

Non-syndromic retinal ciliopathies: translating gene discovery into therapy

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Homozygosity mapping and exome sequencing have accelerated the discovery of gene mutations and modifier alleles implicated in inherited retinal degeneration in humans. To date, 158 genes have been found to be mutated in individuals with retinal dystrophies. Approximately one-third of the gene defects underlying retinal degeneration affect the structure and/or function of the ‘connecting cilium’ in photoreceptors. This structure corresponds to the transition zone of a prototypic cilium, a region with increasing relevance for ciliary homeostasis. The connecting cilium connects the inner and outer segments of the photoreceptor, mediating bi-directional transport of phototransducing proteins required for vision. In fact, the outer segment, connecting cilium and associated basal body, forms a highly specialized sensory cilium, fully dedicated to photoreception and subsequent signal transduction to the brain. At least 21 genes that encode ciliary proteins are implicated in non-syndromic retinal dystrophies such as cone dystrophy, cone–rod dystrophy, Leber congenital amaurosis (LCA), macular degeneration or retinitis pigmentosa (RP). The generation and characterization of vertebrate retinal ciliopathy animal models have revealed insights into the molecular disease mechanism which are indispensable for the development and evaluation of therapeutic strategies. Gene augmentation therapy has proven to be safe and successful in restoring long-term sight in mice, dogs and humans suffering from LCA or RP. Here, we present a comprehensive overview of the genes, mutations and modifier alleles involved in non-syndromic retinal ciliopathies, review the progress in dissecting the associated retinal disease mechanisms and evaluate gene augmentation approaches to antagonize retinal degeneration in these ciliopathies.

INTRODUCTION

Up to just a few years ago, identifying the genetic basis of an inherited human disease was a long journey, requiring many years of work in the ascertainment of families for linkage analysis, followed by fine mapping of the locus and, finally, sequencing of candidate genes one by one until the likely causative gene was identified. Until now, at least 158 genes (RetNet; <http://www.sph.uth.tmc.edu/retnet/>) have been associated with several types of inherited retinal dystrophies, and the arrival of new genetic technologies such as single-nucleotide polymorphism (SNP) microarrays and next-generation sequencing (NGS) have increased the pace of disease gene identification. For example, during the past

year (2011–2012), the use of SNP arrays and/or NGS have enabled the identification of 10 new retinal dystrophy genes. Remarkably, four of these genes [*C8orf37* (1), *IFT140* (2), *MAK* (3,4) and *TMEM237* (5)] encode ciliary proteins, which underlines the importance of disrupted ciliary processes in the pathogenesis of retinal dystrophies.

The primary cilium functions as the antenna of the cell, allowing the effective transduction of various forms of sensory information from the extracellular environment. The cilium is present on many different types of cells throughout the human body, and the importance of this organelle in biology and in medicine has been highlighted by the growing number of genetic diseases that have been linked to primary cilium defects. The connecting cilium at the base of

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the photoreceptor outer segment corresponds to the transition zone of a prototypic cilium, a region with increasing relevance for ciliary homeostasis. The connecting cilium connects the inner and outer segments of the photoreceptor cell, mediating bi-directional transport of phototransducing proteins required for vision. In fact, the outer segment, connecting cilium and associated basal body, forms a highly specialized sensory cilium, fully dedicated to photoreception and subsequent signal transduction to the brain. Ciliary databases, such as CiliaProteome (6) or Cildb (7), have integrated bioinformatic, genomic and proteomic ciliary data from numerous high-throughput studies. Of the 158 identified genes implicated in retinal dystrophies, at least 53 (33%) encode proteins that localize to the cilium and/or basal body of the cell.

Mutations in these ciliary genes can lead to a wide range of clinical features involving the eye, kidney, heart, liver, central nervous system, adipose tissue, gonads and bones. Various syndromes have been defined based on the combination of clinical features involving these organs (8,9). Syndromic ciliopathies include Alström syndrome (ALMS, MIM 203800), Bardet–Biedl syndrome (BBS, MIM 209900), Ellis–van Creveld syndrome (EVC, MIM 225500), asphyxiating thoracic dystrophy (ATD, MIM 208500), Joubert syndrome (JBTS, MIM 213300), McKusick–Kaufman syndrome (MKKS, MIM 236700), Meckel–Gruber syndrome (MKS, MIM 249000), orofacioidigital syndrome type 1 (OFD1, MIM 311200), Senior–Løken syndrome (SLSN, MIM 266900) and Usher syndrome type 2A (USH2A, MIM 276901). These syndromes are genetically heterogeneous, involving mutations in a large number of genes. They can show considerable clinical and genetic overlap, as mutations in a number of these genes can cause various syndromes.

Retinal dystrophy can present as one of the clinical features of these syndromes, but is more often present as an isolated disease without additional features. One-third of these non-syndromic retinal dystrophies involve a defect in a ciliary protein. This group of retinal dystrophies, the non-syndromic retinal ciliopathies, is the scope of this review.

NON-SYNDROMIC RETINAL CILIOPATHIES

Non-syndromic retinal ciliopathies can have various clinical presentations, depending on the type of photoreceptors that are primarily affected by the disease. Retinitis pigmentosa (RP, MIM 268000) is the most common inherited retinal degeneration with an estimated worldwide prevalence of 1:4,000 individuals (10). RP is initially characterized by rod photoreceptor dysfunction, giving rise to night blindness, followed by progressive midperipheral vision loss, tunnel vision, and at advanced stages when cones also are affected, eventually can progress to blindness. The disease is genetically heterogeneous and displays all Mendelian patterns of inheritance. In addition, some cases have been linked to mitochondrial mutations or displayed digenic inheritance (11,12).

