

# Whole-Exome Sequencing Identifies *KIZ* as a Ciliary Gene Associated with Autosomal-Recessive Rod-Cone Dystrophy

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Rod-cone dystrophy (RCD), also known as retinitis pigmentosa, is a progressive inherited retinal disorder characterized by photoreceptor cell death and genetic heterogeneity. Mutations in many genes have been implicated in the pathophysiology of RCD, but several others remain to be identified. Herein, we applied whole-exome sequencing to a consanguineous family with one subject affected with RCD and identified a homozygous nonsense mutation, c.226C>T (p.Arg76\*), in *KIZ*, which encodes centrosomal protein kizuna. Subsequent Sanger sequencing of 340 unrelated individuals with sporadic and autosomal-recessive RCD identified two other subjects carrying pathogenic variants in *KIZ*: one with the same homozygous nonsense mutation (c.226C>T [p.Arg76\*]) and another with compound-heterozygous mutations c.119\_122delAACT (p.Lys40Ilefs\*14) and c.52G>T (p.Glu18\*). Transcriptomic analysis in mice detected mRNA levels of the mouse ortholog (*Plk1s1*) in rod photoreceptors, as well as its decreased expression when photoreceptors degenerated in *rd1* mice. The presence of the human *KIZ* transcript was confirmed by quantitative RT-PCR in the retina, the retinal pigment epithelium, fibroblasts, and whole-blood cells (highest expression was in the retina). RNA in situ hybridization demonstrated the presence of *Plk1s1* mRNA in the outer nuclear layer of the mouse retina. Immunohistology revealed *KIZ* localization at the basal body of the cilia in human fibroblasts, thus shedding light on another ciliary protein implicated in autosomal-recessive RCD.

Rod-cone dystrophy (RCD), also known as retinitis pigmentosa (MIM 268000), is a heterogeneous group of inherited retinal disorders affecting rod photoreceptors in the majority of cases and causing secondary cone degeneration.<sup>1</sup> Population-based studies have indicated that there are one million affected individuals worldwide.<sup>1</sup> Subjects diagnosed with RCD initially complain of night blindness due to rod dysfunction, as well as subsequent progressive constriction of their visual field, abnormal color vision, and eventually loss of central vision due to cone photoreceptor involvement.<sup>1</sup> RCD is inherited as a Mendelian trait in most cases; 30%–40% is autosomal dominant, 50%–60% is autosomal recessive, and 5%–15% is X-linked.<sup>1</sup>

Candidate-gene approaches—for example, those comparing human phenotypes to similar phenotypes observed in animal models—are widely used for identifying gene defects leading to inherited retinal diseases.<sup>2–5</sup> However, with the emergence of massively parallel sequencing techniques, considerable efforts are now being made to report known and novel genes implicated in the pathophysiology of inherited retinal disease.<sup>6–9</sup> In the present study, we applied whole-exome sequencing (WES) to five members (including one affected by RCD) of a consanguineous

family (family A [128], Figure 1) to identify the underlying gene defect. We detected a homozygous nonsense mutation, c.226C>T (p.Arg76\*), in the third exon of *KIZ*, coding for centrosomal protein kizuna (*KIZ*).

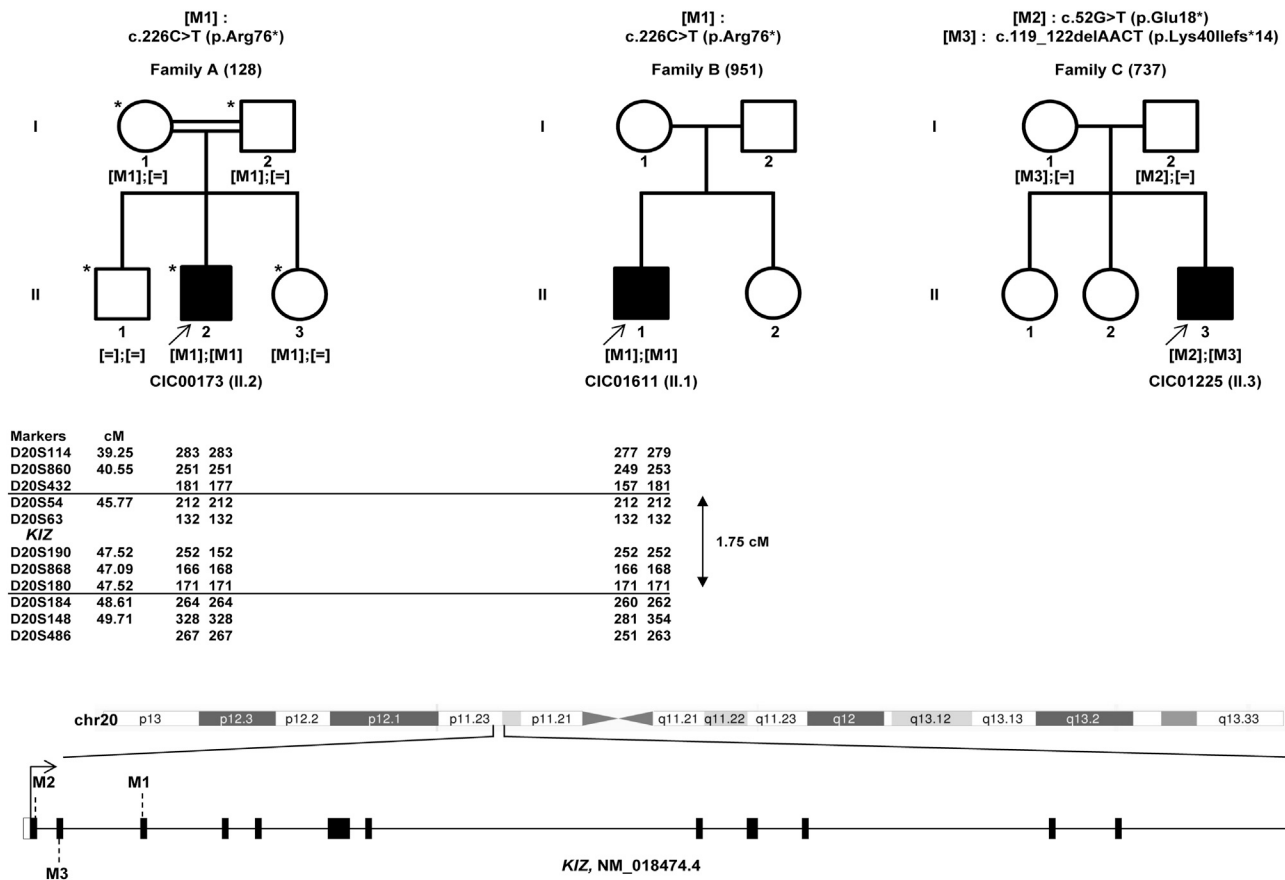
The study protocol was conducted in accordance with the Declaration of Helsinki, national guidelines, and the regional ethics committee. Prior to testing and after explanation of the study and its potential outcome, informed consent was obtained from RCD subjects and their family members. Each subject underwent an ophthalmic examination with clinical assessment as previously described.<sup>10</sup> At a time when next-generation sequencing (NGS) approaches were not commonly used, index subject CIC00173 II.2 (family A, Figure 1) was excluded upon screening by microarray analysis and direct Sanger sequencing for known mutations in *EYS* and *C2orf71* (a major and a minor gene, respectively, implicated in RCD).<sup>11,12</sup> Because exon ORF15 in *RPGR* (MIM 312610) is not targeted by existing NGS panels,<sup>13</sup> we also analyzed it by Sanger sequencing but found no pathogenic variant. Subsequently, we performed targeted NGS on the index subject's DNA by using a panel of 120 genes previously found to carry mutations in retinal diseases (this panel

