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Tatyana N. Kuznetsova

Barbara Zangerl
University of Pennsylvania, bzangerl@vet.upenn.edu

Gustavo D. Aguirre
University of Pennsylvania, gda@vet.upenn.edu

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Abstract
Cone–rod dystrophies (*crd*) represent a group of progressive inherited blinding diseases characterized by primary dysfunction and loss of cone photoreceptors accompanying or preceding rod death. Recessive *crd* type 1 was described in dogs associated with an *RPGRIP1* exon 2 mutation, but with lack of complete concordance between genotype and phenotype. This review highlights role of the RPGRIP1, a component of complex protein networks, and its function in the primary cilium, and discusses the potential mechanisms of genotype–phenotype discordance observed in dogs with the *RPGRIP1* mutation.

Keywords
RPGRIP1, polymorphism, cone-rod dystrophy, protein network, photoreceptor cilia

Disciplines
Eye Diseases | Geriatrics | Medical Biotechnology | Medical Genetics | Medical Immunology | Ophthalmology | Veterinary Medicine

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RPGRIP1 and Cone-Rod Dystrophy in Dogs

Tatyana Kuznetsova[1], **Barbara Zangerl**[1], and **Gustavo Aguirre**[1]
[1]Section of Ophthalmology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

Keywords

RPGRIP1; Polymorphism; Cone-rod dystrophy; Protein network; Photoreceptor cilia

42.1 Introduction

Cone-rod dystrophies (*crd*) affect the cone and rod photoreceptors resulting in reduced visual acuity followed by severe loss of central and color vision that often progresses to complete blindness. The onset of clinical disease in man ranges from early to late adulthood, and inherited as X-linked, autosomal dominant or, most commonly, autosomal recessive. Mutations in over 20 genes have been identified to cause *crd* (RetNet db). Canine models have been developed for several human retinal degenerations (Aguirre and Acland, 2006); in terms of *crd*, the standard wire-haired dachshund (SWHD), miniature long-haired dachshund (MLHD), pit bull and Glen of Imaal terriers are the only dog breeds thus far affected with *crd*, and the involved genes have been identified in all except pit bull terriers (Goldstein et al., 2010; Kijas et al., 2004; Mellersh et al., 2006; Ropstad et al., 2007).

A canine autosomal recessive, early-onset *crd* (*crd1*) was described in MLHDs (Mellersh et al., 2006). The *crd1* locus was mapped to a region of CFA15 syntenic to HSA14 and containing *RPGRIP1* (the retinitis pigmentosa GTPase interacting protein 1). This gene encodes a ciliary protein that plays an important role in maintenance and function of the cilium. In man, mutations in *RPGRIP1* are associated with Leber congenital amaurosis (Dryja et al., 2001), and cone-rod dystrophy (Hameed et al., 2003). Sequence analysis of canine *RPGRIP1* revealed a 44-nucleotide insertion in exon 2 that was proposed to be responsible for the disease in MLHD as it altered the reading frame by introducing a premature stop-codon (Mellersh et al., 2006). Subsequent studies, however, indicate that only ~80% of homozygous mutant MLHDs had the disease (Miyadera et al., 2009). The genotype-phenotype discordance suggests that another mutation in the same or different gene may contribute to the disease. In this review, we examine the RPGRIP1 protein interactome with the view of selecting possible interacting genes that could be associated with the *crd1* phenotype.

42.2 Focus on the *RPGRIP1*. What Makes it Special?

Human *RPGRIP1* encompasses 25 (24 coding) exons (Dryja et al., 2001), and is expressed in amacrine neurons, photoreceptors and, at reduced levels, in many other eye tissues (Roepman et al., 2000). Knockout studies indicated RPGRIP1 is required for morphogenesis of the outer segment (OS) discs (Zhao et al., 2003), and plays an important role in OS formation, particularly in rods (Won et al., 2009). Moreover, *RPGRIP1* is subject to multiple

Corresponding Author: Tatyana Kuznetsova, 3900 Delancey Street, VHUP, Philadelphia, PA 19104-6010; Phone: (215) 573-9586; tatyanak@vet.upenn.edu.

splicing leading to numerous isoforms with species-specific subcellular localization patterns, e.g connecting cilium, photoreceptor inner and OS (Castagnet et al., 2003; Mavlyutov et al., 2002), and the basal body of cells with primary cilia (Shu et al., 2005), suggesting that different isoforms may perform cell-specific functions.

