

PRIMARY CILIARY DYSKINESIA: GENETICS

1: Am J Respir Crit Care Med. 2006 Apr 20;

DNAH5 Mutations are a Common Cause of Primary Ciliary Dyskinesia with Outer Dynein Arm Defects.

Hornef N, Olbrich H, Horvath J, Zariwala MA, Fliegau M, Loges NT, Wildhaber J, Noone PG, Kennedy M, Antonarakis SE, Blouin JL, Bartoloni L, Nublein T, Ahrens P, Griese M, Kuhl H, Sudbrak R, Knowles MR, Reinhardt R, Omran H.

Rationale: Primary ciliary dyskinesia (PCD) is characterized by recurrent airway infections and randomization of left-right body asymmetry. To date, autosomal recessive mutations have only been identified in a small number of patients involving DNAI1 and DNAH5, which encode outer dynein arm components. Methods: We screened 109 Caucasian PCD families originating from Europe and North America for presence of DNAH5 mutations by haplotype analyses and/or sequencing. Results: Haplotype analyses excluded linkage in 26 families. In 30 PCD families we identified 33 novel (12 nonsense, 8 frameshift, 5 splicing and 8 missense mutations), and two known DNAH5 mutations. We observed clustering of mutations within five exons harbouring 27 mutant alleles (52%) out of the 52 detected mutant alleles. Interestingly, six (32%) of 19 PCD families with DNAH5 mutations from North America carry the novel founder mutation 10815delT. Electron microscopic analyses in 22 PCD patients with mutations detected invariably outer dynein arm ciliary defects. High-resolution immunofluorescence imaging of respiratory epithelial cells from eight patients with DNAH5 mutations showed mislocalization of mutant DNAH5 and accumulation at the microtubule organizing centers. Mutant DNAH5 was absent throughout the ciliary axoneme in seven patients and remained detectable in the proximal ciliary axoneme in one patient carrying compound heterozygous splicing mutations at the 3'-end (IVS75-2A>T, IVS76+5G>A). In a pre-selected subpopulation with documented outer dynein arm defects (n=47), DNAH5 mutations were identified in 53%. Conclusions: DNAH5 is frequently mutated in PCD patients exhibiting outer dynein arm defects and mutations cluster in five exons.

2: Trends Genet. 2006 Mar 22;

Novel tools to unravel molecular mechanisms in cilia-related disorders.

Fliegau M, Omran H.

Cilia are hair-like organelles extending from the cell surface that execute motile (e.g. respiratory cilia) and/or sensory functions (e.g. renal monocilia). The basic ultrastructure of cilia and flagella has been well established by electron microscopy. Several recent reports have now provided intriguing new insights into the complex molecular composition of cilia and flagella. These

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data from genome, proteome and transcriptome analyses will facilitate the systematic discovery and understanding of genes responsible for human cilia-related diseases, such as primary ciliary dyskinesia, polycystic kidney disease and male sterility.

3: J Cell Sci. 2006 Mar 15;119(Pt 6):1165-74.

Radial spoke proteins of *Chlamydomonas* flagella.

Yang P, Diener DR, Yang C, Kohno T, Pazour GJ, Dienes JM, Agrin NS, King SM, Sale WS, Kamiya R, Rosenbaum JL, Witman GB.

The radial spoke is a ubiquitous component of '9+2' cilia and flagella, and plays an essential role in the control of dynein arm activity by relaying signals from the central pair of microtubules to the arms. The *Chlamydomonas reinhardtii* radial spoke contains at least 23 proteins, only 8 of which have been characterized at the molecular level. Here, we use mass spectrometry to identify 10 additional radial spoke proteins. Many of the newly identified proteins in the spoke stalk are predicted to contain domains associated with signal transduction, including Ca²⁺-, AKAP- and nucleotide-binding domains. This suggests that the spoke stalk is both a scaffold for signaling molecules and itself a transducer of signals. Moreover, in addition to the recently described HSP40 family member, a second spoke stalk protein is predicted to be a molecular chaperone, implying that there is a sophisticated mechanism for the assembly of this large complex. Among the 18 spoke proteins identified to date, at least 12 have apparent homologs in humans, indicating that the radial spoke has been conserved throughout evolution. The human genes encoding these proteins are candidates for causing primary ciliary dyskinesia, a severe inherited disease involving missing or defective axonemal structures, including the radial spokes.

