Biallelic Mutation of *ARHGEF18*, Involved in the Determination of Epithelial Apicobasal Polarity, Causes Adult-Onset Retinal Degeneration

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Mutations in more than 250 genes are implicated in inherited retinal dystrophy; the encoded proteins are involved in a broad spectrum of pathways. The presence of unsolved families after highly parallel sequencing strategies suggests that further genes remain to be identified. Whole-exome and -genome sequencing studies employed here in large cohorts of affected individuals revealed biallelic mutations in *ARHGEF18* in three such individuals. *ARHGEF18* encodes ARHGEF18, a guanine nucleotide exchange factor that activates RHOA, a small GTPase protein that is a key component of tight junctions and adherens junctions. This biological pathway is known to be important for retinal development and function, as mutation of *CRB1*, encoding another component, causes retinal dystrophy. The retinal structure in individuals with *ARHGEF18* mutations resembled that seen in subjects with *CRB1* mutations. Five mutations were found on six alleles in the three individuals: c.808A>G (p.Thr270Ala), c.1617+5G>A (p.Asp540Glyfs*63), c.1996C>T (p.Arg666*), c.2632G>T (p.Glu878*), and c.2738_2761del (p.Arg913_Glu920del). Functional tests suggest that each disease genotype might retain some ARHGEF18 activity, such that the phenotype described here is not the consequence of nullizygosity. In particular, the p.Thr270Ala missense variant affects a highly conserved residue in the DBL homology domain, which is required for the interaction and activation of RHOA. Previously, knock-out of *Arhgef18* in the medaka fish has been shown to cause larval lethality which is preceded by retinal defects that resemble those seen in zebrafish *Crumbs* complex knock-outs. The findings described here emphasize the peculiar sensitivity of the retina to perturbations of this pathway, which is highlighted as a target for potential therapeutic strategies.

Inherited retinal dystrophy (IRD) encompasses a clinically and genetically heterogeneous group of disorders characterized by retinal dysfunction or degeneration. Variants in more than 250 genes encoding proteins essential to a wide range of biological pathways including mRNA splicing, posttranslational protein modification, ciliogenesis, cilia protein transport, retinoid recycling in the visual cycle, phototransduction, and retinal development have been found causative of IRD (RetNet).

This report describes mutation of *ARHGEF18* (MIM: 616432) as a likely cause of human IRD. The gene encodes ARHGEF18 (also known as p114RhoGEF),[1] the Rho/Rac guanine nucleotide exchange factor 18. It has been shown to be involved in the determination of apicobasal (AB) polarity in epithelia and cell-cell junction formation through its action on the small GTPase RHOA.[2] The gene is widely expressed, with expressed sequence tags identified in many human tissues including the neurosensory retina (NCBI-UniGene).

The study protocol adhered to the tenets of the Declaration of Helsinki and received approval from the local ethics committee. Written, informed consent was obtained from all participants prior to their inclusion in this study. To gain further insight into the genetic pathology of inherited retinal dystrophy, whole-exome sequencing (WES) has been performed on 230 individuals and whole-genome sequencing (WGS) on a further 599 probands, ascertained from the inherited retinal disease clinics at Moorfields Eye Hospital (MEH), London. The latter cohort forms part of the NIHR-Bioresource Rare Disease consortium in the UK.[3]

Biallelic mutations in *ARHGEF18* were identified in three individuals (Table S1), presenting as simplex cases, each with a retinal dystrophy sharing features with that seen in retinal disease caused by mutation in *CRB1* (MIM: 604210).[4] For this reason, in all three individuals, Sanger sequencing of *CRB1* had been performed but did not identify any potential disease-associated variants. WGS was performed on individuals 1 and 2; the remaining individual (individual 3) underwent WES as previously described.[5] In the first instance, resulting coding variant calls were filtered using a list of 236 genes previously implicated in retinal dystrophy.[6] No convincing causal variants were identified in these affected individuals (Table S2).

