, van Adelsberg J, Hou H, Jr., Kucherlapati R, Edelmann W, Somlo S (2000) Cardiac defects and renal failure in mice with targeted mutations in *Pkd2*. Nat Genet 24:75-78
62. Kim K, Drummond I, Ibraghimov-Beskrovnaya O, Klinger K, Arnaout MA (2000) Polycystin 1 is required for the structural integrity of blood vessels. Proc Natl Acad Sci USA 97:1731-1736
63. Lu W, Shen X, Pavolva A, Lakkis M, Ward CJ, Pritchard L, Harris PC, Genest DR, Perez-Atayde AR, Zhou J (2001) Comparison of *Pkd1*-targeted mutants reveals that loss of polycystin-1 causes cystogenesis and bone defects. Hum Mol Genet 10:2385-2396
64. Muto S, Aiba A, Saito Y, Nakao K, Nakamura K, Tomita K, Kitamura T, Kurabayashi M, Nagai R, Higashihara E, Harris PC, Katsuki M, Horie S (2002) Pioglitazone improves the phenotype and molecular defects of a targeted *Pkd1* mutant. Hum Mol Genet 11:1731-1742
65. Wu G, Tian X, Nishimura S, Markowitz GS, D'Agati V, Park JH, Yao L, Li L, Gent L, Zhao H, Edelmann W, Somlo S (2002) *Trans*-heterozygous

Pkd1 and *Pkd2* mutations modify expression of polycystic kidney disease. Hum Mol Genet 11:1845-1854

66. Zerres K, Rudnik-Schoneborn S, Steinkamm C, Becker J, Mucher G (1998) Autosomal recessive polycystic kidney disease. J Mol Med 76:303-309
67. Guay-Woodford LM, Desmond RA (2003) Autosomal recessive polycystic kidney disease; the clinical experience in North America. Pediatrics 111:1072-1080
68. Ward CJ, Hogan MC, Rossetti S, Walker D, Sneddon T, Wang X, Kubly V, Cunningham JM, Bacallao R, Ishibashi M, Milliner DW, Torres VE, Harris PC (2002) The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. Nat Genet 30:259-269
69. Onuchic LF, Furu L, Nagasawa Y, Hou X, Eggermann T, Ren Z, Bergmann C, Senderek J, Esquivel E, Zeltner R, Rudnik-Schoneborn S, Mrug M, Sweeney W, Avner ED, Zerres K, Guay-Woodford LM, Somlo S, Germino GG (2002) *PKHD1*, the polycystic kidney and hepatic disease 1 gene, encodes a novel large protein containing multiple immunoglobulin-like plexin-transcription factor domains and parallel beta-helix 1 repeats. Am J Hum Genet 70:1305-1317
70. Furu L, Onuchic L, Gharavi A, Hou X, Esquivel E, Nagasawa Y, Bergmann C, Senderek J, Avner E, Zerres K, Germino G, Guay-Woodford L, Somlo S (2003) Milder presentation of recessive polycystic kidney disease requires presence of amino acid substitution mutations. J Am Soc Nephrol 14:2004-2014
71. Bergmann C, Senderek J, Sedlacek B, Pegiazoglou I, Puglia P, Eggermann T, Rudnik-Schoneborn S, Furu L, Onuchic LF, De Baca M, Germino G, Guay-Woodford L, Somlo S, Moser M, Buttner R, Zerres K (2003) Spectrum of mutations in the gene for autosomal recessive polycystic kidney disease (ARPKD/PKHD1). J Am Soc Nephrol 13:76-89
72. Rossetti S, Torra R, Coto E, Consugar M, Kubly V, Malaga S, Navarro M, El-Youssef M, Torres VE, Harris P (2003) A complete mutation screen of *PKHD1* in autosomal-recessive polycystic kidney disease (ARPKD) pedigrees. Kidney Int 64:391-403
73. International PKD Consortium (1995) Polycystic kidney disease: The complete structure of the *PKD1* gene and its protein. Cell 81:289-298
74. Sandford R, Sgotto B, Aparicio S, Brenner S, Vaudin M, Wilson RK, Chissoe S, Pepin K, Bateman A, Chothia C, Hughes J, Harris PC (1997) Comparative analysis of the polycystic kidney disease 1 (PKD1) gene reveals an integral membrane glycoprotein with multiple evolutionary conserved domains. Hum Mol Genet 6:1483-1489
75. Hughes J, Ward CJ, Peral B, Aspinwall R, Clark K, San Millan JL, Gamble V, Harris PC (1995) The polycystic kidney disease1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. Nat Genet 10:151-159
76. Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, Reynolds DM, Cai Y, Gabow PA, Pierides A, Kimberling WJ, Breuning

MH, Deltas CC, Peters DJM, Somlo S (1996) *PKD2*, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 272:1339-1342

