Next-generation sequencing-based comprehensive molecular analysis of 43 Japanese patients with cone and cone-rod dystrophies

Maho Oishi,1 Akio Oishi,1 Norimoto Gotoh,2 Ken Ogino,1 Koichiro Higasa,2 Kei Iida,3 Yukiko Makiyama,1 Satoshi Morooka,1 Fumihiko Matsuda,2 Nagahisa Yoshimura1

1Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan; 2Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; 3Medical Research Support Center, Kyoto University Graduate School of Medicine, Kyoto, Japan

Purpose: To investigate the efficacy of targeted exome sequencing for mutational screening of Japanese patients with cone dystrophy (CD) or cone-rod dystrophy (CRD).

Methods: DNA samples from 43 Japanese patients with CD or CRD were sequenced using an exome-sequencing panel targeting all 193 known inherited eye disease genes and next-generation sequencing methodologies. Subsequently, candidate variants were screened using systematic data analyses, and their potential pathogenicity was assessed using distinct filtering approaches, which included the frequency of the variants in normal populations, in silico prediction tools, and cosegregation.

Results: Causative mutations were detected in 12 patients with CD or CRD (27.9%). In total, 14 distinct mutations were identified in the genes ABCA4, CDHR1, CRB1, CRX, GUCY2D, KCNV2, PROM1, PRPH2, and RDH5, including four novel mutations, c.3050+1G>A in ABCA4, c.386A>G in CDHR1, c.652+1_652+4del in CRB1, and c.454G>A in KCNV2. Moreover, a putative pathogenic mutation was identified in RGS9BP, a gene recognized as the source of bradypsia.

Conclusions: Targeted exome sequencing effectively identified causative mutations in Japanese patients with CD or CRD. The results confirmed the heterogeneity of the genes responsible for CD and CRD in Japanese populations, as well as the efficacy of targeted exome sequencing-based screening of patients with inherited retinal degeneration.

Cone dystrophy (CD) and cone-rod dystrophy (CRD) are members of a heterogeneous group of inherited progressive retinal disorders featuring predominant cone cell dysfunction [1]. The prevalence of CD and CRD is approximately 1:40,000 worldwide, and the two diseases are considerably similar clinically and genetically: Both diseases are characterized by a loss of visual acuity, disturbance in color vision, and photophobia and result in a central scotoma. Furthermore, cone photoreceptor responses observed using electroretinography (ERG) are impaired or extinguished in CD and CRD cases. Patients with CD exhibit normal rod responses initially, but many of these patients develop rod dysfunction later in life; thus, definitively distinguishing between CD and CRD is not straightforward [2,3].

In the case of CD and CRD, all modes of gene inheritance have been detected, and to date, 30 genes have been reported as causative genes of these retinal diseases in the RetNet database (accessed on March 20, 2015). In previous studies, mutational analysis was performed mainly on single genes or on a set of genes by using Sanger sequencing or microarray techniques [1,4,5]. However, with the recent development of next-generation sequencing, all of the exons of multiple genes can be sequenced in parallel. Thus far, only a few studies have used systematic exome-sequencing analysis for patients with CD or CRD, but the studies suggest that the distribution of the causative genes might differ according to ethnicity [6-9]. Moreover, certain studies have shown that genes previously reported to be associated with other retinal dystrophies might cause CD or CRD [10].

In this study, we conducted a comprehensive molecular analysis of 43 Japanese patients with CD or CRD by using a targeted exome-sequencing approach. We analyzed all retinal and optic-nerve disease genes reported in the RetNet database at the time this study was designed (193 genes), including all causative genes of CD or CRD. We expect our results to contribute to the catalog of genetic variations in Japanese patients with CD and CRD.

METHODS

All procedures used in this study adhered to the tenets of the Declaration of Helsinki. The institutional review board and the ethics committee of each institution approved the
study protocols. All patients and their relatives were fully informed of the purpose and procedures of this study, and written consent was obtained from each participant.

Study participants: We recruited 43 Japanese patients with CD or CRD who visited the Department of Ophthalmology and Visual Sciences at the Kyoto University Graduate School of Medicine, Kyoto, Japan, between January 2011 and December 2012 and agreed to participate in the study. An additional 26 patients with other inherited retinal diseases, carrying 33 identified variants, were investigated to evaluate the integrity of the current approach [11].

