SUPPORTING INFORMATION

SUPP. METHODS

DNA library preparation and next generation sequencing (NGS)

1μg of genomic DNA was sheared into 200-500bp fragments. The fragments were processed and amplified as previously described (Wang, et al., 2014). For each capture reaction, we pooled 50 pre-capture DNA libraries together. The targeted DNA was captured with a customized retinal disease gene panel (Supp. Table S1) for target capture sequencing to screen for variants in known disease-causing genes. If no causative variants were identified, we performed whole exome sequencing (WES) by capturing the DNA with NimbleGenSeqCap EZ Hybridization and Wash kit (NimbleGenSeqCap EZ Human Exome Library v.2.0) following the manufacturer's instructions. We used the Illumina HiSeq 2000 (Illumina, San Diego, CA) to sequence captured libraries.

Bioinformatics analysis

Paired-end sequencing reads were obtained for each sample. Reads were mapped to human reference genome hg19 using Burrows-Wheeler Aligner (Li and Durbin, 2009). Base quality recalibration, local realignment and variant calling were performed as previously described (Wang, et al., 2014). We obtained the variant frequencies from a series of public and internal control databases including Exome Aggregation

Consortium (ExAC) database, CHARGE consortium (Psaty, et al., 2009), ESP-6500 (Tennessen, et al., 2012) and 1000 Genome Project (Abecasis, et al., 2010). Since RP is a rare Mendelian disorder, variants with a frequency higher than 1/200 (for a recessive model) or 1/10,000 (for a dominant model) were filtered out. After frequency-based filtering, we filtered out synonymous variants, identified known retinal disease-causing variants and predicted the pathogenicity of variants using SIFT (Ng and Henikoff, 2003), PolyPhen-2 (Adzhubei, et al., 2010), LRT (Chun and Fay, 2009), MutationTaster (Schwarz, et al., 2010) (Supp. Table S2) as previously described (Xu, et al., 2015a; Xu, et al., 2015b).

Sanger sequencing

A 500bp sequence flanking both sides of candidate variants were retrieved from the UCSC genome browser (hg19 assembly). We masked the repetitive sequences with RepeatMasker (Smit, 1996-2010). We used Primer3 (Untergasser, et al., 2012) to design primers to amplify a 500bp PCR product to sequence the variant and at least 50bp region surrounding it. After PCR, ABI 3730xl was used to sequence the amplicons.

Immunohistochemistry

Eyes of adult albino wild-type mice were fixed overnight in modified Davidson's fixative for paraffin embedding. Eye sections of 7 µm were cut (Microtome, Leica). Slides were de-paraffinized, and antigen retrieval was performed by boiling sections in 0.01 M Tris-EDTA buffer (pH 9.0) for 30 minutes, followed by cooling for 30 minutes at room

temperature. Slides were washed in phosphate-buffered saline (PBS), incubated for 1 hour at room temperature in hybridization buffer (10% normal goat serum, 0.1% Triton X-100, PBS), and then incubated overnight in primary antibody (anti-mouse ADIPOR1; 1:100 dilution; Santa Cruz, Dallas, TX, USA) diluted in hybridization buffer. Slides were then washed in PBS, incubated with secondary antibody (Alexa 488 anti-rabbit; 1:500 dilution; Invitrogen, Carlsbad, CA, USA) diluted in hybridization buffer at room temperature for 2 hours, washed in PBS, mounted with anti-fade medium (Prolong; Invitrogen) to reduce bleaching, and cover-slipped. 4’,6-Diamidino-2-phenylindole with 1:1000 dilution (DAPI) (Life Technology, Carlsbad, CA, USA) was used for nuclear counterstaining. Fluorescent images were produced with an Apotome 2 microscope (Zeiss, Pleasanton, CA, USA) and processed with ZEN software and Photoshop CS4 software (Adobe, San Jose, CA, USA).