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**Figure 1. Identification of Homozygous and Compound-Heterozygous *KIZ* Variants in Three Unrelated Families Affected by Autosomal-Recessive RCD**

Family A (left), family B (middle), and family C (right) were analyzed in the present study. Individuals highlighted with an asterisk were screened with WES. Affected individuals are marked with an arrow. A homozygous nonsense mutation, c.226C>T (p.Arg76\*), in *KIZ* was identified in the affected index subject from family A and cosegregated with the phenotype. Subsequent direct Sanger sequencing of *KIZ* in 340 individuals with autosomal-recessive RCD identified two other subjects with *KIZ* mutations: the affected index subject in family B had the homozygous c.226C>T (p.Arg76\*) mutation, and the affected index subject in family C had compound-heterozygous mutations c.52G>T (p.Glu18\*) and c.119\_122delAACT (p.Lys40Ilefs\*14). Haplotype analysis performed for investigating whether the c.226C>T (p.Arg76\*) variant represents a founder mutation in families A and B is shown under each pedigree symbol. Microsatellite marker locations according to Marshfield genetic maps (Marshfield Laboratories) are shown in cM. Numbers indicate the allele size in nucleotides for each microsatellite. Both subjects were found to share a common haplotype of five polymorphic microsatellites (i.e., DS20S54, DS20S63, DS20S190, DS20S868, and DS20S180; delineated by two horizontal lines) flanking *KIZ* and spanning  $\approx 1.75$  cM (1.18 Mb). Filled and unfilled symbols indicate affected and unaffected status, respectively. Square boxes indicate males, and circles indicate females. In the schematic representation of the structure of *KIZ* (RefSeq NM\_018474.4; harboring 13 exons), filled and unfilled boxes represent coding and noncoding exonic regions, respectively. M1, M2, and M3 depict the positions of the mutations identified in the current study.

was modified and improved since our previous study).<sup>13</sup> This survey did not reveal any pathogenic variant, and we therefore proceeded to WES.

Exons of DNA samples were captured and investigated as reported before with in-solution enrichment methodology (SureSelect Human All Exon Kits version 3, Agilent) and NGS (Illumina HiSeq, Illumina). Image analysis and base calling were performed with real-time analysis software (Illumina).<sup>6,9,13</sup> Bioinformatic analysis of sequencing data was based on a pipeline (Consensus Assessment of Sequence and Variation 1.8, Illumina) that performs alignment, variant calling (single-nucleotide variants [SNVs] and indels), and coverage analysis. Annotation of genetic variation was done by an in-house pipeline (IntegraGen). To rapidly identify the pathogenic variant, we applied

WES to all five members of the consanguineous family A (Figure 1). For all subjects, the overall sequencing coverage of the captured regions was 94% and 85% for a 10 $\times$  and 25 $\times$  depth of coverage, respectively, resulting in a mean sequencing depth of 80 $\times$  per base.

Filtering approaches were subsequently applied for identification of candidate mutation(s). Referenced variants that occurred homozygously or heterozygously with a minor allele frequency (MAF)  $\geq 0.005$  in dbSNP137, HapMap,<sup>14</sup> 1000 Genomes,<sup>15</sup> and the NHLBI Exome Sequencing Project Exome Variant Server (EVS)<sup>16</sup> were removed.<sup>6,7,9</sup> This step reduced the number of variants from 4,572 to 0 indels and from 55,051 SNVs to two compound-heterozygous missense variants in *OBSCN* (MIM 608616), one homozygous missense variant in four

**Table 1. KIZ Mutations Causing RCD**

Index individual	Family	Exon	Nucleotide Exchange <sup>a</sup>	Allele State	Protein Effect
CIC00173 (II.2)	A (128)	3	c.226C>T	homozygous	p.Arg76*
CIC01611 (II.1)	B (951)	3	c.226C>T	homozygous	p.Arg76*
CIC01225 (II.1)	C (737)	1	c.52G>T	heterozygous	p.Glu18*
		2	c.119_122delAACT	heterozygous	p.Lys40lefs*14

<sup>a</sup>RefSeq NM\_018474.4.

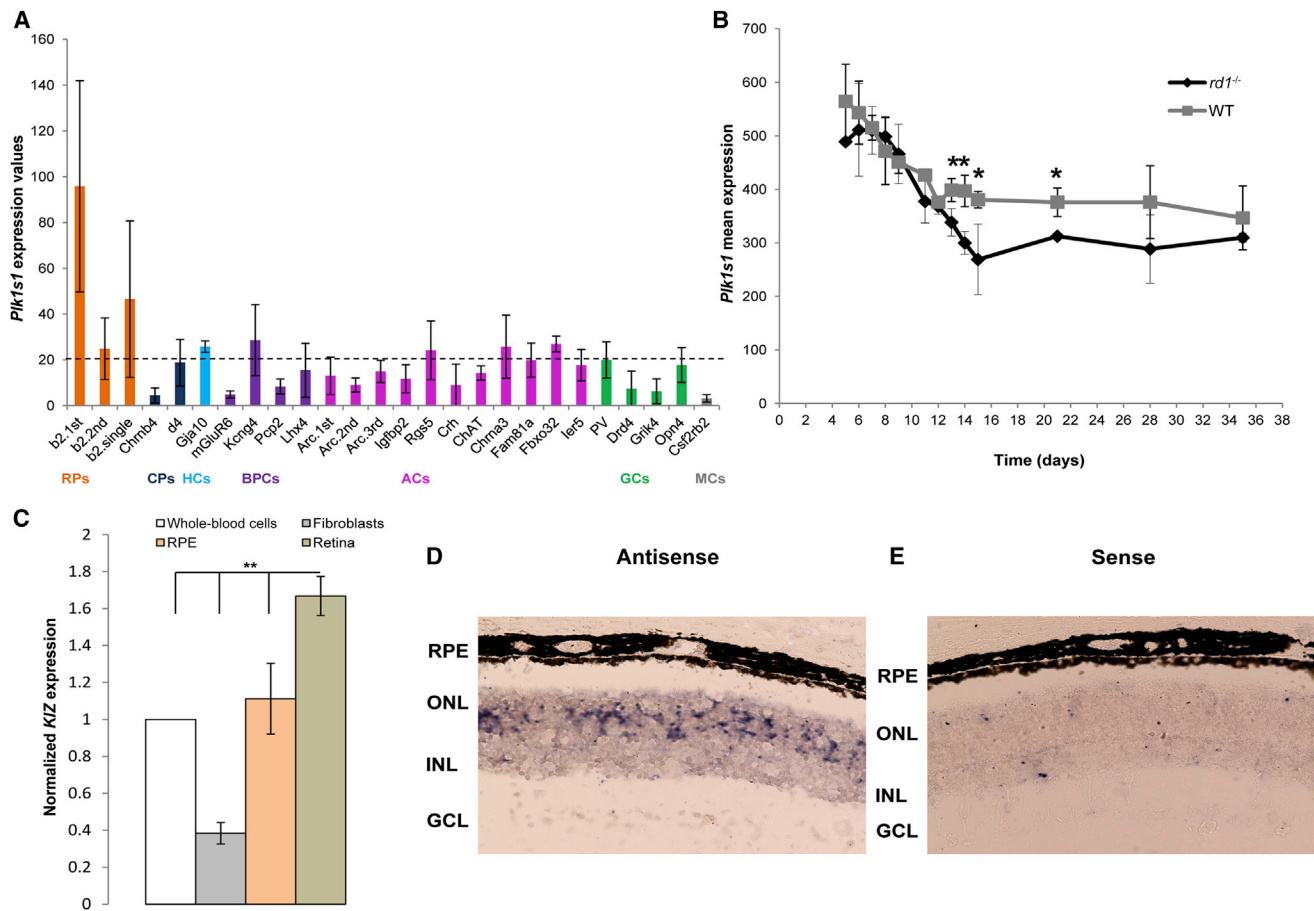
different genes (*CTNNA3* [MIM 607667], *PRRX2* [MIM 604675], *UNCX*, and *PTCD3* [MIM 614918]), and one nonsense exchange in *KIZ*. Sanger sequencing confirmed all variants except the *UNCX* mutation, which turned out to be a false positive. In order to identify a potential disease-causing effect of missense substitutions, we investigated their species conservation and their predicted impact on the protein structure.<sup>17,18</sup> All missense variants, except the ones in *OBSCN*, were excluded after consideration of the previously mentioned characteristics. The compound-heterozygous mutations in *OBSCN* were absent in genetic public databases, conserved across species, and predicted to be probably damaging and deleterious by PolyPhen-2 and SIFT. However, given that consanguinity among parents was reported for family A (Figure 1), homozygous variants represent the most likely candidate, although this does not totally exclude underlying causative compound-heterozygous mutations (K.M. Bujakowska et al., 2011, ARVO, abstract).

