

Zebrafish as models to study ciliopathies of the eye and kidney

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Cilia are highly-conserved organelles projecting from the cell surface of nearly every cell type in vertebrates. Ciliary proteins have essential functions in human physiology, particularly in signaling and organ development. As cilia are a component of almost all vertebrate cells, cilia dysfunction can manifest as a constellation of features that characteristically include, retinal degeneration, renal disease and cerebral anomalies. The terminology "Ciliopathies" refers to inherited human

disorders caused by genetic mutations in ciliary genes, leading to cilia dysfunctions that form an important and ever expanding multi-organ disease spectrum. Ciliopathies are a diverse class of congenital diseases, with twenty-four recognized syndromes caused by mutations in at least ninety different genes. In order to start to dissect the phenotypes of each disease associated with ciliary dysfunction it is necessary to understand the mechanisms underlying the phenotype using suitable animal models. Here, we review the advantages of the zebrafish as a vertebrate model for human ciliopathies, with a focus on ciliopathies affecting the eye and the kidney.

Key Words: Cilia, Ciliopathies, Retina, Kidney, Zebrafish

ABBREVIATIONS

AHI1: Abelson helper integration site 1 (ORF1; AHI-1; JBTS3; dJ71N10.1); ARL13B: ADP ribosylation factor like GTPase 13 (JBTS8; ARL2L1); ARL6: ADP ribosylation factor like GTPase 6 (BBS3; RP55); ARMC9: Arrowhead Regional Medical Center 9; BBS5: Bardet-Biedl syndrome 5; CC2D2A: coiled-coil and C2 domain containing 2A (MKS6; JBTS9); Cdc42: cell division cycle 42 (TKS; G25K; CDC42Hs); CEP41: centrosomal protein 41 (JBTS15; TSGA14); CEP290: centrosomal protein 290 (CT87; MKS4; POC3; rd16; BBS14; JBTS5; LCA10; NPHP6; SLSN6; 3H11Ag); CSPP1: centrosome and spindle pole associated protein 1 (CSPP; JBTS21); C8ORF37: chromosome 8 open reading frame 37 (RP64; BBS21; CORD16; smalltalk); Exoc5: exocyst complex component 5 (SEC10; HSEC10; SEC10P; PRO1912; SEC10L1); IFT122: intraflagellar transport 122 (CED; SPG; CED1; WDR10; WDR10P; WDR140); IFT81: intraflagellar transport81 (DV1; CDV1; CDV-1; CDV1R; CDV-1R); INPP5e: inositol polyphosphate 5-phosphatase E (CPD4; CORS1; JBTS1; MORMS; PPI5PIV; pharbin); KIAA0556: KIAA0556 (JBTS26); NBCe1: electrogenic Na⁺/nHCO₃⁻ cotransporter (SLC4A4); POC1B: POC1 centriolar protein B (PIX1; CORD20; TUWD12; WDR51B); PDE6D: phosphodiesterase 6D (PDED; JBTS22); RP2: RP2, ARL3 GTPase activating protein provided (XRP2; NME10; TBCCD2; NM23-H10; DELXp11.3); RPGRIP1: retinitis pigmentosa GTPase regulator interacting protein 1; SDCCAG8: serologically defined colon cancer antigen 8 (BBS16; CCCAP; SLSN7; NPHP10; hCCCAP; HSPC085; NY-CO-8; CCCAP SLSN7); TMEM67: transmembrane protein 67 (MKS3; JBTS6; NPHP11; TNEM67; MECKELIN); TTC26: tetratricopeptide repeat domain 26 (DYF13; IFT56; dyf-13).

INTRODUCTION

Cilia are thin rod-like microtubule-based organelles, which are found on most vertebrate cell types. Cilia can be classified as motile or non-motile (more commonly referred to as primary) cilia which arise from a common origin, the centrosome [1]. Motile cilia function mainly as motor organelles and are also found in larger organisms, including humans. For example, motile cilia are present on cells that line the trachea, where their coordinated wave-like motions carry mucus along with the inhaled dust, bacteria, and other small particles towards the mouth to be removed from the body. Primary cilia play a key role in the receptor cells of sensory systems and are responsible for cell communication [2-4]. The outer segment of the rod *photoreceptor* cell in the human eye is connected to its