CD and CRD were diagnosed by two retina specialists (authors A.O. and M.O.) based on comprehensive ophthalmologic examinations. When the two diagnoses disagreed, another retina specialist (author K.O.) arbitrated. The criteria for inclusion in the CD diagnostic group were a progressive decline in visual acuity, the presence of a central scotoma, and reduced cone responses from full-field ERG but normal rod responses. Full-field ERG was performed according to the protocol of the International Society for Clinical Electrophysiology of Vision (ISCEV) [12]. Inclusion in the CRD diagnostic group was based on the following criteria: a progressive decline in visual acuity, the presence of a central scotoma, and reduced responses of cones and rods in full-field ERG, with cone functions equally or more severely impaired than rod function. Atrophic changes in the macula were confirmed in most of the patients by using ophthalmoscopy and optical coherence tomography (OCT) imaging. We excluded patients with presumed Stargardt disease who exhibited subretinal yellow-white flecks or the typical dark choroid sign in fluorescein angiograms. Patients with central areolar choroidal dystrophy, pattern dystrophy, vitelliform macular dystrophy, age-related macular degeneration, and syndromic disorders were also excluded from this study. A detailed family history was obtained through patient interviews, and the presumed inheritance traits were determined using this information. Genomic DNA was extracted and purified from the peripheral blood of the patients and their available family members by using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan).

Target capture and next-generation sequencing: A capture panel of inherited retinal-disease genes was previously designed and assessed by our group [11]. The capture panel comprised 2,433,298 bp that covered all exons together with the flanking exon and intron boundaries (±25 bp) of 365 genes, including all 193 genes related to retinal and optic diseases that had been reported in RetNet at the time this study was designed (December 26, 2012). Precapture Illumina libraries were generated as previously described [11].

A custom-designed HaloPlex Target Enrichment Kit 2.5 Mb (Agilent Technologies, Santa Clara, CA) was used for target enrichment, according to the manufacturer’s protocol. Subsequently, DNA libraries were quantified and sequenced as 100 bp paired-end reads by using the Illumina HiSeq 2500 system (Illumina, San Diego, CA) according to the manufacturer’s protocols.

Data analysis and interpretation of genetic variants: Data were processed using our in-house pipeline, and several filtering steps were applied to determine the pathogenicity of the variants as previously described [11]. The analysis was focused specifically on the variants of the 193 genes reported in the RetNet database, which included all identified causative genes of CD or CRD. Because CD and CRD are rare Mendelian genetic disorders, we excluded variants that exhibited an allele frequency >0.5% (for recessive variants) or >0.1% (for dominant variants) in any of the following databases: 1000 Genomes [13], NHLBI GO Exome Sequencing Project (ESP6500), and Human Genetic Variation Database, which contains the genetic variations of 1,208 Japanese patients. To predict the effect of missense variants, we used dbNSFP [14], which contains seven well-established in silico prediction programs (Scale-Invariant Feature Transform [SIFT], Polyphen2, LRT, MutationTaster, MutationAssessor, PhyloP, and GERP++); only variants that were predicted to be pathogenic by at least five of the seven algorithms are reported here. For splice-site variants, we used the prediction program MaxEntScan [15], and variants whose scores differed by >5 between the wild-type and mutant sequences were considered pathogenic. The Human Gene Mutation Database (HGMD) was used to screen mutations reported in published studies.

All mutations and potential pathogenic variants were validated using conventional Sanger sequencing methods. Segregation analysis was performed if DNA from family members was available. As for novel mutations, we checked the Exome Aggregation Consortium (ExAC) database (Cambridge, MA; accessed November 2015) and confirmed that none of them were registered at the time of analysis.

RESULTS

Before the sequence analysis, CD and CRD were diagnosed in 17 and 26 of the 43 participants, respectively. The inheritance patterns of the causative genes were determined to be autosomal dominant (ad) in nine patients, autosomal recessive (ar) in eight patients, and simplex in 26 patients. These cases included one consanguineous pair of patients with adCRD (a parent–child relationship).

Targeted sequencing and data processing: The targeted gene-capture and sequencing analysis described in the preceding
section was applied to the 43 Japanese patients with CD or CRD, and the data were processed using our bioinformatics pipeline [11]. In a few cases (7/43, 16.3%), the coverage obtained was lower than that in others, but the mean and median coverages of the 193 RetNet genes in all samples were 269X and 260X, respectively. Within the targeted region, 93.2% of the bases showed >10X coverage and 90.1% showed >20X coverage, which suggested that sufficient coverage was achieved [11]. Among the 3,619 targeted exons, only 1.46% were covered at <5X (Appendix 1).

After automated variant detection, a mean of 507 raw variants and small insertions or deletions per sample were identified in the exons and corresponding splice junctions. When we used our systematic data-filtering method, we detected an average of 6.88 rare variants that were likely to cause a deleterious protein-coding change. All variants detected an average of 6.88 rare variants that were likely to cause a deleterious protein-coding change. All variants detected after the stringent filtering steps, which excluded putative false-positive variants and reconfirmed the integrity of our approach [11].