After WGS, individual 1 (GC18203), a 37-year-old female with simplex retinitis pigmentosa (RP [MIM: 268000]), the second of two siblings born to unrelated parents with no family history of eye disease, had 20,863 coding (±8 bp splice region) variants passing standard quality filters. Of

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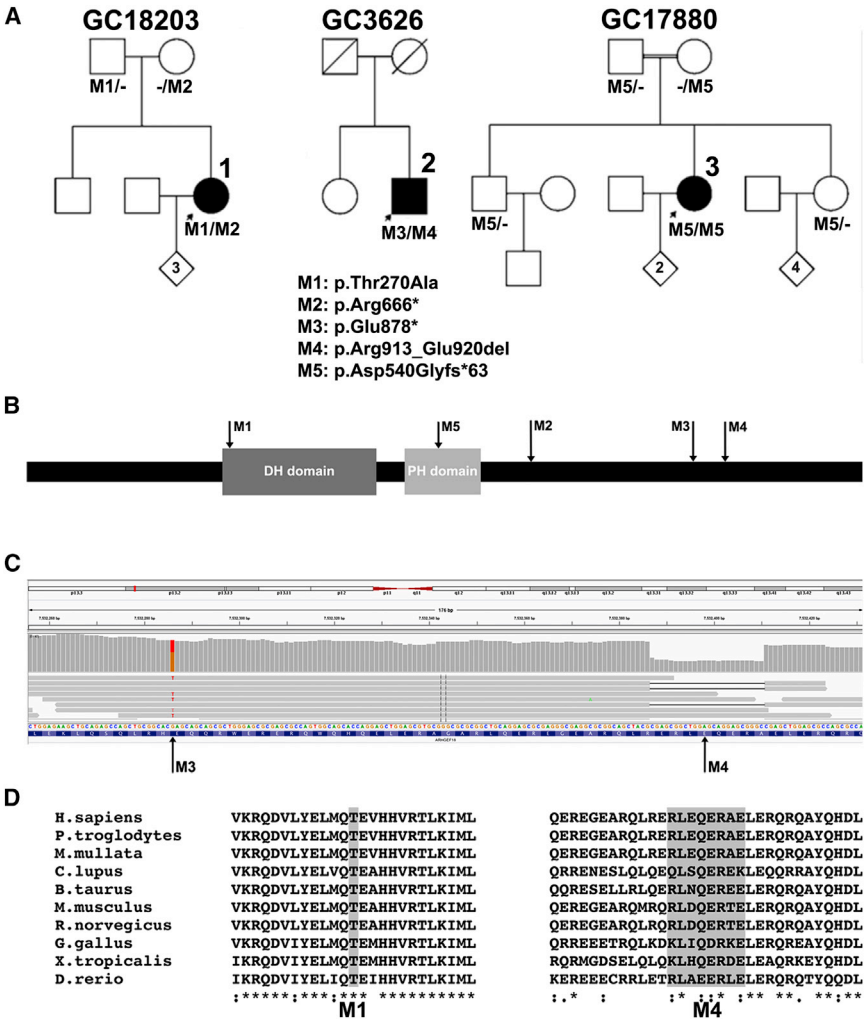

Figure 1. Variant Analysis of *ARHGEF18* in Individuals 1–3

(A) Pedigrees and cosegregation of mutations M1–M5 in families 1–3.

(B) Schematic representation of mutation location in full-length ARHGEF18 including DBL homology (DH) and Plekstrin homology (PH) domains.

(C) IGV visualization of 150 bp paired end reads spanning mutations *ARHGEF18*, c.2632G>T, and c.2738_2761del in individual 2, showing biallelic state.

(D) Clustal Omega alignment of amino acid residues affected by M1 (missense) and M4 (in-frame deletion) mutations throughout vertebrate orthologues.

these, 360 had a minor allele frequency (MAF) ≤ 0.01 in the publicly available dataset (Exome Aggregation Consortium database [ExAC]). Assuming autosomal-recessive inheritance, five genes contained ≥2 variants (Table S3). Variants were further manually interrogated for variant call quality, MAF in publicly available datasets and our own in-house exome-sequencing dataset (UCL exome project of more than 5,000 individuals), predicted protein impact, and biological plausibility (including protein function, expression profile, and pathway analysis). Of these, two variants were identified in *ARHGEF18*. The variants were a missense and nonsense absent from ExAC: GRCh37 (hg19) chr19: g.7509101A>G, GenBank: NM_001130955, c.808A>G (p.Thr270Ala) and chr19: g.7527145C>T, GenBank: NM_001130955, c.1996C>T (p.Arg666*). The missense variant p.Thr270Ala was predicted to be damaging by in silico prediction algorithms (sorting the intolerant from tolerant [SIFT], Polymorphism Phenotyping v2 [PolyPhen-2])[7,8] and affects a highly conserved amino acid residue in the DBL homology (DH) domain (Figure 1).