4: Pediatr Res. 2006 Mar;59(3):418-22.

Axonemal localization of the dynein component DNAH5 is not altered in secondary ciliary dyskinesia.

Olbrich H, Horvath J, Fekete A, Loges NT, van's Gravesande KS, Blum A, Hormann K, Omran H.

Primary ciliary dyskinesia (PCD) is a heterogeneous genetic disorder characterized by recurrent airway infections and situs inversus in half of affected individuals. Diagnosis currently relies on demonstration of abnormal ciliary ultrastructure or altered ciliary beat. Alterations encountered in

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secondary ciliary dyskinesia (SCD) caused by inflammation often complicate the diagnostic workup. We have recently shown that in respiratory epithelial cells from PCD patients with outer dynein arm defects the dynein protein DNAH5 is mislocalized and either completely or partially absent from the ciliary axoneme. In this study, we addressed the question whether SCD might affect axonemal DNAH5 localization in respiratory cells. To induce SCD in vitro, we treated primary human respiratory epithelial cell cultures with interleukin-13 (IL-13). Ciliary function and ultrastructure were assessed by high-speed videomicroscopy and transmission electron microscopy, respectively. For in vivo localization of DNAH5, we performed nasal brushing biopsies in patients with evidence of SCD. Expression of DNAH5 was analyzed by immunofluorescence microscopy. IL-13-treated cells showed evidence of SCD. Ciliary beat frequency was significantly reduced and ultrastructural analyses showed axonemal disorganization compared with control cells. High-resolution immunofluorescence studies of respiratory epithelial cells with SCD identified in vitro and in vivo normal axonemal DNAH5 localization. DNAH5 localization is not altered by SCD, indicating a high potential for immunofluorescence analysis as a novel diagnostic tool in PCD.

5: J Med Genet. 2006 Jan;43(1):e1.

Linkage analysis localises a Kartagener syndrome gene to a 3.5 cM region on chromosome 15q24-25.

Geremek M, Zietkiewicz E, Diehl SR, Alizadeh BZ, Wijmenga C, Witt M.

METHODS: In a genome-wide search for PCD loci performed in 52 KS families and in 18 PCD families with no situs inversus present (CDO, ciliary dysfunction-only), the maximal pairwise LOD score of 3.36 with D15S205 in the KS families indicated linkage of a KS locus to the long arm of chromosome 15. In the follow-up study, 65 additional microsatellite markers encompassing D15S205 were analysed.

RESULTS: A maximal pairwise LOD score of 4.34 was observed with D15S154, further supporting linkage of the KS, but not the CDO, families to 15q24-25. Analysis of heterogeneity and haplotypes suggested linkage to this region in 60% of KS families.

CONCLUSIONS: Reinforced by the results of multipoint linkage, our analyses indicate that a major KS locus is localised within a 3.5 cM region on 15q, between D15S973 and D15S1037.

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6: J Med Genet. 2006 Apr;43(4):326-33. Epub 2005 Jul 31.

RPGR is mutated in patients with a complex X linked phenotype combining primary ciliary dyskinesia and retinitis pigmentosa.

Moore A, Escudier E, Roger G, Tamalet A, Pelosse B, Marlin S, Clement A, Geremek M, Delaisi B, Bridoux AM, Coste A, Witt M, Duriez B, Amselem S.

INTRODUCTION: Primary ciliary dyskinesia (PCD) is a rare disease classically transmitted as an autosomal recessive trait and characterised by recurrent airway infections due to abnormal ciliary structure and function. To date, only two autosomal genes, DNAI1 and DNAH5 encoding axonemal dynein chains, have been shown to cause PCD with defective outer dynein arms. Here, we investigated one non-consanguineous family in which a woman with retinitis pigmentosa (RP) gave birth to two boys with a complex phenotype combining PCD, discovered in early childhood and characterised by partial dynein arm defects, and RP that occurred secondarily. The family history prompted us to search for an X linked gene that could account for both conditions.

