Exome Sequencing on 298 Probands With Early-Onset High Myopia: Approximately One-Fourth Show Potential Pathogenic Mutations in RetNet Genes

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Purpose. To investigate mutations in 234 genes associated with retinal dystrophies in a cohort of 298 probands with early-onset high myopia using whole exome sequencing.

Methods. Genomic DNA from 298 probands with early-onset high myopia was analyzed by whole exome sequencing. Variants from 234 genes were selected and analyzed by multistep bioinformatics analyses.

Results. Systematic analysis of variants in the 234 genes identified potential pathogenic mutations in 34 of 234 genes in 71 of 298 (23.8%) probands. Of the 71 probands, 44 (62.0%) had mutations in 11 genes responsible for ocular diseases accompanied by high myopia, including *COL2A1*, *COL11A1*, *PRPH2*, *FBN1*, *GNAT1*, *OPA1*, *PAX2*, *GUCY2D*, *TSPAN12*, *CACNA1F*, and *RPGR*. Initial clinical records of the 71 patients with mutations did not show recognizable signs of original diseases other than high myopia.

Conclusions. Mutations in genes known to be responsible for retinal diseases were found in approximately one-fourth of the probands with early-onset high myopia. The high mutation frequency of RetNet genes in these patients can provide clues for genetic screening and further specific clinical examinations of high myopia to promote long-term follow-up assessment and prompt treatment of some diseases.

Keywords: high myopia, RetNet, whole exome sequencing, mutation, frequency

High myopia, defined as a refractive error of at least –6.0 diopters (D) or an axial length of at least 26 mm,[1,2] is a leading cause of blindness.[3] Genetic factors are well known to play an important role in the development of high myopia, as confirmed by a number of studies.[4] Most are complex traits governed by both genetics and environment, while some are Mendelian traits with autosomal dominant (AD),[5] autosomal recessive (AR),[6] and X-linked (XL) inheritance patterns.[7]

Early-onset high myopia (eoHM), occurring before school age, is an ideal model for monogenic studies of high myopia because of the minimum influence of environment (e.g., such as near work). Six genes have been identified in patients with high myopia, including *SCO2* (MIM 602474),[8] *ZNF644* (MIM 614159),[9] *LRPAP1* (MIM 104225),[10] *SLC39A5* (MIM 608730),[11] *LEPREL1* (MIM 610341),[12,13] and *CTSH* (MIM 116820).[10] Mutations in these six known genes have been identified in only a few families with eoHM.[8–13] In our previous studies, based on whole exome sequencing analysis of 298 probands with eoHM, mutations in the six known causative genes of eoHM were also identified in only a few families.[14] We then analyzed variants in 12 genes from a genome-wide association study (GWAS) on complex myopia in the same cohort of 298 probands with eoHM and found no significant association of eoHM with variants in these 12 genes from GWAS.[15] Therefore, for most patients with eoHM, the causal genetic influence remains unclear.

High myopia has also been identified as a symptom of various forms of retinal dystrophies as well as systemic syndromes caused by a series of known genes, including *RP2* (MIM 300757)[16,17] and *RPGR* (MIM 312610)[18,19] responsible for retinitis pigmentosa (RP); *NYX* (MIM 300278),[20] *CACNA1F* (MIM 300110),[21] *GRM6* (MIM 604096),[22] and *LRIT3* (MIM 615004)[23] responsible for congenital stationary night blindness (CSNB); *COL2A1* (MIM 120140),[24] *COL11A1* (MIM 120280),[25] *COL9A1* (MIM 120210),[26] and *COL9A2* (MIM 120260)[27] responsible for Stickler syndrome; *FBN1* (MIM 134797)[28,29] responsible for Marfan syndrome; and *COL18A1* (MIM 120328)[30] responsible for Knobloch syndrome. Interestingly, the mutations in *NYX* are found not only in patients with CSNB accompanied by high myopia but also in patients with high myopia without CSNB.[31,32] Therefore, other genes responsible for retinal dystrophies as well as high myopia-related syndromes might be candidates for screening for mutations in patients with eoHM.

In this study, we used data obtained from whole exome sequencing in 298 patients with eoHM to attempt to verify mutations in all the genes responsible for retinal diseases and genes responsible for systemic diseases accompanied by high myopia. Our study expands the list of candidate genes associated with eoHM.

