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Development and application of a next-generation-sequencing (NGS) approach to detect known and novel gene defects underlying retinal diseases

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Abstract

Background: Inherited retinal disorders are clinically and genetically heterogeneous with more than 150 gene defects accounting for the diversity of disease phenotypes. So far, mutation detection was mainly performed by APEX technology and direct Sanger sequencing of known genes. However, these methods are time consuming, expensive and unable to provide a result if the patient carries a new gene mutation. In addition, multiplicity of phenotypes associated with the same gene defect may be overlooked.

Methods: To overcome these challenges, we designed an exon sequencing array to target 254 known and candidate genes using Agilent capture. Subsequently, 20 DNA samples from 17 different families, including four patients with known mutations were sequenced using Illumina Genome Analyzer IIx next-generation-sequencing (NGS) platform. Different filtering approaches were applied to identify the genetic defect. The most likely disease causing variants were analyzed by Sanger sequencing. Co-segregation and sequencing analysis of control samples validated the pathogenicity of the observed variants.

Results: The phenotype of the patients included retinitis pigmentosa, congenital stationary night blindness, Best disease, early-onset cone dystrophy and Stargardt disease. In three of four control samples with known genotypes NGS detected the expected mutations. Three known and five novel mutations were identified in NR2E3, PRPF3, EYS, PRPF8, CRB1, TRPM1 and CACNA1F. One of the control samples with a known genotype belongs to a family with two clinical phenotypes (Best and CSNB), where a novel mutation was identified for CSNB. In six families the disease associated mutations were not found, indicating that novel gene defects remain to be identified.

Conclusions: In summary, this unbiased and time-efficient NGS approach allowed mutation detection in 75% of control cases and in 57% of test cases. Furthermore, it has the possibility of associating known gene defects with novel phenotypes and mode of inheritance.

Keywords: NGS, retinal disorders, diagnostic tool.
be related to the dysfunction of the same gene [2-4]. Furthermore, there may be additional phenotype-genotype associations that are still not recognized. The state-of-the-art phenotypic characterization including precise family history and functional as well as structural assessment (i.e. routine ophthalmic examination, perimetry, color vision, full field and multifocal electroretinography (ERG), fundus autofluorescence (FAF) imaging and optical coherence tomography (OCT)) allows targeted mutation analysis for some disorders. However, in most cases of inherited retinal diseases, similar phenotypic features can be due to a large number of different gene defects. Various methods can be used for the identification of the corresponding genetic defect. All these methods have advantages and disadvantages. Sanger sequencing is still the gold-standard in determining the gene defect, but due to the heterogeneity of the disorders it is time consuming and expensive to screen all known genes. Mutation detection by commercially available APEX genotyping microarrays (ASPER Ophthalmics, Estonia) [5,6] allows the detection of only known mutations. In addition, a separate microarray has been designed for each inheritance pattern, which tends to escalate the costs especially in simplex cases, for which inheritance pattern cannot be predetermined. Indirect methods with single nucleotide polymorphism (SNP) microarrays for linkage and homozygosity mapping are also powerful tools, which has proven its reliability in identifying novel and known gene defects [7-12]. However, in case of homozygosity mapping the method can only be applied to consanguineous families or inbred populations. To overcome these challenges, we designed a custom sequencing array in collaboration with a company (IntegraGen, Evry, France) to target all exons and part of flanking sequences for 254 known and candidate retinal genes. This array was subsequently applied through NGS to a cohort of 20 patients from 17 families with different inheritance pattern and clinical diagnosis including RP, CSNB, Best disease, early-onset cone dystrophy and Stargardt disease.

Methods
Clinical investigation
The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local Ethics Committee (CPP, Ile de France V). Informed written consent was obtained from each study participant. Index patients underwent full ophthalmic examination as described before [13]. Whenever available, blood samples from affected and unaffected family members were collected for co-segregation analysis.

Previous molecular genetic analysis
Total genomic DNA was extracted from peripheral blood leukocytes according to manufacturer’s recommendations (Qiagen, Courtaboeuf, France). DNA samples from some patients with a diagnosis of RP were first analyzed and excluded for known mutations by applying commercially available microarray analysis (arRP and adRP ASPER Ophthalmics, Tartu, Estonia). In some cases, pathogenic variants in EYS, C2orf71, RHO, PRPF31, PRPH2 and RP1 were excluded by direct Sanger sequencing of the coding exonic and flanking intronic regions of the respective genes [13-17]. Conditions used to amplify PRPH2 can be provided on request.

Molecular genetic analysis using NGS
A custom-made SureSelect oligonucleotide probe library was designed to capture the exons of 254 genes for different retinal disorders and candidate genes according to Agilent’s recommendations (Table 1). These genes include 177 known genes underlying retinal dysfunction (http://www.sph.uth.tmc.edu/retnet/sum-dis.htm, October 2010, Table 1) and 77 candidate genes associated with existing animal models and expression data (Table 2). The eArray web-based probe design tool was used for this purpose https://earray.chem.agilent.com/earray. The following parameters were chosen for probe design: 120 bp length, 3x probe-tiling frequency, 20 bp overlap in restricted regions, which were identified by the implementation of eArray’s RepeatMasker program. A total of 27,430 probes, covering 1177 Mb, were designed and synthesized by Agilent Technologies (Santa Clara, CA, USA). Sequence capture, enrichment, and elution were performed according to the manufacturer’s instructions (SureSelect, Agilent). Briefly, three µg of each genomic DNA were fragmented by sonication and purified to yield fragments of 150-200 bps. Paired-end adaptor oligonucleotides from Illumina were ligated on repaired DNA fragments, which were then purified and enriched by six PCR cycles. 500 ng of the purified libraries were hybridized to the SureSelect oligo probe capture library for 24 h. After hybridization, washing, and elution, the eluted fraction underwent 14 cycles of PCR-amplification. This was followed by purification and quantification by qPCR to obtain sufficient DNA template for downstream applications. Each eluted-enriched DNA sample was then sequenced on an Illumina GAIIx as paired-end 75 bp reads. Image analysis and base calling was performed using Illumina Real Time Analysis (RTA) Pipeline version 1.10 with default parameters. Sequence reads were aligned to the reference human genome (UCSC hg19) using commercially available software (CASA1A.7, Illumina) and the ELANDv2 alignment algorithm. Sequence variation annotation was performed using the IntegraGen in-house pipeline, which consisted of gene annotation (RefSeq), detection of known polymorphisms (dbSNP 131, 1000 Genome) followed by mutation characterization (exonic, intronic,
| Number | Gene name   |
|-------|-------------|
| 1     | ABCA4       |
| 2     | ABCC6       |
| 3     | ADAM9       |
| 4     | AHI1        |
| 5     | AIPL1       |
| 6     | ALMS1       |
| 7     | ARL6        |
| 8     | ARMS2       |
| 9     | ATXN7       |
| 10    | BBS10       |
| 11    | BBS12       |
| 12    | BBS2        |
| 13    | BBS4        |
| 14    | BBS5        |
| 15    | BBS7        |
| 16    | BBS9        |
| 17    | BEST1       |
| 18    | C1QTNF5     |
| 19    | C2          |
| 20    | C2orf71     |
| 21    | C3          |
| 22    | CA4         |
| 23    | CABP4       |
| 24    | CACNA1F     |
| 25    | CACNA2D4    |
| 26    | CC2D2A      |
| 27    | CDH23       |
| 28    | CDH3        |
| 29    | CEP290      |
| 30    | CERKL       |
| 31    | CFB         |
| 32    | CFH         |
| 33    | CHM         |
| 34    | CLN3        |
| 35    | CLRN1       |
| 36    | CNGA1       |
| 37    | CNGA3       |
| 38    | CNGB1       |
| 39    | CNGB3       |
| 40    | CNNM4       |
| 41    | COL11A1     |
| 42    | COL2A1      |
| 43    | COL9A1      |
| 44    | CRB1        |
| 45    | CRX         |
| 46    | CYP4V2      |
| 47    | DFNB31      |
| 48    | DMD         |
| 49    | DPP3        |
| 50    | EFEMP1      |
| 51    | ELOVL4      |
| 52    | ERCC6       |
| 53    | EYS         |
| 54    | FAM161A     |
| 55    | FBLN5       |
| 56    | FSCN2       |
| 57    | FZD4        |
| 58    | GNAT1       |
| 59    | GNAT2       |
| 60    | GPR98       |
| 61    | GRK1        |
| 62    | GRM6        |
| 63    | GUCA1A      |
| 64    | GUCA1B      |
| 65    | GUCY2D      |
| 66    | HMCN1       |
| 67    | HTRA1       |
| 68    | IDH3B       |
| 69    | IMPDH1      |
| 70    | IMPG2       |
| 71    | INPP5E      |
| 72    | INVS        |
| 73    | IQCB1       |
| 74    | JAG1        |
| 75    | KCNU13      |
| 76    | KCNV2       |
| 77    | KLHL7       |
| 78    | LCA5        |
| 79    | LRAT        |
| 80    | LRPS        |
| 81    | MERTK       |
| 82    | MFRP        |
| 83    | MKS5        |
| 84    | MKS1        |
| 85    | MTND1       |
| 86    | MTND6       |
| 87    | MT-AP6      |
| 88    | MTND2       |
| 89    | MTND5       |
| 90    | MTND4       |
| 91    | MYO7A       |
| 92    | NDP         |
| 93    | NPHP1       |
| 94    | NPHP3       |
| 95    | NPHP4       |