RESULTS: We found perfect segregation of the disease phenotype with RP3 associated markers (Xp21.1). Analysis of the retinitis pigmentosa GTPase regulator gene (RPGR) located at this locus revealed a mutation (631_IVS6+9del) in the two boys and their mother. As shown by study of RPGR transcripts expressed in nasal epithelial cells, this intragenic deletion, which leads to activation of a cryptic donor splice site, predicts a severely truncated protein.

CONCLUSION: These data provide the first clear demonstration of X linked transmission of PCD. This unusual mode of inheritance of PCD in patients with particular phenotypic features (that is, partial dynein arm defects and association with RP), which should modify the current management of families affected by PCD or RP, unveils the importance of RPGR in the proper development of both respiratory ciliary structures and connecting cilia of photoreceptors.

7: J Med Genet. 2006 Jan;43(1):62-73. Epub 2005 Jun 3.

Identification of predicted human outer dynein arm genes: candidates for primary ciliary dyskinesia genes.

Pazour GJ, Agrin N, Walker BL, Witman GB.

BACKGROUND: Primary ciliary dyskinesia (PCD) is a severe inherited disorder characterised by chronic respiratory disease, male infertility, and, in approximately 50% of affected individuals, a left-right asymmetry defect called situs inversus. PCD is caused by defects in substructures of the ciliary and

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flagellar axoneme, most commonly loss of the outer dynein arms. Although PCD is believed to involve mutations in many genes, only three have been identified. METHODS: To facilitate discovery of new PCD genes, we have used database searching and analysis to systematically identify the human homologues of proteins associated with the *Chlamydomonas reinhardtii* outer dynein arm, the best characterised outer arm of any species. RESULTS: We find that 12 out of 14 known *Chlamydomonas* outer arm subunits have one or more likely orthologues in humans. The results predict a total of 24 human genes likely to encode outer dynein arm subunits and associated proteins possibly necessary for outer arm assembly, plus 12 additional closely related human genes likely to encode inner dynein arm subunits. CONCLUSION: These genes, which have been located on the human chromosomes for easy comparison with known or suspected PCD loci, are excellent candidates for screening for disease-causing mutations in PCD patients with outer and/or inner dynein arm defects.

8: Am J Respir Cell Mol Biol. 2005 Jul;33(1):41-7. Epub 2005 Apr 21.

Identification and analysis of axonemal dynein light chain 1 in primary ciliary dyskinesia patients.

Horvath J, Fliegauf M, Olbrich H, Kispert A, King SM, Mitchison H, Zariwala MA, Knowles MR, Sudbrak R, Fekete G, Neesen J, Reinhardt R, Omran H.

Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder characterized by chronic infections of the upper and lower airways, randomization of left/right body asymmetry, and reduced fertility. The phenotype results from dysfunction of motile cilia of the respiratory epithelium, at the embryonic node and of sperm flagella. Ultrastructural defects often involve outer dynein arms (ODAs), that are composed of several light (LCs), intermediate, and heavy (HCs) dynein chains. We recently showed that recessive mutations of DNAH5, the human ortholog of the biflagellate *Chlamydomonas* ODA gamma-HC, cause PCD. In *Chlamydomonas*, motor protein activity of the gamma-ODA-HC is regulated by binding of the axonemal LC1. We report the identification of the human (DNAL1) and murine (Dnal1) orthologs of the *Chlamydomonas* LC1-gene. Northern blot and in situ hybridization analyses revealed specific expression in testis, embryonic node, respiratory epithelium, and ependyma, resembling the DNAH5 expression pattern. In silico protein analysis showed complete conservation of the LC1/gamma-HC binding motif in DNAL1. Protein interaction studies demonstrated binding of DNAL1 and DNAH5. Based on these findings, we considered DNAL1 a candidate for PCD and sequenced all exons of DNAL1 in 86 patients. Mutational analysis was negative, excluding a major role of DNAL1 in the pathogenesis of PCD.

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9: Am J Respir Crit Care Med. 2005 Jun 15;171(12):1343-9. Epub 2005 Mar 4.

