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Genotypes and phenotypes of genes associated with achromatopsia: A reference for clinical genetic testing

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Purpose: Achromatopsia is a congenital autosomal recessive cone disorder, and it has been found to be associated with six genes. However, pathogenic variants in these six genes have been identified in patients with various retinal dystrophies with the exception of achromatopsia. Thus, this study aims to investigate the contribution of these genes in hereditary retinal diseases and the potential genotype–phenotype correlations.
Methods: Biallelic variants in six achromatopsia-related genes, namely, *CNGA3*, *CNGB3*, *GNAT2*, *ATF6*, *PDE6C*, and *PDE6H*, were analyzed based on data obtained from 7,195 probands with different eye conditions. A systematic genotype–phenotype analysis of these genes was performed based on these data, along with the data reported in the literature.
Results: Biallelic potential pathogenic variants (PPVs) in five of the six genes were identified in 119 probands with genetic eye diseases. The variants in *CNGA3* were the most common and accounted for 81.5% (97/119). Of the 119 probands, 62.2% (74/119) have cone-rod dystrophy, whereas only 25.2% (30/119) have achromatopsia. No biallelic pathogenic variants in these genes were identified in patients with rod-dominant degeneration. A systematic review of genotypes and phenotypes revealed certain characteristics of each of the six genes, providing clues for the pathogenicity evaluation of the variants of the genes.
Conclusions: PPVs in the six genes were identified in various inherited retinal degeneration diseases, most of which are cone-dominant diseases but no rod-dominant diseases based on the data from a cohort of 7,195 probands with different eye conditions. The systematic genotype–phenotype analysis of these genes will be useful in drafting guidelines for the clinical genetic diagnostic application for the investigated genes.

Achromatopsia (ACHM, OMIM 216900) is a rare congenital autosomal recessive cone disorder with a prevalence of less than 1 in 30,000 [1]. However, the prevalence of ACHM could be as high as 4–10% in certain regions where consanguinity is common [2]. The clinical features of ACHM include congenital nystagmus, photophobia, reduced visual acuity (VA), color blindness, and severely reduced to nonrecordable cone response but with a normal rod response [1]. Some patients also develop macular dystrophy. ACHM was previously considered a stationary disorder, but follow-up studies have shown that ACHM is characterized by progressive loss of photoreceptor cells [3-5].

Potential pathogenic variants (PPVs) in six genes have been identified in patients with ACHM, including *CNGA3* (OMIM 600053), *CNGB3* (OMIM 605080), *GNAT2* (OMIM 139340), *ATF6* (OMIM 605537), *PDE6C* (OMIM 600827), and *PDE6H* (OMIM 601190). *ATF6* encodes a widely expressed endoplasmic reticulum stress response element-binding protein. The five other genes encode cone-specific expression and function in the G-protein cascade of phototransduction. *CNGA3* and *CNGB3* encode the α- and β-subunits of the cGMP-gated channel, respectively [6]. *GNAT2* encodes the α2-subunit for the G-protein transduction [7]. *PDE6C* and *PDE6H* encode the catalytic subunit and the γ-subunit of cGMP phosphodiesterase, respectively [8,9]. PPVs in these five cone-specific genes (*CNGA3*, *CNGB3*, *GNAT2*, *PDE6C*, and *PDE6H*) have been identified in patients with various retinal dystrophies, including ACHM, cone-rod dystrophy (CORD), and Leber congenital amaurosis (LCA) [10-16]. Studies have also identified PPVs in *CNGA3* in patients with retinitis pigmentosa (RP) [10,14] and congenital stationary night blindness (CSNB) [17]. However, there are several concerns regarding these PPVs in *CNGA3*. First, several of these PPVs have been identified in only a few cases with RP or CSNB, leading to the following question: What are the characteristics of these PPVs and of the rare phenotypes in these few cases? Second, most PPVs in the genes above were identified based on a cohort of patients with a single disease (especially ACHM). Thus, the following questions arise: Are there additional PPVs in the other five genes in patients with rod-dominant degeneration? What is the contribution of the PPVs in these six genes in all inherited retinal dystrophies (IRDs) as well as in different groups? Third, the potential genotype–phenotype correlation has yet to be investigated.

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With the use of whole-exome sequencing and targeted exome sequencing for genetic analysis, variants in a panel of genes can be obtained from individuals with different diseases. These tools are useful in genotype-guided organization of the phenotypic spectrum and in the pathogenicity evaluation of the variants of a single gene. In this study, we systematically analyzed the frequencies, spectra, and phenotypes associated with the PPVs in six genes (*ATF6*, *CNGA3*, *CNGB3*, *GNAT2*, *PDE6C*, and *PDE6H*) based on exome sequencing data from 7,195 probands with different eye conditions. We performed a systematic genotype–phenotype analysis of the six genes based on the present data, along with the data reported in the literature. The results will be useful in establishing guidelines for genetic diagnostic application of the investigated genes.