(Continued)
silent, nonsense etc.). For each position, the exomic frequencies (homozygous and heterozygous) were determined from all the exomes already sequenced by IntegraGen and the exome results provided by HapMap project.

Investigation of annotated sequencing data
We received the annotated sequencing data in the form of excel tables. On average 946 SNPs and 83 insertions and deletions were identified for each sample (Figure 1). By using the filtering system, we first investigated variants (nonsense and missense mutations, intronic

| Table 1 Known retinal disease genes (Continued) |
|-----------------------------------------------|
| 96    | NR2E3  |
| 97    | NRL    |
| 98    | NYX    |
| 99    | OAT    |
| 100   | OFD1   |
| 101   | OPA1   |
| 102   | OPA3   |
| 103   | OPN1LW |
| 104   | OPN1MW |
| 105   | OTX2   |
| 106   | PANK2  |
| 107   | PAX2   |
| 108   | PCDH15 |
| 109   | PCDH21 |
| 110   | PDE6A  |
| 111   | PDE6B  |
| 112   | PDE6C  |
| 113   | PDE6G  |
| 114   | PDZD7  |
| 115   | PEX1   |
| 116   | PEX2   |
| 117   | PEX7   |
| 118   | PGK1   |
| 119   | PHYH   |
| 120   | PITPNM3|
| 121   | PRCD   |
| 122   | PROM1  |
| 123   | PRP3   |
| 124   | PRPF31 |
| 125   | PRPF8  |
| 126   | PRPH2  |
| 127   | RAX2   |
| 128   | RB1    |
| 129   | RBP3   |
| 130   | RBP4   |
| 131   | RD3    |
| 132   | RHOD   |
| 133   | RHOD5  |
| 134   | RGR    |
| 135   | RG59   |
| 136   | RG59BP |
| 137   | RHO    |
| 138   | RIMS1  |
| 139   | RLB1   |
| 140   | ROM1   |
| 141   | RP1    |
| 142   | RS1    |
| 143   | SAG    |
| 144   | SDCCAG8|
| 145   | SEMA4A |
| 146   | SLC24A1|
| 147   | SNRNP200|
| 148   | SPATA7 |
| 149   | TEAD1  |
| 150   | TIMM8A |
| 151   | TIMP3  |
| 152   | TLR3   |
| 153   | TLR4   |
| 154   | TMEM126A|
| 155   | TOPORS |
| 156   | TREX1  |
| 157   | TRIM32 |
| 158   | TRPM1  |
| 159   | TSPAN12|
| 160   | TTC8   |
| 161   | TTPA   |
| 162   | TULP1  |
| 163   | UNC119 |
| 164   | USH1C  |
| 165   | USH1G  |
| 166   | USH2A  |
| 167   | VCAN   |
| 168   | WFS1   |
| 169   | ZNF513 |
| Number | Gene name | Reason | References |
|--------|-----------|--------|------------|
| 1      | ADCY1     | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 2      | ANKRD33   | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 3      | ANXA2     | Promotion of choroidal neovascularization | [36] |
| 4      | ARL13B    | Cilia protein, mutations lead to Joubert Syndrome | [37] |
| 5      | BMP7      | Regulation of Pax 2 in mouse retina | [38] |
| 6      | BSG       | - | Thierry Leveillard personal communication |
| 7      | CAMK2D    | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 8      | CCDC28B   | Modifier for BBS | [39,40] |
| 9      | CLCN7     | Cln7/- mice severe osteopetrosis and retinal degeneration | [41] |
| 10     | COL4A3    | Alport syndrome, with eye abnormalities | [42,43] |
| 11     | COL4A4    | Alport syndrome, with eye abnormalities | [42,44] |
| 12     | COL4A5    | Alport syndrome, with eye abnormalities | [42,45] |
| 13     | CUBN      | - | Personal communication Renata Kozyraki |
| 14     | CYP1B1    | glaucoma | [46] |
| 15     | DOHH      | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 16     | DSCAML1   | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 17     | ESRB      | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 18     | FIZ1      | Interactor of Nrl | [47] |
| 19     | GJA9      | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 20     | GNAZ      | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 21     | GNFT1     | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 22     | GPR152    | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 23     | HCN1      | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 24     | HEATR5A   | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 25     | HIST1H1C  | Expressed in retina | Expression databases |
| 26     | IMPG1     | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 27     | INSL5     | diff. Expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 28     | KCNB1     | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 29     | KCTD7     | Expressed in retina | Expression databases |
| 30     | LASS4     | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 31     | LRIT2     | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 32     | LRP2      | - | Personal communication Renata Kozyraki |
| 33     | MAB21L1   | diff. expression Rd1 mouse | Chalmel et al., manuscript in preparation |
| 34     | MAP2      | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 35     | MAS1      | Degeneration of cones due to expression of Mas1 | [48] |
| 36     | MAST2     | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 37     | MPP4      | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 38     | MYOC      | glaucoma | [49] |
| 39     | NDUFA12   | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 40     | NEUROD1   | BET2/NeuroD1 -/- mouse: photoreceptor degeneration | [50] |
| 41     | NOS2      | glaucoma | [51] |
| 42     | NXNL1     | Rod-derived cone viability factor | [52] |
| 43     | NXNL2     | Rod-derived cone viability factor 2 | [53] |
| 44     | OPN1MW2   | Cone opsin, medium-wave-sensitive2 | [54] |
| 45     | OPTN      | glaucoma | [55] |
| 46     | PFKFB2    | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 47     | PIAS3     | Rod photoreceptor development | [56] |
variants located +/- 5 apart from exon), which were absent in dbSNP and NCBI databases http://ncbi.nlm.nih.gov/. In the absence of known gene defects or putative pathogenic variants (see below) in the first step, we selected known genes, which were previously clinically associated including variants present in dbSNP and NCBI databases (Figure 1). Each predicted pathogenic variant was confirmed by Sanger sequencing.