The homozygous nonsense mutation (c.226C>T [p.Arg76\*]; RefSeq accession number NM\_018474.4) located in exon 3 of *KIZ* cosegregated with the phenotype (family A in Figure 1 and Table 1). It represents a rare variant (rs202210819) that was detected heterozygously in 5 out of 5,920 individuals in the NHLBI EVS (MAF = 0.0004 in European Americans exclusively because it was not found among African Americans). After our discrete filtering approach, which identified candidate mutations in *KIZ* and *OBSCN*, we performed stratification on the basis of functional impact and gave a greater weight to the likelihood that the homozygous stop codon in *KIZ* was the most deleterious.

To further evaluate which of the two genes might be the most likely to carry the pathogenic variants underlying autosomal-recessive RCD, we assessed genetic expression of both genes. Ubiquitous expression of *KIZ*, including in the eye, was found in UniGene. The *KIZ* mouse ortholog (*Plk1s1*, also known as *Gm114*) showed higher expression in rod photoreceptors of the retinal-cell-type comparative transcriptome atlas<sup>20</sup> than in cone photoreceptors and horizontal, bipolar, amacrine, ganglion, and microglia cells (Figure 2A).<sup>20</sup> The in-house *rd1* mouse transcriptomic database revealed a significant decrease in the *Plk1s1* mRNA level from day 12 to day 21 ( $p < 0.05$ ) when photoreceptors degenerated, which was in keeping with rod photoreceptor expression (Figure 2B). In addition, Strunni-

kova et al.<sup>21</sup> reported *KIZ* expression in the human retinal pigment epithelium (RPE).<sup>21</sup> Similarly to *KIZ*, *OBSCN* was found to be ubiquitously expressed in UniGene. In contrast, mRNA expression was not reported in the mouse retinal-cell-type comparative transcriptome atlas.<sup>20</sup> Furthermore, the in-house *rd1* mouse transcriptomic database reported no changes in *Obecn* mRNA levels during photoreceptor degeneration (data available upon request). To support transcriptomic database results and further document the implication of *KIZ* in retinal physiology, we performed quantitative real-time PCR, which revealed that the *KIZ* transcript was most abundant in the retina, followed by the RPE, whole-blood cells, and fibroblasts ( $p \leq 0.01$ , Figure 2C). Subsequent Sanger sequencing of the PCR products confirmed correct *KIZ*-fragment amplification. Because transcriptomic data were only reported in the mouse retina at postnatal day 7 and there was no available information on expression location,<sup>22</sup> we performed RNA in situ hybridization in the adult mouse retina as previously described<sup>23,24</sup> by using a riboprobe encompassing exons 8–14 of *Plk1s1* mRNA (RefSeq NM\_001033298.3). *Plk1s1* was found to be expressed in the outer nuclear layer, corresponding to photoreceptor nuclei, in the mouse retina (Figure 2D and 2E). All together, these results support our hypothesis that the nonsense variant in *KIZ* is the most convincing underlying defect for RCD in subject II.2 of family A (Figure 1).

Further screening of coding and flanking exonic regions of *KIZ* in 340 unrelated individuals with autosomal-recessive and sporadic RCD by direct Sanger sequencing (PCR protocol and primer sequences are available upon request) identified two other subjects with mutations in this gene. Interestingly, another subject (CIC01611 II.1 in family B [951]) had the same nonsense variant (homozygous c.226C>T [p.Arg76\*]) found in subject CIC00173 II.2 (Figure 1). Subject CIC00173 was of North African Sephardic Jewish ancestry, and CIC01611 was of Spanish ancestry, and neither was aware of any family connection. To investigate whether the stop variant represents a founder alteration, we performed haplotype analysis for each of the index subjects (CIC00173 II.2 from family A) and CIC01611 II.1 from family B) by selecting 11 microsatellite DNA markers flanking the *KIZ* locus.<sup>25</sup> These markers were distributed over a physical distance of  $\approx 7.38$  Mb, corresponding to  $\approx 10.5$  cM (genetic distance according to Marshfield genetic maps). Both subjects were found to



**Figure 2. *Pik1s1* Transcriptional Analysis in the Mouse Retina**

(A) *Pik1s1* expression (1455558\_at) in six different cell types from the mouse adult retina: rod photoreceptors (RPs), cone photoreceptors (CPs), horizontal cells (HCs), bipolar cells (BPCs), amacrine cells (ACs), ganglion cells (GCs), and microglia cells (MCs). The graph presents *Pik1s1* normalized expression values. Only values higher than 20 can be considered significantly expressed. Retinal cell types were established from a library composed of 22 transgenic mouse lines.<sup>20</sup> Each value on the x axis corresponds to a specific retinal cell type established from the following abbreviated mouse lines: RPs (b2), CPs (Chnrb4 and d4), HCs (Gja10), BPCs (mGluR6, Kcng4, Pcp2, and Lhx4), ACs (Arc, Igfbp2, Rgs5, Crh, ChAT, Chrna3, Fam81a, Fbxo32, and Ier5), GCs (Pv, Drd4, Grik4, and Opn4), and MCs (Csf2rb2). (According to Siegert et al.,<sup>20</sup> RNA amplification was performed in different batches. For avoiding differences caused by variable amplification across batches, RNA samples of cell groups belonging to the same biological triplicate were amplified in different batches. As such, “1<sup>st</sup>,” “2<sup>nd</sup>,” “single,” and “3<sup>rd</sup>” correspond to the batch numbers.) *Pik1s1*, implicated in autosomal-recessive RCD, showed the highest expression in RPs.

(B) *Pik1s1* expression (1455558\_at) in *rd1* and wild-type mice during RP degeneration. The *rd1* mouse, carrying *Pde6b* mutations, is a naturally occurring RCD model leading to a complete loss of RPs by postnatal day 36 and a preserved inner retina. cDNAs of neural retinas from *rd1* and wild-type mice on identical genetic backgrounds were hybridized to the mouse genome 430 2.0 array (Affymetrix). \**p* < 0.05.

