

PRIMARY CILIARY DYSKINESIA: HETEROTAXY SYNDROMES

1: Cell. 2006 Apr 7;125(1):33-45.

Nodal flow and the generation of left-right asymmetry.

Hirokawa N, Tanaka Y, Okada Y, Takeda S.

The establishment of left-right asymmetry in mammals is a good example of how multiple cell biological processes coordinate in the formation of a basic body plan. The leftward movement of fluid at the ventral node, called nodal flow, is the central process in symmetry breaking on the left-right axis. Nodal flow is autonomously generated by the rotation of cilia that are tilted toward the posterior on cells of the ventral node. These cilia are built by transport via the KIF3 motor complex. How nodal flow is interpreted to create left-right asymmetry has been a matter of debate. Recent evidence suggests that the leftward movement of membrane-sheathed particles, called nodal vesicular parcels (NVPs), may result in the activation of the non-canonical Hedgehog signaling pathway, an asymmetric elevation in intracellular Ca^{2+} and changes in gene expression.

2: Am J Physiol Renal Physiol. 2005 Dec;289(6):F1159-69.

An incredible decade for the primary cilium: a look at a once-forgotten organelle.

Davenport JR, Yoder BK

Since the discovery that numerous proteins involved in mammalian disease localize to the basal bodies and cilia, these organelles have emerged from relative obscurity to the center of intense research efforts in an expanding number of disease- and developmental-related fields. Our understanding of the association between cilia and human disease has benefited substantially from the use of lower organisms such as *Chlamydomonas* and *Caenorhabditis elegans* and the availability of murine models and cell culture. These research endeavors led to the discovery that loss of normal ciliary function in mammals is responsible for cystic and noncystic pathology in the kidney, liver, brain, and pancreas, as well as severe developmental patterning abnormalities. In addition, the localization of proteins involved in rare human disorders such as Bardet-Biedl syndrome has suggested that cilia-related dysfunction may play a role in modern human epidemics such as hypertension, obesity, and diabetes. Although we have made great advances in demonstrating the importance of cilia over the past decade, the physiological role that this organelle plays in most tissues remains elusive. Research focused on addressing this issue will be of critical importance for a further understanding of how ciliary dysfunction can lead to such severe disease and developmental pathologies.

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3: Curr Opin Genet Dev. 2003 Aug;13(4):385-92.

Cilia are at the heart of vertebrate left-right asymmetry.

McGrath J, Brueckner M.

Handed asymmetry of the shape and position of the internal organs is found in all vertebrates, and is essential for normal cardiac development. Recent genetic and embryological experiments in mouse embryos have demonstrated that left-right asymmetry is established by directional flow of extraembryonic fluid surrounding the node, which is driven by motile monocilia.

4: Nat Genet. 2003 Aug;34(4):413-20.

Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination.

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.

Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, leads to chronic renal failure in children. The genes mutated in NPHP1 and NPHP4 have been identified, and a gene locus associated with infantile nephronophthisis (NPHP2) was mapped. The kidney phenotype of NPHP2 combines clinical features of NPHP and polycystic kidney disease (PKD). Here, we identify inversin (INVS) as the gene mutated in NPHP2 with and without situs inversus. We show molecular interaction of inversin with nephrocystin, the product of the gene mutated in NPHP1 and interaction of nephrocystin with beta-tubulin, a main component of primary cilia. We show that nephrocystin, inversin and beta-tubulin colocalize to primary cilia of renal tubular cells. Furthermore, we produce a PKD-like renal cystic phenotype and randomization of heart looping by knockdown of *invs* expression in zebrafish. The interaction and colocalization in cilia of inversin, nephrocystin and beta-tubulin connect pathogenetic aspects of NPHP to PKD, to primary cilia function and to left-right axis determination.

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5: Clin Anat. 2003 May;16(3):269-76.

Isomerism of the right atrial appendages: clinical, anatomical, and microscopic study of a long-surviving case with asplenia and ciliary abnormalities.

Raman R, Al-Ali SY, Poole CA, Dawson BV, Carman JB, Calder L.