METHODS

Subjects: As part of an ongoing study of the genetic basis of inherited eye diseases, this research involved 7,195 families with different eye conditions recruited at the Pediatric and Genetic Eye Clinic of the Zhongshan Ophthalmic Center. Of the 7,195 families, 5,063 were new participants, and 2,132 families had been previously investigated [11,18-25]. The peripheral blood and clinical data of these families were collected after written informed consent was obtained from the participants or from their guardians in accordance with the tenets of the Declaration of Helsinki. Genomic DNA was prepared from leukocytes of the peripheral blood. Diagnoses were made based on their symptoms and ophthalmic examinations, including a VA test, a slit-lamp examination, and ophthalmoscopy, along with other required examinations, such as electroretinogram (ERG), optical coherence tomography (OCT), and fundus fluorescein angiography [26]. This study was approved by the institutional review board of the Zhongshan Ophthalmic Center.

Whole-exome sequencing: Whole-exome sequencing (WES) was performed on genomic DNA obtained from 5,280 unrelated individuals. Of these individuals, 3,735 were newly enrolled, whereas 1,545 had been previously subjected to a systematic variant analysis of a panel of genes, including the ACHM-associated genes above [18-23]. We described the WES process in a previous study [27].

Targeted exome sequencing: Targeted exome sequencing (TES) was performed on genomic DNA obtained from 1,896 probands exhibiting different eye diseases. Of these probands, 1,328 were newly enrolled, and 568 probands had been previously analyzed [24]. The TES was performed as described previously [24].

Evaluation and verification of the variants obtained through WES and TES: The variants of the six ACHM-associated genes were selected from the exome sequencing data of 7,176 probands; the data included the WES data from 5,280 probands and the TES data from 1,896 probands. After the low-certainty variants with coverage of fewer than ten were excluded, the variants detected in the investigated genes were filtered through multistep bioinformatics analyses, as follows: 1) exclusion of variants with minor allelic frequencies (MAFs) of less than 0.01 according to the 1000 Genomes and the Exome Aggregation Consortium (ExAC), 2) exclusion of variants at the noncoding region and of synonymous variants that did not affect the splice sites, 3) exclusion of missense variants that were predicted to be benign by SIFT and PolyPhen-2, and 4) exclusion of single heterozygous variants. The remaining candidate variants were verified in the probands and in the available family members through Sanger sequencing. A variant was excluded if it did not segregate with the disease in the family. In addition, PPVs in *CNGA3*, *CNGB3*, and *PDE6C* were identified in 19 additional probands by using Sanger sequencing as we described in a previous study [11].

Systematic review of the genotypes and phenotypes of the six genes based on the present data combined with the data reported in the literature: The present data and the data on the available PPVs and clinical diagnoses obtained from the Human Gene Mutation Database and through a search in PubMed were combined. A total of 169 *CNGA3* PPVs in 409 families [2,4-6,10,11,13-18,28-70], 119 *CNGB3* PPVs in 829 families [5,6,12,15,16,18,30-32,34,38,39,43,45,49-52,57,62,63,67,71-89], 61 *PDE6C* PPVs in 53 families [8,14,15,18,58,62,79,86,89-94], 17 *GNAT2* PPVs in 17 families [7,12,15,31,50,63,95-99], 16 *ATF6* PPVs in 17 families [15,100-105], and one *PDE6H* PPV in three families [106-108] were identified. The genotypes (including the frequencies, types, and locations) and the phenotypes (including congenital nystagmus, photophobia, impaired color vision, VA, refractive error, and ERG) of the PPVs in the six genes were summarized and serve as a reference in the application of clinical genetic testing.

RESULTS

Mutational frequencies in the six genes in 7,195 Chinese probands with various genetic eye diseases: In total, 92 PPVs in five of the six genes were identified in 119 of the 7,195 probands; these 92 PPVs comprise 33 novel and 59 reported PPVs (Appendix 1) [18]. Moreover, the 92 PPVs comprise 63 variants in *CNGA3*, 16 in *PDE6C*, eight in *CNGB3*, three in *ATF6*, and two in *GNAT2*. For the PPVs in *CNGA3*, the

missense and truncation variants accounted for 65.1% (41/63) and 31.7% (20/63), respectively, while the remaining two PPVs were non-frameshift variants. For *CNGB3* and *PDE6C*, the missense and truncation variants accounted for about half of the total, respectively. The three *ATF6* PPVs included one splicing and two missense variants, whereas the two *GNAT2* PPVs were missense variants. Of the 119 probands with PPVs in the five genes, 51 were newly recruited (Appendix 2), and the remaining 68 probands were included in our previous studies (Appendix 3) [11,18,21,24]. Segregation analysis in available family members of 38 families suggested that the PPVs cosegregated with disease in these families (Appendix 4). The clinical data of the 51 new probands are described in Appendix 2. The PPVs in *CNGA3* were the most common and were identified in 81.5% (97/119) of the probands, whereas the PPVs in *GNAT2*, *ATF6*, *CNGB3*, and *PDE6C* were detected in one, two, 7, and 12 probands, respectively. No biallelic PPVs were identified in *PDE6H* in the 7,195 probands (Appendix 2, Appendix 3).