Identical analysis was performed on the 21,042 coding variants identified by WGS in individual 2 (GC3626), a 51-year-old male simplex RP-affected individual, the second of two siblings born to unrelated parents with no family history of eye disease. 17 genes with ≥2 variants (MAF ≤ 0.01) were identified (Table S4), among which were two variants comprising a nonsense and in-frame deletion in *ARHGEF18*: chr19: g.7532286G>T, GenBank: NM_001130955, c.2632G>T (p.Glu878*) and chr19: g.7532392_7532415del, GenBank: NM_001130955, c.2738_2761del (p.Arg913_Glu920del). The two variants occur within 130 bp in exon 16 of *ARHGEF18*. Interrogation of the 150 bp paired end reads in this region using the Integrative Genomics Viewer (IGV)[9,10] allowed phasing of the variants on seven reads suggesting they were in *trans* (Figure 1C). Familial DNA samples were unavailable for segregation analysis. The in-frame deletion of eight amino acid residues removes part of a highly conserved region of the protein (RLEQERAE) (Figure 1D).

Individual 3 (GC17880), an affected individual with simplex RP, the second of three siblings born to first-cousin parents with no family history of eye disease, underwent WES revealing 21,404 coding variants. Of these, 383 were rare (MAF ≤ 0.01 in the publicly available NHLBI GO Exome Sequencing Project dataset [EVS]). Assuming recessive inheritance due to autozygosity, seven genes had homozygous variants affecting the canonical transcript (Table S5), six of which were located within homozygous regions ≥5 Mb identified by prior SNP array autozygosity mapping data (SNP6, Affymetrix). Of these variants, a splice region substitution (chr19: g.7521294G>A, GenBank: NM_001130955, c.1617+5G>A) in *ARHGEF18* was the most compelling candidate. This variant is predicted to weaken the canonical splice donor site and lead to out-of-frame skipping of *ARHGEF18* exon 8.

The variants in each individual were confirmed to be biallelic by familial segregation analysis in all available