This study describes a case of isomerism of the right atrial appendages (bilateral morphologically right atrial appendages associated with complex congenital cardiac lesions) with ciliary abnormalities. Detailed investigation included gross anatomic dissection, review of the clinical history, and light, confocal, and electron microscopy. Clinically, this 40-year-old, long-surviving male patient had relatively good health until 4 years before death, which was due to cardiac failure. Surgical intervention consisted only of a Blalock-Taussig shunt (anastomosis of the right subclavian artery to the right pulmonary artery) at 6 years of age. Despite the presence of complex cardiac malformations and asplenia, his longevity may be attributed to the connection of the pulmonary veins to the atrium without pulmonary venous obstruction, pulmonary valvar stenosis rather than atresia, no significant atrioventricular valve regurgitation, and no serious infections during his life. Microscopic examination of bronchial epithelium revealed a narrow, disorganized epithelium with abundant goblet cells and short, angulated cilia with a random orientation and possibly an abnormal central microtubule doublet. These abnormalities were not present in controls, and have been noted in primary ciliary dyskinesia (PCD) or Kartagener's syndrome. Because this syndrome has classically been thought to cause random lateralization resulting in a mirror-imaged arrangement of the organs, the occurrence of truly isomeric patterns is not widely recognized. Whereas polysplenia and left bronchial isomerism have been reported to occur in immotile cilia syndrome, this is the first report to present detailed postmortem anatomic evidence of isomerism of the right atrial appendages, right bronchial isomerism, and asplenia in association with microscopy suggesting ciliary abnormalities. Copyright 2003 Wiley-Liss, Inc.

6: Trends Genet. 2003 Mar;19(3):162-7.

Lateralization defects and ciliary dyskinesia: lessons from algae.

El Zein L, Omran H, Bouvagnet P.

Flagella and cilia are two very similar organelles that "beat" to move cells and to propel fluid over tissues. They are highly conserved, being found in organisms ranging from prokaryotes to plant and animal eukaryotes. In humans, cilia are present in almost every organ, and several human conditions involve dysfunctional cilia; for example, lateralization defects, where the positions of organs are reversed, and primary ciliary dyskinesia, a rare

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condition where patients suffer from recurrent respiratory infections. In this article, we will discuss how information gained from studies on algae has aided research into these human diseases. These studies found a variety of functions that was previously unsuspected, renewing interest in cilia.

7: Am J Med Genet. 2001 Jul 15;101(4):339-44.

Cilia propel the embryo in the right direction.

Brueckner M.

Cilia have long been suspected to play a role in the determination of left-right asymmetry. Humans with the dominantly inherited condition Kartagener syndrome have defective cilia and a 50% incidence of mirror-image positioning of their organs (situs inversus). Analysis of mouse mutations affecting ciliary biogenesis and motility has demonstrated that the molecular motors kinesin and dynein are required to establish normal handed organismal asymmetry. The cilia that propel formation of the embryonic left-right axis are not conventional cilia, but monocilia. They are found on the node, or organizer, of the gastrulation-stage mouse embryo where they drive net leftward movement of the fluid surrounding the node, and initiate left-right asymmetry. Copyright 2001 Wiley-Liss, Inc.

8: Am J Respir Cell Mol Biol. 2000 Jul;23(1):45-51.

Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice.

Brody SL, Yan XH, Wuerffel MK, Song SK, Shapiro SD.

Cilia have been classified as sensory or motile types on the basis of functional and structural characteristics; however, factors important for regulation of assembly of different cilia types are not well understood. Hepatocyte nuclear factor-3/forkhead homologue 4 (HFH-4) is a winged helix/forkhead transcription factor expressed in ciliated cells of the respiratory tract, oviduct, and ependyma in late development through adulthood. Targeted deletion of the Hfh4 gene resulted in defective ciliogenesis in airway epithelial cells and randomized left-right asymmetry so that half the mice had situs inversus. In HFH-4-null mice, classic motile type cilia with a 9 + 2 microtubule ultrastructure were absent in epithelial cells, including those in the airways. In other organs, sensory cilia with a 9 + 0 microtubule pattern, such as those on olfactory neuroepithelial cells, were present. Ultrastructural analysis of mutant cells with absent 9 + 2 cilia demonstrated that defective ciliogenesis was due to abnormal centriole migration and/or apical membrane docking, suggesting that HFH-4 functions to direct basal body positioning or anchoring. Evaluation of wild-type embryos at

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gestational days 7.0 to 7.5 revealed Hfh4 expression in embryonic node cells that have monocilium, consistent with a function for this factor at the node in early determination of left-right axis. Analysis of the node of HFH-4 mutant embryos revealed that, in contrast to absent airway cilia, node cilia were present. These observations indicate that there are independent regulatory pathways for node ciliogenesis compared with 9 + 2 type ciliogenesis in airways, and support a central role for HFH-4 in ciliogenesis and left-right axis formation.