Phenotypic spectrum of the 119 Chinese probands with PPVs in five of the investigated ACHM-associated genes: Of the 119 probands with PPVs in five of the investigated genes, 74 were diagnosed with CORD, 30 with ACHM, one with LCA, one with early-onset high myopia (eoHM), three with macular dystrophy (MD), and ten with unclassified IRD (Appendix 2, Appendix 3). ERG recordings were available for 40 of the 51 newly recruited probands, and all had severely reduced or even extinguished cone responses with different rod responses (Appendix 2, Figure 1). The available OCT results from ten newly recruited probands showed different patterns, including normal, irregular or disruption ellipsoid zone, foveal hypoplasia, macular atrophy, and thinning retina (Appendix 2, Appendix 5). No biallelic PPVs in the six genes were identified in patients with rod-dominant retinopathy, such as RP and CSNB. Biallelic PPVs in *CNGA3* had the highest frequency; it was found in 81.1% (60/74) of the probands with CORD and in 86.7% (26/30) of those with ACHM.

Genotypes of the investigated genes: Currently, 321 PPVs in the six genes have been reported in previous literature except the 62 PPVs from the present cohort (Appendix 6). The total 383 PPVs included 169 *CNGA3* PPVs from 409 families, 119 *CNGB3* PPVs from 829 families, 61 *PDE6C* PPVs from 53 families, 17 *GNAT2* PPVs from 17 families, 16 *ATF* PPVs from 17 families, and one *PDE6H* PPV from three families. Regarding the variant types of the six investigated genes, the PPVs in *CNGA3* were predominately missense, accounting for 69.8% (118/169), whereas the PPVs in *CNGB3*, *GNAT2*, and *ATF6* were predominately truncation variants (frameshift, nonsense, splicing change, start loss, and gross deletion/insertion; Figure 2). Missense and truncation PPVs accounted for half of the variants in *PDE6C* (Figure 2), and the lone PPV in *PDE6H* was a truncation variant. The PPVs in the six genes were identified in 1,328 families. In these families, the biallelic PPVs in *CNGB3* were the most common, and they were found in 62.4% (829/1,328) of the families, while the PPVs in *CNGA3* were found in 30.8% (409/1,328) of the families. The PPVs in *PDE6C*, *GNAT2*, *ATF6*, and *PDE6H* were detected in 4.0% (53/1,328), 1.3% (17/1,328), 1.3% (17/1,328), and 0.2% (3/1,328) of the investigated families, respectively.

The functional domains in the investigated genes, except in *GNAT2*, were studied. *CNGA3* and *CNGB3* have six similar transmembrane domains, four loops, one pore region, and one cGMP-binding domain (Figure 3A,D). Most of the missense PPVs in *CNGA3* are located at the regions that encode functional domains, and the four hotspots are as follows: p.Arg277, p.Arg283, p.Val529, and p.Phe547. Among them, p.Arg277 and p.Arg283 are located at the S4 transmembrane domain, whereas p.Val529 and p.Phe547 are located at the cGMP-binding domain. None of the PPVs are located at exon 4 of *CNGA3* that is exclusively present in transcript isoform 1 (NM_001298.2) and is absent in isoform 2 (NM_001079878.1), whereas one splicing variant is located in the upstream region of exon 4. In addition, all but one of the nine PPVs in the first four coding exons and their adjacent intronic regions in *CNGA3* are truncation variants (Figure 3A). The remaining missense variant c.284C>T (p.Pro95Leu) was predicted to be tolerated by SIFT and PolyPhen-2 (Appendix 6). The *CNGB3* gene has five mutation hotspots: p.Arg274Valfs*, c.991–3T>G, p.Glu336*, p.Thr383Ilefs*, and c.1578+1G>A. These five hotspots, as well as most truncation variants in *CNGA3* and *CNGB3*, are located before the regions that encode the last functional domain (cGMP-binding domain). This pattern indicates that these truncation PPVs could affect at least one functional domain (Figure 3A,D). In addition, the PPVs in the three other genes (*PDE6C*, *ATF6*, and *PDE6H*) have similar locations, and all their truncation variants affect at least one functional domain (Appendix 7).

The combined number of PPVs in the literature and identified in the present data is 383, and four of these PPVs showed an MAF higher than 0.1% according to the ExAC database. These PPVs are as follows: c.682G>A (p.Glu228Lys) and c.1618G>A (p.Val540Ile) in *CNGA3* and c.1148del (p.Thr383Ilefs*) and c.1208G>A (p.Arg403Gln) in *CNGB3*. The MAFs of the other PPVs were all lower than 0.1%. The allele frequencies of the reported PPVs in the general population based on ExAC are shown in Appendix 6. Two of the four PPVs, namely, c.1148del (p.Thr383Ilefs*) and c.1208G>A