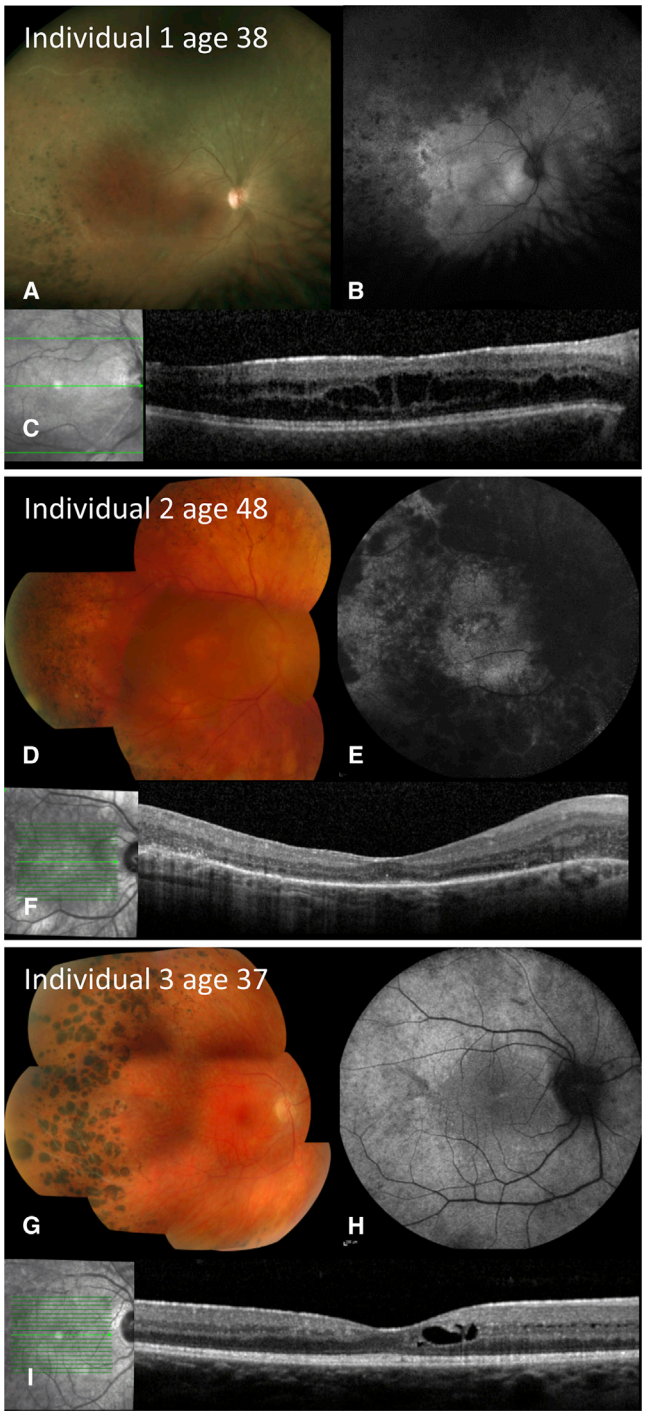

Figure 2. Retinal Abnormalities in *ARHGEF18*-Related Retinal Dystrophy

Color fundus photographs, 55 degree fundus autofluorescence imaging, and optical coherence tomography (OCT).

(A–C) Individual 1, right eye at age 38 years, showing (A) optic disc pallor, peripheral retinal pigment epithelium (RPE) atrophy, and nummular pigmentation; (B) peripheral patchy reduction of autofluorescence; and (C) reasonably preserved retinal layers on OCT with disruption of the inner segment ellipsoid band and intra-retinal cysts within the inner nuclear layer.

(D–F) Individual 2, right eye at 48 years, showing (D) disc pallor and vessel attenuation with RPE atrophy within the macula and mid-periphery as well as peripheral nummular and dot lesions of hyperpigmentation; (E) extensive loss of autofluorescence in periphery and in a central ring in macula; and (F) loss of outer retina and RPE throughout the macula with small foci of preserved photoreceptors centrally.

(G–I) Individual 3, right eye at 37 years, showing (G) vascular attenuation and occlusion, peripheral RPE atrophy, white dots, and nummular pigmentation; (H) loss of autofluorescence in periphery; and (I) reasonably preserved retinal layers on OCT with disruption of the inner segment ellipsoid band and intra-retinal cysts within the inner nuclear layer.

relatives (Figure 1A); no unaffected family member available for screening carried two disease alleles. Subsequent direct Sanger sequencing of all coding exons of *ARHGEF18* in ten individuals with a similar phenotype and no detectable mutation in *CRB1*[4] revealed no further mutations in *ARHGEF18*. In a cohort of 5,695 individuals who underwent WES (UCL-exome cohort), no rare (MAF $\leq$ 0.01) loss-of-function (LOF) variants were identified. Four individuals with unrelated phenotypes had predicted biallelic rare missense variants (Table S6).

The affected individuals reported here all presented in their third to fourth decades with central visual disturbance, visual field defects, and mild nyctalopia (Table S7). At last review at ages 37, 51, and 38 years for individuals 1, 2, and 3, respectively, visual acuity ranged from 0.18 log MAR (Snellen 20/30) to 1.8 log MAR (Snellen 20/1250); the worst was in the oldest individual. Fundus examination revealed optic disc pallor, attenuated retinal vessels, and irregular mid-peripheral intra-retinal pigment migration. Fundus autofluorescence (FAF) imaging revealed widespread, irregular, peripheral hypo-autofluorescence (Figure 2). Optical coherence tomography (OCT) demonstrated intra-retinal cysts in all affected individuals. Such imaging produces an in vivo cross-section of the retina. A useful landmark to gauge the degree of retinal degeneration is a contiguous line, parallel with the inner retinal surface, that is thought to be formed by reflection from mitochondria in photoreceptor inner segments, the so-called inner-segment ellipsoid line (ISe).[11] Individuals 1 and 3 had a preserved ISe throughout the macula; for individual 2 the ISe was retained only in the foveal region. The irregularity of the autofluorescence is distinct from that occurring in primary rod photoreceptor disease and instead resembles that seen in *CRB1* retinopathy. Moreover, peripheral nummular pigment was similar to *CRB1* retinopathy. In most degenerative dystrophies the retina is thinner than normal, and in these individuals retinal thickness instead resembled that seen in *CRB1* retinopathy. Full-field electroretinography (ERG), performed in all individuals at a similar age (29–30 years),[12] demonstrated severe generalized retinal dysfunction affecting rod more than cone photoreceptors (Figure S1). The pattern ERG[13] was subnormal in individuals 1 and 3, indicating relatively mild macular involvement, but was undetectable in individual 2, consistent with severe macular involvement. There was no clinical evidence of other systemic, neurological, or other epithelial disease in any of the individuals.