Mislocalization of DNAH5 and DNAH9 in respiratory cells from patients with primary ciliary dyskinesia.

Fliegau M, Olbrich H, Horvath J, Wildhaber JH, Zariwala MA, Kennedy M, Knowles MR, Omran H.

RATIONALE: Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder characterized by recurrent infections of the airways and situs inversus in half of the affected offspring. The most frequent genetic defects comprise recessive mutations of DNAH5 and DNAI1, which encode outer dynein arm (ODA) components. Diagnosis of PCD usually relies on electron microscopy, which is technically demanding and sometimes difficult to interpret. **METHODS:** Using specific antibodies, we determined the subcellular localization of the ODA heavy chains DNAH5 and DNAH9 in human respiratory epithelial and sperm cells of patients with PCD and control subjects by high-resolution immunofluorescence imaging. We also assessed cilia and sperm tail function by high-speed video microscopy. **RESULTS:** In normal ciliated airway epithelium, DNAH5 and DNAH9 show a specific regional distribution along the ciliary axoneme, indicating the existence of at least two distinct ODA types. DNAH5 was completely or only distally absent from the respiratory ciliary axoneme in patients with PCD with DNAH5- (n = 3) or DNAI1- (n = 1) mutations, respectively, and instead accumulated at the microtubule-organizing centers. In contrast to respiratory cilia, sperm tails from a patient with DNAH5 mutations had normal ODA heavy chain distribution, suggesting different modes of ODA generation in these cell types. Blinded investigation of a large cohort of patients with PCD and control subjects identified DNAH5 mislocalization in all patients diagnosed with ODA defects by electron microscopy (n = 16). Cilia with complete axonemal DNAH5 deficiency were immotile, whereas cilia with distal DNAH5 deficiency showed residual motility. **CONCLUSIONS:** Immunofluorescence staining can detect ODA defects, which will possibly aid PCD diagnosis.

10: J Appl Genet. 2004;45(3):347-61.

Primary ciliary dyskinesia: genes, candidate genes and chromosomal regions.

Geremek M, Witt M.

Primary ciliary dyskinesia (PCD) is a multisystem disease characterized by recurrent respiratory tract infections, sinusitis, bronchiectasis and male subfertility, associated in about 50% patients with situs inversus totalis (the

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Kartagener syndrome). The disease phenotype is caused by ultrastructural defects of respiratory cilia and sperm tails. PCD is a heterogenetic disorder, usually inherited as an autosomal recessive trait. So far, mutations in two human genes have been proved to cause the disease. However, the pathogenetics of most PCD cases remains unsolved. In this review, the disease pathomechanism is discussed along with the genes that are or may be involved in the pathogenesis of primary ciliary dyskinesia and the Kartagener syndrome.

11: Paediatr Respir Rev. 2004 Mar;5(1):69-76.

Cilia, primary ciliary dyskinesia and molecular genetics.

Chodhari R, Mitchison HM, Meeks M.

Primary ciliary dyskinesia (PCD) is a phenotypically and genetically heterogeneous condition in which three genetic mutations have already been identified. The primary defect is in the ultrastructure or function of cilia, highly complex organelles that are structurally related to the flagella of sperm and protozoa. The clinical features of PCD include recurrent sinopulmonary infections, subfertility and laterality defects; the latter due to ciliary dysfunction at the embryological node. Completion of the human genome sequence has accelerated the identification and characterisation of disease genes, and the current molecular strategy in PCD includes candidate gene analysis, positional cloning, model organism analysis and proteomic analysis. The identification of these genes will provide new insights into the molecular mechanisms involved in the assembly and function of cilia and the pathway that determines left-right axis in man. This may also allow the development of new methods for diagnosis, prevention and treatment of PCD.

12: Pediatr Pulmonol. 2004 Jul;38(1):88-9.

PCD and RP: X-linked inheritance of both disorders?

Krawczynski MR, Witt M.