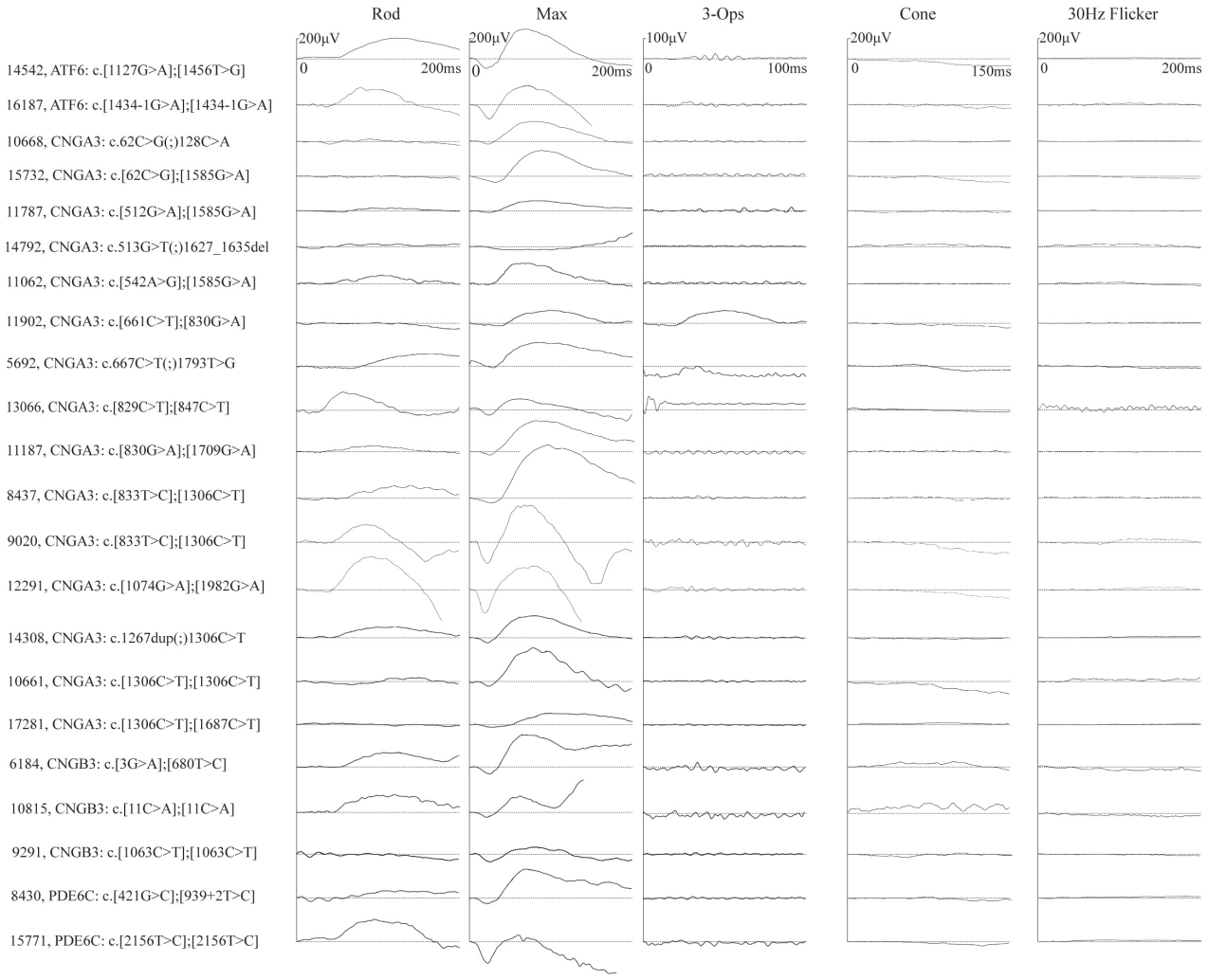

Figure 1. The available ERG data of newly recruited probands. The proband IDs and their genotypes are indicated to the left. The electroretinogram (ERG) recordings from the probands all show severely reduced or even extinguished cone responses with different rod responses.

(p.Arg403Gln) in *CNGB3*, showed an allele frequency of 224/120,952 and 618/120,874 in ExAC, respectively. However, these allele frequencies were statistically significantly higher than the controls based on ExAC ($p<0.01$), whereas the allele frequencies for the other two variants did not differ statistically significantly from the controls (Appendix 8). Additionally, all three missense PPVs were predicted by SIFT and PolyPhen-2 to be damaging (Appendix 6).

Diseases associated with PPVs in the investigated genes: Of the 1,328 families with PPVs in the investigated genes (Appendix 9), 85.8% (1139/1,328) had ACHM, and 9.3% (124/1,328) had CORD (Figure 4A). The highest percentage of ACHM in all cases with PPVs in the six genes was caused by biallelic PPVs in *CNGB3* (Figure 4B). The PPVs in *CNGA3* were most common in Asian patients with ACHM and CORD, whereas the PPVs in *CNGB3* were mostly identified in Caucasian patients with ACHM. Thus, the phenotypic spectrum and the distribution of the *CNGA3* and *CNGB3* PPVs differed between Asian and Caucasian patients (Figure 5).

The patients carrying the PPVs in the six genes displayed the ACHM-associated phenotypes, including congenital nystagmus, photophobia, color blindness, and extinguished or severely reduced cone response but with normal rod response by ERG. Moreover, some cases showed refractive error, abnormal OCT results, and fundus changes in MD [52].