Cell-cell junctions (tight junctions [TJ] and adherens junctions [AJ]) are important in the establishment of AB polarity. During vertebrate eye morphogenesis, AB polarity of epithelial cells forming the optic vesicle is established and maintained by the migration and accumulation of specific polarity proteins and lipid complexes and the regulation of the actomyosin network in distinct apical and basal membrane domains and the formation of TJ and AJ.[14,15] Interkinetic nuclear migration (IKNM) along the AB polarity axis results in specific positioning of nuclei in the single-cell neuroepithelium and a pseudostratified appearance, and in turn contributes to the cell fate determination during differentiation into the three nuclear layers of the retina.[16]

Three major classes of protein complexes have been implicated in the establishment and maintenance of AB polarity: the Crumbs, Par, and Scribble complexes that serve as either apical or basolateral determinants. Rho small GTPase family members RHOA, RAC1, and CDC42 are central to the regulation of cell migration, contact adhesion, and the regulation of these apical and basolateral determinants.[15] The genes encoding these GTPase family members have not been implicated in retinal disease.[15,17] The activation status of Rho GTPases is determined by the guanine nucleotide bound to them (GTP-active, GDP-inactive), which is, in turn, regulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs).

Regulation of RHOA activation through ARHGEF18 is important for tissue morphogenesis and migration and in the assembly and maintenance of cell-cell junctions, TJ and AJ.[14,15] Cell junctions form intracellular connections essential for control of cell proliferation and morphology and maintenance of tissue integrity. In epithelia, TJ are formed at the apical/lateral border and control the movement of molecules along the paracellular space.[18] Molecular mechanisms regulating RHOA activation are crucial components of the pathways that guide TJ assembly and function. ARHGEF18 drives RHOA activation at TJ and thereby regulates actomyosin activity and TJ assembly, epithelial morphology, and dynamics.[2,19,20]

Cell-cell junctions and AB polarity are essential in the function and maintenance of retinal architecture.[21,22] In particular, the outer limiting membrane (OLM) is formed of AJ between Müller glia cells and photoreceptors and the inner/outer segments of photoreceptors are formed from the apical membrane of developing photoreceptors.

All of the individuals harbored genotypes of *ARHGEF18* that might conceivably produce some protein function, rather than being definite biallelic nulls. Individuals 1 and 2 had a nonsense mutation in *trans* with a missense or in-frame deletion, respectively. Reverse transcription-PCR (RT-PCR) and direct sequencing analysis of the *ARHGEF18* transcript from lymphocytes of individual 3 (c.1617+5G>A) using PCR primers spanning exons 6–9 identified differently spliced transcripts (Figure S2). Direct sequencing of the PCR-generated products identified a short transcript lacking exon 8 and a weaker band corresponding to the wild-type (WT) transcript (including exon 8). Hence, a low proportion of WT transcript and full-length WT protein is likely to be produced despite this splice-site alteration. Guanine to adenine transitions at position +5 in splice donor sites are recognized pathogenic mutations but have been reported previously to produce some normal mRNA product, for example in the context of cystic fibrosis.[23] The downstream consequence of exon 8 skipping would be a termination codon following 62 out-of-frame codons (p.Asp540Glyfs*63) and a transcript that is likely to undergo nonsense-mediated decay (NMD). The in-frame deletion in individual 2 is predicted to abolish several putative exonic splice enhancer (ESE) motifs.[24] However, RT-PCR and direct sequencing of the *ARHGEF18* transcript from lymphocytes of individual 2 using PCR primers spanning exons 13–17 identified no alteration in splicing (Figure S2) as a consequence of the deletion.

In order to determine the functional consequence and potential pathogenicity of the missense and in-frame deletion variants, HEK293T cells were transfected with expression vectors encoding WT *ARHGEF18* (GenBank: NM_001130955)[2] or with the p.Thr270Ala substitution or the p.Arg913_Glu920del deletion generated using the Q5 site-directed mutagenesis kit (New England Biolabs) according to manufacturers' instructions and propagated, purified, and sequenced using standard procedures. A previously characterized catalytically inactive mutant was included as a control (p.Tyr418Ala, previously referred to as p.Tyr260Ala).[2] RHOA activation is essential for ARHGEF18 to stimulate TJ assembly. This was tested by measuring RHOA-GTP levels in transfected HEK293T cells using a biochemical 96-well assay that measures binding of RHOA-GTP to the Rho binding domain of Rhotekin (G-LISA, Cytoskeleton, Inc.[2]). Ectopic expression of the WT protein led to a more than 5-fold stimulation of RHOA-GTP level (Figure 3A). The p.Thr270Ala mutant retained some activity compared to the catalytically inactive p.Tyr418Ala construct as it led to a 3-fold increase in RHOA-GTP level, but was less than 50% of the WT level (Figure 3A). The deletion mutant (p.Arg913_Glu920del) had a similar increase in RHOA-GTP level to the WT protein (Figure 3A).