Assessment of the pathogenicity of variants
Following criteria were applied to evaluate the pathogenic nature of novel variations identified by NGS: 1) stop/frameshift variants were considered as most likely to be disease causing; 2) co-segregation in the family; 3) absence in control samples; 4) for missense mutations amino acid conservation was studied in the UCSC Genome Browser http://genome.ucsc.edu/ across species from all different evolutionary branches. If the amino acid residue did not change it was considered as “highly conserved”. If a different change was seen in fewer than five species and not in the primates then it was considered as “moderately conserved” and if a change was present in 5-7, it was considered as “weakly conserved”, otherwise the amino acid residue was considered as “not conserved”, 5) pathogenicity predictions with bioinformatic tools (Polyphen: Polyorphism Phenotyping, http://genetics.bwh.harvard.edu/pph/ and SIFT: Sorting Intolerant From Tolerant, http://blocks.fhcrc.org/sift/SIFT.html) if at least one of the program predicted the variant to be possibly damaging, it was considered to be pathogenic; 6) presence of the second mutant allele in the case of autosomal recessive inheritance. Mutations were described according to the HGVS website http://www.hgvs.org/mutnomen. In accordance with this nomenclature, nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG.

Table 2 Candidate genes for retinal disorders (Continued)

| No. | Gene | Function | Expression | Reference |
|-----|------|----------|------------|-----------|
| 48  | PKD2L1 | Diff. expression in human retinal detachment | Delyfer et al. 2011 submitted |
| 49  | PLEKHA1 | Age-related macular degeneration | [57] |
| 50  | PPEF2 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 51  | RABBA | Interacts with RPGR, role in cilia biogenesis and maintenance | [58] |
| 52  | RABGEF1 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 53  | RCVRN | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 54  | RG5 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 55  | RNF144B | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 56  | RORB | Rod photoreceptor development in mice | [59] |
| 57  | RXRG | Retinoic acid receptor, highly expressed in the eye | Expression databases |
| 58  | SGIP1 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 59  | SLC16A8 | Altered visual function in ko-mice | [60] |
| 60  | SLC17A7 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 61  | STAM2 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 62  | STK35 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 63  | STX3 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 64  | SV2B | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 65  | TBC1D24 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 66  | THR8 | Essential for M-cone development in rodents | [61] |
| 67  | TMEM216 | Cilia protein, mutations lead to Joubert and Meckel syndrome | [62] |
| 68  | TMEM67 | Cilia protein, mutations lead to Joubert | [63] |
| 69  | TRPC1 | diff. expression rd1 mouse | diff. expression Rd1 mouse |
| 70  | UHMK1 | diff. expression rd1 mouse | diff. expression Rd1 mouse |
| 71  | VSX1 | Stimulator for promoter NXNL1 | [64] |
| 72  | VSX2 | Stimulator for promoter NXNL1 | [64] |
| 73  | WDR17 | diff. expression rd1 mouse | diff. expression Rd1 mouse |
| 74  | WDR31 | diff. expression Nxnl1-/- mouse | [65] |
| 75  | WISP1 | diff. expression rd1 mouse | Chalmel et al., manuscript in preparation |
| 76  | XIAP | Protects photoreceptors in animal models of RP | [66] |
| 77  | ZDHHC2 | diff. expression Rd1 mouse | Chalmel et al., manuscript in preparation |
translation initiation codon in the reference sequence. The initiation codon is codon 1. The correct nomenclature for mutation was checked applying Mutalyzer http://www.lovd.nl/mutalyzer/.

Results
The overall sequencing coverage of the captured regions was 98.4% and 90.4% for a 1× and a 10× coverage respectively. The overall sequencing depth was > 120×. The number of reference and variant sequences detected by NGS, reflected the correct zygosity state of the variant; on average if 50% of the sequences represented the variant, then a heterozygous state was called, while if 100% of the sequences represented the variant, then a homozygous or hemizygous state was annotated by IntegraGen.

Validation of the novel genetic testing tool for retinal disorders
To validate the novel genetic testing tool for retinal disorders, we used four DNA samples from families, in which we had previously identified different types of mutations by Sanger sequencing: one 1 bp duplication and one 1 bp deletion in PRPF31 and missense mutations in TRPM1 and BEST1 (Table 3). Three of the four mutations were detectable by NGS, whereas the deletion in PRPF31 was not identified. To validate if this was due to a technical problem of deletion detection in general or low coverage at this position, the sequencing depth was investigated in detail. Indeed the coverage at this position reflected by the mean depth was only ~1-6 for all samples. This indicates that although the coverage in
general was very good, specific probes used here need to be redesigned to improve the capture for specific exons.

Detection of known and novel mutations
Some of the patients from the 14 families with no known gene defect were previously excluded for known mutations using microarray analysis and by Sanger sequencing in the known genes EYS, C2orf71, RHO, PRPF31, PRPH2 and RP1. Other samples were never genetically investigated. In four DNA samples known mutations were detected (Table 4) from three different families with autosomal dominant (ad) or recessive (ar) RP. All mutations co-segregated with the phenotype (Figure 2). In seven samples, novel mutations in known genes were identified. These mutations co-segregated with the phenotype from five different families with adCSNB, x-linked incomplete CSNB, adRP, arRP and x-linked RP (Table 5, Figures 3 and 4). One of the cases from these five families was also used as a control for Best disease carrying a known BEST1 mutation (Table 4, Figure 3).

Unsolved cases
In six of the 14 families with Stargardt disease, adRP, adCD with postreceptoral defects, arRP, early onset arCD with macrocephaly and mental retardation described in affected sister and x-linked cCSNB, the disease associated mutations remain to be elucidated or validated (Table 6, Figure 5).