A Caucasian, seven-generation family of Polish origin with apparently X-linked inheritance of coexisting retinitis pigmentosa (RP) and primary ciliary dyskinesia (PCD), with 14 identified males affected with RP and 14 obligate healthy female carriers, is presented. To our knowledge, four of the RP-affected males were diagnosed with PCD. The cases might imply the presence of one of the PCD loci, influencing neither laterality nor fertility, within the X-chromosome.

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13: J Appl Genet. 2004;45(1):107-10.

Apparent X-linked primary ciliary dyskinesia associated with retinitis pigmentosa and a hearing loss.

Krawczynski MR, Dmenska H, Witt M.

Three brothers, one 10-year-old and a pair of 14-year-old dizygotic twins--expressed the classical, early-onset retinitis pigmentosa (RP) with typical ophthalmoscopic findings, night blindness, visual field constricted to 10 degrees and flat ERG response. All three brothers were also diagnosed with primary ciliary dyskinesia (PCD) and had recurrent respiratory infections, chronic sinusitis and bronchiectasis. In all of them, resection of the middle lobe of the right lung was performed. A similar clinical picture of coexisting RP and PCD was noted in the brother of the probands' mother. All probands displayed situs solitus. Consistent with the X-linked mode of RP inheritance, there were also three obligatory female carriers of the disorder in this family: the mother of the affected boys, her mother and a daughter of her brother. In all of them, retinitis pigmentosa "sine pigmento" was found with milder but clinically significant symptoms (mild night blindness, visual field constricted to 30 degrees, and scotopic and photopic ERG responses reduced to 30-60%). No extraocular symptoms were detected in any of the heterozygous female carriers. This family presents an example of two rare phenomena: X-linked dominant retinitis pigmentosa (with milder expression in females) and a rare combination of RP with recurrent respiratory infections due to PCD.

14: Proc Natl Acad Sci U S A. 2002 Aug 6;99(16):10282-6. Epub 2002 Jul 25.

Mutations in the DNAH11 (axonemal heavy chain dynein type 11) gene cause one form of situs inversus totalis and most likely primary ciliary dyskinesia.

Bartoloni L, Blouin JL, Pan Y, Gehrig C, Maiti AK, Scamuffa N, Rossier C, Jorissen M, Armengot M, Meeks M, Mitchison HM, Chung EM, Delozier-Blanchet CD, Craigen WJ, Antonarakis SE.

Primary ciliary dyskinesia (PCD; MIM 242650) is an autosomal recessive disorder of ciliary dysfunction with extensive genetic heterogeneity. PCD is characterized by bronchiectasis and upper respiratory tract infections, and half of the patients with PCD have situs inversus (Kartagener syndrome). We characterized the transcript and the genomic organization of the axonemal heavy chain dynein type 11 (DNAH11) gene, the human homologue of murine Dnah11 or Ird, which is mutated in the iv/iv mouse model with situs inversus. To assess the role of DNAH11, which maps on chromosome 7p21, we searched for mutations in the 82 exons of this gene in a patient with situs

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inversus totalis, and probable Kartagener syndrome associated with paternal uniparental disomy of chromosome 7 (patUPD7). We identified a homozygous nonsense mutation (R2852X) in the DNAH11 gene. This patient is remarkable because he is also homozygous for the F508del allele of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Sequence analysis of the DNAH11 gene in an additional 6 selected PCD sibships that shared DNAH11 alleles revealed polymorphic variants and an R3004Q substitution in a conserved position that might be pathogenic. We conclude that mutations in the coding region of DNAH11 account for situs inversus totalis and probably a minority of cases of PCD.

15: Hum Mol Genet. 2002 Mar 15;11(6):715-21.

Loss of function of axonemal dynein Mdnah5 causes primary ciliary dyskinesia and hydrocephalus.

Ibanez-Tallon I, Gorokhova S, Heintz N.