Methods

A total of 298 probands from unrelated families with eoHM were enrolled in this study, conducted at the clinic of the Zhongshan Ophthalmic Center. Patients were included if they had high myopia (spherical refraction at least −6.0 D or axial length > 26 mm) before school age (7 years old in China). Patients were excluded if they showed signs of ocular or systemic abnormalities other than high myopia, either by ophthalmic examination or by questionnaire or oral description of family histories. Only the probands were included for analysis; these were the first affected members who sought medical attention for a genetic disorder. Questionnaires were used to determine if other family members were affected. The refractive errors were available if the family members agreed to participate in our study and came to Zhongshan Ophthalmic Center for ophthalmic examination. Ophthalmologic examinations, including best visual acuity test, slit-lamp examination, and direct ophthalmoscopy, were performed on every proband. Written informed consent conforming to the tenets of the Declaration of Helsinki and following the Guidance of Sample Collection of Human Genetic Diseases (863-plan) by the Ministry of Public Health of China were obtained from all participating individuals or their guardians before the study. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. Genomic DNA of each participant was prepared from leukocytes of peripheral venous blood, as previously described.[33]

Whole exome sequencing was performed in the 298 genomic DNA samples by Macrogen (http://www.macrogen.com/eng/ [in the public domain]) as described in our previous study.[14] In brief, the 298 samples were subjected to exome capture using an Agilent SureSelect Human All Exon Enrichment Kit V4 (51M; Agilent, Santa Clara, CA, USA) array. Exome-enriched DNA fragments from all 298 samples underwent high-throughput sequencing by the Illumina (San Diego, CA, USA) HiSeq2000 instrument with an average sequencing depth of 125-fold. The reads were aligned with the consensus sequence (UCSC hg19) to detect variants.

A total of 234 genes were included in this study, including 231 genes (accessed on January 27, 2015) associated with retinal dystrophies from the RetNet database (https://sph.uth.edu/retnet/, a website that provides tables of genes and loci causing inherited retinal diseases [in the public domain]) and three other genes, *COL9A2*, *FBN1*, and *COL18A1*, which are responsible for Stickler syndrome, Marfan syndrome, and Knobloch syndrome, respectively. The *NYX* and *OPN1LW* genes have been analyzed before, so the results are not presented in the current study.

Variants from 234 genes were selected from the whole exome sequencing dataset of the 298 probands with eoHM. Low-certainty variant positions with a depth of coverage less than 10 were ignored. Then variants unlikely to be pathogenic were filtered out in the following order. (1) Single nucleotide polymorphisms (SNPs) and short indels in the exome region were filtered against data from dbSNP, 1000 Human Genome Project, Exome Variant Server, and Exome Aggregation Consortium (ExAC, Cambridge, MA, USA; http://exac.broadinstitute.org [in the public domain], accessed November 2015) excluding minor allele frequency (MAF) ≥ 1/6000 (according to a prevalence of approximately 1/3000 of retinal degeneration[34] and an allele frequency of a variant in AD genes not exceeding 1/6000 in the general population). (2) We excluded variants in noncoding region as well as the synonymous variants that did not affect splice sites according to the Berkeley Drosophila Genome Project (BDGP; http://www.fruitfly.org/ [in the public domain]).[35] (3) We excluded variants predicted to be benign by two online tools: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/ [in the public domain])[36] and SIFT (http://sift.jcvi.org [in the public domain]).[37] (4) We excluded variants not consistent with hereditary patterns, which were only one hit heterozygous variants in AR genes and homozygous variants in AD genes. (5) We excluded variants not consistent with the mutation types or mutant regions of the diseasing-causing mutations in corresponding genes based on the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php [in the public domain]) and previous studies. These included (a) truncation and the glycine replaced missense variants in *COL2A1* (MIM 120140) and *COL11A1* (MIM 120280), which are well known to be pathogenic; several missense mutations in *COL2A1* were also considered to be pathogenic; other variants in the two genes were excluded; (b) *CACNA1F* (MIM 300110), where all mutations listed in HGMD were hemizygous; all heterozygous variants were excluded; (c) *GUCY2D* (MIM 600179), mainly described as AR retinal disease genes,[38] except that mutations at codons 837, 838, and 849 caused AD retinal disease[39–41]; (d) most of the causative mutations in *DMD* (MIM 300377), which were truncation or splice mutations. Only a few missense mutations, which were located in the N-terminal or C-terminal domains, were reported as pathogenic.[42] (6) A total of 312 patients were included as ethnicity-matched relative controls; they had ocular diseases other than high myopia and retinal degeneration. Whole exome sequencing was also performed on the 312 individuals, using the same sequencing platform as used for the 298 probands with eoHM. The variants were excluded if they were found in the 312 controls.