77. Gonzalez-Perrett S, Kim K, Ibarra C, Damiano AE, Zotta E, Batelli M, Harris PC, Reisin IL, Arnaout MA, Cantiello HF (2001) Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca^{2+}-permeable nonselective cation channel. Proc Natl Acad Sci USA 98:1182-1187
78. Vassilev PM, Guo L, Chen XZ, Segal Y, Peng JB, Basora N, Babakhanlou H, Cruger G, Kanazirska M, Ye Cp, Brown EM, Hediger MA, Zhou J (2001) Polycystin-2 is a novel cation channel implicated in defective intracellular Ca^{2+} homeostasis in polycystic kidney disease. Biochem Biophys Res Comm 282:341-350
79. Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, Witzgall R, Ehrlich BE, Somlo S (2002) Polycystin-2 is an intracellular calcium release channel. Nat Cell Biol 4:191-197
80. Hanaoka K, Qian F, Boletta A, Bhunia AK, Piontek K, Tsiokas L, Sukhatme VP, Guggino WB, Germino GG (2000) Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. Nature 408:990-994
81. Pazour GJ, Dickert BL, Vucica Y, Seeley ES, Rosenbaum JL, Witman GB, Cole DG (2000) *Chlamydomonas IFT*88 and its mouse homologue, polycystic kidney disease gene *Tg*737, are required for assembly of cilia and flagella. J Cell Biol 151:709-718
82. Murcia NS, Richards WG, Yoder BK, Mucenski ML, Dunlap JR, Woychik RP (2000) The *Oak Ridge Polycystic Kidney (orpk)* disease gene is required for left-right axis determination. Development 127:2347-2355
83. Taulman PD, Haycraft CJ, Balkovetz DF, Yoder BK (2001) Polaris, a protein involved in left-right axis patterning, localizes to basal bodies and cilia. Mol Biol Cell 12:589-599
84. Hou X, Mrug M, Yoder BK, Lefkowitz EJ, Kremmidiotis G, D'Eustachio P, Beier DR, Guay-Woodford LM (2002) Cystin, a novel cilia-associated protein, is disrupted in the *cpk* mouse model of polycystic kidney disease. J Clin Invest 109:533-540
85. Mochizuki T, Saijoh Y, Tsuchiya K, Shirayoshi Y, Takai S, Taya C, Yonekawa H, Yamada K, Nihei H, Nakatsuji N, Overbeek PA, Hamada H, Yokoyama T (1998) Cloning of *inv*, a gene that controls left/right asymmetry and kidney development. Nature 395:177-181
86. Pennekamp P, Karcher C, Fischer A, Schweickert A, Skryabin B, Horst J, Blum M, Dworniczak B (2002) The ion channel polycystin-2 is required for left-right axis determination in mice. Curr Biol 12:938-943
87. Barr MM, DeModena J, Braun D, Nguyen CQ, Hall DH, Sternberg PW (2001) The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs *lov-1* and *pkd-2* act in the same pathway. Curr Biol 11:1341-1346

88. Pazour GJ, San Agustin JT, Follit JA, Rosenbaum JL, Witman GB (2002) Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. Curr Biol 12:R378-R380
89. Luo Y, Vassilev PM, Li X, Kawanabe Y, Zhou J (2003) Native polycystin 2 functions as a plasma membrane Ca^{2+}-permeable cation channel in renal epithelia. Mol Cell Biol 23:2600-2607.
90. Yoder BK, Hou X, Guay-Woodford L (2002) The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. J Am Soc Nephrol 13:2508-2516
91. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AEH, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat Genet 33:129-137
92. Ward CJ, Yuan D, Masyuk TV, Wang X, Punyashthiti R, Whelan S, Bacallao R, Torra R, LaRusso F, Torres VE, Harris P (2003) Cellular and subcellular localization of the ARPKD protein: fibrocystin is expressed on primary cilia. Hum Mol Genet 12:2703-2710
93. Schwartz EA, Leonard ML, Bizios R, Bowser SS (1997) Analysis and modeling of the primary cilium bending response to fluid shear. Am J Physiol 272:F132-F138
94. Roth KE, Rieder CL, Bowder SS (1988) Flexible-substratum technique for viewing cells from the side: some *in vivo* properties of primary (9+0) cilia in cultured kidney epithelia. J Cell Sci 89:457-466
95. Praetorius HA, Spring KR (2001) Bending the MDCK cell primary cilium increases intracellular calcium. J Membr Biol 184:71-79.
96. Watnick T, Germino G (2003) From cilia to cyst. Nat Genet 34:355-356
97. Cantiello HF (2003) A tale of two tails: ciliary mechanotransduction in ADPKD. Trends Mol Med 9:234-236
98. Lin F, Hiesberger T, Cordes K, Sinclair AM, Goldstein LSB, Somlo S, Igarashi P (2003) Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. Proc Natl Acad Sci USA 100:5286 - 5291
99. Marszalek JR, Goldstein LSB (2000) Understanding the functions of kinesin-II. Biochim Biophys Acta 1496:142-150
100. Marszalek JR, Ruiz-Lozano P, Roberts E, Chien KR, Goldstein LSB (1999) Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. Proc Natl Acad Sci USA 96:5043-5048
101. Ansley S, Badano J, Blacque O, Hill J, Hoskins B, Leitch C, Kim J, Ross A, Eichers E, Teslovich T, Mah A, Johnsen R, Cavender J, Lewis R, Leroux M, Beales P, Katsanis N (2003) Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. Nature 425:628-633