Cone dystrophy (CD) is a progressive cone disorder with an estimated prevalence of 1:30,000 to 1:40,000 (13). Patients have normal cone function initially, but show visual loss and color vision disturbance in the first or second decade (14). Macular abnormalities can be present, and the optic nerve

may show a variable degree of temporal pallor. On electroretinography (ERG), cone responses progressively deteriorate and rod responses are initially normal, but can diminish slightly over time. The visual acuity generally diminishes to legal blindness before the third or fourth decade of life.

Cone–rod dystrophy (CRD, MIM 120970) has an estimated prevalence of 1:30,000 to 1:40,000 (13), and also displays all types of Mendelian inheritance. CRD is characterized by a primary loss of cone photoreceptors and subsequently followed by, or simultaneous with, the loss of rod photoreceptors (14). The disease in most cases becomes apparent during primary school years. The symptoms include photo aversion, decreased visual acuity with or without nystagmus (pendular or roving eye movements), color vision defects and decreased sensitivity of the central visual field. Since rods are also involved, night blindness and peripheral vision loss may occur. The diagnosis of CRD is mainly based on ERG recordings, in which cone responses are more severely reduced than, or equally reduced as, rod responses (15).

Macular dystrophy (MD) affects the central region of the retina called the macula, leading to loss of color and sharp vision. Inherited forms of the disease can be transmitted through autosomal dominant and autosomal recessive mechanisms. Various forms of MD are distinguished based on particular clinical features, such as a dark choroid typical of Stargardt disease and a yellow ‘egg-yolk’ appearance of the macula in Best disease, although the retinal appearance is essentially normal in occult MD.

Leber congenital amaurosis (LCA, MIM 204000) is the most severe form of early-onset retinal blindness and typically becomes evident in the first year of life. The frequency varies between 1:30,000 (16) and 1:81,000 (17). Poor visual function is accompanied by nystagmus, photophobia, absent pupillary responses, hyperopia, extinguished or severely reduced rod and cone signals on ERG, and a highly variable retinal appearance. LCA is generally inherited in an autosomal recessive manner.

GENETIC HETEROGENEITY

Although CD, CRD, LCA, MD and RP are described as distinct clinical entities, they are not always easily distinguished (18) (Fig. 1) and can have overlapping genetic causes (12). To date, 74 genes have been associated with these diseases and it is expected that more will follow soon, since ~50% of autosomal recessive (ar) RP, 90% of arCD, 60% of arCRD and 30% of arLCA cases still need to be solved (18). Of these 74 genes, 21 (~28%) encode ciliary proteins (Table 1). As studies involving comprehensive screening of all these genes in large cohorts are scarce, it is difficult to calculate the prevalence of mutations in these 21 genes in retinal dystrophies. Several gene mutations have so far only been described in one or a few families. If we assign a frequency of 1% to such cases, we estimate that mutations in 16 ciliary genes (*ARL6*, *BBS1*, *BBS9*, *C2orf71*, *C8orf37*, *CLRN1*, *FAM161A*, *MAK*, *OFD1*, *RP1*, *RP2*, *RPGR*, *TOPORS*, *TTC8*, *TULP1*, *USH2A*) represent at least 36% of the genetic causes in RP.

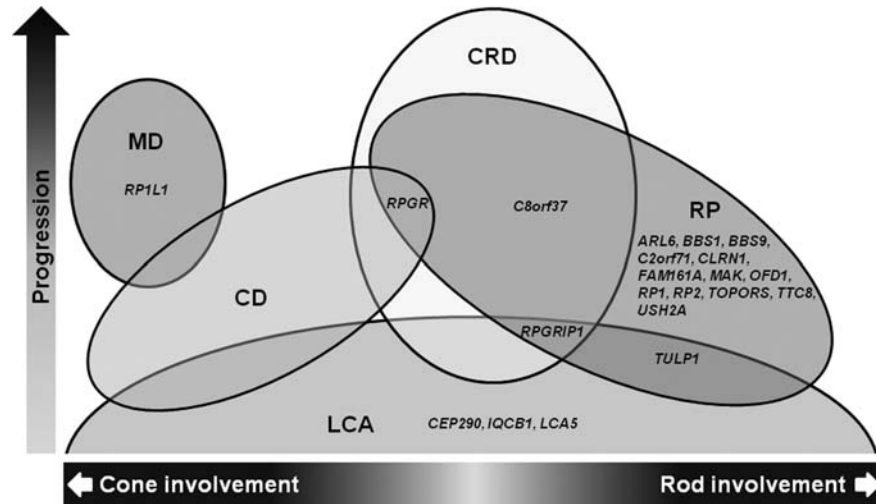


Figure 1. Schematic representation of phenotypic and genetic overlap among non-syndromic retinal ciliopathies. The involvement of cone and rod photoreceptors and progression of diseases are shown in order to match the genetic overlap. At end stage, CD can hardly be distinguished from CRD. Patients with RP initially display tunnel vision due to rod defects that very often progresses to complete blindness when the cones are also affected. In patients with LCA, the defects can occur in both types of photoreceptors, or in RPE cells, and therefore clinical and molecular genetic overlap with CD, CRD or RP can be expected. At early stage, MD can show an overlap with CD. *RPGR* is also associated with MD. CD, cone dystrophy; CRD, cone-rod dystrophy; LCA, Leber congenital amaurosis; MD, macular dystrophy; RP, retinitis pigmentosa [modified from den Hollander *et al.* (18)].

Among the most frequent causes of RP are mutations in the *USH2A* gene, and in particular the p.Cys759Phe mutation (19).