**Mutation analysis of patients:** Pathogenic mutations were identified in 12 of the 43 patients (27.9%). A total of 14 distinct mutations were identified, including ten reported mutations and four novel mutations. Furthermore, one novel heterozygous putative pathogenic mutation was identified in **CRX** (OMIM: 602225; c.284delG), and one novel homozygous nonsense mutation was identified in **RGS9BP** (OMIM: 607814; c.211G>T (p.E71*)), which is a causative gene for bradyopsia (Table 1). Table 2 lists the novel missense mutations predicted to be pathogenic by using a combination of in silico prediction tools. Gene reference numbers are shown in Appendix 2. Figure 1 shows the pedigrees of the 12 families carrying the pathogenic mutations, and the phenotype data of these patients are shown in Appendix 3 and Figure 2, Figure 3, and Figure 4.

**Patients carrying mutations in known CD or CRD genes:** Among the 12 resolved patients, eight known mutations were identified in the genes **ABCA4** (OMIM: 601691), **PRPH2**, **GUCY2D** (OMIM: 600179), **KCNV2** (OMIM: 607604), **PROM1** (OMIM: 604365), and **PRPH2** (OMIM: 179605), whereas four novel mutations were identified in **ABCA4** (OMIM: 609502), **CRBI** (OMIM: 604210), and **KCNV2** (Table 1). The pair of familial-related cases (K6073 and K6205) was included in this group. We included proband K6247, who carried the novel homozygous splice-site mutation c.652+1_652+4del in **CRBI**, in this group because patients harboring the **CRBI** mutation occasionally exhibit CRD or macular dystrophy phenotypes [10,16-18]. Three probands (K1741, K2039, and K6120) were discovered to carry

### TABLE 1. KNOWN MUTATIONS AND NOVEL POTENTIALLY CAUSATIVE CHANGES DETECTED IN PATIENTS.

| ID     | Type       | Phenotype | Gene     | Mutation         | Reference | rs ID       |
|--------|------------|-----------|----------|------------------|-----------|-------------|
| Patients harboring known mutations |
| K1741  | simplex    | CRD       | ABCA4    | c.6445C>T        | [19,21]   | rs61750654 |
| K2022  | ad         | CD        | PRPH2    | c.589A>G         | [38]      | rs62645931 |
| K2039  | simplex    | CRD       | ABCA4    | c.1760+2T>G (homo)| [20,30]   | rs61751385 |
| K3341  | ad         | CD        | GUCY2D   | c.2512C>T        | [39]      | rs61750172 |
| K6073  | ad         | CRD       | PROM1    | c.1117C>T        | [40]      | rs137853006|
| K6120  | ar         | CRD       | ABCA4    | c.1957C>T        | [21,31]   | rs61749420 |
| K6205  | ad         | CRD       | PROM1    | c.1117C>T        | [40]      | rs137853006|
| K6343  | simplex    | CD        | CRX      | c.121C>T         | [41]      | rs104894672|
| Patients harboring at least one novel mutation |
| K2044  | ar         | CRD       | ABCA4    | c.3050+1G>A (homo)| Novel    | NA          |
| K6140  | simplex    | CD        | CDHR1    | c.386A>G         | Novel     | NA          |
| K6247  | ar         | CRD       | CRBI     | c.652+1_652+4del (homo)| Novel | NA |
| K6496  | simplex    | CD        | KCNV2    | c.529T>C         | [42]      | NA          |
| K6345  | simplex    | CD        | CRX      | c.284delG         | Novel     | NA          |
| K3479  | ar         | Bradyopsia| RGS9BP   | c.211G>T         | Novel     | NA          |

CD: cone dystrophy; CRD: cone-rod dystrophy; ad: autosomal dominant; ar: autosomal recessive; hetero: heterozygous; homo: homozygous; NA: not available. K6345 and K3479 were classified as unresolved cases based on the criteria used in this study.
homzygous mutations in \(ABC\)A4 (c.6445C>T (p.R2149*), c.1760+2T>G, and c.1957C>T (p.R653C), respectively) that are known to cause Stargardt disease. The mutations are associated with early onset macular degeneration [6,19,20], vessel attenuation [6], patchy parafoveal atrophy surrounded by numerous yellow-white flecks [21], or retinitis pigmentosa [20] in certain cases. The three patients in this study (K1741, K2039, and K6120) had experienced early onset visual-acuity decline and presented pan-retinal degeneration but did not exhibit yellowish-white flecks; this agrees with previous reports suggesting the phenotypic variation. Additional information is provided in Appendix 4, which lists all the other potentially pathogenic rare variants identified in patients who carried pathogenic mutations in known CD or CRD genes.