RPGRIP1 is a component of the cilia protein network, but details about its molecular function and interacting proteins are incomplete. In retina, RPGRIP1 was identified as a part of different protein complexes including RPGR (Roepman et al., 2000), NPHP4 (Roepman et al., 2005), and/or RanB (Castagnet et al., 2003). It directly interacts with RPGR via its C-terminal domain (RID; Roepman et al., 2000). The C2 domain specifically binds with NPHP4 (Roepman et al., 2005) which is part of a multifunctional complex localized in actin- and microtubule-based structures. Mutations in the NPHP4 gene are linked to nephronophthisis type 4, Senior-Løken syndrome in man (Mollet et al., 2002), and *crd* in dogs (SWHD) (Wiik et al., 2008). To better understand of the potential role and functioning of RPGRIP1 in the cilium, and identify potential candidate proteins that influence the *crd1* phenotype, a putative molecular network was generated based on results of integrated information of protein-protein interactions or co-localization of ciliary proteins.

42.3 Characterization of the RPGRIP1- Associated Protein Network

In spite of the fact that the exact function of the RPGRIP1 is still unclear, interaction with RPGR and NPHP4 establishes connection to several cellular processes (Fig. 42.1). Mutations in many of these genes have been shown to have a retinal phenotype.

42.3.1 RPGRIP1 Is a Component of the Transport Network for Cilia Assembly

RPGRIP1 interacts with RPGR anchoring it to the connecting cilium. RPGR isoforms were found associated with intraflagellar transport polypeptide IFT88 and the p150Glued subunit of the dynactin transport machinery (Khanna et al., 2005) suggesting involvement of RPGR in regulation of transport in primary cilia. IFT88 interacts directly with the molecular chaperone MRJ which acts as a cargo receptor for photoreceptor specific guanylate cyclase (GUCY) and plays a critical role in formation or stabilization of the IFT-cargo complexes (Roepman and Wolfrum, 2007) (Fig. 42.1).

Another RPGR-interacting partner is PDEδ (Linari et al., 1999). PDEδ binds and specifically stabilizes the GTP-bound form of Arl3, which belongs to the ARF small GTPase family, by strongly decreasing the dissociation rate of GTP (Zhang et al., 2004). The retinitis pigmentosa RP2 protein, which links pericentriolar vesicle transport between the Golgi and the primary cilium, is a GTPase activating protein for Arl3. Depletion of RP2 and dysregulation of Arl3 resulted in dispersal of vesicles cycling cargo from the Golgi complex to the cilium.

42.3.2 RPGRIP1 Involvement in Regulating Cytoskeleton Dynamics and Ciliogenesis

There is a group of proteins in the RPGRIP1-associated interactome with different functions that are part of a complex cellular machine regulating cytoskeletal function and integrity (Fig. 42.1). RPGRIP1-interacting partner RPGR was found in a protein complex with CEP290 (NPHP6), a microtubule motor protein (Khanna et al., 2005), suggesting involvement of RPGR in microtubule organization. Although a centrosomal protein, CEP290 also was localized in the basal bodies of the ciliary apparatus in different cell types, including the photoreceptor cilium. CEP290 interacts with a component of centriolar satellites PCM1 which is implicated in some BBS proteins function (Kim et al., 2008), and localizes to centriolar satellites in a PCM1- and microtubule-dependent manner. PCM1 plays a role in the recruitment of some centrosomal proteins including centrin and

pericentrin (PCNT), and is required for the organization of the cytoplasmic microtubule network. CEP290 interaction with PCM1 is important for the ciliary targeting of RAB8, a small GTPase involved with BBS protein complex to promote ciliogenesis (Kim et al., 2008). Direct or indirect interactions with other proteins, e.g. RABIN8, CP110, BSS1 and others, also are important for ciliogenesis (Nachury et al., 2007; Tsang et al., 2008).