Mutations in five ciliary genes (*CEP290*, *IQCB1*, *LCA5*, *RPGRIP1*, *TULP1*) explain 23% of all solved LCA cases. An intronic mutation (c.2991+1655A→G) in *CEP290*, which creates a strong splice-donor site and inserts a cryptic exon into the mRNA, is by far the most frequent cause of LCA (20).

In CD and CRD, only 4% of the cases are due to mutations in three ciliary genes (*C8orf37*, *RPGR*, *RPGRIP1*), which is unexpected considering the high percentage of mutations in ciliary genes identified in LCA and RP. Possibly, the number of ciliary genes is underrepresented due to the high percentage of unsolved cases in CD and CRD (90 and 60%, respectively) (21–25). Mutations in the ciliary *RP1L1* gene have been described in Japanese patients with occult MD (26–28); other ciliary genes have so far not been associated with MD.

INHERITANCE PATTERNS

In genes associated with non-syndromic retinal ciliopathies, the most predominant patterns of inheritance are autosomal recessive and X-linked. Only two genes, *RP1* and *TOPORS*, have been associated with autosomal dominant RP. It is remarkable that just two genes of a total of 21 adRP genes encode ciliary proteins (29). An explanation for the small number of autosomal dominant non-syndromic retinal ciliopathies could lie in the type of mutations and gene function. Many non-syndromic retinal ciliopathy genes encode proteins that are part of a multiprotein complex, such as the BBSome (BBS1, BBS9, TTC8) (30) or the IQCB1-CEP290 complex (31). Haploinsufficiency due to the loss of one allele could potentially be compensated by another member of the protein complex. *RPGRIP1* forms stable polymers in the connecting

cilium through the coiled-coil domain (32). Eight additional non-syndromic retinal ciliopathy proteins are predicted by SMART (<http://smart.embl-heidelberg.de/>) to have coiled-coil domains which, when mutated, could affect cell fate through a dominant-negative disease mechanism. Therefore, it is possible that dominant-negative mutations in ciliary proteins remain to be identified in non-syndromic retinal dystrophies. Mutations in *RP1* have been suggested to act in a dominant-negative fashion rather than a gain-of-function, as heterozygous mutant *Rp1* mice did not show any phenotype, opposed to RP patients carrying the mutation p.Arg677* in heterozygous state (33,34). On the other hand, *TOPORS* is inherited as an autosomal dominant trait and likely to be due to haploinsufficiency (35).

The occurrence of *de novo* mutations could be relatively frequent, as NGS analysis of retinal dystrophy genes identified *de novo* mutations in four (~14%) of 28 isolated cases in which causative variants were found (36). *De novo* mutations have recently been shown to play a major role in human diseases with reduced reproductive fitness (37–39), suggesting that they may also occur frequently in individuals affected by retinal ciliopathies, particular in syndromic forms involving intellectual disorders.

GENOTYPE-PHENOTYPE CORRELATION

For the majority of mutations, a clear-cut correlation has not been established between genotypes and phenotypes. This holds true for mutations in genes that exclusively cause non-syndromic retinal ciliopathies, in which the clinical presentation can partially overlap (Fig. 1, Table 1). However, among genes that cause both syndromic and non-syndromic retinal ciliopathies (*ARL6*, *BBS1*, *BBS9*, *CLRN1*, *CEP290*, *IQCB1*, *OFD1*, *TTC8*, *USH2A*), there are some interesting examples of genotype-phenotype correlations. In *TTC8*, a splice site

Table 1. Non-syndromic retinal ciliopathy genes

Entrez gene symbol	Entrez gene identifier	Localization of the encoded protein in photoreceptors	Pattern of inheritance	Non-syndromic disease association	Syndromic disease association	References
<i>ARL6</i>	84100	Photoreceptor and ganglion cell layer, nerve fiber layer	AR	RP	BBS	(98–100)
<i>BBS1</i>	582	BB, CC, synapse of the plexiform layer	AR	RP	BBS	(53,101,102) ^a
<i>BBS9</i>	27241	BB, CC, synapse of the plexiform layer	AR	RP	BBS	(41,103) ^a
<i>C2orf71</i>	388939	CC (putative)	AR	RP		(82,83)
<i>C8orf37</i>	157657	BB, ciliary rootlet	AR	CRD, RP		(1)
<i>CEP290</i>	80184	CC	AR	LCA	BBS, JBTS, MKS, SLSN	(20,104–107)
<i>CLRN1</i>	7401	CC, IS, ribbon synapses	AR	RP	USH3	(42,108–110)
<i>FAM161A</i>	84140	Apical part of CC, BB, IS, OPL	AR	RP		(111,112) ^b
<i>IQCB1</i>	9657	CC, OS	AR	LCA	SLSN	(67,113,114)
<i>LCA5</i>	167691	BB, CC	AR	LCA		(71)
<i>MAK</i>	4117	CC, OS axoneme	AR	RP		(3,4,115)
<i>OFD1</i>	8481	CC, IS (putative)	XL	RP	JBTS, OFD, SGBS2	(43,97,116,117)
<i>RP1</i>	6101	CC, OS axoneme	AD, AR	RP		(68,118–121)
<i>RP1L1</i>	94137	CC, OS axoneme	AD with reduced penetrance	MD		(26–28,68)
<i>RP2</i>	6102	BB, Golgi, periciliary region, PM	XL	RP		(80,122,123)
<i>RPGR</i>	6103	BB, CC	XL	CD, CRD, MD, RP, RP and sinorespiratory infections, with or without deafness		(74,78,124–133)
<i>RPGRIP1</i>	57096	CC	AR	CRD, LCA, RP		(24,32,134–136)
<i>TOPORS</i>	10210	BB, nuclei of ganglion cells, periciliary region	AD	RP		(35,72)
<i>TTC8</i>	123016	BB, CC, synapse of the plexiform layer	AR	RP	BBS	(40,137) ^a
<i>TULP1</i>	7287	CC, IS, OLM	AR	LCA, RP		(138–142)
<i>USH2A</i>	7399	CC, IS, OLM, OPL	AR	RP	USH2	(19,143–145)

For all non-syndromic retinal ciliopathy genes, the gene identifier, localization of the encoded protein in the photoreceptor, the type of inheritance, association with non-syndromic and syndromic disease and references are shown.