Discussion
By using NGS in 254 known and candidate genes we were able to detect known and novel mutations in 57% of families tested. In order to achieve this goal, we applied a rigorous protocol (Figure 1). To our knowledge, this is the first report using NGS to investigate all inherited retinal disorders at once. In a study restricted to adRP, Bowne and co-workers used a similar approach including 46 known and candidate genes for adRP [18]. All their cases had previously been screened and excluded for most of the known genes underlying adRP. The authors were able to identify known or novel mutations in five out of 21 cases in genes not included in a pre-screening [18]. This added five patients to their

### Table 3 Patients with known mutations used to validate the novel genetic approach for retinal disorders

| Index     | Phenotype     | Gene          | Mutation          | Allele State | Read reference NGS | Read variant NGS | Mutation detected by NGS | Mean depth |
|-----------|---------------|---------------|-------------------|--------------|--------------------|------------------|--------------------------|------------|
| CIC00034, F28 | adRP          | PRPF31        | c.666dupG         | het          | 11                 | 13               | yes                      | 21.3-22.5  |
| CIC00140, F108 | adRP          | PRPF31        | c.997delG         | het          | -                  | -                | no                       | 5.0-5.2    |
| CIC00238, F165 | arCSNB        | TRPM1         | c.1418G > C       | homo         | 0                  | 38               | yes                      | 36.7       |
| CIC00707, F470 | Best and adCSNB see Table 5 | BEST1        | c.73C > T         | het          | 40                 | 38               | yes                      | 99.4       |

### Table 4 Detection of known mutations by using the novel genetic approach for retinal disorders

| Index     | Phenotype      | Pre-screening | Gene            | Mutation         | Allele State | Read reference NGS | Read variant NGS | Reference Sanger and co-segregation | Mutation verified by Sanger and co-segregation |
|-----------|----------------|---------------|-----------------|------------------|--------------|--------------------|------------------|-------------------------------------|-----------------------------------------------|
| CIC00019, F16 | adRP          | Linkage, RHO, PRPF31, PRPH2, RP1 | PRPF31         | c.1481C > T p.T494M | het          | 25                 | 22               | [67]                                | yes                                           |
| CIC0000893, F574 | adRP          | RHO, PRPF31, PRPH2, RP1 | NR2E3          | c.166G > A p.G56R | het          | 5                  | 3                | [68]                                | yes                                           |
| CIC000128, F100 | arRP, consang | -             | EYS             | c.408_423del p.N137VfsX24 | homo         | -                  | 179              | [13,69]                             | yes                                           |
| CIC0000943, F100 | arRP, consang | -             | EYS             | c.408_423del p. N137VfsX24 | homo         | 0                  | 193              | [13,69]                             | yes                                           |
adRP cohort with known gene defects, indicating that 64% of their patients show known mutations with new genes still to be discovered in the remaining 36%. The current study provides a more exhaustive tool, since it incorporates screening of 254 genes implicated in various retinal disorders of different inheritance patterns and additional candidate genes for these phenotypes. With this approach a cohort of both pre-screened and unscreened samples, was investigated. The mutation detection rate of 57% is high and was never obtained before by high throughput screening methods. Furthermore, this approach is probably less time consuming and expensive than existing methods such as direct sequencing of all known genes or microarray analysis. Of note however is one of the variants detected with the NGS approach (i.e. p.V973L exchange in GLYC2D), which was not confirmed by direct Sanger sequencing, suggesting the possibility of false positive using the high throughput screening. Verification by direct Sanger sequencing of most likely pathogenic variants is therefore essential to validate NGS data, although the false positive rate is assumed to be low (in our study 1/28 verified sequence variants represented a false positive).

Overall, the study of 20 subjects from 17 families by NGS showed that most of the targeted regions are well covered (more than 98%). However, some of the regions showed a lower coverage (GC-rich regions) or were not captured (repetitive regions). This was for instance the case for two genes underlying cCSNB, (i.e. NYX and GRM6) and the repetitive region of ORF15 of RPGR. For GC-rich regions the capture design could be improved in the future by modifying NGS chemistry, as...
it was successfully achieved for Sanger sequencing using different additives, which improved the amplification and subsequent sequencing. If repetitive regions like ORF15 of RPGR remain problematic for sequencing by NGS, direct Sanger sequencing of these targets might be the first screening of choice; in particular for disorders caused only by a few gene defects such as CSNB, and xl-RP.

By applying NGS sequencing to our retinal panel, known and novel mutations were detected in different patients. We believe that our diagnostic tool is particularly important for heterogeneous disorders like RP, for which many gene defects with different prevalence have been associated to one phenotype. It also allows the rapid detection of novel mutations in minor genes which are often not screened as a priority by direct Sanger sequencing. This was the case in our study for three individuals from one family with adRP in which NGS detected a novel PRPF8 mutation in both affected and one unaffected family member (Table 4, Figure 4). In this family, the RP phenotype is mild and therefore it is possible that the unaffected member may develop symptoms later in life or alternatively it may be a case of incomplete penetrance as reported for another splicing factor gene, PRPF31 and recently for PRPF8 as well [19-22]. Interestingly, a novel TRPM1 mutation was identified in a patient with adCSNB, a gene previously only associated with arCSNB [23-26]. This is the first report of a TRPM1 mutation co-segregating with ad Schubert-Bornschein type complete CSNB. Since the location of this mutation is not different compared to other mutations leading to arCSNB, it is not quite clear how TRPM1 mutations might lead to either ad or arCSNB. Functional investigations are needed to validate the pathogenicity of this variant. Furthermore, this finding suggests that TRPM1 heterozygous mutation carriers from arCSNB families should be investigated by electroretinography to determine whether they display similar retinal dysfunction as in affected members of the presented adCSNB family. Detection of a novel RPGR splice site mutation in family 146 presented a challenge. The actual disease causing change was concealed under a wrongly annotated rs62638633, which had previously been clinically associated to RP by a German group [27], but has recently been reported to be a variant of unknown clinical significance [28].

Table 5 Detection of novel mutations by using the novel genetic approach for retinal disorders

| Index  | Phenotype | Pre-screening | Gene | Mutation | Allele State | Read reference | Read variant | Mutation verified by Sanger and co-segregation | Conservation | Polyphen | Sift |
|--------|-----------|---------------|------|----------|--------------|---------------|--------------|-----------------------------------------------|--------------|---------|-----|
| CIC00707, F470 | adCSNB and Best see Table 3 | RHO, PRPH2, RP1, adRP chip | TRPM1 | c.1961A > C, p.H654P | het | 39 | 38 | yes | moderately conserved | possibly damaging | tolerated |
| CIC000348, F232 | adRP, mild | RHO, PRPF31, PRPH2, RP1, adRP chip | PRPF8 | c.6992A > G, p.E2331G | het | 13 | 10 | yes | moderately conserved | possibly damaging | affect protein function |
| CIC000346, F232 | adRP | - | PRPF8 | c.6992A > G, p.E2331G | het | 5 | 9 | yes | moderately conserved | possibly damaging | affect protein function |
| CIC000347, F232 | adRP | - | PRPF8 | c.6992A > G, p.E2331G | het | 15 | 17 | yes | moderately conserved | possibly damaging | affect protein function |
| CIC04240, F2025 | arRP, consang., detailed clinic in [78] | RST | CRB1 | c.2219C > T, p.S740F | homo | 2 | 194 | yes | highly conserved | probably damaging | affect protein function |
| CIC00199, F146 | adRP or x-linked RP with affected carrier | RHO, PRPF31, PRPH2, RP1, adRP chip | RPGR | c.248-2A > G | hetero | 30 | 22 | yes | conserved splice site | n.a. | n.a. |
| CIC04094, F1915 | icCSNB | - | CACNA1F | c.973C > T, p.Q325X | hemi | 0 | 28 | yes | n.a. | n.a. | n.a. |
referenced disease causing variants. Bearing this in mind one can still first investigate unknown variants, but should then examine dbSNP for referenced variants either described to be disease causing, having a low minor allele frequency or present in interesting candidate genes. An accurate discrimination of non-pathogenic polymorphisms versus disease causing polymorphism in SNP databases is warranted to resolve this challenge.