Based on the method of Taylor et al.,[43] we analyzed all the potential pathogenic variants using the Ensemble Variant Effect Predictor (VEP), considering that the variants were originally annotated by ANNOVAR.

To evaluate the false-positive rate of the 34 genes with potential mutations detected in 298 eoHM, variants in these genes from approximately 4300 East Asian samples from ExAC were selected as controls and were analyzed by the same filtering process as described for eoHM. As for genes associated with AR diseases, the probability of encountering either compound heterozygous or homozygous mutations in these genes is equivalent to squaring the sum of all potential pathogenic variant frequencies.

Results

Of the 298 patients in the study, 154 (51.7%) were male and 144 (48.3%) were female. Their mean (±SD) examination age was 18.0 (±15.4) years of age, with the mean (±SD) age of myopia onset of 4.0 (±1.7) years of age. The mean (±SD) spherical refraction was −11.2 D (±5.1 D) for the right eye and −11.2 D (±5.0 D) for the left eye. Of the 298 participants, 124 participated in this study before the age of 7 years. The other 174 patients who participated after 7 years of age were included as eoHM by questionnaires, but no details of their age of myopia onset or their refractive error or axial length before 7 years of age were available. Ophthalmoscopy examination of the 298 patients showed that 184 patients had a leopard fundus; 86 of these had crescent and 16 had retinal detachment.

Systematic analysis of variants in the 234 genes identified 80 potential pathogenic mutations in 71 of 298 (23.8%) probands (Supplementary Table S1); among these, five patients had mutations in two genes (Table). These 80 mutations involved 34 of 234 genes. The 71 probands included 55 (74.6%, 53/71) with heterozygous mutations in the AD genes, 6 (8.5%, 6/71) with compound heterozygous mutations in the AR genes, and