In one simplex case (K6345), we identified a heterozygous putative pathogenic mutation, c.284delG, in CRX, which has been reported to cause CD and CRD in families who exhibit autosomal dominant inheritance patterns. However, this variant was a novel mutation, and segregation analysis was not performed because DNA samples from the patient’s family members were unavailable. Therefore, we classified this case as unresolved, according to the criteria used in this study (see “Data analysis and interpretation of genetic variants” in Materials and Methods and our previous report [11]). Furthermore, probands K3445 and K1909 were discovered to carry mutations in \(ABC\)A4 and RDH5, respectively, which are recognized to cause CD and CRD in families who exhibit recessive inheritance patterns. Although these two variants were reported to be pathogenic mutations, a second copy of the mutations was not detected using the current approach, and the cases were ultimately classified as unresolved.

**Patients carrying mutations in other retinal disease-related genes:** Among the remaining cases, one patient was identified to carry mutations in genes that are known to cause other retinal diseases. Proband K3479 carried a novel homozygous nonsense mutation, c.211G>T (p.E71*), in RGS9BP; mutation of this gene is recognized to cause bradyopsia, which is characterized by difficulty in tracking moving objects or adapting to sudden changes in illuminance. Careful reassessment of the clinical symptoms revealed that the patient showed difficulty in adjusting to illuminant changes, photophobia, a moderately reduced visual acuity (RV=0.6 and LV=0.3), and visual field defects with a loss of central sensitivity. Observation of the fundus with ophthalmoscopy and OCT imaging indicated no apparent retinal degeneration, except drusen and epiretinal membrane. All of these symptoms were compatible with bradyopsia, but because the patient developed a malignant lymphoma after inclusion in the study, we could not complete follow-up confirmation of the characteristic ERG findings, such as a reduced response to consecutive stimuli. However, these clinical symptoms were consistent with those of bradyopsia, and this disorder was molecularly diagnosed in the proband.

**DISCUSSION**

In this study, we performed a comprehensive molecular screening of 43 Japanese patients with CD or CRD by using next-generation sequencing techniques, and we successfully obtained molecular diagnoses for 12 cases (27.9%).

Our results identified causative \(ABC\)A4 mutations in four patients. The \(ABC\)A4 mutation is a major cause of arCD and CRD in Europe: >31–65% of patients with arCD or CRD carry mutations of \(ABC\)A4 [22-24]. In contrast, a study conducted in China reported that the prevalence of \(ABC\)A4 mutations in Chinese patients with CRD was only 2.1% [6]. This discrepancy indicates that ethnic differences exist in the prevalence of causative gene alleles for CD and CRD, as in the case of retinitis pigmentosa [11]. Here, the prevalence of \(ABC\)A4 mutations was 9.3% (4/43), which lies between the prevalence rates measured for European and Chinese populations and confirms the ethnic difference in the prevalence of \(ABC\)A4 mutations.

The results of this study hold implications for the role of CRB1 in CRD. CRB1 is widely recognized as a causative gene for retinitis pigmentosa and Leber congenital amaurosis; moreover, mutations in this gene were reported to be associated with the various phenotypes of retinal dystrophies, including CRD [10,16-18]. Here, proband K6247 carried the homozygous splice-site mutation c.652+1_652+4del in CRB1, and the patient exhibited the CRD phenotype. The patient’s

**Table 2. Results of seven in silico programs of novel missense mutations.**

| ID: mutation | SIFT | Polyphen2 | LRT | Mutation | Mutation | GERP++ | PhyloP |
|-------------|------|-----------|-----|----------|----------|--------|--------|
| c.386A>G (p.N129S) | 1 (D) | 0.885 (P) | 1 (D) | 4.55 (H) | 5.09 | 1.917 |
| c.142C>T (p.R48W) | 1 (D) | 0.996 (D) | 1 (D) | 2.385 (M) | 1.47 | 0.246 |
| c.454G>A (p.D152N) | 1 (D) | 1 (D) | 1 (D) | 3.95 (H) | 5.07 | 2.352 |

D: damaging; P: possibly damaging; H: high; M: medium.
parents were a consanguineous couple, and the brother (not included in this study) harbored the same mutation and developed the same phenotype. The early onset phenotype and autosomal recessive heritability agreed with the results of a previous study [18], and the presence of cystoid macular edema and predominantly nasal involvement agreed with

the results of another report [17]. The current study further supports the notion that mutations in CRB1 cause CRD.

This study revealed that one patient carried a putative disease-causing homozygous mutation in RGS9BP. RGS9BP plays a crucial role together with RGS9 in the recovery phase of visual transduction [25-27]. Mutations in RGS9

Figure 1. Pedigrees of the 12 patients with cone or cone-rod dystrophy carrying pathogenic mutations. The patients’ IDs and the corresponding genes are shown above the pedigrees; +: wild-type allele.
and RGS9BP are known to cause bradyopsia (slow vision), which is characterized by delays in adaptation to changes in light and darkness, photophobia, moderate loss of visual acuity, difficulty in seeing moving objects, normal color vision, and a normal fundus [25,28,29]. Proband K3479 carried the novel homozygous nonsense mutation c.211G>T (p.E71*) in RGS9BP. The symptoms, fundus appearance, and standard ERG findings of the proband were compatible with bradyopsia. Rod responses were within the normal range, whereas cone responses were non-recordable by using ISCEV ERG standards. The patient’s parents were healthy but consanguineous, and three of the six siblings of the patient showed similar disease phenotypes, which suggested an autosomal recessive inheritance pattern. We could not confirm the characteristic ERG findings (i.e., a reduction in cone and rod responses after the first stimulation with prolonged...
recovery) because the proband had died by the time we identified the mutation. However, the clinical symptoms and mode of inheritance were consistent with those of bradyopsia, and thus, in this patient, we diagnosed bradyopsia instead of CRD.