RPGR has been proposed as a regulator of some NPHP proteins complexes in the mammalian retina (Murga-Zamalloa et al., 2010). The RCC1-like domain of RPGR interacts with the N-terminal part of NPHP4, an RPGRIP1-interacting partner. In the retina, RPGR also interacts directly with RPGRIP1L (NPHP8) and NPHP1, a protein that interacts with NPHP4, NPHP2, and tubulin (Mollet et al., 2002; Murga-Zamalloa et al., 2010). RPGR, NPHP5 and CaM were detected in a common multiprotein complex in the retina, and NPHP5 was shown to directly interact with CaM (Otto et al., 2005).

One of NPHP1-interacting protein is AHI1, a cilium-localized protein that shown to cause a form of Joubert syndrome (Eley et al., 2008). AHI1 is required for photoreceptor OS development, and null mice fail to form OS and have abnormal distribution of opsin throughout their photoreceptors. AHI1 binds huntingtin-associated protein 1 (HAP1) which interacts with PCM1 and with the p150Glued subunit of dynactin transport machinery. It was found that BBS4 acts as an adaptor that connects p150Glued with PCM1, and thus assists the centrosomal recruitment of PCM1 and is associated with some centrosomal proteins.

42.3.3 Components of Molecular Networks Regulating Cellular Transport and Cytoskeleton Dynamics Show Connection with Visual Pathway

Fig. 42.1 also includes a group of molecules from the visual signaling cascade, including RHO, GRK1, transducin (Tαβγ), GUCY, PDE, ARR1 and others, that “border” the RPGRIP1-associated network. Interaction of CP110 with different proteins, e.g. CaM and centrin, is an example of intersection of signaling pathways (Tsang et al., 2006). Ca2+ ions are required for the activation of centrin isoforms and for centrin/transducin (T-βγ) complex formation. Centrin functions are not only regulated by Ca2+-binding but also by site-specific phosphorylation/dephosphorylation. A changeover of assembly and disassembly of centrin/transducin complexes may regulate the diffusion of transducin through the connecting cilium (Trojan et al., 2008).

The network represented on the Fig. 42.1 carries limited information and doesn’t show any pathways in details but is presented as an overview of the RPGRIP1 interactome, and to provide clues as to what other proteins may interact with RPGRIP1 and determine the phenotype/disease outcome in the mutant dogs.

42.4 Dogs with Retinal Degeneration Show Significant Phenotypic Variation with RPGRIP1 Mutation Ins44

The *crd1* “causative” *RPGRIP1* Ins44 mutation was initially completely associated with disease status in an inbred research colony (Mellersh et al., 2006). A recent study of a general MLDH population identified substantial genotype-phenotype discordance: 16% of normal controls were homozygous for the insertion, while 20% of dogs with retinal degeneration did not carry the insertion on both chromosomes (Miyadera et al., 2009). Additionally, four other breeds were identified to carry Ins44 including English Springer Spaniels (ESSs; in dogs affected with PRA, ~30% were homozygous for Ins44), Beagles (allelic frequency 27.8%), French Bulldogs (12.5%), and Labrador Retriever (3%), with some variation in the length of the polyA insertion (Miyadera et al., 2009). That result in

ESSs is in good agreement with our own data. Depending on the survey conducted (unpublished), the clinical incidence of retinal degeneration in ESSs is ~3%, yet the number of dogs that are homozygous for the mutation ranges from ~20-40%. These observations resulted in the suggestion that the *RPGRIP1* Ins44 mutation represents a predisposing factor for disease rather than the causative mutation. This possibility could be explained by several different hypotheses.