AD, autosomal dominant; AR, autosomal recessive; BB, basal body; BBS, Bardet–Biedl syndrome; CC, connecting cilium; CD, cone dystrophy; CRD, cone–rod dystrophy; IS, inner segment; JBTS, Joubert syndrome; LCA, Leber congenital amaurosis; MD, macular dystrophy; MKS, Meckel–Gruber syndrome; OFD, orofaciocaudal syndrome; OLM, outer limiting membrane; OMD, occult macular dystrophy; OPL, outer plexiform layer; OS, outer segment; PM, plasma membrane; RP, retinitis pigmentosa; SGBS2, Simpson–Golabi–Behmel syndrome type 2; SLSN, Senior–Løken syndrome; USH2, Usher syndrome type 2; USH3, Usher syndrome type 3; XL, X-linked.

^aLocalization of BBS1, BBS9 and TTC8 in the photoreceptor reported by U. Wolfrum (personal communication).

^bFAM161A localization at the BB and CC reported by S.A. Di Gioia (personal communication).

mutation, leading to the in-frame skipping of a 30 bp retina-specific exon, was identified in a family with non-syndromic RP, whereas most BBS mutations are predicted to significantly impact mRNA stability and/or protein function (40). Interestingly, a deletion in *BBS9* (p.Glu148_Val234del) has been described to cause non-syndromic RP in two affected members of the family, whereas the third affected member showed all the primary features of BBS (41), suggesting that additional factors may contribute to the disease in the latter individual. Two missense mutations in *CLRN1* identified in RP patients are predicted to have a less severe effect on protein function than mutations identified in patients with Usher syndrome (42). A deep intronic mutation in the *OFD1* gene was identified in a large family with RP, which inserts a cryptic exon into the mRNA, but reduced levels of normally spliced gene product remain (43). This disease mechanism of reduced expression of syndromic ciliopathy genes causing isolated retinal dystrophy is reminiscent of an intronic mutation in

CEP290 that causes LCA (20), suggesting that reduced dosage of correctly spliced ciliopathy genes may be a common disease mechanism in retinal degeneration.

A vast number of mutations have been identified in *CEP290*, which can lead to a wide phenotypic spectrum ranging from non-syndromic LCA to MKS. The intronic mutation c.2991+1655A>G, leading to the insertion of a cryptic exon into the mRNA and introduction of a premature stop codon, is the most prevalent mutation in LCA accounting for 15–20% of the cases (22). Since a fraction of wild-type *CEP290* mRNA remains in patients carrying the intronic mutation, it is hypothesized that this hypomorphic character of the mutation could explain why this mutation leads to LCA, instead of a multiorgan disease (20). However, a number of non-syndromic LCA patients have been described to carry nonsense and frameshift mutations on both alleles (44,45), and the question remains why they have not developed syndromic features. One possible molecular mechanism was

demonstrated for the nonsense mutation p.Arg151*, identified in patients with non-syndromic early-onset retinal dystrophy. RNA analysis demonstrated that the exon containing this mutation was differentially spliced, leading to splice products lacking exon 7 or exons 7 and 8, both remaining in-frame (46). It was suggested that the different splice products are the result of nonsense-associated altered splicing, a putative correction mechanism that recognizes an exon with a premature termination codon and excludes that exon from the mature mRNA (47).

GENETIC CILIARY MODIFIERS FOR RETINAL DEGENERATION

It is presumed that the phenotypic variability of *CEP290*-associated disease, and also of other retinal ciliopathies, might be caused by a modifier allele in a second gene. Two missense mutations in *AHII*, p.Asn811Lys and p.His758Pro, have been described as neurological modifiers of *CEP290*-associated disease (45). A third mutation in *AHII*, p.Arg830Trp, was identified as a modifier allele for retinal degeneration in patients with nephronophthisis (NPHP), independent of a primary *NPHP1* mutation (48). A common allele in the *RPGRIP1L* gene, p.Ala229Thr, may be a modifier of retinal degeneration in ciliopathy patients due to other mutations (49). Heterozygosity for a truncating *PDZD7* allele, p.Arg56Profs*24, aggravates retinal disease in a family with Usher syndrome due to *USH2A* variants (50). Polymorphic alleles in *IQCB1* (p.Ile393Asn) and *RPGRIP1L* (p.Arg744Gln) have been associated with severe disease in X-linked RP caused by *RPGR* mutations (51). Intriguingly, a recent study demonstrated that knockout of *Mkks* in *Cep290-Rd16* mice improved ciliogenesis and sensory functions of the eye and the inner ear (52).

An alternative explanation for the variable phenotypes associated with retinal ciliopathy alleles could be *cis*-acting regulators of expression. Different enhancer or promoter variants may result in higher or lower mRNA expression levels of mutated alleles in affected individuals, which may be correlated with the severity of the phenotype as was suggested for the *BBS1* p.Met390Arg allele (53).