The VA of patients with PPVs in *CNGA3*, *CNGB3*, *GNAT2*, *PDE6C*, and ATF6 mostly ranged from 0.05 to 0.20 (Figure 6) and did not show progression with age, whereas

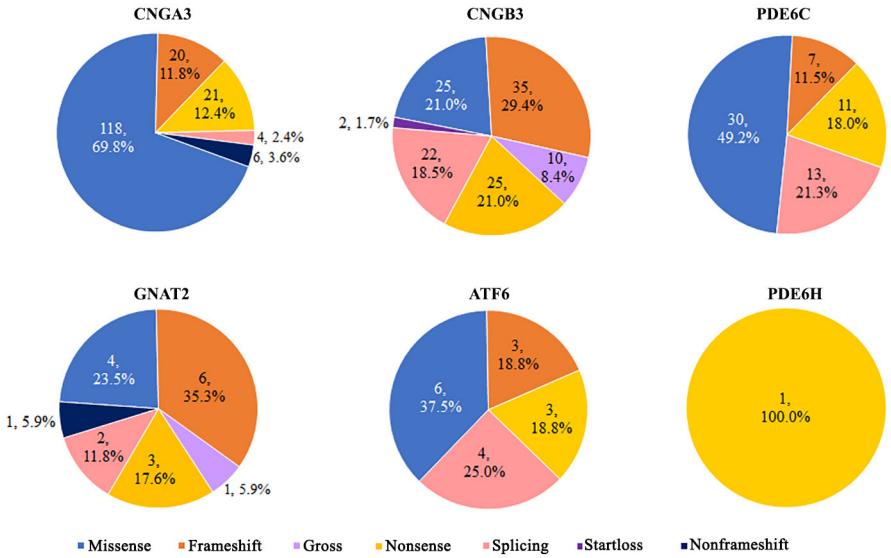

Figure 2. Spectrum of PPV types of the six investigated genes. Only the potential pathogenic variants (PPVs) in *CNGA3* were predominately missense, whereas those in the other five genes were predominately truncation variants.

the VA of the five patients with PPVs in *PDE6H* ranged from 0.1 to 0.4. The presence of nystagmus, photophobia, impaired color vision, and cone response by ERG in patients with PPVs in the six genes are summarized in Table 1. A distinguished or severely reduced cone response by ERG was seen in 98.1% (205/209) of the patients with PPVs in *CNGA3* and in all of the patients with PPVs in the five other genes (Table 1). A mild to moderate reduced cone response by ERG was seen in four of the 209 patients with PPVs in *CNGA3*. Furthermore, a mild to moderate reduced rod response by ERG was seen in nine of the 42 patients with PPVs in *CNGA3* whose rod response descriptions were available; the other 33 patients showed a normal rod response. Additionally, refractive error was observed in patients carrying the PPVs in the six genes. Hyperopia and myopia were present in patients with PPVs in *CNGA3*, *CNGB3*, *PDE6C*, and *ATF6*, whereas myopia alone was present in patients age 5 years and older with PPVs in *GNAT2* and *PDE6H* (Appendix 10).

Genotype–phenotype correlations: The various biallelic variant types of the six genes in patients exhibiting different diseases are summarized in Appendix 11. The biallelic variant types of *CNGA3* differed between families with ACHM and families with CORD (Appendix 12), and the PPVs in *CNGA3* were rare in patients with other diseases. For families with PPVs in *CNGB3*, the biallelic truncation PPVs were the most common in families with all diseases and did not show differences among different diseases. Therefore, the genotype–phenotype correlation of the six genes remains unclear.

DISCUSSION

In this study, a systematic analysis of the variants and the phenotypes of the six ACHM-associated genes was performed based on variants identified from 7,195 probands with different eye conditions. A total of 92 PPVs were identified in 119 probands exhibiting different genetic eye diseases, including CORD, ACHM, LCA, MD, eoHM, and unclassified IRD, whereas no biallelic PPVs were identified in patients with rod-dominant diseases.

The review of genotypes and phenotypes of the six genes based on previous literature and the present data revealed several characteristics of variants in the investigated genes. First, the truncation variants and the missense variants that could affect the functional domains are evidence of the pathogenicity of these variants. Therefore, a missense variant might be tolerated when it is located outside the functional domains of the genes; examples include any of the first four exons of *CNGA3* or any of the five exons of *CNGB3* (e.g., c.284C>T, p.Pro95Leu in *CNGA3*) [43]. Second, different mutation hot spots were identified in Asian and Caucasian patients. The missense variants affecting p.Arg277, p.Arg283, and p.Phe547 were common among Caucasians, whereas those affecting p.Val529 were common among Asians. Five mutational hot spots in *CNGB3* were found in Caucasians, and the hot spots were all truncations; none were identified in Asians. All of the reported PPVs in the six genes were rare in the general population with an MAF of less than 1%, mostly less than 0.1%. Thus, it is difficult to set a cut-off allele frequency in control populations to evaluate the pathogenicity