First, *RPGRIP1* undergoes to multiple splicing, and some its isoforms can delete exon 2 which is the site of the putative mutation (Kuznetsova et al., under review). Moreover, a subset of *RPGRIP1* isoforms can be driven from internal alternative promoter(s) located 3′ of the mutation. In both cases those isoforms could provide protein function independent of the exon 2 Ins44 genotype. This hypothesis could be tested by investigating RPGRIP1 protein expression in dogs with Ins44.

Second, it has been demonstrated that Ins44 contains a prolonged polyA tract. In many cases, including a recent report (Miyadera et al., 2009), such polynucleotide tracts are polymorphic. This suggests that DNA polymerase fidelity defects with the replication of long polynucleotide repeats, some chromosomes carrying the insertion may not consist of a 44, but 43, 45 or 47 nucleotide repeat. In those cases the mutation would not necessarily result in a premature stop-codon, but at a modified RPGRIP1 N-terminus and could partially explain variation in disease phenotype in dogs with Ins44.

Lastly, Ins44 alone may not be sufficient to cause disease, but be dependent on a second mutation in *RPGRIP1* or a different gene. Based on outcross/intercross/backcross studies carried out in our lab, the potential modifier gene is most likely located inside the mapped 1.74 Mb region of canine chromosome 15 containing *RPGRIP1* (Miyadera et al., 2009). In this case cosegregation of a haplotype with *crd1* would explain better the cases of the disease and be in agreement that Ins44 alone highly associated with *crd1* in the inbred population.

In summary, RPGRIP1 is an important signaling component inside the cilia. *RPGRIP1* primary mRNA undergoes an extensive splicing resulting in the formation of multiple isoforms. To date, the exact role of RPGRIP1 protein isoforms in cilia function is unknown, but published data about RPGRIP1 interacting molecules support the role of this protein in the cytoskeleton dynamic and transport of ciliary proteins. Reconstruction and analysis of the putative RPGRIP1-associated molecular network offers opportunities to further investigate the molecular mechanisms of cone-rod dystrophy in dogs.

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References

Aguirre, GD.; Acland, GM. Models, Mutants and Man: Searching for Unique Phenotypes and Genes in the Dog Model of Inherited Retinal Degeneration. The Dog and Its Genome. Ostrander, EA.; Giger, U.; Lindblad-Toh, K., editors. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 2006. p. 291-325.

Castagnet P, Mavlyutov T, Cai Y, et al. RPGRIP1s with distinct neuronal localization and biochemical properties associate selectively with RanBP2 in amacrine neurons. Hum Mol Genet. 2003; 12:1847–1863. [PubMed: 12874105]

Dryja TP, Adams SM, Grimsby JL, et al. Null RPGRIP1 alleles in patients with Leber congenital amaurosis. Am J Hum Genet. 2001; 68:1295–1298. [PubMed: 11283794]

Eley L, Gabrielides C, Adams M, et al. Jouberin localizes to collecting ducts and interacts with nephrocystin-1. Kidney Int. 2008; 74:1139–1149. [PubMed: 18633336]

Goldstein O, Mezey JG, Boyko AR, et al. ADAM9 mutation in Canine Cone-Rod Dystrophy 3 (crd3) establishes homology with human CORD9. Mol Vis. 2010; 16:1549–1569. [PubMed: 20806078]

Hameed A, Abid A, Aziz A, et al. Evidence of RPGRIP1 gene mutations associated with recessive cone-rod dystrophy. J Med Genet. 2003; 40:616–619. [PubMed: 12920076]

Khanna H, Hurd TW, Lillo C, et al. RPGR-ORF15, which is mutated in retinitis pigmentosa, associates with SMC1, SMC3, and microtubule transport proteins. J Biol Chem. 2005; 280:33580–33587. [PubMed: 16043481]