cell body with a specialized *non-motile cilium*. Mutations in cilia proteins have the potential to adversely affect numerous organs and tissues, and may be multifunctional [5]. Ciliopathies, referring to cilia loss and/or dysfunction in cilia development or function, cause a group of disorders associated with genetic mutations encoding defective proteins, resulting in abnormal formation or function of cilia. Clinical manifestations of ciliopathies can arise in nearly all tissue types during development and throughout life. Sensory impairments include the presence or onset of blindness, neurosensory hearing loss, altered nociception and anosmia. In addition, organ defects such as renal and liver cyst formation, airway distress, and hydrocephaly occur. Ciliopathies, phenotypes associated with cilia dysfunction, are often syndromes, such as Bardet-Biedl syndrome (BBS), Joubert syndrome (JBTS), Meckel-Gruber syndrome (MKS), Senior-Loken syndromes (SLS), Orofaciodigital syndrome (OFD), Leber's congenital amaurosis (LCA), Ellis van Creveld syndrome, Sensenbrenner syndrome, Nephronophthisis (NPHP), Renal dysplasia, and Autosomal Polycystic kidney disease (APKD) affect multiple organs, resulting in central nervous system malformation, cystic kidney disease, polydactyly, situs inversus obesity, encephalocele and retinal dystrophy [6-8]. While disease manifestation in any organ can occur in the context of ciliopathic dysfunction, the predominant organs affected include the kidney, eye, liver and brain. Currently there is a ciliary proteome database that is an integrated community resource for the genetic and functional dissection of cilia [9]. Although ciliopathies are conveniently classified into specific syndromes, their phenotypes are best viewed as a continuum that spans a phenotypic spectrum from embryonic lethality to isolated late onset retinal degeneration [10]. Several studies support this view by demonstrating that individual ciliopathy disease genes are expressed broadly rather than discretely across the spectrum, and that mutations within the same gene can display marked phenotypic differences across and even within families [11,12]. In the ensuing text, we will provide an overview of cilia protein and ciliopathies of the kidney and eye function, highlight an ideal animal model, zebrafish, and, importantly, discuss the future direction of research into ciliopathies".

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Over the past decade zebrafish has proven to be an excellent vertebrate model for genetic analysis and imaging of cilia-related processes. The developing zebrafish larvae are largely transparent, and differentiate cilia

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at early stages of embryogenesis. Thus, immunostaining for ciliary proteins combined with confocal microscopy makes it easy to examine the morphology and movement of cilia during organ development in zebrafish [13-15]. Zebrafish are vertebrates, and zebrafish eyes are well-laminated structures that are functionally very similar to the eyes of other vertebrates, including humans. The eye shape of the zebrafish begins at 11.5 hours post fertilization (hpf), and the eyecup is well formed by 24 hpf. Most of the retina is subdivided into its characteristic subcellular structure by 48 hpf. The internal connecting cilia and basal body of the inner segment are observed at 50 hpf, and the outer segment is visible at 54 hpf. The first visual response can be seen around 70 hpf, and the photoreceptor cells reach an adult size of 576 hpf (24 days) [16].

Primary cilia are found in developing and mature human kidneys, which extend from the apical surface of the epithelial cells lining the nephron tubule and collecting duct. Cilia are present on endothelial cells in the developing zebrafish vasculature [14]. Zebrafish kidney vascularization and glomerular filtration occurred between 40 and 48 hpf [17]. The pronephric ducts are completely formed and patent to the exterior by 24 hours post fertilization (hpf). Cilia have been known for decades to exist, and have recently been recognized as sensory antennas that are involved in physiological functions. Nodal cilia, for example, propagate fluid flow across the embryonic node, and thereby are thought to function in the determination of left-right asymmetry. In mutations, the mis-orientation and shortening of kidney duct cilia suggest that pronephric fluid flow may be affected [15]. As a dynamic organelle, the presence, length, and composition of primary cilia are under constant regulation in order to fulfill essential functions such as signaling transduction.



Figure 1: Common ciliopathy phenotypes in *exoc5*-mutant zebrafish. Lateral view of WT and *exoc5* homozygous mutants zebrafish at 3.5 days post fertilization (dpf). *Exoc5* mutants showed cilia defects, which included; *tail curvature **hydrocephaly; ***smaller eyes and ****pericardial edema.

A notable feature of the zebrafish model is that cilia homozygote mutants usually manifest a curly-body axis, a phenotype that is very easy to detect during genetic screens (Figure 1) [16,18,19]. Recent advances in targeted genomic mutagenesis using TALEN and CRISPR/Cas9 nuclease systems make the zebrafish an attractive model to study reverse genetics. These approaches are valuable as tools to study the genetic bases of cilia function in a living embryo. For multiple ciliopathies, zebrafish mutants are available, including AHI1, ARL13B, ARL6, ARMC9, BBS5, CC2D2A, Cdc42, CEP41, CEP290, CSPP1, C8ORF37, Exoc5, IFT122, IFT81, INPP5e, KIAA0556, NBCe1, POC1B, PDE6D, RPGR1P1, RP2, SDCCAG8, TMEM6, TTC26, which have kidney and retina phenotypes that suggest a common mechanism underlying these defects [18,20-31] (Table 1).