In six families from the investigated cohort the disease causing mutations still remain to be identified. In the Stargardt patient with no pathogenic ABCA4 mutations two variants in CFH were detected, one of which (rs1061170) had previously been reported to predispose to age related macular degeneration (AMD) [27-29]. The second CFH change is a novel variant, affecting a highly conserved residue, not found in NGS data from the other 19 samples and never associated with a disease. The variants co-segregated in the only available family members, which were the patient’s parents. Apart from the association with AMD, CFH mutations have been previously associated with renal diseases, the most common being membranoproliferative glomerulonephritis and hemolytic uremic syndrome, which can be also associated with an eye phenotype [30,31]. No renal dysfunction was present in our patient. To validate if the two variants identified in CFH are indeed disease causing, the DNA samples from other available family members for co-segregation analysis as well as characterization of functional consequences of the novel variant are needed. One patient with complete CSNB had an affected nephew and thus x-linked inheritance was assumed. However, neither Sanger nor NGS detected a mutation in the only known x-linked gene, NYX, causing cCSNB. To exclude recessive inheritance TRPM1 and GRM6 were investigated in detail. Indeed the patient carried a novel heterozygous

Figure 3 Best disease and CSNB co-segregating in one family  a) Sanger and NGS detected in all patients with Best disease a BEST1 mutation. b) NGS detected in all patients with a cCSNB phenotype a novel TRPM1 mutation. c) Fundus colour photographs (above) and fundus autofluorescence (below) of patient 707 showing multiple yellow deposits within the posterior pole which are hyper autofluorescent d) Electro-oculogram of patient 707 showing no slight rise after illumination in keeping with the diagnosis of Best disease e) Full Field Electroretinogram of patient 707 showing ON-bipolar cell pathway dysfunction in keeping with the diagnosis of cCSNB.
TRPM1 variant, which affects a highly conserved amino acid and was not identified in the other 19 samples investigated here (Table 6). However, direct Sanger sequencing of lower covered regions did not identify a second mutation in this gene. Similarly no mutations in GRM6 were identified. These findings outline the need for additional family members to determine, through co-segregation, the pathogenicity of the numerous variants identified by NGS. This was also true for two other families with nonsense mutations in CUBN (Fam795) and RP1L1 (Fam761) (Table 6). The nonsense mutation in CUBN, co-segregated with the phenotype in most of the family members (Figure 5). Had we not had access to additional family members, we might have retained this gene defect as the underlying cause for adCD and considered CUBN as a new gene involved in adCD. None of the other putatively pathogenic mutations identified in CUBN, TRPM1 and GUCY2D co-segregated with the phenotype in this family (Table 6, Figure 5). RP1L1 was already a candidate for adRP [32] but was previously associated with occult macular dystrophy [33]. In our study, this variant did not co-segregate with the phenotype in other affected family members (data not shown).

This NGS study ended with six genetically unresolved families, which can be further investigated with whole exome sequencing. Although, no clear information about the actual percentage of missing gene defects underlying each group of inherited retinal disorders exists, previous studies have reported that in many cases the genetic cause still needs to be determined [18,34]. Whole exome sequencing approaches allow the detection of both, novel and known gene defects, but also generate numerous variants and therefore require the inclusion of more than one DNA sample for each family to rapidly exclude non-pathogenic variants. Due to the higher costs of exome sequencing for one sample compared to targeted sequencing, we propose to initially perform targeted sequencing in the index patient and proceed only after exclusion of a known gene defect to whole exome sequencing.

Figure 4 Detection of novel mutations using NGS in 254 retinal genes. Novel mutations in PRPF8, CRB1, RPGR and CACNA1F co-segregated in affected and asymptomatic carriers with the adRP, arRP, x-linked dominant and X-linked icCSNB phenotypes respectively. Asymptomatic individuals are marked with a question mark.
| Index      | Phenotype                      | Pre-screening | Gene     | Mutation                           | Allele State | Read reference NGS | Read variant NGS | Mutation verified by Sanger and co-segregation | Comment                                                                 |
|------------|-------------------------------|---------------|----------|------------------------------------|--------------|--------------------|------------------|------------------------------------------------|-------------------------------------------------------------------------|
| CIC03282, F1388 | Stargardt                | ABCA4 microarray | ABCA4    | c.1268A > G p.H423R               | het          | 77                 | 61               | yes                                                           | but reported as polymorphism [71]                                       |
|            |                               |               |          | c.6764G > T p.S2255I              |              |                    |                  |                                                               | but reported as polymorphism [72]                                       |
|            |                               |               |          |                                    |              |                    |                  |                                                               |                                                                          |
|            |                               |               |          |                                    |              |                    |                  |                                                               |                                                                          |
| CFH        |                               |               |          | c.3482C > A p.P1161Q             | het          | 77                 | 52               | yes                                                           | conserved, probably damaging                                           |
|            |                               |               |          | c.1204C > T p.H402Y             |              | 94                 | 87               | yes                                                           | AMD                                                                      |
| CIC01269, F761 | adRP                    |               | RP1L1    | c.5959C > T p.Q1987X            | het          | 145                | 150              | yes, did not co-segregate                                    | pass to whole exome sequencing                                          |
| CIC01312, F795 | adCD with post-receptor defects | RHO, PDE6B, GNAT1 | CUBN    | c.127C > T p.R43X              | het          | 139                | 102              | yes, did not co-segregate                                    | pass to whole exome sequencing                                          |
| CUBN       |                               |               |          | c.9340G > A p.G3114S            |              | 61                 | 44               | yes, did not co-segregate                                    |                                                                          |
| GUCY2D     |                               |               |          | c.1499C > T p.S500L             |              | 41                 | 34               | yes, did not co-segregate                                    |                                                                          |
| TRPM1      |                               |               |          | c.3904T > C p.C1302R            | het          | 102                | 99               | yes, did not co-segregate                                    |                                                                          |
| CIC03225, F1362 | arRP consang.       | arRP chip    | PROM1    | c.314A > G p.Y105C             | het          | 120                | 115              | yes, but no additional mutation                             | no homo, no compound hets, pass to whole exome sequencing               |
| GUCY2D     |                               |               |          | c.2917G > A p.V973L            |              | 6                  | 2                | false positive, not found by Sanger                        |                                                                          |
| DSCAML1    |                               |               |          | c.592C > T p.R198C             | het          | 70                 | 81               | yes, but no additional mutation                             |                                                                          |
| TBC1D24    |                               |               |          | c.641G > A p.R214H             | het          | 27                 | 12               | yes, but no additional mutation                             |                                                                          |
| TMEM67     |                               |               |          | c.1700A > G p.Y567C            | het          | 80                 | 58               | yes, but no additional mutation                             |                                                                          |
| CIC04757, F2364 | Index and affected sister early onset drCD, macrocephaly and mental retardation in affected sister consang. |               | IMPG2    | c.3439C > T p.P1147S          | homo         | 0                  | 140              | no                                                            | Polyphen and Sift benign, not conserved                                 |
| PKD2L1     |                               |               |          | c.1027C > T p.R343C            | het          | 63                 | 68               |                                                               | appeared also het in 11 of our samples                               |
|            |                               |               |          | c.1202T > G p.V401G            |              | 25                 | 19               |                                                               | appeared also het in affected sister but no other mutation in less covered exons |
Conclusions
In summary, our diagnostic tool is an unbiased time efficient method, which not only allows detecting known and novel mutations in known genes but also potentially associates known gene defects with novel phenotypes. This genetic testing tool can now be applied to large cohorts of inherited retinal disorders and should rapidly deliver the prevalence of known genes and the percentage of cases with missing genetic defect for underlying forms of retinal disorders.