(C) *KIZ* expression in four human tissues: retina, retinal pigment epithelium (RPE), fibroblasts, and whole-blood cells. Quantitative real-time PCR, normalized to the expression of *18S*, revealed that *KIZ* had higher expression in the retina than in the RPE, whole-blood cells, and fibroblasts (*n* = 3, \*\**p* ≤ 0.01).

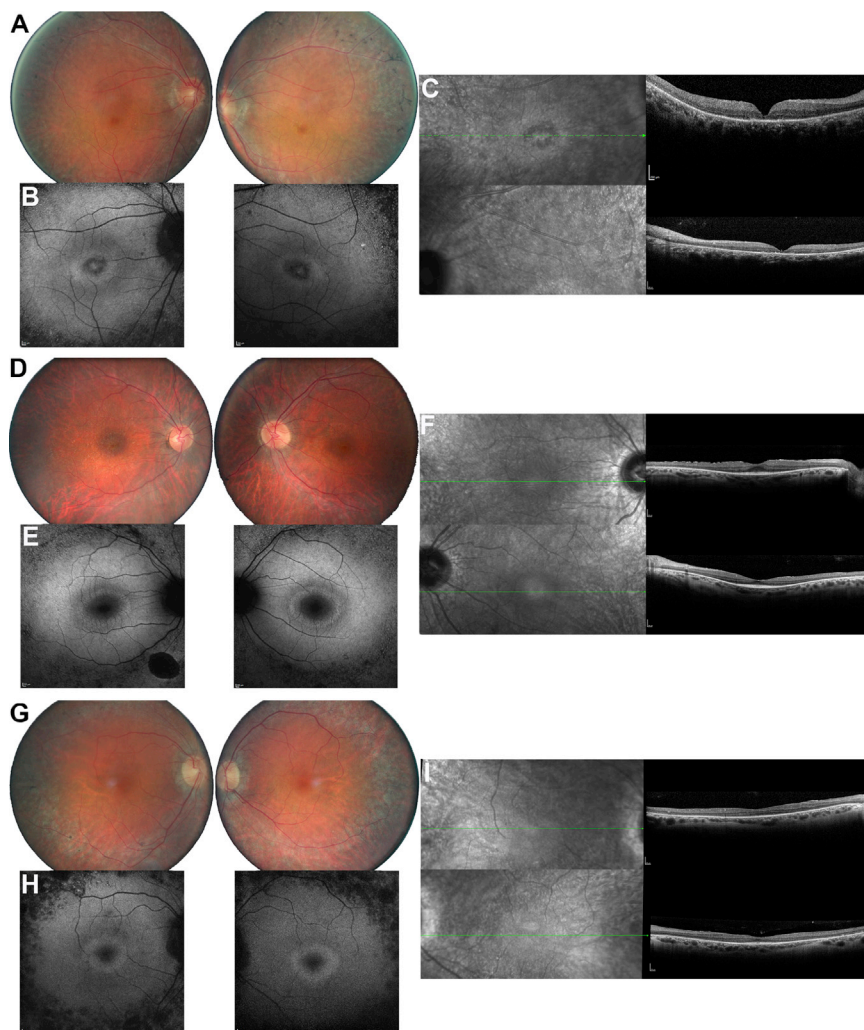
(D and E) An RNA in situ hybridization assay for *Pik1s1* expression in the mouse retina. A riboprobe encompassing exons 8–14 of mouse *Pik1s1* mRNA (RefSeq NM\_001033298.3) was used. Antisense (D) and sense (E) probes are shown. Abbreviations are as follows: GCL, ganglion cell layer; INL, inner nuclear layer; ONL: outer nuclear layer; and RPE, retinal pigment epithelium.

share a common haplotype of five polymorphic microsatellites (DS20S54, DS20S63, DS20S190, DS20S868, and DS20S180) flanking *KIZ* and spanning ≈1.75 cM (1.18 Mb) (Figure 1). This result suggests that c.226C>T (p.Arg76\*) is most likely a founder mutation causing autosomal-recessive RCD in the southern European population.

C1C01225 II.3 in family C (737), an additional individual with sporadic RCD, carried compound-heterozygous

mutations: nonsense mutation c.52G>T (p.Glu18\*) in exon 1 and deletion c.119\_122delAACT (p.Lys40Ilefs\*14) in exon 3 (Table 1). Cosegregation analysis revealed that the father was heterozygous for the nonsense mutation and the mother was heterozygous for the frameshift deletion (Figure 1). The identified defects most likely result in nonsense-mediated mRNA decay or truncated *KIZ* with a loss of function as a putative disease mechanism. Additional *KIZ* polymorphisms identified from screening the





**Figure 3. Retinal Imaging of Autosomal-Recessive-RCD Individuals Carrying *KIZ* Variants**

(A–C) Fundus imaging of the right and left eye of CIC00173 from family A at 50 years of age. Fundus color photographs (FCPs; A), fundus autofluorescence (FAF; B), and macular horizontal scans of spectral domain optical coherence tomography (SD-OCT; C) show not only pigmentary changes in the peripheral retina but also atrophic changes in the central macula with a ring of hypoautofluorescence and foveal thinning with loss of outer-segment structures on SD-OCT.

(D–F) Fundus imaging of the right and left eye of CIC01611 from family B at 34 years of age. FCPs (D), FAF (E), and SD-OCT (F) show mild pigmentary changes in the peripheral retina in association with slight changes in FAF outside the vascular arcades and a perifoveal ring of increased autofluorescence (larger than observed for CIC01225), as well as normal foveal structure on OCT in keeping with normal central vision.

(G–I) Fundus imaging of the right and left eye of CIC01225 from family C at 51 years of age. FCPs (G), FAF (H), and SD-OCT (I) show pigmentary changes in the peripheral retina in association with a loss of FAF outside the vascular arcades and a perifoveal ring of increased autofluorescence and moderate thinning of the fovea on OCT.

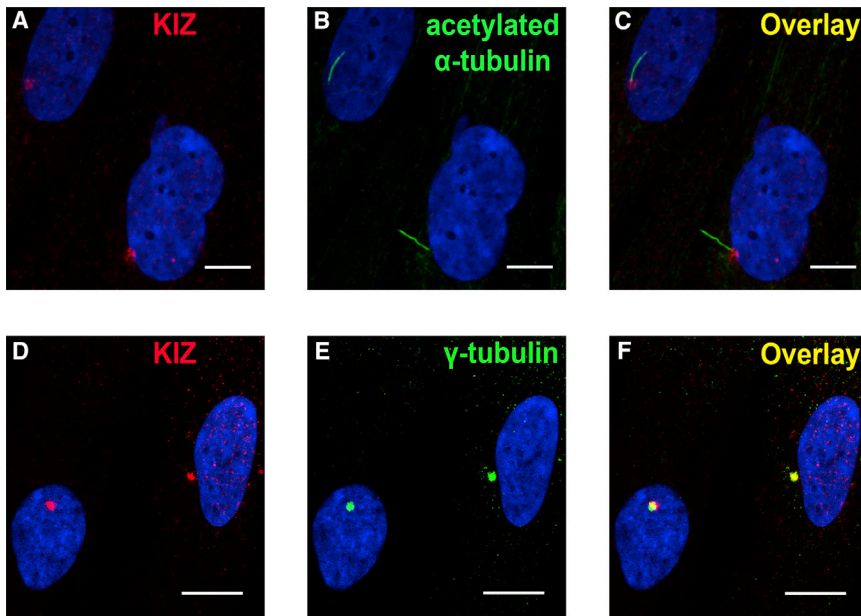
RCD cohort and their respective frequencies are provided in [Table S1](#).