TRANSCRIPTOMICS

The majority of the genes that cause retinal dystrophies have been shown to be specifically expressed, or highly enriched, in photoreceptors (54). A range of transcription factors have been shown to contribute to photoreceptor gene regulation, with crucial roles attributed to CRX, NRL and NR2E3 (55–57). CRX is a key photoreceptor regulator required for the expression of many rod and cone genes, including ~50% of genes known to be mutated in retinal dystrophies (RetNet) (54,58–60). CRX-binding sites were identified in the promoter of 13 (~61%) of 21 genes that cause non-syndromic retinal ciliopathies (61). Considering that many genes for CD, CRD, LCA, MD and RP have not been identified yet, ciliary genes that have CRX-binding sites are excellent candidate genes for the unsolved cases. Notably, prioritizing candidate genes in exome data based on these

CRX-binding sites was recently used successfully to identify *MAK* as a novel RP-associated gene (4).

PROTEOMICS

Non-syndromic retinal ciliopathy proteins are located at the ciliary base (basal body and associated centriole), ciliary axoneme or membrane of the connecting cilium of photoreceptors (Fig. 2). At these specific sites of action, this class of proteins and associated complexes is essential for photoreceptor function by guiding the transport of newly formed proteins which reside in vesicles from the Golgi apparatus toward the base of the connecting cilium (62), transport of phototransduction proteins such as rhodopsin through the connecting cilium toward the outer segment and the transport of turnover products back toward the inner segment of the photoreceptor cell (63,64). Given the specific site of expression of ciliopathy proteins, quite a few protein–protein interaction studies have been carried out to examine the connectivity and mechanism of action on a molecular level. This approach has proven to be successful and has led to the identification of multiple protein complexes, of which the individual molecules functionally overlap and, when mutated, result in similar phenotypes in humans. Examples are the BBSome complex, which is comprised of BBS proteins [BBS1, 2, 4, 5, 7, 9 and TTC8 (30)], in which mutations have been identified in BBS (65) and non-syndromic forms of RP [BBS1 (53), BBS9 (41), TTC8 (40)], and the NPHP–JBTS–MKS complex (31), which is composed of proteins involved in NPHP, JBTS, MKS (66) and non-syndromic forms of LCA [*CEP290* (20), *IQCB1* (67)]. In addition, protein interaction studies have uncovered the biochemical fundament for known genetic interactions between non-syndromic retinal ciliopathy genes [e.g. *RP1-RP1L1* (68) or *IQCB1-CEP290* (69)], and have proven to be a powerful tool in prioritizing candidate disease genes for syndromic ciliopathies [e.g. *ATXN10* and *TCTN2* (31)] and non-syndromic retinal ciliopathies [e.g. *RPGRIP1* (70)].

Importantly, a comparison between the interactome of full-length and truncated LCA-associated lebercilin has led to the identification of the molecular disease mechanism for LCA type 5 (64,71). A homozygous mutation in the last exon of *LCA5*, predicted to escape nonsense-mediated decay, results in a truncated protein that displays loss of interaction with the intraflagellar transport (IFT) machinery. This IFT complex bi-directionally transports ciliary proteins in all types of cilia, including connecting cilia of the photoreceptor, suggesting that defective transport through the connecting cilium is the underlying cause for LCA. Studies have shown that transport defects could be an underlying cause for RP as well, since RP-associated BBS1, RPGR and TOPORS have been found to interact with IFT and/or the dynein complex (72–74), which is required for the dynein-dependent retrograde transport (75).

ANIMAL STUDIES

Most of the current knowledge of the biological roles for genes associated with non-syndromic retinal ciliopathies has come