List of abbreviations
ad: autosomal dominant; ar: autosomal recessive; as: asymptomatic; het: heterozygous; homo: homozygous; hemi: hemizygous; - not noted; consang.

Table 6 Patients with unsolved genotype and unlikely disease causing mutations (Continued)

| Gene     | Mutation | Genotype | Case | Control | Phenotype | Comment                                      |
|----------|----------|----------|------|---------|-----------|----------------------------------------------|
| DFNB31   | c.1943C > A p.S648Y | het | 7    | 7       | yes       | affected sister also both variants from father, no other variant in lower covered region. |
|          | c.2644C > A p.R882S | het  | 27   | 14      | yes       |                                               |
| EYS      | c.7597A > G p.K2533E | het | 151  | 149     | yes       | Affected sister does not carry this variant   |
| RPGRIP1  | c.2417C > T p.T806I | het | 138  | 132     | no        | not conserved                                 |
| CIC04152, F1953 | male x-linked | NYX |     |         |           |                                               |
|          | cCSNB, has affected nephew | c.470C > T p.S157F | het | 118     | 130       | yes, no other het mutation. x-linked inheritance and phenotype verification |

Index patients and respective gene defect are highlighted in bold. In some cases also family members were used for NGS.

Figure 5 Detection of novel mutation by using NGS in 254 retinal genes. Family 795 reveals autosomal dominant cone dystrophy with post-receptoral defects. Four putative disease causing mutations were investigated on the basis of co-segregation. However, none of them co-segregated in all affected family members with the phenotype and thus are not considered to be disease causing. Individuals marked with a star were clinically investigated, patients with a question mark are asymptomatic and patients with a plus sign show high myopia.

Fam 795:
M1: CUBN: c.127C>T p.R43X
M2: CUBN: c.9340G>A p.G311S
M3: GUCY2D: c.1499C>T p.P500L
M4: TRPM1: c.3904T>C p.C1302R
consanguinity was reported; n.a.: not applicable, CSNB: congenital stationary night blindness, RP: retinitis pigmentosa.

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Authors’ contributions

IA was involved in the study design, participated in the choice of genes, interpreted the NGS data, clinically investigated patients, collected DNA samples, and has been involved in drafting the manuscript. KB participated in the choice of genes, interpreted the NGS data and has been involved in drafting the manuscript. TL was involved in the study design, participated in the choice of genes and has been involved in drafting the manuscript. SM-S clinically investigated patients and collected DNA samples. M-EL confirmed the NGS data by Sanger sequencing, performed control and co-segregation analysis. AG extracted DNA, confirmed the NGS data by Sanger sequencing, and performed control and co-segregation analysis. AA extracted DNA, confirmed the NGS data by Sanger sequencing, and performed control and co-segregation analysis. CM confirmed the NGS data by Sanger sequencing, and performed control and co-segregation analysis. J-PS performed NGS. ML performed the bioinformatic interpretation of NGS. J-AS clinically investigated patients and participated in the study design. SSB participated in the study design and has been involved in drafting the manuscript. CZ has made the study design, participated in the choice of genes, interpreted the NGS data and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Sahoo LJ, Hider RN, Emerich DF, et al: The spectrum of retinal dystrophies caused by mutations in the peripherin/RDS gene. Prog Retin Eye Res 2008, 27:213-235.
2. Boon CJ, Klevering BJ, Leroy BP, Huygen CJM, Smit ME, Luttikhuizen EP, Keuning JE: The spectrum of ocular phenotypes caused by mutations in the BEST1 gene. Prog Retin Eye Res 2008, 27:187-205.
3. Schorle WC, Green P, Naderi S, et al: Novel C2orf71 mutations account for approximately 1% of cases in a large French adRP cohort. Hum Mutat 2011, 32:E2091-2103.
4. Bandah-Rozenfeld D, Collin RW, Banin E, van den Born LI, Coene KL, Smit ME, Luttikhuizen EP, Keuning JE: The spectrum of rhodopsin mutations in French autosomal dominant rod-cone dystrophies. Hum Mutat 2010, 31:E1406-1435.
5. Boon CJ, Klevering BJ, Keuning JE: Expression of PRPF31 mRNA in the human retina. Exp Eye Res 2009, 88:499-505.
6. Boon CJ, Klevering BJ, Keuning JE: Expression of PRPF31 mRNA in cones and photoreceptors of the retina. Exp Eye Res 2009, 88:499-505.
7. Boon CJ, Klevering BJ, Keuning JE: Expression of PRPF31 mRNA in the human retina. Exp Eye Res 2009, 88:499-505.
8. Boon CJ, Klevering BJ, Luttikhuizen EP, Keuning JE: The spectrum of ocular phenotypes caused by mutations in the BEST1 gene. Prog Retin Eye Res 2008, 27:213-235.
9. Schorle WC, Green P, Naderi S, et al: Novel C2orf71 mutations account for approximately 1% of cases in a large French adRP cohort. Hum Mutat 2011, 32:E2091-2103.
10. Boon CJ, Klevering BJ, Luttikhuizen EP, Keuning JE: The spectrum of ocular phenotypes caused by mutations in the BEST1 gene. Prog Retin Eye Res 2008, 27:213-235.
11. Bandah-Rozenfeld D, Collin RW, Banin E, van den Born LI, Coene KL, Smit ME, Luttikhuizen EP, Keuning JE: The spectrum of rhodopsin mutations in French autosomal dominant rod-cone dystrophies. Hum Mutat 2010, 31:E1406-1435.
12. Boon CJ, Klevering BJ, Keuning JE: Expression of PRPF31 mRNA in the human retina. Exp Eye Res 2009, 88:499-505.
13. Boon CJ, Klevering BJ, Luttikhuizen EP, Keuning JE: The spectrum of ocular phenotypes caused by mutations in the BEST1 gene. Prog Retin Eye Res 2008, 27:213-235.
Bernd A, Antonio A, Moskova-Doumanova V, Lancelot ME, Poloschek CM, Bertrand JY, Cantagrel V, Silhavy JL, Bielas SL, Swistun D, Marsh SE, Beales PL, Dietz HC, Fisher S, Katsanis N. Dissection of epistasis in oligogenic Bardet-Biedl syndrome. Nature 2006; 439:326-330.

