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.[1] Population-based studies have indicated that there are one million affected individuals worldwide.[1] 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.[1] 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.[1]

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.[2–5] 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.[6–9] 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.[10] 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).[11,12] Because exon ORF15 in *RPGR* (MIM 312610) is not targeted by existing NGS panels,[13] 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
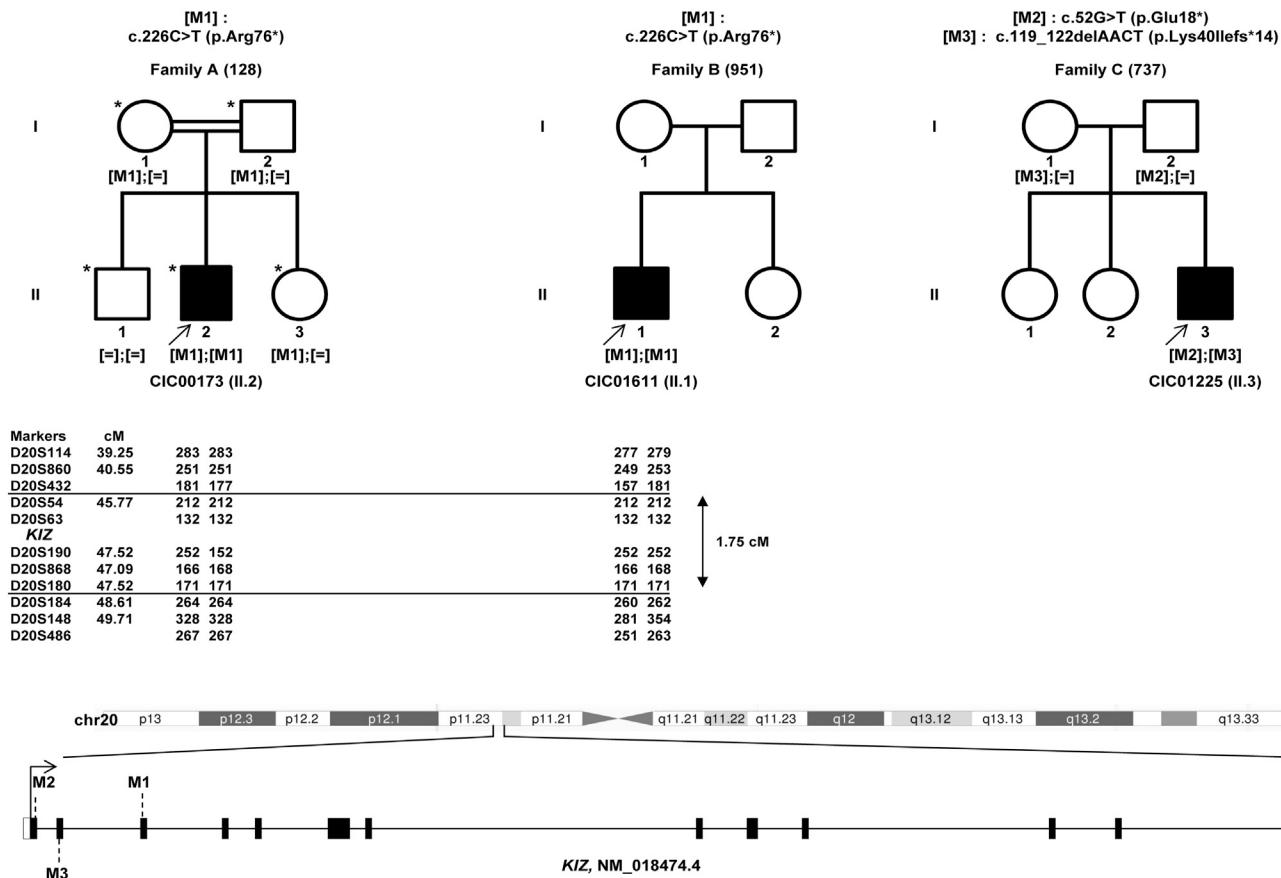
Figure 1. Identification of Homozygous and Compound-Heterozygous *KIZ* Variants in Three Unrelated Families Affected by Autosomal-Recessive RCD