The transcriptional activity of serum response factor (SRF) was measured in transfected cells using a double luciferase reporter assay[25] to monitor signaling output of the mutant ARHGEF18 constructs. Similar to the RHOA activation assay, the WT construct led to a strong stimulation of SRF-driven luciferase expression while the missense mutant (p.Thr270Ala) led to a 3-fold reduction in the level of luciferase expression but, as in the RHOA-GTP assay, showed significant activity also if compared to the inactive p.Tyr418Ala mutant; the deletion mutant (p.Arg913_Glu920del) level was unaltered (Figure 3B). Thus, the p.Thr270Ala mutant led to reduced but not abolished RHOA activation and signaling, and the

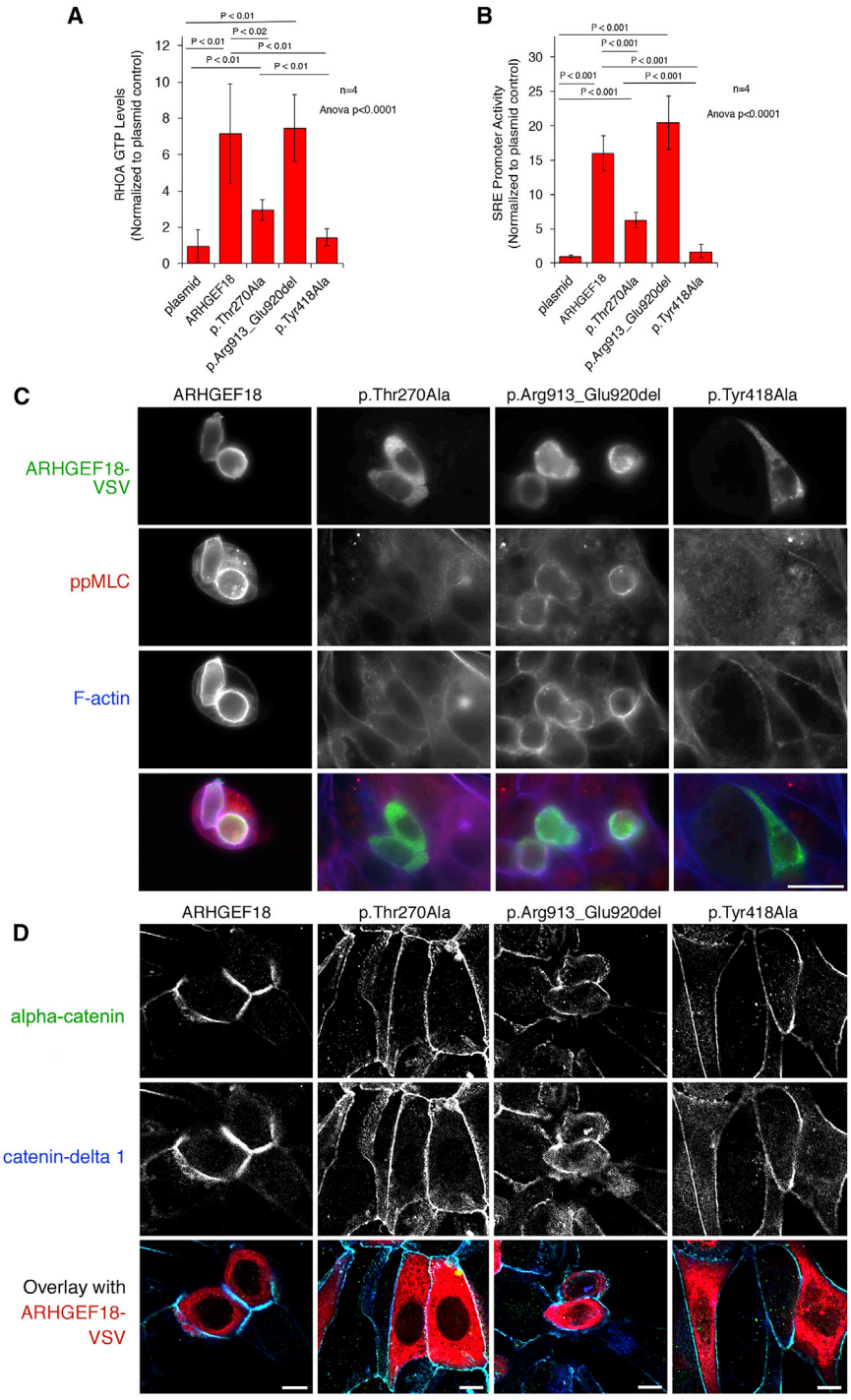

Figure 3. Signalling Activity of ARHGEF18 Variants

HEK293T or HCE cells were transfected with cDNAs encoding wild-type or mutant VSV-tagged ARHGEF18.

(A) Lysates of transfected HEK293T cells were assayed for RHOA-GTP levels by G-LISA assay, which measures binding of active RHOA to the GTPase binding domain of Rhotekin.

(B) Serum response element (SRE) element activity was measured using a double luciferase assay with an SRE-containing promoter driving firefly luciferase expression and a CMV promoter expression of renilla luciferase. Firefly to renilla luciferase ratios were calculated and normalized to a plasmid control performed by co-transfecting an empty expression vector. The graphs show averages ± 1 standard deviations, n = 4, indicated are p values from ANOVA and t tests.

(C) Transfected HCE cells were fixed and stained with anti-VSV and anti-phosphorylated myosin regulatory light chain (ppMLC) antibodies along Ato-647-labeled phalloidin to visualize F-actin and imaged by epifluorescence.

(D) Cells transfected as in (C) were stained for the junctional markers alpha-catenin and Catenin delta-1 and imaged by confocal microscopy.

Scale bars represent 20 μm in (C) and 10 μm in (D).

p.Arg913_Glu920del mutant did not affect RHOA activation.

As ARHGEF18 stimulates cortical actomyosin activation leading to TJ assembly and cell rounding in epithelial cells, human corneal epithelial cells (HCEs) were transfected with the WT or mutant expression vectors, including the p.Tyr418Ala catalytically inactive control. Transfection of WT but not the p.Tyr418Ala control led to rounded morphology of the normally flat cells, increased cortical phospho-myosin (pp-MLC), and F-actin staining (Figure 3C). Similar to the GEF inactive mutant (p.Tyr418Ala), the p.Thr270Ala mutant was not recruited to the cell cortex and failed to induce the cortical actomyosin cytoskeleton enrichment (Figure 3C), supporting the conclusion that its activity was strongly reduced. The deletion mutant (p.Arg913_Glu920del) induced some cortical actomyosin reorganization but appeared to do so less efficiently than WT. In addition, its distribution was more patchy and irregular than WT. Both mutations thus affect the normal subcellular localization of ARHGEF18. Only the WT protein induced a strong stimulation of recruitment of junctional proteins when overexpressed (Figure 3D). The two point mutations (p.Thr270Ala and p.Tyr418Ala) failed to induce a response, and the deletion mutation led to cell rounding but only a weak increase in junctional recruitment of TJ markers alpha-catenin and Catenin delta-1, suggesting that the deletion inhibits the normal cellular activity of ARHGEF18 despite showing normal catalytic activity.

The missense variant p.Thr270Ala resides within the DH domain of ARHGEF18, which is the catalytic domain

required for guanine nucleotide exchange.[26] Thr270 is located within the first alpha-helix of the highly conserved DH domain. This residue is conserved as a threonine or serine in virtually all DH domains throughout nature. In the *C. elegans* Rac GTPase activating protein, UNC-73, serine or threonine at this residue maintain the catalytic activity whereas mutation to alanine abolishes its activity.[26,27] The hydroxyl group of the serine or threonine at this position in the DH domain is thought to mediate GTPase interaction; hence, substitution of Thr270 of ARHGEF18 may inhibit RHOA activation in this way.

The in-frame deletion occurs in exon 16, downstream of the DH and PH module, and does not directly interfere with the catalytic activity. The STK11 binding domain has been mapped to the C-terminal region of the murine Arhgef18 protein encompassing these deleted amino acid residues, and interaction of STK11 and ARHGEF18 is essential in AJ formation.[28] Despite being catalytically active, the in-frame deletion mutant appeared less potent for induction of cortical actomyosin organization than the WT GEF, suggesting that the deletion may indeed affect interactions required for normal cellular ARHGEF18 function, possibly by removing residues required for interactions or for normal folding of the C-terminal domain.