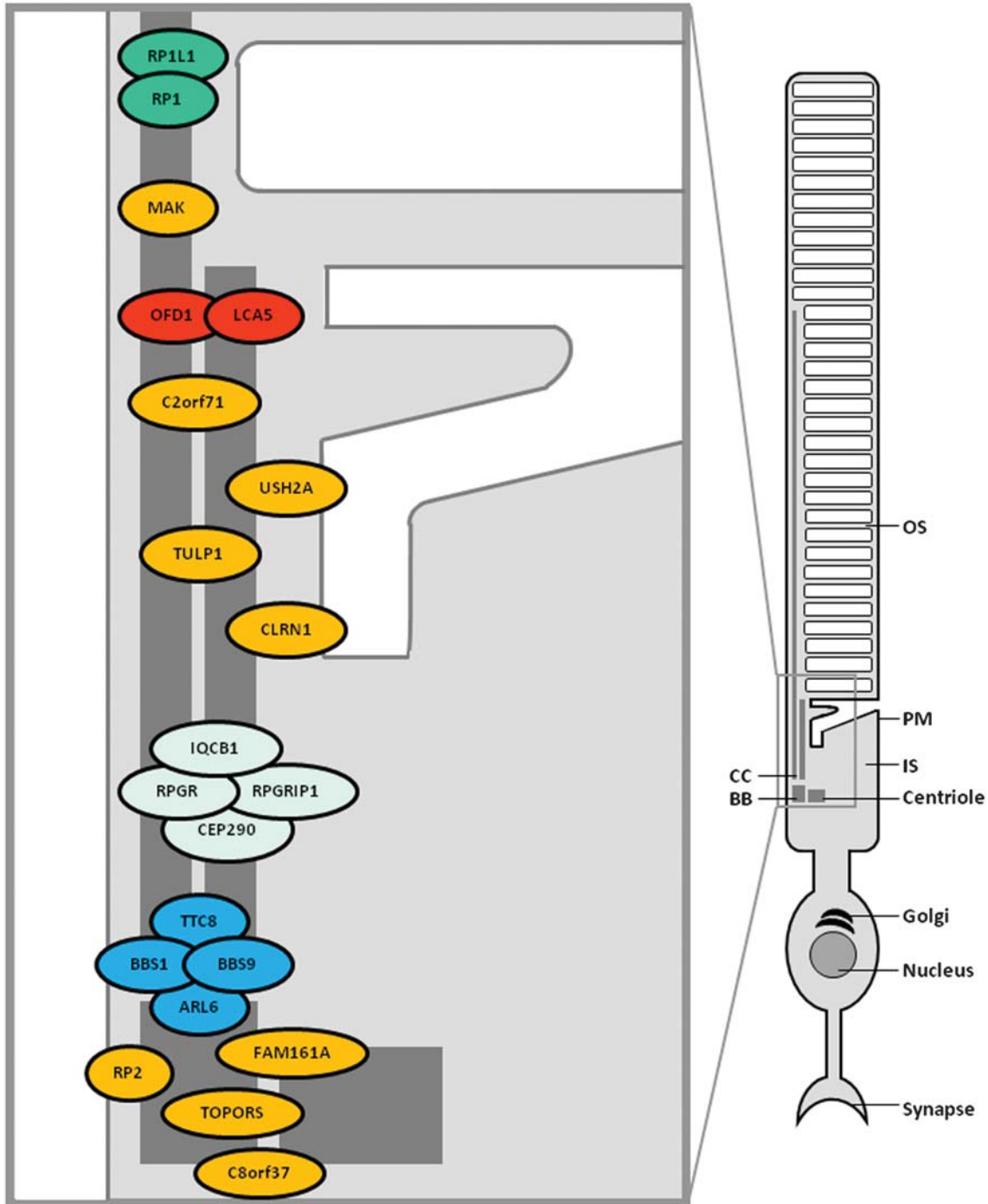


Figure 2. Model of the localization of non-syndromic retinal ciliopathy proteins in the ciliary compartment of the photoreceptor. For each protein, the corresponding gene name has been used. At least four protein complexes have been reported in literature which localize to the connecting cilium (Table 1). The BBSome consisting of BBS1, BBS9 and TTC8, and recruited by ARL6, forms a coat at the base of the CC (63). A complex consisting of nephronophthisis-associated proteins CEP290/IQCB1 connected to RPGR by RPGRIP1 (96) localizes to CC, like OFD1 bound to lebercilin (97). In the OS axonemes, localization of RP1L1 and RP1 is observed (68). BB, basal body; CC, connecting cilium; IS, inner segment; OS, outer segment; PM, plasma membrane.

from studies using vertebrate animal models, which have proven to be powerful tools to study the effect of specific gene inactivation on vertebrate photoreceptor function. In addition, these models have created excellent opportunities for therapeutic studies. Genetically modified mouse models have

been reported for the majority of known non-syndromic retinal ciliopathy genes (Table 2). Common retinal features in these mice are reduced ERG responses to light stimulation, opsin mislocalization in photoreceptors and progressive photoreceptor degeneration. The onset and progression of

Table 2. Overview of published vertebrate animal models for non-syndromic retinal ciliopathy genes, including morpholino studies in zebrafish