Supp. Table S1. List of known retinal disease genes screened in this study

ABCA4	*CDH3*	*GNAT2*	***MKKS***	*PLA2G5*	*SAG*
ABCC6	*CDHR1*	*GNPTG*	***MKS1***	*PRCD*	***SDCCAG8***
ABHD12	*CEP164*	*GPR179*	*MPV17*	*PRD*	*SEMA4A*
ACBD5	***CEP290***	*GRK1*	*MTTP*	*PROM1*	*SLC24A1*
ADAM9	*CERKL*	*GRM6*	*MYO7A*	*PRPF3*	*SLC6A5*
ADAMTS18	*CFB*	*GUCA1A*	*NDP*	*PRPF31*	*SNRNP200*
ADGRA3	*CFH*	*GUCA1B*	*NEK2*	*PRPF6*	*SPATA7*
ADGRV1	*CHM*	*GUCY2D*	*NMNAT1*	*PRPF8*	*TEAD1*
AHI1	*CIB2*	*HARS*	***NPHP1***	*PRPH2*	*TIMM8A*
AIPL1	*CISD2*	*HMCN1*	*NPHP3*	*PVRL1*	*TIMP3*
ALMS1	*CLN3*	*HTRA1*	*NPHP4*	*RAB28*	*TK2*
ARL2BP	*CLRN1*	*IDH3B*	*NR2E3*	*RAX2*	*TLR3*
ARL6	*CNGA1*	*IFT140*	*NRL*	*RB1*	*TLR4*
ARMS2	*CNGA3*	***IFT172***	*NYX*	*RBP3*	*TLR6*
ATXN7	*CNGB1*	***IFT27***	*OAT*	*RBP4*	*TMEM126A*
BBIP1	*CNGB3*	*IMPDH1*	*OFD1*	*RCD1*	*TMEM216*
BBS1	*CNNM4*	*IMPG1*	*OPA1*	*RD3*	*TMEM237*
BBS10	*COL11A1*	*IMPG2*	*OPA3*	*RDH12*	*TOPORS*
BBS12	*COL2A1*	***INPP5E***	*OPN1LW*	*RDH5*	*TREX1*
BBS2	*COL9A1*	*INVS*	*OPN1MW*	*RGR*	***TRIM32***
BBS4	*CRB1*	*IQCB1*	*OPN1MW2*	*RGS9*	*TRPM1*
BBS5	*CRX*	*ITM2B*	*OPN1SW*	*RGS9BP*	*TSPAN12*
BBS7	*CYP4V2*	*JAG1*	*OTX2*	*RHO*	***TTC8***
BBS9	*DFNB31*	***KCNJ13***	*PANK2*	*RIMS1*	*TTPA*
BEST1	*DHDDS*	*KCNV2*	*PAX2*	*RLBP1*	*TULP1*
C1QTNF5	*DMD*	*KIAA1549*	*PCDH15*	*ROM1*	*UNC119*
C2	*DTHD1*	*KIF11*	*PDE6A*	*RP1*	*USH1C*
C21ORF2	*EFEMP1*	*KLHL7*	*PDE6B*	*RP1L1*	*USH1G*
C2ORF71	*ELOVL4*	*LCA5*	*PDE6C*	*RP2*	*USH2A*
C3	*EMC1*	*LRAT*	*PDE6G*	*RP8*	*VCAN*
C8ORF37	*ERCC6*	*LRIT3*	*PDE6H*	*RP9*	*VPS13B*
CA4	*EYS*	*LRP1B*	*PDZD7*	*RPE65*	*WDPCP*
CABP4	*FAM161A*	*LRP5*	*PEX1*	*RPGR*	*WDR19*
CACNA1F	*FBLN5*	***LZTFL1***	*PEX2*	*RPGRIP1*	*WFS1*
CACNA2D4	*FLVCR1*	*MAK*	*PEX7*	*RPGRIP1L*	*ZNF408*
CAPN5	*FSCN2*	*MERTK*	*PGK1*	*RRM2B*	*ZNF423*
CC2D2A	*FZD4*	*MFN2*	*PHYH*	*RS1*	*ZNF513*
CDH23	*GNAT1*	*MFRP*	*PITPNM3*		

Bardet-Biedl syndrome-associated genes are in bold font (information from RetNet, the the Retinal Information Network).

Supp. Table S2. Information of variant prediction algorithms used

Program	SIFT	PolyPhen-2	LRT	MutationTaster
URL	http://sift.jcvi.org/	http://genetics.bwh.harvard.edu/pph2/	http://www.genetics.wustl.edu/jflab/	http://www.mutationtaster.org/
Version	1.0.3	2.2.2	Web version	Web version
Datasets	NCBI: GRCh37	NCBI: GRCh37	NCBI: GRCh36	NCBI: GRCh37
Outputs and cut-off	[0,1]; Damaging [0.95,1]; Tolerant [0, 0.949]	[0,1]; Probably damaging [0.957,1]; possibly damaging [0.453, 0.956]; Benign [0, 0.452]	Deleterious; Neutral; Unknown	disease_causing_automatic; disease_causing; polymorphism; polymorphism_automatic

SUPP. REFERENCES

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