Probands K1741, K2039, and K6120 were identified to carry the homozygous mutations c.6445C>T, c.1760+2T>G, and c.1957C>T in \(ABCA4\), respectively, which have been reported to cause Stargardt disease [19-21,30,31]. \(ABCA4\) is a causative gene for autosomal recessive CD, CRD, retinitis pigmentosa, and Stargardt disease. Before the era of genetic diagnostics, these diseases were differentiated based on clinical findings; however, Stargardt disease, CD,
and CRD are currently recognized to show considerable overlap. For instance, the same mutation is associated with Stargardt disease and CRD [24,32]. Furthermore, the mutation c.1760+2T>G identified in proband K2039 was reported to cause retinitis pigmentosa [20]. These three cases in this study highlight the phenotypic variations, and these patients should be treated as patients with \(ABCA4\)-associated retinopathy and not designated arbitrarily as patients with Stargardt disease or CRD [33-35].

In this study, molecular diagnoses were made in 27.9% of the patients, and 62.1% of the cases remained unresolved. The detection rate was slightly higher than that for the Chinese population (21.28%) but lower than that for the European population (62.1%), which indicates the ethnic differences in the prevalence of causative gene alleles for CD and CRD [6,9]. The methodological limitations of our approach and the technical limitations of next-generation sequencing technology might be one of the reasons for not determining the molecular cause of disease in these patients [11]. Here, we did not assess intronic mutations, synonymous mutations, and small insertions or deletions that do not cause frameshift. For instance, some intronic mutations in \(ABCA4\) reported in European populations were not screened in this study [36]. Furthermore, next-generation sequencing technology occasionally does not allow easy reading of certain regions such as GC-rich regions, repeated sequences, copy-number variations, and large deletions or insertions. Moreover, the sequencing of seven samples (16.9%) yielded lower coverage.

![Figure 4](http://www.molvis.org/molvis/v22/150/)

**Figure 4.** Optical coherence tomography of the patients carrying pathogenic mutations. Images were obtained by using Spectralis (Heidelberg Engineering, Heidelberg, Germany). Most of the examinations for K6205, the mother of K6073, were performed at another institution and were not available.
than that in the case of other samples due to a technical reason: generation of the precapture libraries. Although the mean coverage of these samples was sufficient for the detection of genetic variants (233X), the percentage of bases showing >10X coverage was 81%, and low-coverage variants were excluded. To compensate for the deficiencies of our approach, additional target screening of low-depth regions could be conducted using conventional direct sequencing, and this might serve as an effective tool for improving genetic diagnosis [37]. Another reason for the limited detection rate could be our stringent criteria for determining the pathogenicity of variants; we included only those variants that were predicted to be pathogenic by five out of the seven in silico programs used. When less stringent criteria were employed, such as prediction by one out of five in silico programs, two additional patients were assessed as carrying disease-causing mutations.

In conclusion, this study screened the largest sample of Japanese patients with CD and CRD to date and indexed the genetic constitution of this cohort. Our results confirmed the efficacy of next-generation sequencing-based molecular diagnosis of patients with CD or CRD.

APPENDIX 1. LOW-COVERAGE REGIONS IN THE 193 CAUSATIVE GENES OF HERITABLE EYE DISORDERS.
CD: cone dystrophy; CRD: cone-rod dystrophy; Chr: chromosome. To access the data, click or select the words “Appendix 1.”

APPENDIX 2. LIST OF GENE REFERENCE NUMBERS.
To access the data, click or select the words “Appendix 2.”

APPENDIX 3. PHENOTYPE DATA OF THE PATIENTS WHO CARRIED MUTATIONS IN KNOWN CD OR CRD GENES.
VA: visual acuity; NR: non-recordable; NA: not available. *Visual acuity at the current age. †Data of the right eyes. To access the data, click or select the words “Appendix 3.” K6205 is a mother of K6073 and most examinations were performed in another institution. K6140 underwent electroretinogram more than 15 years before and the current data is not available.