Kornak U, Kasper D, Boul MR, Kaiser E, Schweizer M, Schuler A, Friedman W, Delling G, Jentsch TJ. Loss of the CIC-7 chloride channel leads to osteopetrosis in mice and man. Cell 2001; 104:205-215.

Colville DJ, Savage J. Alport syndrome. A review of the oculomacular manifestations. Ophthalm Genet 1997; 18:161-173.

Lemmink HH, Hochzikul T, van den Heuvel LP, Schroder CH, Barontos N, Monnens LA, van Oost BA, Brunner HG, Reinders ST, Smets HJ. Mutations in the type IV collagen alpha 3 (COL4A3) gene in autosomal recessive Alport syndrome. Hum Mol Genet 1994; 3:1293-1273.

Jefferson JA, Lemmink HH, Hughes AE, Hill CM, Smets HJ, Doherty CC, Maxwell AP. Autosomal dominant Alport syndrome linked to the type IV collagen alpha 3 and alpha 4 genes (COL4A3 and COL4A4). Nephrol Dial Transplant 1997; 12:1595-1599.

Lemmink HH, Klijtmans LA, Brunner HG, Schroder CH, Kriebelmann B, Jelinikova E, van Oost BA, Monnens LA, Smets HJ. Abrantial splicing of the COL4A3 gene in patients with Alport syndrome. Hum Mol Genet 1994; 3:317-322.

Stoilov I, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. Hum Mol Genet 1997; 6:641-647.

Mitton RP, Swain PK, Khanna H, Dowd M, Apel I, Swaroop A. Interaction of retinal BZIP transcription factor NRL with IT12-interacting zinc-finger protein Fiz1: possible role of Fiz1 as a transcriptional repressor. Hum Mol Genet 2003; 12:365-373.

Xu X, Quinlao AB, Robin L, Pardue MT, Max JL, Rohlich P, Peayը, Al-Ubaidi MR. Degeneration of cone photoreceptors induced by expression of the Mas1 protooncogene. Exp Neurol 2000; 163:207-219.

Kubota R, Kodou J, Maitima Y, Asakawa S, Minoshima S, Hejtmancik JF, Oguchi Y, Shimizu N. Genomic organization of the human myocilin gene (MYOC) responsible for primary open angle glaucoma (GLC1A). Biochim Biophys Acta 1998; 1425:1-20.

Bhattarayya SS: A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 1q9-13.4 (RP11). Mol Cell Biol 2001; 21:375-381.

Maubaret CG, Vaclavik V, Muhopadhyay R, Waseem NH, Churchill A, Holder GE, Moore AT, Bhattarayya SS, Webster AR. Autosomal Dominant Retinitis Pigmentosa with Intralaminar Variability and Incomplete Penetration in Two Families carrying Mutations in PRP8. Invest Ophthalmol Vis Sci 2011.

Li Z, Sergouniotis PI, Michaelides M, Mackay DS, Wright GA, Devery S, Moore AT, Holder GE, Robson AG, Webster AR. Recessive mutations of the gene TRPM1 abrogate ON bipolar cell function and cause complete congenital stationary night blindness in humans. Am J Hum Genet 2009; 85:711-719.

van Gendersen MM, Bijveel MM, Claassen YB, Florijn Rj, Pearing JN, Meire FM, McCall MA, Riemsag FC, Gregg RG, Bergeren AG, Kammens M. Mutations in TRPM1 are a common cause of complete congenital stationary night blindness. Am J Hum Genet 2009; 85:730-736.

Audo I, Kohl S, Lenoy BP, Munier FL, Guillonneau X, Mohand-Said S, Bujakowska K, Nandrot EF, Lorence B, Preising M, Kellner U, Renner AB, Bernd A, Antonio A, Moskova-Doumanova V, Lancret ME, Poloscek CM, Dunmire J, Defoe-Delhommeres S, Wissinger B, Leveillard T, Harpal CP, Schorster DF, DeBaere E, Berger W, Jacobson SG, Zierner E, Sahel J, Bhattarayya SS, Dietz HC. TRPM1 is mutated in patients with autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 2009, 85:720-729.

Nakamura M, Sanuki R, Yasuma TR, Onishi A, Nishiguchi KM, Koike C, Kadozaki M, Kondo M, Miyake Y, Furutaka T. TRPM1 mutations are associated with the complete form of congenital stationary night blindness. Mol Vis 2010; 16:4-15.

Klein RJ, Geiz C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. Science 2005; 308:385-389.

Edwards AO, Ritter R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science 2005; 308:421-424.

Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Edwards AO, Ritter R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science 2005; 308:421-424.

Bowen SJ, Daiger SP, Malone KA, Heckenlively JR, Kennan A, Humphries P, Factor H and the pathogenesis of renal diseases. Pediatr Nephrol 2004, 19:1045-1053.

Boon CJ, van de Kar NC, Klevering BJ, Keunen JE, Cremers FP, Klaver CC, Hoyng CB, Daha MR, den Hollander AI. The spectrum of phenotypes caused by variants in the CFH gene. Mol Immunol 2009; 46:1573-1594.

Bovine SJ, Daiger SP, Malone KA, Heckenlively JR, Kennan A, Humphries P, Hugbikents-Wheaton D, Birch DG, Liu Q, Piente EA, Zuo J, Huang G, Donovan DD. Characterization of RP1L1, a highly polymorphic paralog of the retinitis pigmentosa 1 (RP1) gene. Mol Vis 2003; 9:147-153.

Akahori M, Tsunoda K, Miyake Y, Fukuda Y, Ishiura H, Tsuji S, Usui T, Hatase T, Nakamura M, Odate In, Ibatachi T, Okamoto H, Takada Y, Iwata T. Dominant mutations in RP1L1 are responsible for occult macular dystrophy. Am J Hum Genet 2010; 87:242-429.

Berger W, Kloeckener-Gruissem B, Nendhurt J. The molecular basis of human retinal and vitreoretinal diseases. Prog Retin Eye Res 2010; 29:335-375.

Vaclavik V, Gaillard MC, Tsai L, Schorster DF, Munier FL. Variable phenotypic expressivity in a Swiss family with autosomal dominant retinitis pigmentosa due to a T949M mutation in the RPFR3 gene. Mol Vis 2010; 16:467-475.

Zhao SH, Pan DY, Zhang Y, Wu JH, Lu X, Xu Y. Annexin A2 promotes chondrocyte neovascularization by increasing vascular endothelial growth factor expression in a rat model of argon laser coagulation-induced chondrocyte neovascularization. Chin Med J (Engl) 2010; 123:713-721.