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 ≈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).[13] 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).[6,9,13] 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× and 25× depth of coverage, respectively, resulting in a mean sequencing depth of 80× 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) ≥ 0.005 in dbSNP137, HapMap,[14] 1000 Genomes,[15] and the NHLBI Exome Sequencing Project Exome Variant Server (EVS)[16] were removed.[6,7,9] 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[a]	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.Lys40Ilefs*14

[a]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.[17,18] 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[20] than in cone photoreceptors and horizontal, bipolar, amacrine, ganglion, and microglia cells (Figure 2A).[20] 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.[21] reported _KIZ_ expression in the human retinal pigment epithelium (RPE).[21] 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.[20] Furthermore, the in-house _rd1_ mouse transcriptomic database reported no changes in _Obscn_ 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 ≤ 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,[22] we performed RNA in situ hybridization in the adult mouse retina as previously described[23,24] 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.[25] These markers were distributed over a physical distance of ≈7.38 Mb, corresponding to ≈10.5 cM (genetic distance according to Marshfield genetic maps). Both subjects were found to
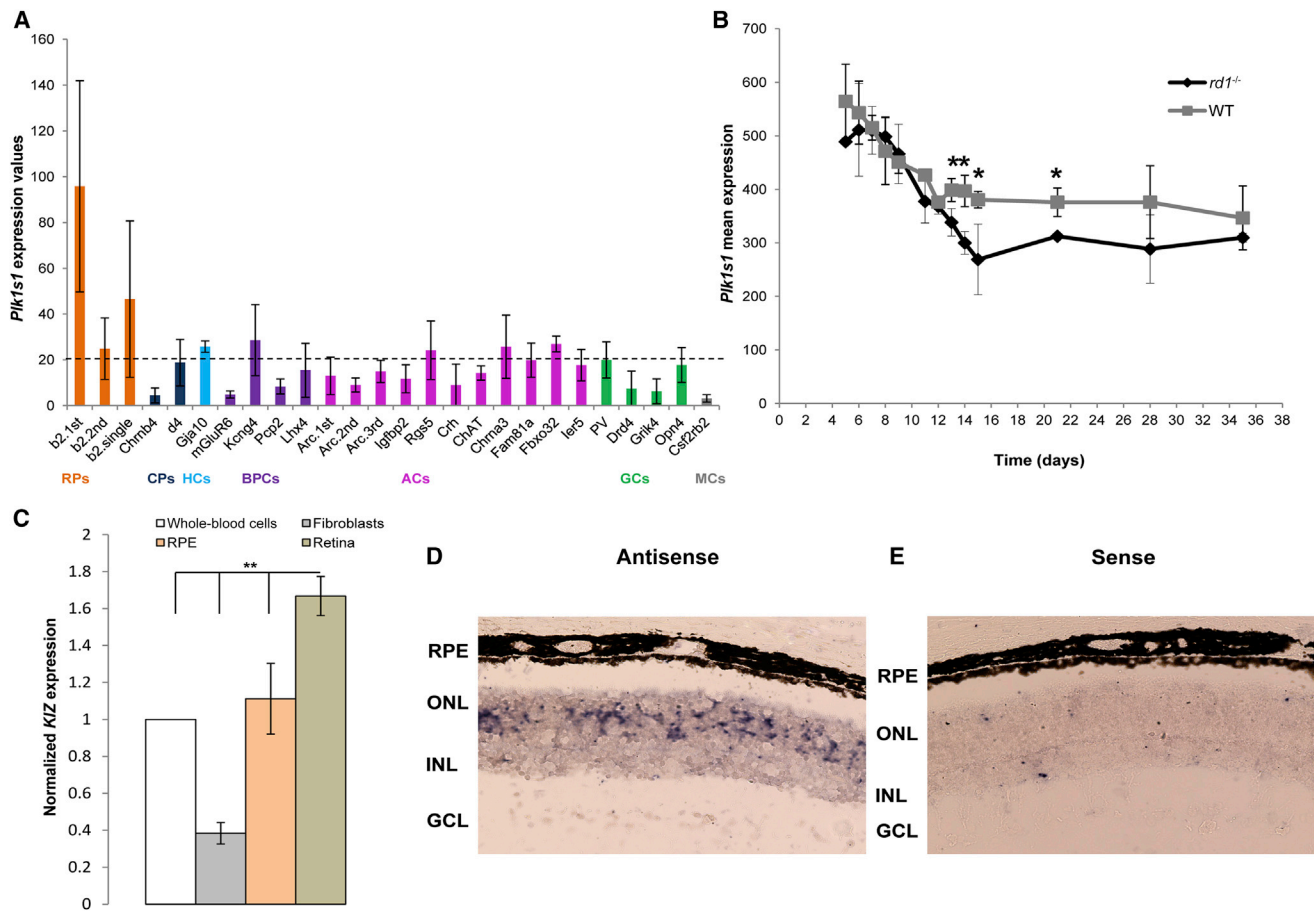
Figure 2. *Plk1s1* Transcriptomic Analysis in the Mouse Retina