Primary ciliary dyskinesia (PCD), also known as Kartagener's syndrome, is a human syndrome that results from ciliary dysfunction. This syndrome is characterized by recurrent respiratory infections, situs inversus and infertility. In some cases, hydrocephalus is also observed. We have characterized an insertional mutation in a mouse axonemal dynein heavy chain gene (Mdnah5) that reproduces most of the classical features of PCD, including recurrent respiratory infections, situs inversus and ciliary immotility. These mice also suffer from hydrocephalus and die perinatally. Electron microscopic studies demonstrate the loss of axonemal outer arms. These results show that mutations in Mdnah5 are a primary cause of PCD and provide direct evidence that mutations in an axonemal dynein can cause hydrocephalus. Mutations in the human DNAH5 have recently been identified in PCD patients. Comparison of the mouse model and the human data suggests that the degree of ciliary dysfunction is causally related to the severity of human PCD, particularly the presence of hydrocephalus.

16: Mol Cell Biol. 2002 Apr;22(8):2769-76.

Hydrocephalus, situs inversus, chronic sinusitis, and male infertility in DNA polymerase lambda-deficient mice: possible implication for the pathogenesis of immotile cilia syndrome.

Kobayashi Y, Watanabe M, Okada Y, Sawa H, Takai H, Nakanishi M, Kawase Y, Suzuki H, Nagashima K, Ikeda K, Motoyama N.

A growing number of DNA polymerases have been identified, although their

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physiological function and relation to human disease remain mostly unknown. DNA polymerase lambda (Pol lambda; also known as Pol beta2) has recently been identified as a member of the X family of DNA polymerases and shares 32% amino acid sequence identity with DNA Pol beta within the polymerase domain. With the use of homologous recombination, we generated Pol lambda(-/-) mice. Pol lambda(-/-) mice develop hydrocephalus with marked dilation of the lateral ventricles and exhibit a high rate of mortality after birth, although embryonic development appears normal. Pol lambda(-/-) mice also show situs inversus totalis and chronic suppurative sinusitis. The surviving male, but not female, Pol lambda(-/-) mice are sterile as a result of spermatozoal immobility. Microinjection of sperm from male Pol lambda(-/-) mice into oocytes gives rise to normal offspring, suggesting that the meiotic process is not impaired. Ultrastructural analysis reveals that inner dynein arms of cilia from both the ependymal cell layer and respiratory epithelium are defective, which may underlie the pathogenesis of hydrocephalus, situs inversus totalis, chronic sinusitis, and male infertility. Sensitivity of Pol lambda(-/-) cells to various kinds of DNA damage is indistinguishable from that of Pol lambda(+/+) cells. Collectively, Pol lambda(-/-) mice may provide a useful model for clarifying the pathogenesis of immotile cilia syndrome.

17: Chest. 2002 Mar;121(3 Suppl):97S.

Mutations in DNAI1 (IC78) cause primary ciliary dyskinesia.

Noone PG, Zariwala M, Sannuti A, Minnix S, Leigh MW, Carson J, Knowles MR.

18: J Biol Chem. 2002 May 17;277(20):17906-15. Epub 2002 Mar 4.

Identification of dynein heavy chain 7 as an inner arm component of human cilia that is synthesized but not assembled in a case of primary ciliary dyskinesia.

Zhang YJ, O'Neal WK, Randell SH, Blackburn K, Moyer MB, Boucher RC, Ostrowski LE.

Although the basic structure of the axoneme has been highly conserved throughout evolution, the varied functions of specialized axonemes require differences in structure and regulation. Cilia lining the respiratory tract propel mucus along airway surfaces, providing a critical function to the defense mechanisms of the pulmonary system, yet little is known of their molecular structure. We have identified and cloned a dynein heavy chain that is a component of the inner dynein arm. Bronchial epithelial cells were obtained from normal donors and from a patient with primary ciliary dyskinesia (PCD)

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whose cilia demonstrated an absence of inner dynein arms by electron microscopy. Cilia from normal and PCD cells were compared by gel electrophoresis, and mass spectrometry was used to identify DNAH7 as a protein absent in PCD cilia. The full-length DNAH7 cDNA was cloned and shares 68% similarity with an inner arm dynein heavy chain from *Drosophila*. DNAH7 was induced during ciliated cell differentiation, and immunohistochemistry demonstrated the presence of DNAH7 in normal cilia. In cilia from PCD cells, DNAH7 was undetectable, whereas intracellular DNAH7 was clearly present. These studies identify DNAH7 as an inner arm component of human cilia that is synthesized but not assembled in a case of PCD.