9: Curr Opin Genet Dev. 2000 Jun;10(3):257-61.

Left-right axis malformations in man and mouse.

Casey B, Hackett BP.

The study of left-right axis malformations in man and mouse has greatly advanced understanding of the mechanisms regulating vertebrate left-right axis formation. Recently, the roles of the TGF-beta family, Sonic hedgehog and fibroblast growth factor signaling, homeobox genes, and cilia in left-right axis determination have been more clearly defined. The identification of genes and environmental factors affecting left-right axis formation has important implications for understanding human laterality defects.

10: Mol Cell. 1999 Oct;4(4):459-68.

Abnormal nodal flow precedes situs inversus in *iv* and *inv* mice.

Okada Y, Nonaka S, Tanaka Y, Saijoh Y, Hamada H, Hirokawa N.

We examined the nodal flow of well-characterized mouse mutants, *inversus viscerum* (*iv*) and inversion of embryonic turning (*inv*), and found that their laterality defects are always accompanied by an abnormality in nodal flow. In a randomized laterality mutant, *iv*, the nodal cilia were immotile and the nodal flow was absent. In a situs inversus mutant, *inv*, the nodal cilia was motile but could only produce very weak leftward nodal flow. These results consistently support our hypothesis that the nodal flow produces the gradient of putative morphogen and triggers the first L-R determination event.

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11: Int J Dev Biol. 1999 Jul;43(4):283-6.

Asymmetry of cilia and of mice and men.

Afzelius BA.

Evidence is given for the opinion that cilia in the early embryo, by their work, determine the laterality of the body; without ciliary work body laterality would be randomized. More exactly, monocilia in the primitive node are responsible for this determination. They have been described as being of the 9+0 type, but with dynein arms and with a gyrating movement. The orientation of the monocilia on the epithelium is of no importance but the direction of their gyration is, as may also be the shape of the node. The chirality of the cilia is thus reflected directly in the asymmetry of the body. The dynein arms go clockwise as seen from the base to tip and the ciliary rotation is in the same direction. The resulting waterflow is towards the left and so is the movement of the forming heart. In most subgroups of the immotile-cilia syndrome this mechanism does not work and equally many individuals will be born with situs inversus as with situs solitus. An exception is the immotile-cilia subgroup, named 'microtubule transposition', which is characterized by all cilia having a 9+0 structure throughout most of their length.

12: Am J Med Genet. 1999 Jan 15;82(2):155-60.

Discordant organ laterality in monozygotic twins with primary ciliary dyskinesia.

Noone PG, Bali D, Carson JL, Sannuti A, Gipson CL, Ostrowski LE, Bromberg PA, Boucher RC, Knowles MR.

Primary ciliary dyskinesia (PCD) is a genetic disease characterized by abnormal ciliary structure and function, impaired mucociliary clearance, and chronic middle ear, sinus, and lung disease. PCD is associated with situs inversus in approximately 50% of the patients. One proposed explanation for this relationship is that normal ciliary function plays a role in normal organ orientation, whereas organ orientation in PCD is a random event because of dysfunctional cilia in early embryonic development. Another hypothesis for the association between PCD and situs inversus is that mutated genes in PCD not only cause defective cilia, but are also linked to the control of organ laterality, such that abnormalities in this molecular pathway result in random left-right asymmetry. We report on a set of monozygotic twin women with PCD. In both patients, deficiency of the inner dynein arms was noted on ciliary ultrastructural analysis, associated with a clinical syndrome of bronchiectasis, chronic sinusitis, and middle ear disease. One of the twins has situs solitus, the other has situs inversus totalis. DNA analysis confirmed that the twins are monozygotic. This is consistent with the hypothesis that situs inversus occurring in patients with primary ciliary dyskinesia is a random but "complete" event in the fetal development of patients with PCD.