APPENDIX 4. LIST OF ADDITIONAL POTENTIALLY PATHOGENIC RARE VARIANTS IDENTIFIED IN PATIENTS WHO CARRIED MUTATIONS IN KNOWN CD OR CRD GENES.
To access the data, click or select the words “Appendix 4.” CD: cone dystrophy; CRD: cone-rod dystrophy; ad: autosomal dominant; ar: autosomal recessive; hetero: heterozygous; NA: not available.

ACKNOWLEDGMENTS
The authors thank the following colleagues: Jun Zhu, Ph.D., Yoshiyuki Wakabayashi, Ph.D., and Yanqin Yang, M.D., M.Sc., at the DNA Sequencing and Computational Biology Core of the NIH NHLBI, for advice on technical matters; the staff at the Medical Research Support Center of the Graduate School of Medicine at Kyoto University (Chief Director: Masatoshi Hagiwara M.D., Ph.D.), for help in DNA sequencing analysis performed using the Sanger method; and Hatsue Hamanaka, for technical support in the preparation and sequencing of the genetic samples. Funding/Support: This study was supported in part by the Japan Ministry of Health, Labor and Welfare (No. 12,103,069), a Grant-in-Aid for scientific research (No. 26,861,445), and the Japanese Retinitis Pigmentosa Society. The funding organizations had no role in the design or execution of this research.

REFERENCES
1. Thiadens AA, Phan TM, Zekveld-Vroon RC, Leroy BP, van den Born LI, Hoyng CB, Klaver CC. Writing Committee for the Cone Disorders Study Group Consortium. Roosing S, Pott JW, van Schooneveld MJ, van Moll-Ramirez N, van Genderen MM, Boon CJ, den Hollander AI, Bergen AA, De Baere E, Cremers FP, Lotery AJ. Clinical course, genetic etiology, and visual outcome in cone and cone-rod dystrophy. Ophthalmology 2012; 119:819-26. [PMID: 22264887].
2. Traboulsi EI. Cone dysfunction syndromes, cone dystrophies, and cone-rod degenerations. In: Traboulsi EI, editor. Genetic diseases of the eye. 2nd ed. New York: Oxford University Press; 2012. p. 410–20.
3. Michaelides M, Hardecastle AJ, Hunt DM, Moore AT. Progressive cone and cone-rod dystrophies: phenotypes and underlying molecular genetic basis. Surv Ophthalmol 2006; 51:232-58. [PMID: 16644365].
4. Klevering BJ, Yzer S, Rohrschneider K, Zonneveld M, Allikmets R, van den Born LI, Mauger A, Hoyng CB, Cremers FP. Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. Eur J Hum Genet 2004; 12:1024-32. [PMID: 15494742].
5. Fishman GA, Stone EM, Eliasen DA, Taylor CM, Lindeman M, Derlacki DJ. ABCA4 gene sequence variations in
patients with autosomal recessive cone-rod dystrophy. Arch Ophthalmol 2003; 121:851-5. [PMID: 12796258].

6. Huang L, Zhang Q, Li S, Guan L, Xiao X, Zhang J, Jia X, Sun W, Zhu Z, Gao Y, Yin Y, Wang P, Guo X, Wang J, Zhang Q. Exome sequencing of 47 Chinese families with cone-rod dystrophy: mutations in 25 known causative genes. PLoS One 2013; 8:e65546- [PMID: 23776498].

7. Shanks ME, Downes SM, Copley RR, Lise S, Broxholme J, Hudspith KA, Kwansiewska A, Davies WI, Hankins MW, Packham ER, Clouston P, Seller A, Wilkie AO, Taylor JC, Ragoussis J, Németh AH. Next-generation sequencing (NGS) as a diagnostic tool for retinal degeneration reveals a much higher detection rate in early-onset disease. Eur J Hum Genet 2013; 21:274-80. [PMID: 22968130].

8. Huang XF, Huang F, Wu KC, Wu J, Chen J, Pang CP, Lu F, Qu J, Jin ZB. Genotype–phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. Genet Med 2015; 17:271-8. [PMID: 25356976].

9. Boulanger-Scemama E, El Shamieh S, Demontant V, Bujakowska K, Audo I, Mohand-Saïd S, Lancelot ME, Antonio Boulanger-Scemama E, El Shamieh S, Demontant V, Bujakowska K, Audo I. Next-generation sequencing applied to a large French cone and cone-rod dystrophy cohort: mutation spectrum and new genotype-phenotype correlation. Orphanet J Rare Dis 2015; 10:85- [PMID: 26103963].

10. Bujakowska K, Audo I, Mohand-Saïd S, Lancelot ME, Antonio A, Germain A, Léveillard T, Letexier M, Saraiva JP, Lonjou C, Carpentier W, Sahel JA, Bhattacharya SS, Zeitz C. CRBl mutations in inherited retinal dystrophies. Hum Mutat 2012; 33:306-15. [PMID: 22065545].