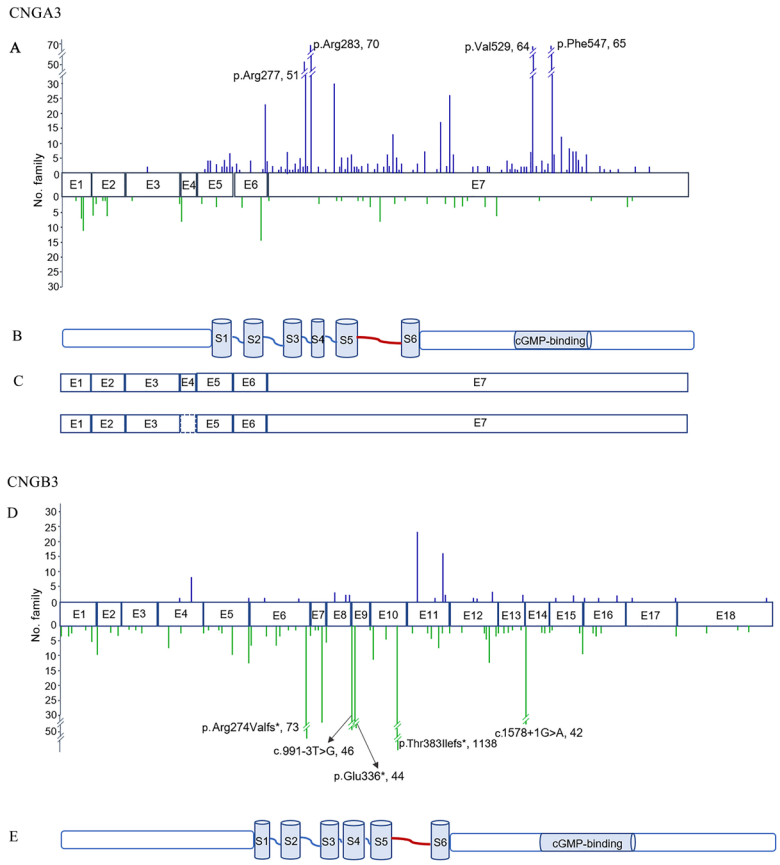

Figure 3. Variant locations in *CNGA3* and *CNGB3*. The blue bar above and the green bar below represent the missense and truncation variants, respectively. B and E represent the functional domains of *CNGA3* and *CNGB3,* respectively. C represents the two alternative transcripts of *CNGA3*. The NM_001298.2 transcript above is longer than the NM_001079878.1 transcript, which lacks exon 4. S1–6, six transmembrane helix domains; E, exon. The vertical axis represents the number of families.

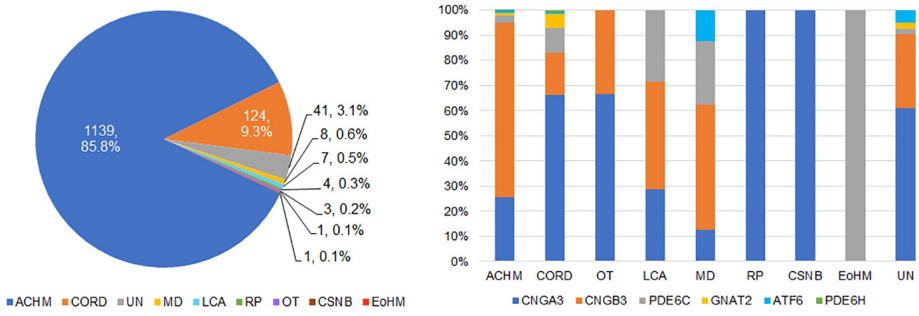

Figure 4. Proportion of diseases associated with the six genes. **A**: ACHM is the most common disease in families carrying potential pathogenic variants (PPVs) in the six genes. **B**: Frequency of each gene in families with different diseases. PPVs in *CNGB3* were the most common in families with ACHM. PPVs in *CNGA3* were most common in families with CORD. ACHM, achromatopsia; CORD, cone-rod dystrophy; UN, unclassified retinopathy; LCA, Leber congenital amaurosis, OT, oligocone trichromacy; MD, macular degeneration; RP, retinitis pigmentosa; eoHM, early-onset high myopia; CSNB, congenital stationary night blindness.

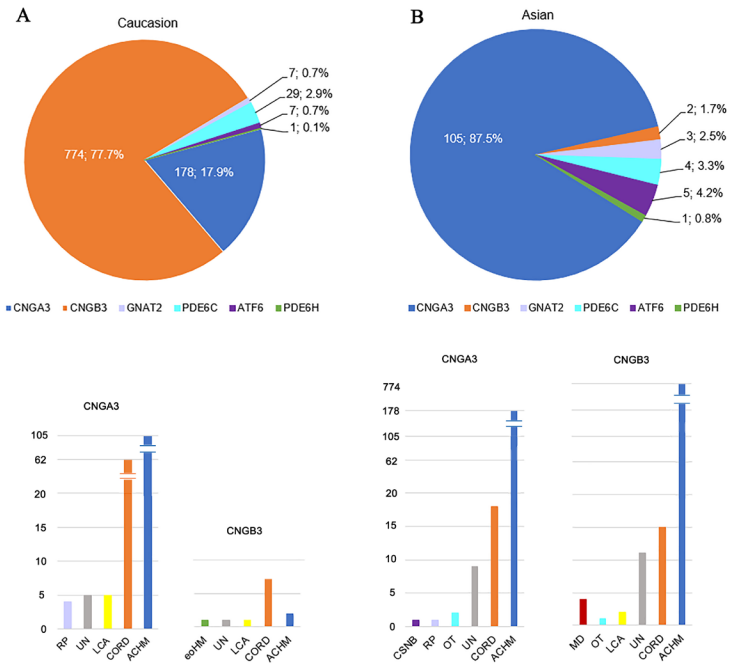

Figure 5. Frequencies of variants in the six genes in Caucasian and Asian patients. **A**: Potential pathogenic variants (PPVs) in *CNGA3* were the most common among Asians and involved the common achromatopsia (ACHM) and cone-rod dystrophy (CORD), as well as the rare Leber congenital amaurosis (LCA), unclassified retinopathy (UN), and retinitis pigmentosa (RP). **B**: Among Caucasians, PPVs in *CNGB3* were the most common, and these variants are associated with ACHM, which is the most common disease, as well as with CORD, UN, LCA, oligocone trichromacy (OT), and macular dystrophy (MD).

of a variant in the six genes. However, an MAF that is significantly higher in patients than in the controls would strongly indicate the pathogenicity of a variant, as is the case for the most common c.1148del variant in *CNGB3*.