Overall, *KIZ* mutations in the studied cohort would account for about 1% of autosomal-recessive RCD. This might be a slight overestimation given that a majority of the 340 affected individuals included in this work had already been investigated and excluded for carrying defects in known genes implicated in retinal diseases.

The three affected individuals with *KIZ* variants in this study were all diagnosed with RCD in their late teens on the basis of night blindness followed by changes in mid-peripheral visual fields and undetectable responses in a full-field electroretinogram by approximately 35 years of age. Index II.2 of family A (CIC00173, [Figure 1](#)) was a 50-year-old male subject of North African Jewish Sephardic descent and had unaffected first-cousin parents. The index subject was overweight and complained of moderate hearing difficulties. Best-corrected visual acuity (BCVA) was 20/800 in the right eye and 20/640 in the left eye. A kinetic visual-field test revealed decreased central retinal sensitivity in addition to bilateral peripheral-field constriction. Fundus changes were typical of RCD with additional macular thinning ([Figures 3A–3C](#)). Index II.1 of family B

(CIC01611, [Figure 1](#)) was a 34-year-old subject of Spanish descent. His medical and familial history was noncontributory. BCVA was 20/20 in both eyes. A binocular kinetic visual field using the III4e stimulus showed an annular scotoma in the midperiphery and preservation of the peripheral isopter. Fundus changes were typical of RCD with macular preservation ([Figures 3D–3F](#)). Index II.3 of family C (CIC01225, [Figure 1](#)) was a 51-year-old male subject of a mixed Italian and French descent. His history was significant for a congenital ichthyosis that was well tolerated. There was no familial history of systemic or ocular disease. BCVA was 20/40 in the right eye and 20/32 in the left. A binocular kinetic visual field using the III4e stimulus was reduced to the central 10° with bitemporal islands of perception peripherally. Fundus changes were typical of RCD with relative macular preservation ([Figures 3G–3I](#)).

*KIZ* is a 12 kb gene clustering on the short arm of chromosome 20 at 20p11.23 ([Figure 1](#)). It harbors 13 exons and encodes a 673 aa protein that belongs to the kizuna family ([Figure 1](#)). It plays a critical role during cell-cycle progression while it undergoes sequential phosphorylation.<sup>26,27</sup> Oshimori et al.<sup>27</sup> demonstrated that *KIZ* is a centrosomal substrate for PLK1, given that the latter mediates its phosphorylation at amino acid residue Thr379. During



#### Figure 4. Subcellular Localization of KIZ in Monocilia from Human Fibroblasts

Primary fibroblasts derived from human skin biopsies were cultured via standard conditions. To induce formation of monocilia in human fibroblasts, we deprived cell culture from fetal bovine serum for 24 hr. Serum-starved fibroblasts were fixed 5 min with methanol at  $-20^{\circ}\text{C}$ , and immunofluorescence staining was performed as previously described.<sup>31</sup> Human fibroblasts were immunolabeled with a KIZ antibody (PLK1S1 rabbit-polyclonal antibody, 1/250, Sigma Aldrich SAB2700541; A, C, D, and F), an acetyl  $\alpha$ -tubulin mouse-monoclonal antibody (1/1,000, Sigma-Aldrich; B and C), and a  $\gamma$ -tubulin mouse-monoclonal antibody (1/1,000, Sigma-Aldrich; E and F), visualized with standard secondary antibodies, and counterstained with nuclear DAPI (blue, 1/1,000, Euromedex, Souffelweyersheim). The overlay view in (C) shows KIZ localization (A, red) at the bottom of the cilia (B, green) and colocalization (F) of KIZ (D, red) to the basal body of the cilium-associated centriole (E, green). Scale bars represent 20  $\mu\text{m}$ .

mitosis, KIZ localizes to mature centrioles and interacts with PLK1 in order to protect the centrosome from collapsing during spindle formation in a Thr379-phosphorylation-dependent manner.<sup>27</sup> Stabilized centrosomes resist microtubule-mediated pulling and pushing forces and thus ensure the spindle bipolarity required for accurate separation of chromosomes during mitosis.<sup>28,29</sup> At the plasma membrane, the mother centriole can serve as the basal body where ciliogenesis begins, thus giving rise to either motile or immotile cilia, which exist on most cells in the human body.<sup>30</sup> Ciliogenesis can be either linked to the cell cycle or associated with cell differentiation.<sup>30</sup> To investigate a potential association between KIZ and cilia in humans, we induced cilia formation in a serum-free human fibroblast cell culture as described elsewhere.<sup>31</sup> Colocalization with a cilium marker (acetylated  $\alpha$ -tubulin) (Figures 4A–4C) and a basal body marker ( $\gamma$ -tubulin) (Figures 4D–4F) demonstrated that KIZ (SAB2700541, Sigma Aldrich) localizes to the basal body of monocilia in human fibroblasts. This might suggest its location at the connecting cilium of photoreceptors. Interestingly, in the in-house *rd1* mouse transcriptomic database, *Rab28*, which encodes a protein localized at the connecting cilium of photoreceptors, shows a profile similar to that of KIZ. In rod and cone photoreceptors, the connecting cilium bridges the inner segment to the outer segment, which harbors the photopigment.<sup>32</sup> All components necessary for assembly, maintenance, and continuous turnover of the outer segments are synthesized in the cell body and are moved through the connecting cilium. KIZ might have a critical role in this trafficking process, and its dysfunction or absence would compromise molecular transport and therefore outer-segment homeostasis. Future studies are needed for

documenting this potential role for KIZ in human and mouse retinas.

An increasing number of Mendelian and complex diseases are associated with defects in ciliogenesis.<sup>29,33</sup> These comprise a diverse group of pathologies, including isolated or syndromic retinal degeneration (e.g., Bardet-Biedl syndrome), cystic kidney disease, infertility, chronic respiratory problems, hypertension, and obesity.<sup>29,34</sup> To date, mutations in at least 24 ciliary genes have been implicated in retinal degenerations, such as RCD, cone dystrophy, cone-rod dystrophy, Leber congenital amaurosis, and macular degeneration.<sup>35–39</sup> For the majority of mutations, a clear genotype-phenotype correlation cannot be established, given that mutations in the same ciliary gene can cause both syndromic and nonsyndromic forms.<sup>37</sup> However, retina-restricted disease was reported for mutations in autosomal-recessive RCD (in *C2ORF71* [MIM 613425],<sup>35,40</sup> *FAM161A* [MIM 613596],<sup>41,42</sup> *MAK* [MIM 154235],<sup>43,44</sup> *RP1* [MIM 603937],<sup>45</sup> and *ARL2BP* [MIM 615407]<sup>36</sup>), in X-linked RCD (in *RPGR* and *RP2* [MIM 300757]<sup>46,47</sup>), and in autosomal-dominant RCD (in *RP1* [MIM 603937]<sup>45</sup> and *TOPORS* [MIM 609507]<sup>48,49</sup>). The most common inheritance patterns are autosomal recessive and X-linked.<sup>37</sup>

In conclusion, our study has identified KIZ mutations leading to autosomal-recessive RCD. We speculate that these defects are loss-of-function alleles, are sensitive to nonsense-mediated mRNA decay, or result in a truncated KIZ in photoreceptors and thus lead to a defect in photoreceptor-connecting cilia.<sup>32</sup> The data presented here suggest a role for KIZ in photoreceptor homeostasis. However, further functional studies are needed for elucidating the exact role of KIZ in the retina and the underlying pathogenic mechanism(s).