(A) *Plk1s1* 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 *Plk1s1* 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.[20] Each value on the x axis corresponds to a specific retinal cell type established from the following abbreviated mouse lines: RPs (b2), CPs (Chrnb4 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.,[20] 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, "1st," "2nd," "single," and "3rd" correspond to the batch numbers.) *Plk1s1*, implicated in autosomal-recessive RCD, showed the highest expression in RPs.

(B) *Plk1s1* 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 *Plk1s1* expression in the mouse retina. A riboprobe encompassing exons 8–14 of mouse *Plk1s1* 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.

CIC01225 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
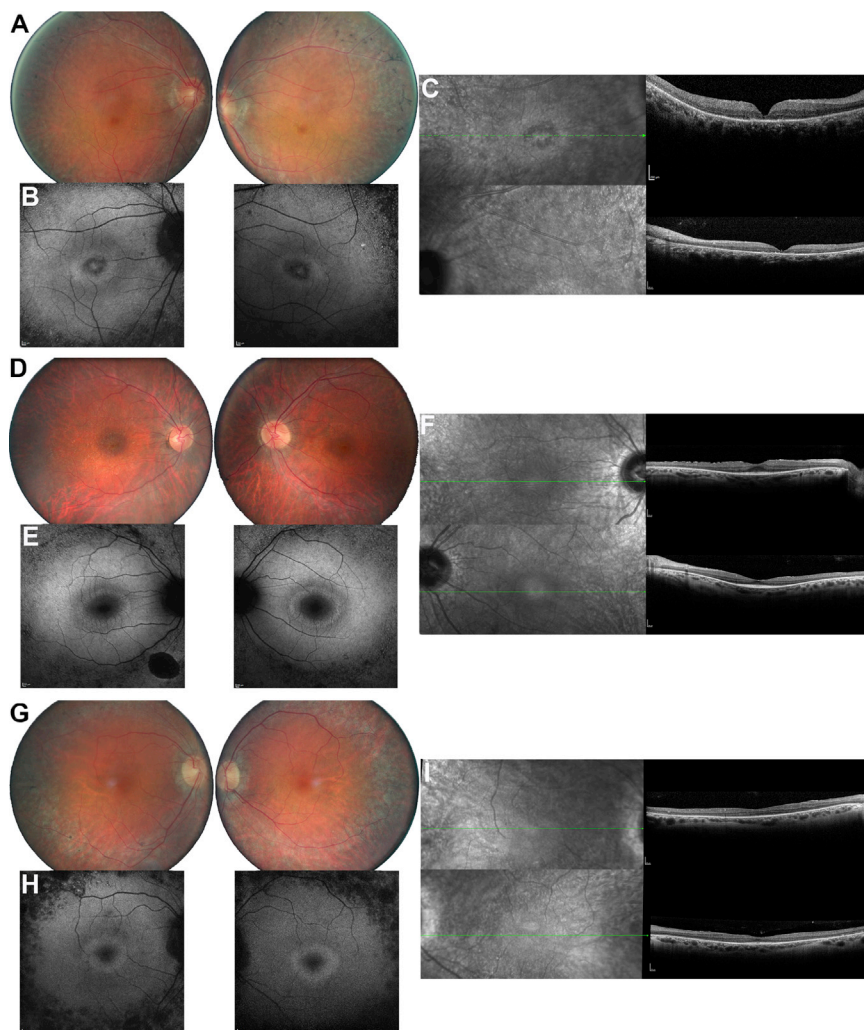
Figure 3. Retinal Imaging of Autosomal-Recessive-RCD Individuals Carrying *KIZ* Variants