The data indicate that all affected individuals retain some exchange factor activity or native protein. The strong reduction of ARHGEF18 function observed leads to the development of retinal dystrophy in these individuals but heterozygous carriers of LOF mutations are unaffected. The absence of a confirmed biallelic null in the cohort or indeed in the ExAC dataset suggests that complete loss of ARHGEF18 function could be developmentally severe or lethal or may have a more syndromic phenotype. The hypothesis of an embryonic lethal phenotype is supported by the effect of null alleles in medaka fish.[29]

Perturbation of the AB polarity of epithelial cells is recognized in tumorigenesis and cancer progression[17,30,31] but to date, only *CRB1* of the AB polarity complex encoding genes[32] has been implicated in human Mendelian disease. Mutation of *CRB1* causes a wide spectrum of retinal disease including Leber congenital amaurosis (LCA), early-onset retinal dystrophy (EORD), RP, and more recently maculopathy and foveal retinoschisis.[33–36] Age of onset and severity are variable, affected individuals often presenting with early-onset severe loss of vision with characteristic sub-retinal white dots, deep nummular pigmentary lesions, and a thickened, disorganized retina with an undetectable ERG in the most severe cases.[4,37] It is of interest that the three individuals reported here resembled clinically those with *CRB1* retinopathy although the age of onset was later,[4] and this suggests that perturbation of this pathway produces a distinctive human retinal phenotype. The phenotypes of the murine *Crb1* knockout, $Crb1^{-/-}$, and naturally occurring *Rd8* truncating mutant are characterized by disruption of AJ between Müller cells and photoreceptors at the OLM, photoreceptor dysplasia, and consequent focal areas of disorganized lamination and degeneration, although the remaining retina provides functional vision.[22,38,39] The knock-in missense RP mutant Crb^{C249W} has a late-onset degenerative phenotype and can partially rescue the phenotype in $Crb1^{-/-}$ mice.[40]

Mutation of the apical domain essential Crumbs complex proteins *epb41l5* (*moe*), *mpp5a* (*nok*), and *crb2a* (*ome*) in the zebrafish (*mosaic eyes*, *nagie oko*, and *oko meduzy*, respectively) all result in AB polarity defects leading to retinal dystrophy characterized by retinal developmental and lamination abnormalities.[41–45] Similarly, the larval lethal medaka fish retinal differentiation mutant (*medeka* is Japanese for "large eyes") exhibits disorganization of retinal lamination during embryonic development consequent on a LOF mutation resulting in absence of the ArhGEF18 protein product.[29] The phenotype is consequent upon abrogation of ArhGEF18 activity in the developing embryo resulting in disruption of RHOA activation and perturbation of AB polarity characterized by mislocalization of TJ, disorganization of the actin cytoskeleton, and cell proliferation morphology alterations.

The disease mechanism of human *ARHGEF18* retinopathy is not yet fully understood and may include developmental and/or degenerative mechanisms. Disruption of ARHGEF18 function in retinal development seems unlikely, as a severe early-onset retinal dystrophy would be a more probable consequence and our three individuals all experienced normal visual function in early life. A more plausible hypothesis is that photoreceptors are peculiarly sensitive to the failure of AJ maintenance than other cells, causing onset of retinal degeneration in adulthood. Similar clinical features and variable age of onset seen in *CRB1* retinopathy strengthens the assertion that maintenance of this complex is required for continued photoreceptor viability in humans. The phenotypic similarity of ArhGEF18 and crumbs complex protein knockouts in lower vertebrates reflects the similarity of these in humans. Taken together, these observations suggest that other proteins essential in AB polarity maintenance should be regarded as candidate genes in retinal dystrophies. Furthermore, the pathway provides a target for therapeutic intervention with the potential to ameliorate visual impairment due to this type of retinal dystrophy.

Supplemental Data

Supplemental Data include two figures and seven tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.12.014.

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Web Resources

Clustal Omega, http://www.ebi.ac.uk/Tools/msa/clustalo/
ExAC Browser, http://exac.broadinstitute.org/
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
OMIM, http://www.omim.org/
RetNet – Retinal Information Network, https://sph.uth.edu/retnet/home.htm
UniGene, http://www.ncbi.nlm.nih.gov/unigene

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