## Supplemental Data

Supplemental Data include one table and can be found with this article online at <http://www.cell.com/ajhg>.

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## Web Resources

The URLs for data presented herein are as follows:

GeneCards, <http://www.genecards.org/>

Human Gene Mutation Database (HGMD), <http://www.hgmd.org/>

Leiden Open Variation Database (LOVD), <http://www.lovd.nl/3.0/home>

Mammalian Genotyping Service, Marshfield genetic maps, <http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp>

NCBI, <http://www.ncbi.nlm.nih.gov/>

NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/EVS/>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>

RefSeq, <http://www.ncbi.nlm.nih.gov/RefSeq>

Roska, <http://fmi.ch/roska.data/>

SIFT, <http://sift.bii.a-star.edu.sg/>

UCSC Genome Browser, <http://genome.ucsc.edu/>

UniGene, <http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.187635>

UniProt, <http://www.uniprot.org/>

## References

- Hartong, D.T., Berson, E.L., and Dryja, T.P. (2006). Retinitis pigmentosa. *Lancet* 368, 1795–1809.
- Audo, I., Kohl, S., Leroy, B.P., Munier, F.L., Guillonnet, X., Mohand-Saïd, S., Bujakowska, K., Nandrot, E.F., Lorenz, B., Preising, M., et al. (2009). TRPM1 is mutated in patients with autosomal-recessive complete congenital stationary night blindness. *Am. J. Hum. Genet.* 85, 720–729.
- Zeit, C., van Genderen, M., Neidhardt, J., Luhmann, U.F., Hoeben, F., Forster, U., Wycisk, K., Mátyás, G., Hoyng, C.B., Riemsdag, F., et al. (2005). Mutations in GRM6 cause autosomal recessive congenital stationary night blindness with a distinctive scotopic 15-Hz flicker electroretinogram. *Invest. Ophthalmol. Vis. Sci.* 46, 4328–4335.
- Wycisk, K.A., Zeit, C., Feil, S., Wittmer, M., Forster, U., Neidhardt, J., Wissinger, B., Zrenner, E., Wilke, R., Kohl, S., and Berger, W. (2006). Mutation in the auxiliary calcium-channel subunit CACNA2D4 causes autosomal recessive cone dystrophy. *Am. J. Hum. Genet.* 79, 973–977.
- Zeit, C., Kloeckener-Gruissem, B., Forster, U., Kohl, S., Magyar, I., Wissinger, B., Mátyás, G., Borruat, F.X., Schorderet, D.F., Zrenner, E., et al. (2006). Mutations in CABP4, the gene encoding the Ca<sup>2+</sup>-binding protein 4, cause autosomal recessive night blindness. *Am. J. Hum. Genet.* 79, 657–667.
- Audo, I., Bujakowska, K., Orhan, E., El Shamieh, S., Sennlaub, F., Guillonnet, X., Antonio, A., Michiels, C., Lancelot, M.E., Letexier, M., et al. (2014). The familial dementia gene revisited: a missense mutation revealed by whole-exome sequencing identifies ITM2B as a candidate gene underlying a novel autosomal dominant retinal dystrophy in a large family. *Hum. Mol. Genet.* 23, 491–501.
- Audo, I., Bujakowska, K., Orhan, E., Poloschek, C.M., Defoort-Dhellemmes, S., Drumare, I., Kohl, S., Luu, T.D., Lecomte, O., Zrenner, E., et al. (2012). Whole-exome sequencing identifies mutations in GPR179 leading to autosomal-recessive complete congenital stationary night blindness. *Am. J. Hum. Genet.* 90, 321–330.
- Siemiatkowska, A.M., van den Born, L.I., van Hagen, P.M., Stoffels, M., Neveling, K., Henkes, A., Kipping-Geertsema, M., Hoefsloot, L.H., Hoyng, C.B., Simon, A., et al. (2013). Mutations in the mevalonate kinase (MVK) gene cause non-syndromic retinitis pigmentosa. *Ophthalmology* 120, 2697–2705.
- Zeit, C., Jacobson, S.G., Hamel, C.P., Bujakowska, K., Neuillé, M., Orhan, E., Zanlonghi, X., Lancelot, M.E., Michiels, C., Schwartz, S.B., et al.; Congenital Stationary Night Blindness Consortium (2013). Whole-exome sequencing identifies LRIT3 mutations as a cause of autosomal-recessive complete congenital stationary night blindness. *Am. J. Hum. Genet.* 92, 67–75.
- Audo, I., Manes, G., Mohand-Saïd, S., Friedrich, A., Lancelot, M.E., Antonio, A., Moskova-Doumanova, V., Poch, O., Zanlonghi, X., Hamel, C.P., et al. (2010). Spectrum of rhodopsin mutations in French autosomal dominant rod-cone dystrophy patients. *Invest. Ophthalmol. Vis. Sci.* 51, 3687–3700.
- Audo, I., Sahel, J.A., Mohand-Saïd, S., Lancelot, M.E., Antonio, A., Moskova-Doumanova, V., Nandrot, E.F., Doumanov, J., Barragan, I., Antinolo, G., et al. (2010). EYS is a major gene for rod-cone dystrophies in France. *Hum. Mutat.* 31, E1406–E1435.
- Audo, I., Lancelot, M.E., Mohand-Saïd, S., Antonio, A., Germain, A., Sahel, J.A., Bhattacharya, S.S., and Zeit, C. (2011). Novel C2orf71 mutations account for ~1% of cases in a large French arRP cohort. *Hum. Mutat.* 32, E2091–E2103.
- Audo, I., Bujakowska, K.M., Léveillard, T., Mohand-Saïd, S., Lancelot, M.E., Germain, A., Antonio, A., Michiels, C., Saraiva, J.P., Letexier, M., et al. (2012). Development and application of a next-generation-sequencing (NGS) approach to detect known and novel gene defects underlying retinal diseases. *Orphanet J. Rare Dis.* 7, 8.
- Altshuler, D.M., Gibbs, R.A., Peltonen, L., Altshuler, D.M., Gibbs, R.A., Peltonen, L., Dermitzakis, E., Schaffner, S.F., Yu, F., Peltonen, L., et al.; International HapMap 3 Consortium