Kijas JW, Zangerl B, Miller B, et al. Cloning of the canine ABCA4 gene and evaluation in canine cone-rod dystrophies and progressive retinal atrophies. Mol Vis. 2004; 10:223–232. [PubMed: 15064680]

Kim J, Krishnaswami SR, Gleeson JG. CEP290 interacts with the centriolar satellite component PCM-1 and is required for Rab8 localization to the primary cilium. Hum Mol Genet. 2008; 17:3796–3805. [PubMed: 18772192]

Kuznetsova T, Zangerl B, Goldstein O, et al. Structural organization and expression pattern of the canine RPGRIP1 isoforms in retinal tissue. Invest Ophthalmol Vis Sci. 2011; 52:2989–2998. [PubMed: 21282582]

Linari M, Ueffing M, Manson F, et al. The retinitis pigmentosa GTPase regulator, RPGR, interacts with the delta subunit of rod cyclic GMP phosphodiesterase. Proc Natl Acad Sci USA. 1999; 96:1315–1320. [PubMed: 9990021]

Mavlyutov TA, Zhao H, Ferreira PA. Species-specific subcellular localization of RPGR and RPGRIP isoforms: Implications for the phenotypic variability of congenital retinopathies among species. Hum Mol Genet. 2002; 11:1899–1907. [PubMed: 12140192]

Mellersh CS, Boursnell ME, Pettitt L, et al. Canine RPGRIP1 mutation establishes cone-rod dystrophy in miniature longhaired dachshunds as a homologue of human Leber congenital amaurosis. Genomics. 2006; 88:293–301. [PubMed: 16806805]

Miyadera K, Kato K, Aguirre-Hernandez J, et al. Phenotypic variation and genotype phenotype discordance in canine cone-rod dystrophy with an RPGRIP1 mutation. Mol Vis. 2009; 15:2287–2305. [PubMed: 19936303]

Mollet G, Salomon R, Gribouval O, et al. The gene mutated in juvenile nephronophthisis type 4 encodes a novel protein that interacts with nephrocystin. Nat Genet. 2002; 32:300–305. [PubMed: 12244321]

Murga-Zamalloa CA, Desai NJ, Hildebrandt F, et al. Interaction of ciliary disease protein retinitis pigmentosa GTPase regulator with nephronophthisis-associated proteins in mammalian retinas. Mol Vis. 2010; 16:1373–1381. [PubMed: 20664800]

Nachury MV, Loktev AV, Zhang Q, et al. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. Cell. 2007; 129:1201–1213. [PubMed: 17574030]

Otto EA, Loeys B, Khanna H, et al. Nephrocystin-5, a ciliary IQ domain protein, is mutated in senior-loken syndrome and interacts with RPGR and calmodulin. Nat Genet. 2005; 37:282–288. [PubMed: 15723066]

Roepman R, Wolfrum U. Protein networks and complexes in photoreceptor cilia. Subcell Biochem. 2007; 43:209–235. [PubMed: 17953396]

Roepman R, Letteboer SJ, Arts HH, et al. Interaction of nephrocystin-4 and RPGRIP1 is disrupted by nephronophthisis or Leber congenital amaurosis-associated mutations. Proc Natl Acad Sci USA. 2005; 102:18520–18525. [PubMed: 16339905]

Roepman R, Bernoud-Hubac N, Schick DE, et al. The retinitis pigmentosa GTPase regulator (RPGR) interacts with novel transport-like proteins in the outer segments of rod photoreceptors. Hum Mol Genet. 2000; 9:2095–2105. [PubMed: 10958648]

Ropstad EO, Bjerkas E, Narfstrom K. Clinical findings in early onset cone-rod dystrophy in the standard wire-haired dachshund. Vet Ophthalmol. 2007; 10:69–75. [PubMed: 17324160]