Table 1: Ciliopathy Genes modeled in Zebrafish and showing Eye and Kidney phenotypes.

Cilia Gene modeled in Zebrafish	Eye Phenotype	Kidney Phenotype	Disease	PMID
AHI1 mutant	Shortened cone outer segments Cone degeneration Rhodopsin mislocalization	Kidney cysts	JBTS	28118669
ARL13B mutant	Shortened photoreceptor outer segments retinal defects	Renal cysts	JBTS	27571019 25138100 27153923
ARL6 mutant	Retinopathy microphthalmia	Polydactyl renal malformations	BBS	15314642 15258860
ARMC9 mutant	Retinal dystrophy	Fibrocystic kidney disease	JBTS	28625504
BBS5 Morphant	Morphants displayed retinal layering defects	Dilated cystic pronephric ducts	PKD,BBS NPHP,MKS	24559376 18604564
CC2D2A mutant	Shortened outer segments, Mislocalization of opsins and accumulation of vesicles	Pronephric cyst	JBTS MKS	26485645 18950740
Cdc42 Morphants	Smaller eyes	Cystic kidney	PKD	23766535
CEP41 Morphants	Smaller eyes	Cystic kidney	JBTS	22246503
CEP290 Morphants	Rod-cone dystrophy	Renal abnormalities	JBTS, LCA	26301811
CSPP1 Morphants	Smaller eyes	Pronephric cysts	JBTS	24360808
C8ORF37 morphants	Retinal degeneration	Renal cysts	JBTS	27008867
Exoc5 Mutants and Morphants	Smaller eyes Retinal lamination was lost Disorganization and lack of photoreceptor outer segments	Glomerular expansion left-right patterning defects	PKD	28729419 21490950

IFT122 mutation	Photoreceptor degeneration	Cystic kidney	RP	27681595
IFT81 mutation	Retinal dystrophy	Kidney cyst	Nonsyndromic retinal dystrophies	28460050
INPP5e Morphants	Smaller eyes	Cystic kidney	JBTS	27401686
KIAA0556 Morphants	Oculomotor apraxia nystagmus photoreceptor outer segments	Dysmorphic Kidney cysts	JBTS	27245168
NBCe1 mutation	Smaller eyes retinal distention	Pronephric ducts defect	renal tubular acidosis, glaucoma and cataracts	19625604
POC1B Mutation	Smaller eyes Retinal degeneration photoreceptor connecting cilia	Reduce Cystic kidney	JBTS, PKD, LCA	25044745
PDE6D Morphants	Disorganized retinal cell layers	Cloacal cysts distended pronephric tubules polydactyly and kidney hypoplasia	JBTS	24166846
RP2 Morphants	Affected the shedding of membrane discs from the distal end of the photoreceptor outer segment	Pronephric cysts	renal-retinal ciliopathies	20729296
RPGRIP1 Morphants	Smaller and underdeveloped eyes	Pronephric cyst formation	NPHP, RP	20200501
SDCCAG8 Morphants	Smaller eyes	Pronephric cysts	NPHP	20835237
TMEM67 mutation	Smaller eyes	Bilateral pronephric cysts	MKS	23393159
TTC26 Morphants	Eye morphology altered; outer segments of photoreceptor cells appeared shortened or absent	Tubule dilation; distended/dilated pronephric tubes and ducts	renal-retinal ciliopathies	22718903

DISCUSSION & FUTURE DIRECTIONS

Although many mechanistic aspects of ciliogenesis are now better understood, numerous questions revolving round the pathogenesis have yet to be answered. Animal models, including zebrafish in particular, will be indispensable in this regard. Cilia are well characterized in a number of organs, but the understanding of what they do varies greatly depending on the context. Photoreceptor cilia are among the best understood in terms of function and structure. In contrast to the eye, very little is known about the role of cilia in the brain, heart or the bone. The understanding of cilia function in these organs will benefit from live imaging of intact animals at developmental stages. Such imaging experiments are the strength of the zebrafish model. The zebrafish has proven to be an excellent model to study many aspects of cilia function. The ease of generating zebrafish mutants in ciliary genes using forward and reverse genetic approaches has led to a number of important findings [32-35]. Advances in imaging, such as light sheet microscopy and the use of ever more sophisticated combinations of mutant genotypes and transgenic tools to monitor cell behavior in live animals have created a fertile ground for the zebrafish model to continue generating insights into the mechanisms of ciliogenesis.

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