

Title: Primary Ciliary Dyskinesia *GeneReview* – Model Organisms
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Model Organisms

The axonemal structure of a biflagellated unicellular alga, *Chlamydomonas reinhardtii*, is similar to the structure of human cilia. Several motility-defective mutant strains of *Chlamydomonas* are known; their human counterparts could be candidates for PCD.

Homozygous deletion of the mouse *Mdnh5* gene (ortholog of *DNAH5*) resulted in the PCD phenotype including situs abnormalities, recurrent respiratory symptoms, ciliary immotility, hydrocephalus, and outer dynein arm defects by ultrastructural analysis [Ibanez-Tallon et al 2002]. Ethyl nitrosourea induced mutagenesis generated another mouse model with inframe 593 amino acid deletion. The mouse had a PCD phenotype including situs abnormalities and outer dynein arm defects [Tan et al 2007]

Additionally, homozygous deletion of the motor domain of mouse *Ird* gene (ortholog of *DNAH11*) caused only situs inversus without the respiratory phenotype and normal ultrastructure of respiratory cilia [Supp et al 1999].

Ethyl nitrosourea-mediated mutant screening in medaka fish identified *mii* (mirror image of internal organ) mutant with laterality defects. Further analysis revealed that *mii* locus encodes *Dnai2a* that is ortholog of human *DNAI2* and is expressed in the Kupffer's vesicles. The mutant *mii* displayed loss of nodal flow leading to the laterality defects. Ultrastructure analysis of Kupffer's vesicles from *mii* showed absence of outer dynein arms that was consistent with the axonemal defects seen in PCD patient with *DNAI2* mutations and *oda6* mutant of *Chlamydomonas* due to loss of ortholog *IC69*, however, renal cilia of *mii* had normal outer dynein arms and function. A paralog *Dnai2b* was found to be expressed in the prospective kidneys (pronephros and mesonephros) and not in the Kupffer's vesicles, however, it was able to rescue *mii* mutant suggesting its compensatory role. Knock-down of *Dnai2b* as well as double knock-down with *Dnai2a* led to the renal cysts suggesting that *Dnai2b* functions as a complementary molecule of *Dnai2a* and is important component of outer dynein arms in the prospective kidneys for the intratubular flow required for the formation and maintenance of the kidney.

Studies in medaka fish revealed that *KTU* truncating mutations that cause disease in humans result in laterality defects, polycystic kidney disease, and sperm motility defects. The sperm flagella and Kupffer's vesicle (functionally equivalent to mouse node) had both outer and inner dynein arm defects on ultrastructural analysis. Interestingly, mutation of *DNAAF2* (*C14orf104/KTU*), which results in PCD in humans, caused polycystic kidney disease in fish. The difference may be attributable to the different origins of the kidneys: mesonephric in fish and metanephric in mammals. Similar motility and ultrastructural defects were found in the *Chlamydomonas* motility-

defective mutant strain *pf13* which has a mutation in the orthologous gene *PF13* [Omran et al 2008].

Deletion of 1.2 kb including exon 1 of *ODA7* (ortholog of *DNAAF1 (LRRC50)* gene caused motility defects in *Chlamydomonas oda7* mutant. Biochemical analysis indicated that *ODA7* protein interacts with both outer row dyneins and I1 inner row dynein by forming the bridge between these two motors that suggests its role in coordination of dynein isoforms during flagellar motility [Freshour et al 2007]. Additionally, silencing of *DNAAF1 (LRRC50)* ortholog (*Tb11.01.5550*) in *Trypanosoma brucei* (flagellated protest) using RNAi led to an abnormal motility phenotype that was consistent with the 53% decrease in outer dynein arms by flagellar ultrastructural analysis [Duquesnoy et al 2009].

Multiple bobtail dog littermates that were traced to the same female Champion dog presented with PCD phenotype, lateralization defects and ultrastructural defects including eccentric central pair, abnormal radial spokes and nexin links, reduced number of inner dynein arms and displacement of outer doublet that is consistent with axonemal disorganization. Using v2 Canine array, ~15Mb autozygous segment of on dog Chromosome 34 was identified. Within this region 10 genes of the 151 annotated genes were considered interesting based on their presence in the known proteome or cilia protein databases. Sequence analyses were performed for 6 genes and homozygous stop mutation (p.Arg86X) was identified in *CCDC39*. All of the affected bobtail littermates were homozygous and obligate carriers were heterozygous for the mutation. Additionally, this mutation was observed in 8 of the 216 (3.7%) control bobtail alleles, while never seen on 160 alleles from other healthy breeds [Merveille et al 2011]. The ortholog *FAP59* in *Chlamydomonas* is also predicted to be essential for motility ciliary function. *Ccdc39* showed strong RNA expression in mouse tissues that were rich in ciliated cell including upper and lower airways, and ependymal choroid plexus. Suppression of *ccdc39* in zebrafish embryo resulted in heart looping defects and bilateral or lack of expression of southpaw that is expected at the left lateral plate mesoderm in 14 somite embryo. This zebrafish phenotype was rescued by co-injection with wildtype human *CCDC39* mRNA. These findings are consistent with laterality defects that have been seen in other PCD morphants such as *ktu* and *lrrc50* [Merveille et al 2011].

To identify genes required for normal development of mouse embryo, genetic screening identified a mutant *lnks (links)* with laterality defects. Total of 172 embryos were checked and 39% presented with laterality defects including 8% with situs inversus and 19% with left isomerism. Majority of the homozygous mutant mice died before weaning but some had hydrocephalus which was similar to the other PCD mouse model of *Mdnah5*-deficient mouse. Mapping led to the identification of 0.3 Mb segment on mouse chromosome 11 that included *Ccdc40* and mutation analysis identified homozygous nonsense mutation (p.Ser792X). Additionally, *ccdc40* is expressed in zebrafish tissues that contain motile cilia. Knock-down of *ccdc40* using antisense morpholino oligonucleotides resulted in zebrafish mutant with laterality defect; phenotype, that was rescued by the co-injection of wild type *ccdc40* mRNA. Zebrafish mutant *locke (lok)* that was described previously had the similar phenotype as *ccdc40* knock-down, hence *lok* mutant was checked and a nonsense mutation (p.Gln778X) in *ccdc40* was identified.

The laterality defects in mouse and zebrafish mutant together with expression pattern as well as shortened cilia in nodal pit cells in *Inks* mutant and Kupffer's vesicle suggest that Ccdc40 may act to regulate ciliary function [Becker-Heck et al 2011].

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