11. Iishi M, Oishi A, Gotoh N, Ogino K, Higasa K, Iida K, Makiyama Y, Morooka S, Matsuda F, Yoshimura N. Comprehensive molecular diagnosis of a large cohort of Japanese retinitis pigmentosa and Usher syndrome patients by next-generation sequencing. Invest Ophthalmol Vis Sci 2014; 55:7369-75. [PMID: 25324289].

12. Marmor MF, Fulton AB, Holder GE, Miyake Y, Briggel M, Bach M. ISCEV Standard for full-field clinical electoretinography (2008 update). Doc Ophthalmol 2009; 118:69-77. [PMID: 19030905].

13. 1000 Genomes Project Consortium, Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, Hurles ME, McVean GA. A map of human genome variation from population-scale sequencing. Nature 2010; 467:1061-73. [PMID: 20981092].

14. Liu X, Jian X, Boerwinkle E. dbNSFP v2.0: a database of human non-synonymous SNVs and their functional predictions and annotations. Hum Mutat 2013; 34:E2393-402. [PMID: 23843252].

15. Yeo G, Burge CB. Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. J Comput Biol 2004; 11:377-94. [PMID: 15285897].

16. Tsang SH, Burke T, Oll M, Yzer S, Lee W, Xie YA, Allikmets R. Whole exome sequencing identifies CRBl defect in an unusual maculopathy phenotype. Ophthalmology 2014; 121:1773-82. [PMID: 24811962].

17. Khan AO, Aldahmesh MA, Abu-Safieh L, Alkuraya FS. Childhood cone-rod dystrophy with macular cystic degeneration from recessive CRBl mutation. Ophthalmic Genet 2014; 35:130-7. [PMID: 23767994].

18. Henderson RH, Mackay DS, Li Z, Moradi P, Sergouniotis P, Russell-Eggitt I, Thompson DA, Robson AG, Holder GE, Webster AR, Moore AT. Phenotypic variability in patients with retinal dystrophies due to mutations in CRBl. Br J Ophthalmol 2011; 95:811-7. [PMID: 20956273].

19. Lewis RA, Shroyer NF, Singh N, Allikmets R, Hutchinson A, Li Y, Lupsik JR, Leppert M, Dean M. Genotype/phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. Am J Hum Genet 1999; 64:422-34. [PMID: 9973280].

20. Fukui T, Yamamoto S, Nakano K, Tsujikawa M, Morimura H, Nishida K, Ohguro N, Fujikado T, Irie H, Kuniyoshi K, Okada AA, Hiramatsu A, Miyake Y, Tono Y. ABCA4 gene mutations in Japanese patients with Stargardt disease and retinitis pigmentosa. Invest Ophthalmol Vis Sci 2002; 43:2819-24. [PMID: 12202497].

21. Fujinami K, Sergouniotis PI, Davidson AE, Wright G, Chana RK, Tsuboda K, Tsubota K, Egan CA, Robson AG, Moore AT, Holder GE, Michaelsides M, Webster AR. Clinical and molecular analysis of Stargardt disease with preserved foveal structure and function. Am J Ophthalmol 2013; 156:487-501. el. [PMID: 23953153].

22. Maugeri A, Klevering BJ, Rohrschneider K, Blankenagel A, Brunner HG, Deutman AF, Hoyng CF, Cremers FP. Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy. Am J Hum Genet 2000; 67:960-6. [PMID: 10958761].

23. Kitiratschky VB, Grau T, Ordás E, Züger H, Züger A, Kellner U, Rudolph G, Jacobson SG, Cideciyan AV, Schaich S, Kuhl S, Wissinger B. ABCA4 gene analysis in patients with autosomal recessive cone and cone rod dystrophies. Eur J Hum Genet 2008; 16:812-9. [PMID: 18285826].

24. Riveiro-Alvarez R, Lopez-Martinez MA, Züger H, Barthe J, Aguirre-Lamban J, Cantalapiedra D, Avila-Fernandez A, Gimenez A, Lopez-Molina MI, Garcia-Sandoval B, Trujillo-Tiebas MJ, Ramos C, Allikmets R, Ayuso C. Outcome of children with autosomal recessive cone-rod dystrophy: retrospective analysis in 420 Spanish families. Orphanet J Rare Dis 2013; 8:e65546- [PMID: 23776498].
27. Arshavsky VY, Wensel TG. Timing is everything: GTPase regulation in phototransduction. Invest Ophthalmol Vis Sci 2013; 54:7725-33. [PMID: 24265205].

28. Hartong DT, Pott JW, Kooijman AC. Six patients with bradyopsia (slow vision): clinical features and course of the disease. Ophthalmology 2007; 114:2323-31. [PMID: 17826834].