- (2010). Integrating common and rare genetic variation in diverse human populations. *Nature* 467, 52–58.
15. Abecasis, G.R., Altshuler, D., Auton, A., Brooks, L.D., Durbin, R.M., Gibbs, R.A., Hurles, M.E., and McVean, G.A.; 1000 Genomes Project Consortium (2010). A map of human genome variation from population-scale sequencing. *Nature* 467, 1061–1073.
  16. Tennessen, J.A., Bigham, A.W., O'Connor, T.D., Fu, W., Kenny, E.E., Gravel, S., McGee, S., Do, R., Liu, X., Jun, G., et al.; Broad GO; Seattle GO; NHLBI Exome Sequencing Project (2012). Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science* 337, 64–69.
  17. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R. (2010). A method and server for predicting damaging missense mutations. *Nat. Methods* 7, 248–249.
  18. Kumar, P., Henikoff, S., and Ng, P.C. (2009). Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat. Protoc.* 4, 1073–1081.
  20. Siegert, S., Cabuy, E., Scherf, B.G., Kohler, H., Panda, S., Le, Y.Z., Fehling, H.J., Gaidatzis, D., Stadler, M.B., and Roska, B. (2012). Transcriptional code and disease map for adult retinal cell types. *Nat. Neurosci.* 15, 487–495, S1–S2.
  21. Strunnikova, N.V., Maminishkis, A., Barb, J.J., Wang, F., Zhi, C., Sergeev, Y., Chen, W., Edwards, A.O., Stambolian, D., Abecasis, G., et al. (2010). Transcriptome analysis and molecular signature of human retinal pigment epithelium. *Hum. Mol. Genet.* 19, 2468–2486.
  22. Magdaleno, S., Jensen, P., Brumwell, C.L., Seal, A., Lehman, K., Asbury, A., Cheung, T., Cornelius, T., Batten, D.M., Eden, C., et al. (2006). BGEM: an in situ hybridization database of gene expression in the embryonic and adult mouse nervous system. *PLoS Biol.* 4, e86.
  23. Di Meglio, T., Nguyen-Ba-Charvet, K.T., Tessier-Lavigne, M., Sotelo, C., and Chédotal, A. (2008). Molecular mechanisms controlling midline crossing by precerebellar neurons. *J. Neurosci.* 28, 6285–6294.
  24. Orhan, E., Prézéau, L., El Shamieh, S., Bujakowska, K.M., Michiels, C., Zagar, Y., Vol, C., Bhattacharya, S.S., Sahel, J.A., Sennlaub, F., et al. (2013). Further insights into GPR179: expression, localization, and associated pathogenic mechanisms leading to complete congenital stationary night blindness. *Invest. Ophthalmol. Vis. Sci.* 54, 8041–8050.
  25. Goldstein, D.B., Ruiz Linares, A., Cavalli-Sforza, L.L., and Feldman, M.W. (1995). An evaluation of genetic distances for use with microsatellite loci. *Genetics* 139, 463–471.
  26. Dephoure, N., Zhou, C., Villén, J., Beausoleil, S.A., Bakalarski, C.E., Elledge, S.J., and Gygi, S.P. (2008). A quantitative atlas of mitotic phosphorylation. *Proc. Natl. Acad. Sci. USA* 105, 10762–10767.
  27. Oshimori, N., Ohsugi, M., and Yamamoto, T. (2006). The Plk1 target Kizuna stabilizes mitotic centrosomes to ensure spindle bipolarity. *Nat. Cell Biol.* 8, 1095–1101.
  28. Bettencourt-Dias, M., and Glover, D.M. (2007). Centrosome biogenesis and function: centrosomes brings new understanding. *Nat. Rev. Mol. Cell Biol.* 8, 451–463.
  29. Conroy, P.C., Saladino, C., Dantas, T.J., Lalor, P., Dockery, P., and Morrison, C.G. (2012). C-NAP1 and rootletin restrain DNA damage-induced centriole splitting and facilitate ciliogenesis. *Cell Cycle* 11, 3769–3778.
  30. Dawe, H.R., Farr, H., and Gull, K. (2007). Centriole/basal body morphogenesis and migration during ciliogenesis in animal cells. *J. Cell Sci.* 120, 7–15.
  31. Schmid, F., Glaus, E., Barthelmes, D., Fliegau, M., Gaspar, H., Nürnberg, G., Nürnberg, P., Omran, H., Berger, W., and Neidhardt, J. (2011). U1 snRNA-mediated gene therapeutic correction of splice defects caused by an exceptionally mild BBS mutation. *Hum. Mutat.* 32, 815–824.
  32. Fliegau, M., Benzing, T., and Omran, H. (2007). When cilia go bad: cilia defects and ciliopathies. *Nat. Rev. Mol. Cell Biol.* 8, 880–893.
  33. Afzelius, B.A. (1988). Microtubules in the spermatids of stick insects. *J. Ultrastruct. Mol. Struct. Res.* 98, 94–102.
  34. Ansley, S.J., Badano, J.L., Blacque, O.E., Hill, J., Hoskins, B.E., Leitch, C.C., Kim, J.C., Ross, A.J., Eichers, E.R., Teslovich, T.M., et al. (2003). Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature* 425, 628–633.
  35. Collin, R.W., Safieh, C., Littink, K.W., Shalev, S.A., Garzoni, H.J., Rizel, L., Abbasi, A.H., Cremers, F.P., den Hollander, A.I., Klevering, B.J., and Ben-Yosef, T. (2010). Mutations in C2ORF71 cause autosomal-recessive retinitis pigmentosa. *Am. J. Hum. Genet.* 86, 783–788.
  36. Davidson, A.E., Schwarz, N., Zelinger, L., Stern-Schneider, G., Shoemark, A., Spitzbarth, B., Gross, M., Laxer, U., Sosna, J., Sergouniotis, P.I., et al. (2013). Mutations in ARL2BP, encoding ADP-ribosylation-factor-like 2 binding protein, cause autosomal-recessive retinitis pigmentosa. *Am. J. Hum. Genet.* 93, 321–329.
  37. Estrada-Cuzcano, A., Roepman, R., Cremers, F.P., den Hollander, A.I., and Mans, D.A. (2012). Non-syndromic retinal ciliopathies: translating gene discovery into therapy. *Hum. Mol. Genet.* 21 (R1), R111–R124.
  38. Nishiguchi, K.M., Tearle, R.G., Liu, Y.P., Oh, E.C., Miyake, N., Benaglio, P., Harper, S., Koskiniemi-Kuendig, H., Venturini, G., Sharon, D., et al. (2013). Whole genome sequencing in patients with retinitis pigmentosa reveals pathogenic DNA structural changes and NEK2 as a new disease gene. *Proc. Natl. Acad. Sci. USA* 110, 16139–16144.
  39. Roosing, S., Rohrschneider, K., Beryozkin, A., Sharon, D., Weisschuh, N., Staller, J., Kohl, S., Zelinger, L., Peters, T.A., Neveling, K., et al.; European Retinal Disease Consortium (2013). Mutations in RAB28, encoding a farnesylated small GTPase, are associated with autosomal-recessive cone-rod dystrophy. *Am. J. Hum. Genet.* 93, 110–117.
  40. Nishimura, D.Y., Baye, L.M., Perveen, R., Searby, C.C., Avila-Fernandez, A., Pereiro, I., Ayuso, C., Valverde, D., Bishop, P.N., Manson, F.D., et al. (2010). Discovery and functional analysis of a retinitis pigmentosa gene, C2ORF71. *Am. J. Hum. Genet.* 86, 686–695.
  41. Bandah-Rozenfeld, D., Mizrahi-Meissonnier, L., Farhy, C., Obolensky, A., Chowers, I., Pe'er, J., Merin, S., Ben-Yosef, T., Ashery-Padan, R., Banin, E., and Sharon, D. (2010). Homozygosity mapping reveals null mutations in FAM161A as a cause of autosomal-recessive retinitis pigmentosa. *Am. J. Hum. Genet.* 87, 382–391.
  42. Langmann, T., Di Gioia, S.A., Rau, I., Stöhr, H., Maksimovic, N.S., Corbo, J.C., Renner, A.B., Zrenner, E., Kumaramanickavel, G., Karlstetter, M., et al. (2010). Nonsense mutations in FAM161A cause RP28-associated recessive retinitis pigmentosa. *Am. J. Hum. Genet.* 87, 376–381.
  43. Özgül, R.K., Siemiatkowska, A.M., Yücel, D., Myers, C.A., Collin, R.W., Zonneveld, M.N., Beryozkin, A., Banin, E., Hoyng,