The PPVs in the six genes were all initially identified in patients with ACHM and subsequently identified in patients with other autosomal recessive IRDs, most of which were related to cone-predominant dystrophy, including ACHM and CORD. For phenotypic characteristics, congenital nystagmus or photophobia or both were common symptoms among patients with PPVs in the six genes. Congenital nystagmus or photophobia or both with a normal-like fundus would suggest pathogenic mutations in the six genes. The ERG test is strongly suggested for the function evaluation of cones and rods. Additionally, extinguished or severely reduced cone response with or without mild to moderate reduced rod response would additionally indicate the pathogenicity of the variants in the investigated genes.

Several PPVs in these genes were reported to cause LCA or MD, apart from ACHM and CORD, which are common diseases; in some rare cases, PPVs even caused rod-predominant diseases, including RP and CSNB [17,60].

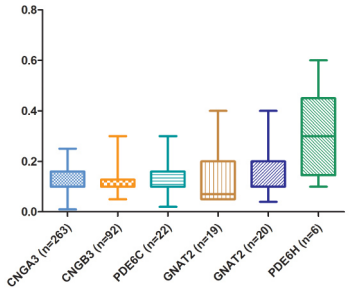

Figure 6. Distribution of available visual acuity in patients with PPVs in the investigated genes.

Table 1. The available clinical manifestations in patients with PPVs in the six genes.

Gene	Congenital nystagmus	Photophobia	Impaired color vision	SR cone response by ERG
CNGA3	92.8% (180/194)	97.8% (175/179)	99.1% (216/218)	98.1% (205/209)
CNGB3	93.8% (60/64)	93.8% (60/64)	96.1% (49/51)	100% (76/76)
GNAT2	83.3% (15/18)	93.8% (15/16)	91.7% (11/12)	100% (14/14)
PDE6C	96.9% (31/32)	94.1% (32/34)	100% (27/27)	100% (28/28)
ATF6	85.7% (18/21)	85.7% (18/21)	100% (19/19)	100% (32/32)
PDE6H	60% (3/5)	40% (2/5)	100% (5/5)	100% (18/18)

Note: SR, severely reduced.

RP and CSNB were identified in several patients with PPVs in *CNGA3*, and LCA was identified in patients with PPVs in *CNGA3* and *CNGB3* [13,15,62]. Seven PPVs in *CNGA3* and four PPVs in *CNGB3* were identified in families with LCA. All of the 11 known PPVs in the two genes are pathogenic because of truncation variants, at functional domains, or with significantly higher frequencies in patients than in the controls. Additionally, there were two PPVs identified in *PDE6C* for patients with LCA: One was a truncation variant, and the other was a missense, which was located outside any functional domains but still predicted to be damaging. Five PPVs in *CNGA3* were identified in patients with RP. Among these PPVs, three were likely pathogenic, whereas the other two located outside the functional domains were identified only in patients with RP and not in patients with ACHM or CORD. However, the pathogenicity of these variants could not be excluded due to their low frequencies in the controls, and because the variants were predicted to be damaging. Two PPVs in *CNGA3* were identified in patients with CSNB: One was a truncation variant, and the other was located at the cGMP-binding domain [17]. In the present data, one missense variant in *PDE6C* was identified in a proband with eoHM. The proband with eoHM was identified to have biallelic missense PPVs in *PDE6C* and showed a bilateral corrected VA of 0.2 at the age of 5 years [21]. Unfortunately, the patient's ERG examination was unavailable. This variant was not identified in previous studies and was located at the functional domain of *PDE6C*.

In all the families affected by rare diseases, the clinical phenotype of only one patient with LCA was described. This patient, with a homozygous c.1579C>A (p.Leu 527Met) variant, exhibited congenital nystagmus and no visual responses with nonrecordable ERG together which indicated LCA [13]. A similar condition was observed in the proband with LCA from the present cohort. Unfortunately, the clinical descriptions of the five patients with RP or CSNB were not mentioned, except the clinical diagnoses. However, none of the biallelic PPVs in the six genes were identified in probands with RP or CSNB based on the present data from 7,195 probands with different eye conditions.

In summary, a systematic genotype–phenotype analysis of the six genes associated with ACHM was performed based on the present data from 7,195 probands with different eye conditions, along with data reported in the literature. The PPVs in the six genes were identified in various IRDs, most of which are cone-dominant diseases. Clear genotype–phenotype correlations have yet to be established in these genes although the truncation variants of *CNGA3* were initially found to be considerably more common in patients with CORD than in patients with ACHM. These results will be valuable for clinical genetic testing involving the investigated genes.

