Supplement S1
Method section – genetic analysis

In all families, genomic DNA was extracted from peripheral lymphocytes according to standard procedures. The proband of family A was initially screened for variants in the ABCA4, CNGB3 (exon 10), MFSD8 (p.E336Q/p.D368H/p.E381*), and PRPH2 genes. Sanger sequencing of these genes revealed no disease-associated variants. Subsequently, whole exome sequencing (WES) was performed in the 3 affected siblings and their unaffected brother. WES capture was obtained using Nimblegen SeqCap EZ Exome v2 kit (Roche NimbleGen, Inc., Madison WI) on Illumina HiSeq sequencer using TruSeq V3 chemistry (Illumina, Inc. San Diego, CA), followed by downstream quality control and genotyping of the samples, as previously described. We selected candidate variants based on their segregation with the disease phenotype, their presence in all three affected siblings, their function (nonsynonymous, presumed loss of function or splicing variants located in coding or near-splice site regions), and minor allele frequency less than 1% in the public databases dbSNP, 1000 Genomes Project, Exome Variant Server, and GoNL. Sanger sequencing was performed to confirm all single-nucleotide variants of interest. Finally, we used restriction enzymes to confirm that variants were located on different alleles. Exome sequencing in patients B-II:8, H-II:2, I-II:2, M-II:2, P-II:13 and Q-III:4 was performed in a certified diagnostic laboratory. In patients B-II:8, M-II:2, P-II:13 and Q-III:4, the exome was enriched using Agilent’s SureSelectXT Human All Exon 50Mb Kit (Agilent Technologies, Santa Clara, CA, USA). Subsequently, next-generation sequencing using an Illumina HiSeq sequencer (Illumina, Inc. San Diego, CA), read alignment to the human reference genome (Genome Reference Consortium Human Reference 37/hg19) using Burrows-Wheeler Aligner (BWA), and variant calling with the Genome Analysis Toolkit (GATK) were performed at BGI-Europe (Copenhagen, Denmark). After the copy number variants were detected using CoNIFER 0.2.0, variants were annotated with a custom designed in-house annotation strategy. The exome data was analyzed using a vision gene filter consisting of 342 (version DG-2.4.1), 366 (version DG-2.8), or 395 genes (version DG-2.11), in patients P-II:13, B-II:8 and M-II:2/Q-III:4, respectively (https://www.radboudumc.nl/en/patientenzorg/onderzoeken/exome-sequencing-diagnostics/exomepanelspreviousversions/vision-disorders; Supplemental Table 1). Segregation analysis of the candidate variants of patient B-II:8 was performed in the son and affected sister. In patients H-II:2 and I-II:2 the DNA was enriched using Roche/NimbleGen’s SeqCap EZ Human Exome Library v.3.0 (Roche NimbleGen, Basel, Switzerland). Subsequently, next-generation sequencing was performed on a Illumina HiSeq2500TM sequencer, read sequences were mapped to the human reference genome (GRCh37/hg19) using BWA version 0.7.5, and variants called with GATK. Detected variants were analysed in Cartagenia 3.0 applying a vision-related gene filter consisting of 220 genes (https://sph.uth.edu/RetNet/sum-dis.htm#B-diseases; June 2014).

In patients O-II:1, R-II:2 and S-III:4, targeted panel sequencing covering 256 vision-related genes (patient R-II:2) or 266 genes (patients O-II:1 and S-III:4) was performed in a certified DNA diagnostic laboratory (Supplemental Table 1). The DNA was enriched using the Nimblegen SeqCap easy choice (OID 42193, version BHv2 or OID 43443, version BHv3), after which next-generation sequencing was performed on a MiSeq sequencer using MiSeq Reagent Kit v2 (Illumina, Inc. San Diego, CA), reads were aligned to the human reference genome (GRCh37/hg19) using BWA, and variants were called using the GATK. Sanger sequencing was performed for all areas with a coverage below 30 reads.

Mutational screening in patient N-III:9 was performed using an arrayed primer extension (APEX) microarray chip (Asper, Biotech, Tartu, Estonia) for autosomal dominant RP (version 3.0: containing 414 variants in 16 genes), according to a previously described protocol. All variants detected by microarray analysis were verified by direct sequencing. Patients C–G and J–L had their RP1 open reading frame screened by means of Sanger sequencing as previously reported. In addition, except for patient G-II:2, molecular inversion probes (MIPs) were used to exclude variants in 109 other known inherited retinal dystrophy genes in patient C–F, J and K, and targeted resequencing containing 83 non-syndromic RP genes was performed in patient L-II:2 (Supplemental...
Table 1). The pathogenicity of novel missense variants was assessed combining co-segregation analysis and \textit{in silico} prediction tools, including SIFT and Polyphen-2, and by using the PhyloP, CADD-PHRED and Grantham scores.

In addition, we performed an extensive review of the literature (PubMed: accessed April 12, 2018) to identify all known mutations in the \textit{RP1} gene (including those reported herein) that have been associated with hereditary retinal dystrophies.

References