Zebrafish as models to study ciliopathies of the eye and kidney

Yi Shi1,2, Yanhui Su1, Joshua H. Lipschutz1 and Glenn P. Lobo1,3*

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

**ABSTRACT**

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 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), Orofaciocutaneous 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, ephelalgia 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”.

**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), Orofaciocutaneous 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, ephelalgia 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.

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

<table>
<thead>
<tr>
<th>Cilia Gene modeled in Zebrafish</th>
<th>Eye Phenotype</th>
<th>Kidney Phenotype</th>
<th>Disease</th>
<th>PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH1 mutant</td>
<td>Shortened cone outer segments Cone degeneration Rhodopsin mislocalization</td>
<td>Kidney cysts</td>
<td>JBTS</td>
<td>28118669</td>
</tr>
<tr>
<td>ARL13B mutant</td>
<td>Shortened photoreceptor outer segments retinal defects</td>
<td>Renal cysts</td>
<td>JBTS</td>
<td>25135023</td>
</tr>
<tr>
<td>ARL6 mutant</td>
<td>Retinopathy microphthalmia</td>
<td>Polydactylyrenal malformations</td>
<td>BBS</td>
<td>15314642</td>
</tr>
<tr>
<td>ARM69 mutant</td>
<td>Retinal dystrophy</td>
<td>Fibrocystic kidney disease</td>
<td>JBTS</td>
<td>28625504</td>
</tr>
<tr>
<td>BBS5 Morphants</td>
<td>Morphants displayed retinal layering defects</td>
<td>Dilated cystic pronephric ducts</td>
<td>PKD,BBS,NPHP,MKS</td>
<td>24559376</td>
</tr>
<tr>
<td>CC2D2A mutant</td>
<td>Shortened outer segments, Mislocalization of opsins and accumulation of vesicles</td>
<td>Pronephric cyst</td>
<td>JBTS</td>
<td>26455645</td>
</tr>
<tr>
<td>Cdo42 Morphants</td>
<td>Smaller eyes</td>
<td>Cystic kidney</td>
<td>PKD</td>
<td>23766535</td>
</tr>
<tr>
<td>CEP41 Morphants</td>
<td>Smaller eyes</td>
<td>Cystic kidney</td>
<td>JBTS</td>
<td>22246503</td>
</tr>
<tr>
<td>CEP290 Morphants</td>
<td>Rod-cone dystrophy</td>
<td>Renal abnormalities</td>
<td>JBTS,LCA</td>
<td>26301611</td>
</tr>
<tr>
<td>CSPP1 Morphants</td>
<td>Smaller eyes</td>
<td>Pronephric cysts</td>
<td>JBTS</td>
<td>24360808</td>
</tr>
<tr>
<td>C8ORF37 morphants</td>
<td>Retinal degeneration</td>
<td>Renal cysts</td>
<td>JBTS</td>
<td>27008867</td>
</tr>
<tr>
<td>Exoc5 Mutants and Morphants</td>
<td>Smaller eyes Retinal lamination was lost Disorganization and lack of photoreceptor outer segments</td>
<td>Glomerular expansion left-right patterning defects</td>
<td>PKD</td>
<td>28729419</td>
</tr>
</tbody>
</table>

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 AH11, ARL13B, ARL6, ARM69, 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).
DISCUSSION & FUTURE DIRECTIONS

Although many mechanistic aspects of ciliogenesis are now better understood, numerous questions revolving around 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 to continue generating insights into the mechanisms of ciliogenesis.

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