APPENDIX 1. RARE VARIANTS IN BIALLELIC STATUS IN FIVE OF THE SIX GENES DETECTED IN THE 119 PROBANDS WITH GENETIC EYE DISEASES.

To access the data, click or select the words "Appendix 1." Note: IVS, intron; D, damaging; B, benign; P, possibly damaging; PHH2, Polyphen-2; /, not available. The nomenclature of variants were according to the reference sequence including NM_007348.3 of ATF6, NM_005272 of GNAT2, NM_001298 of CNGA3, NM_019098 of CNGB3, and NM_006204 of PDE6C.

APPENDIX 2. CLINICAL INFORMATION OF 51 NEW PROBANDS WITH PATHOGENIC VARIANTS IN ACHM-ASSOCIATED GENES.

To access the data, click or select the words "Appendix 2." ARA= Attenuated retinal arterioles; PFR= Poor foveal reflex; EC= Early childhood; FMB= First few months after birth; NYS= Nystagmus; PV= Poor vision; PP= Photophobia; PFR= Poor foveal reflex; MD= Macular Dtrophy; NA= Not

available; PL=Persuing light; PM= Pigmentation in macular; PO= Pursuing object; NFR= No foveal reflex; TDP= Temporal disc pallor; TDC=PIG= Temporal disc coloboma; WPD= Waxy pale discs; RA= Retina atrophy; LF= leopard fundus; CR, crescent; FH= Foveal hypoplasia; EZ= Ellipsoid zone; TR= Thinning retina; MA= Macular atrophy.

APPENDIX 3. THE 68 REPORTED PROBANDS WITH POTENTIAL PATHOGENIC VARIANTS IN THREE OF THE SIX GENES.

To access the data, click or select the words "Appendix 3."

APPENDIX 4. PEDIGREES OF 51 NEW FAMILIES WITH PPVS IN THE ACHM-ASSOCIATED GENES.

To access the data, click or select the words "Appendix 4." The red asterisk indicates that the genomic DNA sample of the individual is available for segregation analysis.

APPENDIX 5. THE TRANSFOVEAL OCT IMAGE OF SEVEN NEWLY RECRUITED PROBANDS.

To access the data, click or select the words "Appendix 5." The proband IDs are indicated to the left and the genotypes are indicated below.

APPENDIX 6. PATHOGENIC VARIANTS IN THE SIX GENES FROM PREVIOUS LITERATURE EXCEPT OUR COHORT.

To access the data, click or select the words "Appendix 6." Note: D, damaging; B, benign; P, possibly damaging; PHH2, Polyphen-2; /, not available. The nomenclature of variants were according to the reference sequence including NM_007348.3 of ATF6, NM_005272 of GNAT2, NM_001298 of CNGA3, NM_019098 of CNGB3, NM_006204 of PDE6C, and NM_006205.3 of PDE6H.

APPENDIX 7. VARIANT LOCATIONS IN *PDE6C* AND *ATF6*.

To access the data, click or select the words "Appendix 7." The blue bar above and the green bar below represent the missense variants and the truncation variants, respectively.

APPENDIX 8. COMPARISON OFFREQUENCIES BETWEEN PATIENTS AND CONTROLS FROM EXAC

To access the data, click or select the words "Appendix 8." Note: † Variants with no significant difference between patients and controls.

APPENDIX 9. BIALLELIC PATHOGENIC VARIANTS IN THE SIX GENES AND THEIR ASSOCIATED PHENOTYPES REPORTED SO FAR.

To access the data, click or select the words "Appendix 9." Note: ACHM, achromatopsia; COArgD, cone-rod dystrophy; OT, Oligocone trichromacy; LCA, Leber congenital amaurosis; MD, macular degeneration; ArgP, retinitis pigmentosa; CSNB, congenital stationary night blindness; EoHM, early-osnet high myopia; UN: phenotype unclassified.

APPENDIX 10. DISTRIBUTION OF THE AVAILABLE REFRACTIVE ERROR IN RELATION TO AGE IN PATIENTS WITH PPVS IN THE INVESTIGATED GENES.

To access the data, click or select the words "Appendix 10." Patients with PPVs in five of the six genes (*CNGA3*, *CNGB3*, *PDE6C*, *GNAT2*, and *ATF6*) could have both myopia and hyperopia, whereas all of the five patients with PPVs in *PDE6H* showed high myopia (< −6.00D).

APPENDIX 11. VARIANT TYPES OF GENES IN FAMILIES WITH DIFFERENT DISEASES.

To access the data, click or select the words "Appendix 11." Note: Trun = truncation variants; Mis = missense variants; ACHM = achromatopsia; CORD = cone-rod dystrophy; OCT = Oligocone trichromacy; LCA = Leber congenital amaurosis; IRD = inherited retinal degeneration; RP = retinitis pigmentosa; CSNB = congenital stationary night blindness; MD = macular dystrophy.

APPENDIX 12. BIALLELIC VARIANT TYPES IN *CNGA3* IN PATIENTS WITH ACHM AND CORD.

To access the data, click or select the words "Appendix 12." Biallelic missense variants were more common in patients with ACHM than in those with CORD, whereas missense and truncation variants and biallelic truncation variants were more common in patients with CORD than in those with ACHM. Trun, truncation variants; Mis, missense variants; ACHM, achromatopsia; CORD, cone-rod dystrophy.

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