Cantagrel V, Silhavy JL, Schwitters D, Marsh SE, Bertrand JY, Audolent S, Attie-Bitach T, Holder KR, Dobyns WB, Traver D, Aiazli L, Ali BR, Lindner TH, Caspary T, Otto HA, Hildebrandt F, Glass IA, Logan CV, Johnson CA, Bennett C, Biancata F, Valente EM, Woods CG, Gleeson JG. Mutations in the cilia gene ARL13B lead to the classical form of Joubert syndrome. Am J Hum Genet 2008, 83:710-719.

Sehgal R, Sheibani N, Rhodes SJ, Belely Adams TL. BMP7 and SHH regulate Pax2 in mouse retinal astrocytes by relieving TLX repression. Dev Biol 2009; 322:429-443.

Beales PL, Badano JL, Ross AJ, Andsey SJ, Hooiens BE, Kirsten B, Mein CA, Trope ML, Scambler PJ, Lewis RA, Lupski JR, Katsanis N. Genetic interaction of BS51 mutations with alleles at other BS5 loci can result in non-Mendelian Bardet-Biedl syndrome. Am J Hum Genet 2003; 72:1187-1199.

Badano JL, Leitch CC, Andsey SJ, May-Simera H, Lawson S, Lewis RA, Beales PL, Dietz HC, Fisher S, Katsanis N. Dissection of epistasis in oligogenic Bardet-Biedl syndrome. Nature 2006; 439:326-330.

Kornak U, Kasper D, Boul MR, Kaiser E, Schweizer M, Schuler A, Friedrich W, Delling G, Jentsch TJ. Loss of the CIC-7 chloride channel leads to osteopetrosis in mice and man. Cell 2001; 104:205-215.
implications for cilia dysfunction and photoreceptor degeneration. Hum Mol Genet 2010, 19:3591-3598.
59. Jia L, Oh EC, Ng L, Srinivas M, Brooks M, Swaroop A, Forrest D: Retinoid-related orphan nuclear receptor RORbeta is an early-acting factor in rod photoreceptor development. Proc Natl Acad Sci USA 2009, 106:17354-17359.
60. Daniele L, Sauer B, Gallagher SM, Pugh EN Jr, Philip NJ: Altered visual function in monocarboxylate transporter 3 (Slc16a8) knockout mice. Am J Physiol Cell Physiol 2008, 295:C451-457.
61. Ng L, Hurley JB, Dierks B, Srinivas M, Salto C, Venstrom B, Reh TA, Forrest D: A thyroid hormone receptor that is required for the development of green cone photoreceptors. Nat Genet 2001, 27:94-98.
62. Valente EM, Logan CV, Mougou-Zerelli S, Lee JH, Silhavy JL, Brancati F, Iannicelli M, Travaglini L, Romani S, Illi B, Adams M, Szymanska K, Mazzotta A, Lee JE, Tolentino JC, Swistun D, Salpietro CD, Fede C, Gabriel S, Russ C, Cibulskis K, Soutoqho C, Hildebrandt F, Otto EA, Held S, Dipla BH, Davis EE, Mikula M, Strom CM, Ben-Zeke B, et al: Mutations in TMEM216 perturb cilogenesis and cause Joubert, Meckel and related syndromes. Nat Genet 2010, 42:619-625.
63. Baala L, Romano S, Khaddour R, Saunier S, Smith UM, Audollent S, Ozbol C, Faivre L, Laurent N, Folletier B, Munnich A, Lyonnet S, Salomon R, Encha-Razavi F, Guider MC, Boddart N, de Lonlay P, Johnson CA, Vekemans M, Antignac C, Attie-Bitach T: The Meckel-Gruber syndrome gene, MKS3, is mutated in Joubert syndrome. Am J Hum Genet 2007, 80:186-194.
64. Reichman S, Kalathur RK, Lambard S, Ait-Ali N, Yang Y, Lardenois A, Ripp R, Poch O, Zick DJ, Sahel JA, Leveillard T: The homeobox gene CHX10/VSX2 regulates RdCVF promoter activity in the inner retina. Hum Mol Genet 2010, 19:250-261.
65. Cronin T, Raffelsberger W, Lee-Rivera J, Jallard C, Niepomniasz ML, Kinzel B, Clerin E, Petroian A, Picaud S, Poch O, Sahel JA, Leveillard T: The disruption of the rod-derived cone viability gene leads to photoreceptor dysfunction and susceptibility to oxidative stress. Cell Death Differ 2010, 17:1199-1210.
66. Leonard KC, Perlin D, Coupland SG, Baker AN, Leonard BC, LaCasse EC, Hauwirth WW, Korneluk RG, Tsilfidis C: XIAP protection of photoreceptors in animal models of retinitis pigmentosa. PLoS ONE 2007, 2:e314.
67. Chakarova CF, Hims MM, Bozic H, Abu-Safieh L, Patel RJ, Papaioannou MG, Inglehearn CF, Keen TJ, Willis C, Moore AT, Rosenberg T, Webster AR, Bird AC, Gal A, Hunt D, Vithana EN, Bathe Shataya SS, Zeitz C: Mutations in HPRP3, a third member of pre-mRNA splicing factor genes, implicate in autosomal dominant retinitis pigmentosa. Hum Mol Genet 2002, 11:87-92.
68. Cooper Net, Leroy BP, Belayen D, Helfemans M, De Bosscher K, Haegebarth A, Koppereau L, Lejeune M, Coucke PJ, De Baere E: Recurrent mutation in the first zinc finger of the orphan nuclear receptor NR2E3 causes autosomal dominant retinitis pigmentosa. Am J Hum Genet 2007, 81:147-157.
69. Bandah-Rozenfeld D, Littin KW, Ban-Yosef T, Strom TM, Chewers I, Collin RW, hen Holland, AI, van den Born LI, Zonneveld MN, Merin S, Banin E, Cremers FP, Sharon D: Novel null mutations in the EYS gene are a frequent cause of autosomal recessive retinitis pigmentosa in the Israeli population. Invest Ophthalmol Vis Sci 2010, 51:4387-4394.
70. Bujakowska K, Audo I, Audo S, Lancelot ME, Bovman A, Germain A, Mann G, Leveillard T, Lettenoi M, Saranne JP, Lonjou C, Carpenter W, Sahel J, Hackett Shataya SS, Zet C: CRB1 mutations in inherited retinal dystrophies. Hum Mutat 2011, 33:306-315.
71. Rivera A, White K, Stothar H, Steiner K, Hymens N, Grimm T, Jurkies B, Lorenz B, Scholl HP, Apfelstedt-Sylla E, Weber BH: A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. Am J Hum Genet 2000, 67:800-813.
72. Shroyer NF, Lewis RA, Lupski JR: Analysis of the ABCR (ABCR) gene in 4-aminooxquinoline retinopathy: is retinal toxicity by chloroquine and hydroxychloroquine related to Stargardt disease? Am J Ophthalmol 2001, 131:761-766.