29. Michaelides M, Li Z, Rana NA, Richardson EC, Hykin PG, Moore AT, Holder GE, Webster AR. Novel mutations and electrophysiologic findings in RGS9- and R9AP-associated retinal dysfunction (Bradyopsia). Ophthalmology 2010; 117:120-127.e1. [PMID: 19818506].

30. Ogino K, Oishi A, Makiyama Y, Nakagawa S, Kurimoto M, Otani A, Yoshimura N. Genotype screening of retinal dystrophies in the Japanese population using a microarray Article in Japanese. Nippon Ganka Gakkai Zasshi 2013; 117:12-8. [PMID: 23424971].

31. Rivera A, White K, Stöhr H, Steiner K, Hemmrich N, Grimm T, Jurklies 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; 67:800-13. [PMID: 10958763].

32. Klevering BJ, Mauger A, Wagner A, Go SL, Vink C, Cremers FP, Hoyng CB. Three families displaying the combination of Stargardt’s disease with cone-rod dystrophy or retinitis pigmentosa. Ophthalmology 2004; 111:546-53. [PMID: 15019334].

33. Cideciyan AV, Swider M, Aleman TS, Sumaroka A, Schwartz SB, Roman MI, Milam AH, Bennett J, Stone EM, Jacobson SG. ABCA4-associated retinal degenerations spare structure and function of the human parapapillary retina. Invest Ophthalmol Vis Sci 2005; 46:4739-46. [PMID: 16303974].

34. Fujinami K, Zernant J, Chana RK, Wright GA, Tsunoda K, Ozawa Y, Tsubota K, Webster AR, Moore AT, Allikmets R, Michaelides M. ABCA4 gene screening by next-generation sequencing in a British cohort. Invest Ophthalmol Vis Sci 2013; 54:6662-74. [PMID: 23982839].

35. Duncker T, Tsang SH, Lee W, Zernant J, Allikmets R, Delori FC, Sparrow JR. Quantitative fundus autofluorescence distinguishes ABCA4-associated and non-ABCA4-associated bull’s-eye maculopathy. Ophthalmology 2015; 122:345-55. [PMID: 25283059].

36. Braun TA, Mullins RF, Wagner AH, Andorf JL, Johnston RM, Bakall BB, Deluca AP, Fishman GA, Lam BL, Weleber RG, Cideciyan AV, Jacobson SG, Sheffield VC, Tucker BA, Stone EM. Non-exonic and synonymous variants in ABCA4 are an important cause of Stargardt disease. Hum Mol Genet 2013; 22:5136-45. [PMID: 23918662].

37. Huang XF, Wu J, Lv JN, Zhang X, Jin ZB. Identification of false-negative mutations missed by next-generation sequencing in retinitis pigmentosa patients: a complementary approach to clinical genetic diagnostic testing. Genet Med 2015; 17:307-11. [PMID: 25569437].

38. Kohl S, Christ-Adler M, Apfelstedt-Sylla E, Kellner U, Eckstein A, Zrenner E, Wissinger B. RDS/peripherin gene mutations are frequent causes of central retinal dystrophies. J Med Genet 1997; 34:620-6. [PMID: 9279751].

39. Wilkie SE, Newbold RJ, Deery E, Walker CE, Stinton I, Ramamurthy V, Hurley JB, Bhattacharya SS, Warren MJ, Hunt DM. Functional characterization of missense mutations at codon 838 in retinal guanylate cyclase correlates with disease severity in patients with autosomal dominant cone-rod dystrophy. Hum Mol Genet 2000; 9:3065-73. [PMID: 11115581].

40. Michaelides M, Gaillard MC, Escher P, Tiab L, Bedell M, Borruat FX, Barthelmes D, Carmona R, Zhang K, White E, McClements M, Robson AG, Holder GE, Bradshaw K, Hunt DM, Webster AR, Moore AT, Schorderet DF, Munier FL. The PROM1 mutation p.R373C causes an autosomal dominant bull’s eye maculopathy associated with rod, rod-cone, and macular dystrophy. Invest Ophthalmol Vis Sci 2010; 51:4771-80. [PMID: 20393116].

41. Swain PK, Chen S, Wang QL, Affatigato LM, Coats CL, Brady KD, Fishman GA, Jacobson SG, Swaroop A, Stone E, Sieving PA, Zack DJ. Mutations in the cone-rod homeobox gene are associated with the cone-rod dystrophy photoreceptor degeneration. Neuron 1997; 19:1329-36. [PMID: 9427255].

42. Fujinami K, Tsunoda K, Nakamura N, Kato Y, Noda T, Shinoda K, Tomita K, Hatase T, Usui T, Akahori M, Itabashi T, Iwata T, Ozawa Y, Tsubota K, Miyake Y. Molecular characteristics of four Japanese cases with KCNV2 retinopathy: Report of novel disease-causing variants. Mol Vis 2013; 19:1580-90. [PMID: 23885164].