Gene	Vertebrate model	Gene targeting	Reference	Eye phenotype
<i>ARL6</i>	Mouse	Deletion of exon 8 (specific for long isoform)	(100)	Late onset, slowly progressive retinal degeneration
<i>ARL6</i>	Mouse	Deletion of exons 6 and 7	(146)	Rapidly progressive retinal degeneration
<i>ARL6</i>	Zebrafish	Morpholino (translation and splice blocker)	(147)	No eye phenotype recorded
<i>ARL6</i>	Zebrafish	Morpholino (splice blocker for long isoform and translation blocker)	(100)	Impaired visual function and opsin mislocalization for splice blocker; translation blocker: no eye phenotype recorded
<i>BBS1</i>	Mouse	Gene trap in intron 11	(148)	Retinal degeneration
<i>BBS1</i>	Mouse	Targeted knock-in M390R allele	(149)	Rapidly progressive photoreceptor degeneration
<i>BBS1</i>	Zebrafish	Morpholino (translation and splice blocker)	(147,150)	No eye phenotype recorded
<i>BBS9</i>	Zebrafish	Morpholino (splice blocker)	(151)	Defective eye development, smaller eyes
<i>C2orf71</i>	Dog	Spontaneous frameshift mutation c.3149_3150insC	(152)	Late-onset, slowly progressive retinal atrophy
<i>C2orf71</i>	Zebrafish	Morpholino (translation blocker)	(83)	Smaller eyes, shorter outer segments
<i>CEP290</i>	Cat (rdAc)	IVS50 + 9T>G (creates splice donor site)	(153)	Photoreceptor degeneration
<i>CEP290</i>	Mouse (Rd16)	Spontaneous in frame deletion of amino acids 1599–1897	(107)	Early-onset, rapidly progressive photoreceptor degeneration
<i>CEP290</i>	Mouse	<i>Rd16</i> crossed with <i>Nrl</i> null (all-cone retina)	(76)	Loss of photoreceptor function
<i>CEP290</i>	Zebrafish	Morpholino (translation and splice blocker)	(69,90,154)	Smaller eyes, altered visual function
<i>CLRN1</i>	Mouse	Deletion of exon 1	(79,155)	No eye phenotype recorded
<i>IQCB1</i>	Zebrafish	Morpholino (splice blocker)	(69)	Defective eye development
<i>LCA5</i>	Mouse	Gene trap in intron 3	(64)	Early onset, rapidly progressive photoreceptor degeneration
<i>MAK</i>	Mouse	Deletion of exons 5 to 8	(115,156)	Normal photoreceptor development, rapidly progressive photoreceptor degeneration
<i>OFD1</i>	Mouse	Conditional knock-out; exons 4 and 5 flanked by loxP sites	(157)	No eye phenotype recorded
<i>OFD1</i>	Zebrafish	Morpholino (translation and splice blocker)	(158)	Incomplete fusion of the choroid fissure of the eye (coloboma)
<i>RP1</i>	Mouse	Deletion of exons 2 and 3	(159)	Early onset, rapidly progressive photoreceptor degeneration
<i>RP1</i>	Mouse	Truncation after codon 662 in exon 4	(33)	Early onset, rapidly progressive photoreceptor degeneration
<i>RP1</i>	Mouse	Point mutation resulting in R522X	(160)	Early onset, rapidly progressive photoreceptor degeneration
<i>RP1L1</i>	Mouse	(tvrn64)		
<i>RP1L1</i>	Mouse	Deletion of exons 2–3 and part of exon 4	(68)	Normal photoreceptor development, slowly progressive photoreceptor degeneration
<i>RP2</i>	Zebrafish	Morpholino (translation and splice blocker)	(81,161)	Defective lamination, lacking outer segments, apoptotic retinal neurons, smaller eyes
<i>RPGR</i>	Dog	Spontaneous 5 bp deletion (del1028-1032) in exon ORF15	(162)	Normal photoreceptor development, slowly progressive photoreceptor degeneration
<i>RPGR</i>	Dog	Spontaneous 2 bp deletion (del1084-1085) in exon ORF15	(162)	Early onset, rapidly progressive photoreceptor degeneration, early inner retina remodeling
<i>RPGR</i>	Mouse	In-frame deletion of exons 4–5–6	(78)	Normal photoreceptor development, slowly progressive photoreceptor degeneration
<i>RPGR</i>	Mouse	In-frame deletion of exon 4	(163)	Normal photoreceptor development, slowly progressive photoreceptor degeneration
<i>RPGR</i>	Mouse (Rd9)	Spontaneous 32 bp duplication in exon ORF15	(77)	Normal photoreceptor development, slowly progressive photoreceptor degeneration
<i>RPGR</i>	Zebrafish	Morpholino (translation blocker)	(164)	No eye phenotype recorded
<i>RPGR</i>	Zebrafish	Morpholino (translation blocker)	(165)	Abnormal development of the retina (lamination defects, failure to form outer segments, smaller eyes)
<i>RPGR</i>	Zebrafish	Morpholino (translation blocker)	(96)	Smaller eyes
<i>RPGRIP1</i>	Dog	Spontaneous 44 bp insertion in exon 2	(166)	Cone–rod dystrophy
<i>RPGRIP1</i>	Mouse	Insertion targeting construct between exon 14 and 15	(32)	Early onset, rapidly progressive photoreceptor degeneration
<i>RPGRIP1</i>	Mouse	(tm1Tili)		
<i>RPGRIP1</i>	Mouse	Point mutation in splice acceptor site in intron 6	(167)	Early onset, rapidly progressive photoreceptor degeneration
<i>RPGRIP1</i>	Mouse	(nmf247)		
<i>TOPORS</i>	Zebrafish	Morpholino (translation blocker)	(72)	Abnormal development of the retina (lamination defects, failure to form outer segments, smaller eyes)
<i>TTC8</i>	Mouse	Deletion of exons 1 and 2	(168)	Photoreceptor degeneration
<i>TTC8</i>	Zebrafish	Morpholino (translation blocker)	(169)	Smaller eyes
<i>TULP1</i>	Mouse	Removal of exons 8–9	(170)	Early onset, rapidly progressive photoreceptor degeneration
<i>TULP1</i>	Mouse	Point mutation in splice donor site in intron 3	(160)	Early onset, rapidly progressive photoreceptor degeneration
<i>TULP1</i>	Mouse	(tvrn124)		
<i>USH2A</i>	Mouse	Targeted disruption of exon 5	(171)	Late onset, slowly progressive photoreceptor degeneration
<i>USH2A</i>	Zebrafish	Morpholino (splice blocker)	(50)	Abnormal apoptotic photoreceptors

Currently, no vertebrate animal models have been published for *C8orf37* or *FAM161A*.

photoreceptor degeneration, however, is variable, which in most cases reflects the human situation. For example, mouse models for non-syndromic LCA in humans, such as the *rd16* mouse model which carries a hypomorphic *Cep290* allele, and the *Lca5* gene-trap mouse model, show early-onset (P14 and P12, respectively) and rapid degeneration of outer and inner segments, and the outer nuclear layer accompanied with low-ERG responses (47,64,76).

Compared with this early-onset phenotype of LCA models, mouse models for RP show a delayed onset and slower progression of photoreceptor degeneration. The *Rd9* mouse strain, harboring a spontaneous frameshift mutation in ORF15 of *Rpgr*, show mildly reduced ERG responses at 6 weeks of age, which develops progressively but slowly over a 2 year period (77). Another mutated *Rpgr* mouse strain shows normal retinal morphology and ERG function at P30. Photoreceptor loss in these mice becomes substantial after 6 months of age, showing reduced outer segment length, outer nuclear layer thickness and abnormal ERG responses (78).

Unfortunately, mouse models for retinal ciliopathy genes do not always match the human phenotype, such as *Clrn1* mutant mice, which do not develop a retinal phenotype (79) (Table 2). Morpholino-mediated silencing of different orthologs for non-syndromic retinal ciliopathy genes in zebrafish shows largely overlapping retinal phenotypes. Knockdown of zebrafish *topors*, the ortholog for autosomal dominant RP-associated *TOPORS* (35), shows microphthalmia, defective retinal development and failure to form outer segments (72). Similar phenotypes (microphthalmia, defective retinal lamination and absence of outer segments) are observed after *rp2* knockdown, the ortholog of X-linked RP-associated *RP2* (80,81). In addition, knockdown of *c2orf71*, the ortholog for arRP-associated *C2orf71* (82,83), results in microphthalmia and shorter outer segments (83). These studies indicate that knockdown of orthologs for retinal ciliopathy genes in zebrafish results in microphthalmia and loss of outer segments. An overview of published animal models for each non-syndromic retinal ciliopathy gene is shown in Table 2.