(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.[26,27] Oshimori et al.[27] demonstrated that KIZ is a centrosomal substrate for PLK1, given that the latter mediates its phosphorylation at amino acid residue Thr379. During
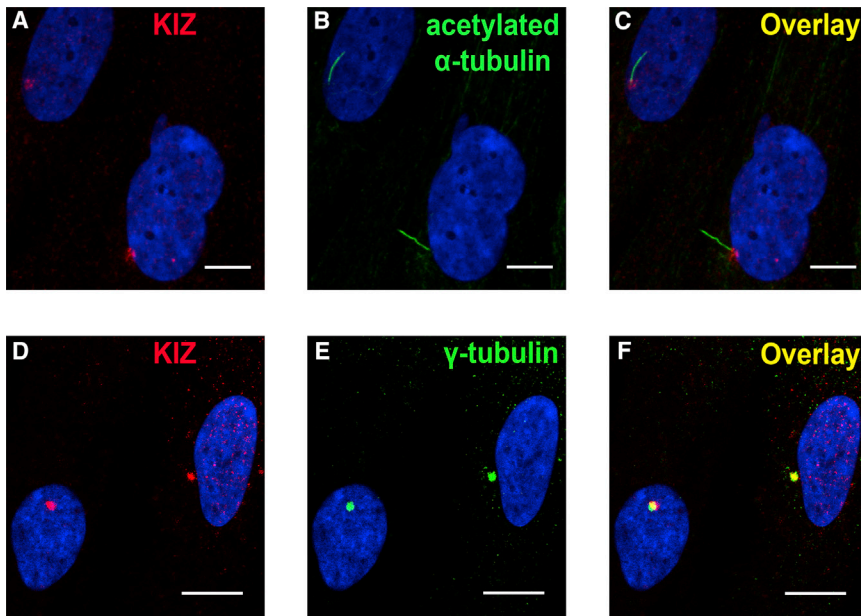
Figure 4. Subcellular Localization of KIZ in Monocilia from Human Fibroblasts

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°C, and immunofluorescence staining was performed as previously described.[31] Human fibroblasts were immunolabeled with a KIZ antibody (PLK1S1 rabbit-polyclonal antibody,1/250, Sigma Aldrich SAB2700541; A, C, D, and F), an acetyl α-tubulin mouse-monoclonal antibody (1/1,000, Sigma-Aldrich; B and C), and a γ-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 μm.

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.[27] Stabilized centrosomes resist microtubule-mediated pulling and pushing forces and thus ensure the spindle bipolarity required for accurate separation of chromosomes during mitosis.[28,29] 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.[30] Ciliogenesis can be either linked to the cell cycle or associated with cell differentiation.[30] 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.[31] Colocalization with a cilium marker (acetylated α-tubulin) (Figures 4A–4C) and a basal body marker (γ-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.[32] 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.[29,33] 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.[29,34] 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.[35–39] 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.[37] However, retina-restricted disease was reported for mutations in autosomal-recessive RCD (in *C2ORF71* [MIM 613425],[35,40] *FAM161A* [MIM 613596],[41,42] *MAK* [MIM 154235],[43,44] *RP1* [MIM 603937],[45] and *ARL2BP* [MIM 615407][36]), in X-linked RCD (in *RPGR* and *RP2* [MIM 300757][46,47]), and in autosomal-dominant RCD (in *RP1* [MIM 603937][45] and *TOPORS* [MIM 609507][48,49]). The most common inheritance patterns are autosomal recessive and X-linked.[37]

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.[32] 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.

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/

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