- C.B., van den Born, L.I., et al.; European Retinal Disease Consortium (2011). Exome sequencing and cis-regulatory mapping identify mutations in MAK, a gene encoding a regulator of ciliary length, as a cause of retinitis pigmentosa. *Am. J. Hum. Genet.* *89*, 253–264.
44. Tucker, B.A., Scheetz, T.E., Mullins, R.F., DeLuca, A.P., Hoffmann, J.M., Johnston, R.M., Jacobson, S.G., Sheffield, V.C., and Stone, E.M. (2011). Exome sequencing and analysis of induced pluripotent stem cells identify the cilia-related gene male germ cell-associated kinase (MAK) as a cause of retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* *108*, E569–E576.
45. Pierce, E.A., Quinn, T., Meehan, T., McGee, T.L., Berson, E.L., and Dryja, T.P. (1999). Mutations in a gene encoding a new oxygen-regulated photoreceptor protein cause dominant retinitis pigmentosa. *Nat. Genet.* *22*, 248–254.
46. Schwahn, U., Lenzner, S., Dong, J., Feil, S., Hinzmann, B., van Duijnhoven, G., Kirschner, R., Hemberger, M., Bergen, A.A., Rosenberg, T., et al. (1998). Positional cloning of the gene for X-linked retinitis pigmentosa 2. *Nat. Genet.* *19*, 327–332.
47. Evans, R.J., Schwarz, N., Nagel-Wolfrum, K., Wolfrum, U., Hardcastle, A.J., and Cheetham, M.E. (2010). The retinitis pigmentosa protein RP2 links pericentriolar vesicle transport between the Golgi and the primary cilium. *Hum. Mol. Genet.* *19*, 1358–1367.
48. Chakarova, C.F., Khanna, H., Shah, A.Z., Patil, S.B., Sedmak, T., Murga-Zamalloa, C.A., Papaioannou, M.G., Nagel-Wolfrum, K., Lopez, I., Munro, P., et al. (2011). TOPORS, implicated in retinal degeneration, is a cilia-centrosomal protein. *Hum. Mol. Genet.* *20*, 975–987.
49. Chakarova, C.F., Papaioannou, M.G., Khanna, H., Lopez, I., Waseem, N., Shah, A., Theis, T., Friedman, J., Maubaret, C., Bujakowska, K., et al. (2007). Mutations in TOPORS cause autosomal dominant retinitis pigmentosa with perivascular retinal pigment epithelium atrophy. *Am. J. Hum. Genet.* *81*, 1098–1103.

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Supplemental Data

## **Whole-Exome Sequencing Identifies *KIZ***

**as a Ciliary Gene Associated with**

## **Autosomal-Recessive Rod-Cone Dystrophy**

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SNP	Exon	Nucleotide Exchange	Allele State	Protein Effect	Minor Allele Frequency (n=340 subjects)	Alleles Frequency
rs150243168	1	c.48C>T	heterozygous	p.(=)	T= 0.006	dbSNP: C: 99.862% (2175 / 2178); T: 0.138% (3 / 2179), <b>EVS</b> (EA) T=15/C=7753 (AA) T=2/C=3528
rs16982513	4	c.348A>G	5 heterozygous  /1  homozygous	p.(=)	G= 0.001	dbSNP: A: 98.320% (2282 / 2321); G: 1.680% (39 / 2321), <b>EVS</b> (EA) G=6/A=7834 (AA) G=170/A=3356
rs4815025	5	c.417C>G	148 heterozygous  /136  homozygous	p.His139Gln	C=0.38	dbSNP: C: 45.454% (1668.785 / 3671); G: 54.546% (2002.625 / 3671), <b>EVS</b> (EA) G=5594/C=2636 (AA) G=1445/C=2365
rs200388739	5	c.505A>G	heterozygous	p.Ser169Gly	G= 0.001	No frequency in dbSNP or 1000Genome, found in Exome chip, NHLBI-ESP , <b>EVS</b> (EA) G=1/A=8237
rs75984134	5	c.509T>G	3 heterozygous	p.Met170Arg	G= 0.004	dbSNP: G: 1.437% (33 / 2296); T: 98.563% (2263 / 2296), <b>EVS</b> (AA)= G=107/T=3733
rs34305929	5	c.698G>A	heterozygous	p.Gly233Asp	A= 0.001	dbSNP: C: 99.822% (2250 / 2254); T: 0.178% (4 / 2254), <b>EVS</b> (AA) A=50/G=3886
rs2236178	5	c.707T>C	148	p.Met236Thr	T=0.34	dbSNP: C: 64.369% (3526.800 / 5479); N: 0.018% (1 / 5464); T: 35.612% (1951.200 / 5479), <b>EVS</b> (EA)

			heterozygous  /153  homozygous			C=5702/T=2592 (AA) C=2660/T=1280
Not known	5	c.709C>A	heterozygous	p.Pro237Thr	A= 0.001	not known SNP
rs35460260	5	c.929A>T	heterozygous	p.Glu310Val	T= 0.001	dbSNP: A: 0.444% (10 / 2254); T: 99.556% (2244 / 2254) , <b>EVS</b> (AA) G=51/C=3695
rs151319642	5	c.1024C>G	heterozygous	p.Pro342Ala	G= 0.006	dbSNP: C: 99.770% (2173 / 2178); G: 0.230% (5 / 2178), <b>EVS</b> (AA) G=51/C=3695
Not known	6	c.1061A>G	heterozygous	p.E354G	G= 0.001	not known SNP
rs80251208	6	c.1331C>G	heterozygous	p.Ser444Cys	G= 0.001	No frequency in dbSNP or 1000Genome, found in Exome chip, Illumina, NHLBI-ESP , <b>EVS</b> (AA) G=26/C=3648
Not known	7	c.1383G>A	heterozygous	p.(=)	A= 0.001	not known SNP
rs36064635	8	c.1488C>T	heterozygous	p.(=)	T= 0.001	dbSNP: A: 0.444% (10 / 2254); G: 99.556% (2244 / 2254), <b>EVS</b> (EA) T=1/C=8209 (AA) T=83/C=3679
rs201638838	10	c.1745G>A	heterozygous	p.Arg582Lys	A= 0.003	No frequency in dbSNP or 1000Genome, found in Exome chip, NHLBI-ESP , <b>EVS</b> (AA) A=5/G=3805
rs34011504	11	c.1809C>T	heterozygous	p.(=)	T= 0.003	dbSNP: A: 0.975% (22 / 2256); G: 99.025% (2234 / 2256), <b>EVS</b> (EA) T=1/C=8305 (AA) T=104/C=3792
rs34269420	11	c.1870C>T	heterozygous	p.Pro624Ser	T= 0.006	dbSNP: A: 0.886% (20 / 2256); G: 99.114% (2236 / 2256), <b>EVS</b> (AA) T=106/C=3656
rs115272132	IVS12	c.1924+3G>A	heterozygous	.....	A= 0.004	dbSNP: A: 0.523% (12 / 2296); G: 99.477% (2284 / 2296), <b>EVS</b> (EA) A=1/G=8155 (AA) A=80/G=3544

Table S1: Benign *KIZ* variants identified in autosomal recessive RCD subjects: Ref.: NM\_018474.4