TRANSLATIONAL RESEARCH

Non-syndromic retinal dystrophies have been considered for long to be incurable. The recent identification and characterization of avian, canine, feline and rodent models for many of the non-syndromic retinal diseases has provided an excellent platform for translational research. Translational research has mainly focused on gene augmentation by subretinal injection of virus, which appears to be safe and successful in long-term restoring of vision in models for non-syndromic retinal dystrophies. The successes of gene augmentation studies in a mouse model for childhood blindness (84) or in an *RPE65* null canine model (85) have led to ongoing clinical trials of ocular subretinal delivery of adeno-associated virus (AAV) serotype 2 carrying human *RPE65* in individuals with known *RPE65* mutations (86). For the non-syndromic retinal ciliopathy genes, the efficacy of gene replacement therapy has been evaluated in an *Rpgr* mutant mouse model (*tm1Tili*; Table 2). Subretinal delivery of the human replacement construct, packaged in an AAV serotype 8 vector,

results in the expression of functional RPGRIP1 in connecting cilia of photoreceptors, better preservation of rod and cone photoreceptor function and prolongs photoreceptor survival (87). In addition, AAV-based gene augmentation therapy of human *RPGR* in two blinding canine models for X-linked RP (XLPRA1 and XLPRA2; Table 2) shows preservation of morphology and functionality of rod and cone photoreceptors, while post-receptor remodeling was corrected as well (88).

AAV-based gene augmentation therapies for large genes, such as *CEP290*, which encodes a 2479 amino acid protein and is the most frequently mutated LCA gene, are problematic given the size-restriction for the AAV vector (~5 kb) (89). A mini-gene augmentation therapy, which uses only part of the gene important for gene function in the photoreceptor, would fulfill the AAV size limit criteria. A successful mini-gene augmentation approach for *CEP290* has recently been performed in zebrafish. Expression of only the N-terminal 1059 amino acids of CEP290 resulted in a rescue of visual impairment in *cep290* morpholino-injected fish (90). Additional studies need to be performed to determine the efficiency of mini-gene replacement therapies in higher vertebrate models.

Promising alternatives for gene augmentation in retinal dystrophies are therapeutic approaches using either modified U1 small nuclear (sn)RNAs or antisense oligonucleotides to correct mutation-induced splicing defects. Lentiviral treatment of fibroblasts derived from individuals with RP who carry a c.479G>A splice donor site mutation in exon 5 of *BBS1* partially corrected aberrant splicing of endogenously expressed *BBS1* transcripts in a dose-dependent manner, indicating that U1 snRNAs can correct pathogenic effects of splice donor site mutations in recessive RP (91). Similar results were obtained for X-linked RP, for which splice defects in *RPGR* have been corrected in patient-derived primary fibroblasts, using U1 snRNAs (92). A recent antisense oligonucleotide-based approach for the most frequent LCA-causative mutation (c.2991+1655A>G), which resulted in the inclusion of an aberrant exon in *CEP290* mRNA, has shown to be successful in almost completely redirecting normal *CEP290* splicing (dose-dependent, with a high degree of sequence specificity), using immortalized lymphoblastoid cells of individuals carrying two intronic *CEP290* mutations (93).

In case gene therapy is not applicable or efficient, pharmacological therapy may offer a good alternative to slow down or halt the course of photoreceptor degeneration. Administration of ciliary neurotrophic factor through an encapsulated cell technology device has shown to be effective in slowing photoreceptor degeneration in animal models of RP, and is currently included in clinical trials for RP (94). Another pharmaceutical drug, tauroursodeoxycholic acid, has shown to preserve photoreceptors in a variety of mouse models, including *Bbs1* mutant mice. Subcutaneous administration of tauroursodeoxycholic acid twice a week, from P40 to P120, has shown to preserve photoreceptor morphology (outer segments and outer nuclear layer) and function in mice with a targeted knock-in of the p.Met390Arg variant on both alleles (95) (Table 2).

The most frequently mutated non-syndromic retinal ciliopathy genes, such as *RPGR* and *USH2A*, are associated with disease in ~1:60,000 individuals (11). Other genes associated with non-syndromic retinal ciliopathies carry mutations less frequently, which poses a challenge to identify sufficient

numbers of patients who are eligible for novel treatments, and to attract the interest of industry to fund preclinical studies. Most likely, these studies will take place through public and private funding. Clearly, global efforts are required to register clinical and genetic data of patients with retinal ciliopathies. In the mean time, large national (e.g. LCA3000 in the USA and RP5000 in the Netherlands) or international programs (e.g. the European Retinal Disease Consortium, comprising nine groups in eight European countries) are required to systematically identify persons with mutations in rarely mutated genes. A comprehensive listing of all mutation data in open-access-curated databases, such as the Leiden Open Variant Database (<http://www.lovd.nl/2.0/>), is still lacking. Compulsory registration of all genetic data in such databases, as required by e.g. *Human Mutation*, would result in valuable resources for the entire scientific community. In addition, there is great need to establish a standardized clinical database that can be shared between international groups.

CONCLUSION

The connecting cilium of photoreceptor cells plays a crucial role in the selective transport of proteins and other molecules to and from the outer segments. This cellular structure consists of hundreds of proteins, many of which have specialized roles. Approximately one-third of the genetic defects associated with inherited retinal dystrophies are located in genes encoding ciliary proteins. Protein interaction studies point to the existence of key modules governing the complex transport mechanism within photoreceptors. These studies and the use of vertebrate animal models are instrumental in the development of an increasing number of rational therapies (e.g. based on gene augmentation or the correction of miss-spliced RNAs) for retinal dystrophies.

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