Shu X, Fry AM, Tulloch B, et al. RPGR ORF15 isoform co-localizes with RPGRIP1 at centrioles and basal bodies and interacts with nucleophosmin. Hum Mol Genet. 2005; 14:1183–1197. [PubMed: 15772089]

Trojan P, Krauss N, Choe HW, et al. Centrins in retinal photoreceptor cells: Regulators in the connecting cilium. Prog Retin Eye Res. 2008; 27:237–259. [PubMed: 18329314]

Tsang WY, Bossard C, Khanna H, et al. CP110 suppresses primary cilia formation through its interaction with CEP290, a protein deficient in human ciliary disease. Dev Cell. 2008; 15:187–197. [PubMed: 18694559]

Tsang WY, Spektor A, Luciano DJ, et al. CP110 cooperates with two calcium-binding proteins to regulate cytokinesis and genome stability. Mol Biol Cell. 2006; 17:3423–3434. [PubMed: 16760425]

Wiik AC, Wade C, Biagi T, et al. A deletion in nephronophthisis 4 (NPHP4) is associated with recessive cone-rod dystrophy in standard wire-haired dachshund. Genome Res. 2008; 18:1415–1421. [PubMed: 18687878]

Won J, Gifford E, Smith RS, et al. RPGRIP1 is essential for normal rod photoreceptor outer segment elaboration and morphogenesis. Hum Mol Genet. 2009; 18:4329–4339. [PubMed: 19679561]

Zhang H, Liu XH, Zhang K, et al. Photoreceptor cGMP phosphodiesterase delta subunit (PDEdelta) functions as a prenyl-binding protein. J Biol Chem. 2004; 279:407–413. [PubMed: 14561760]

Zhao Y, Hong DH, Pawlyk B, et al. The retinitis pigmentosa GTPase regulator (RPGR)-interacting protein: Subserving RPGR function and participating in disk morphogenesis. Proc Natl Acad Sci USA. 2003; 100:3965–3970. [PubMed: 12651948]

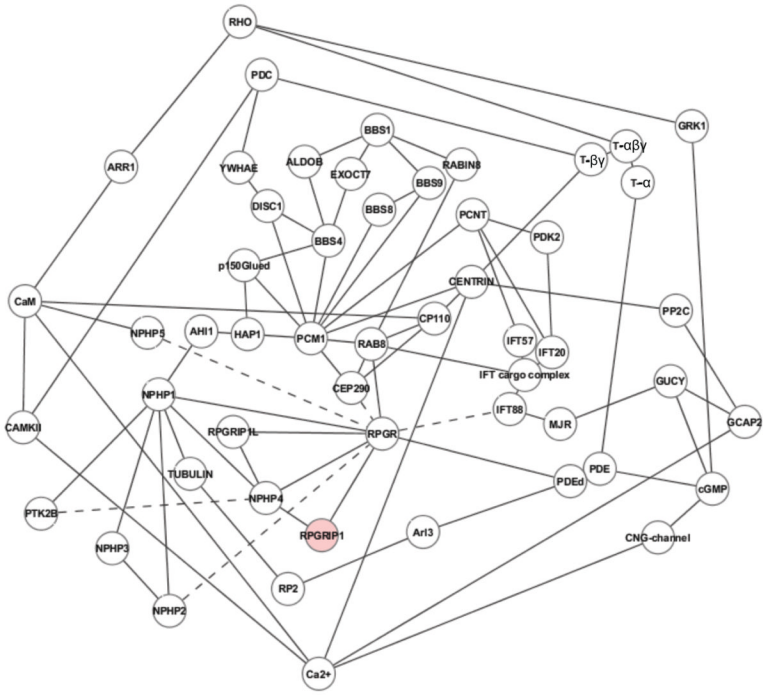

Fig. 42.1.

Putative RPGRIP1-associated protein network. The connections are based on reported protein-protein interactions (for description and references, please see text). Direct interactions are shown by *solid lines*; interactions that are shown by *dotted lines* represent indirect interactions (for example for those that were identified by immunoprecipitation analysis)