Table. Clinical Data of 71 Patients With Potential Pathogenic Mutations

Patient ID	Gene	Mutations	Sex	Age, Years at Exam	Best Visual Acuity		Refraction, D		Axial Length, mm	
					OD	OS	OD	OS	OD	OS
HM845	*COL2A1*	c.[827G>A];[=]	M	7	0.5	0.1	−15.00	−19.50	28.61	30.20
HM820	*COL2A1*	c.[1366-1G>C];[=]	M	3	NA	NA	−7.25	−7.25	25.64	25.45
HM814	*COL2A1*	c.[1597C>T];[=]	M	8	0.1	0.1	−13.50	−16.50	29.22	29.31
HM626	*COL2A1*	c.[1693C>T];[=]	F	7	NA	NA	−16.50	−14.50	NA	NA
HM880	*COL2A1*	c.[1779_1780insG];[=]	F	5	0.1	0.1	−10.00	−10.00	26.25	25.90
HM472	*COL2A1*	c.[1833+1G>A];[=]	F	29	0.6	0.4	−11.00	−11.00	26.60	26.10
HM849	*COL2A1*	c.[1833+1G>A];[=]	M	6	0.3	0.2	−4.50	−3.00	27.10	26.63
HM309	*COL2A1*	c.[1836_1843dup];[=]	M	11	0.5	0.5	−7.75	−10.50	NA	NA
HM842	*COL2A1*	c.[1957C>T];[=]	F	5	0.1	0.1	−20.00	−22.00	29.02	29.34
HM862	*COL2A1*	c.[2794C>T];[=]	M	8	1.0	1.2	−6.75	−6.75	27.29	27.25
HM790	*COL11A1*	c.[1666del];[=]	F	3	NA	NA	−12.50	−11.50	NA	NA
HM713	*COL11A1*	c.[2459G>A];[=]	F	7	0.5	0.5	−6.50	−7.50	25.30	25.68
HM878	*COL11A1*	c.[3782G>T];[=]	M	12	0.5	0.5	−7.00	−5.75	27.87	27.57
HM813	*COL11A1*	c.[4484G>A];[=]	M	6	0.6	0.8	−16.50	−17.00	28.85	28.95
HM838	*PRPH2*	c.[38G>A];[=]	M	3	NA	NA	−14.00	−11.50	26.54	26.78
HM774	*PRPH2*	c.[857T>C];[=]	F	3	NA	NA	−8.00	−6.00	24.97	24.52
HM707	*PRPH2*	c.[863C>A];[=]	M	3	NA	NA	−8.00	−10.00	25.40	25.73
HM381	*EFEMP1*	c.[580C>T];[=]	F	26	0.8	0.8	−8.00	−8.50	NA	NA
HM741	*EFEMP1*	c.[1303G>A];[=]	M	20	0.4	0.2	−17.50	−17.50	30.23	30.43
HM650	*FBN1*	c.[723delG];[=]	M	7	0.4	0.4	−16.50	−16.50	NA	NA
HM769	*FBN1*	c.[3849G>C];[=]	M	4	0.2	0.2	−4.75	−4.75	26.06	26.00
HM679	*FBN1*	c.[6709G>A];[=]	F	30	0.1	0.1	−15.00	−15.00	NA	NA
HM783	*GNAT1*	c.[413G>A];[=]	F	10	1.0	1.0	−6.25	−6.50	26.20	26.42
HM665	*GNAT1*	c.[819G>T];[=]	F	33	1.0	1.0	−10.00	−12.00	NA	NA
HM648	*JAG1*	c.[1442G>A];[=]	F	22	1.0	0.9	−6.50	−6.75	NA	NA
HM864	*OPA1*	c.[275G>C];[=]	M	5	0.2	0.2	−9.50	−8.50	26.42	25.51
HM763	*OPA1*	c.[1397C>G];[=]	M	13	1.0	1.0	−6.25	−6.25	26.69	26.64
HM435	*OPN1SW*	c.[196C>T];[=]	F	33	NA	NA	−10.00	−10.00	NA	NA
HM640	*OPN1SW*	c.[955T>C];[=]	F	32	0.5	0.6	−9.75	−7.25	NA	NA
HM307	*OTX2*	c.[53C>T];[=]	M	7	0.6	0.6	−6.50	−6.50	NA	NA
HM873	*OTX2*	c.[259G>A];[=]	M	5	0.5	0.6	−4.50	−4.50	NA	NA
HM415	*PAX2*	c.[685+2T>C];[=]	F	47	0.9	0.7	−14.00	−15.00	30.69	32.05
HM384	*ROM1*	c.[128T>G];[=]	F	33	0.5	0.0	−17.00	−17.00	29.62	25.89
HM785	*TOPORS*	c.[1946A>G];[=]	M	28	0.3	0.6	−16.00	−9.00	29.74	27.50
HM751	*TREX1*	c.[459dup];[=]	M	3	0.7	0.7	−8.25	−7.50	26.75	26.65
HM762	*BEST1*	c.[868G>A];[=]	F	10	0.7	1.0	−6.50	−6.25	26.19	26.05
HM666	*CAPN5*	c.[2T>C];[=]	F	11	1.0	1.0	−7.75	−7.75	NA	NA
HM691	*CRX*	c.[684G>C];[=]	F	5	0.8	1.0	−9.00	−8.00	26.10	25.46
HM344	*FZD4*	c.[313A>G];[=]	F	1	NA	NA	−8.00	−8.00	23.78	23.91
HM852	*GUCY2D*	c.[2512C>T];[=]	F	12	0.5	0.3	−5.50	−9.50	25.59	26.64
HM883	*IMPG1*	c.[976G>T];[=]	F	8	0.7	0.4	−14.25	−14.25	26.88	26.96
HM327	*OPA3*	c.[509C>T];[=]	F	30	0.4	0.4	−6.00	−6.00	NA	NA
HM693	*PRPF8*	c.[6980C>T];[=]	M	4.5	0.2	0.3	−8.00	−7.00	26.97	26.74
HM639	*RB1*	c.[83C>G];[=]	M	24	1.2	0.9	−8.50	−9.50	NA	NA
HM723	*RGR*	c.[266C>A];[=]	F	45	0.2	0.2	−12.00	−13.50	27.57	28.18
HM244	*RHO*	c.[671G>A];[=]	F	34	NA	NA	−18.00	−18.00	NA	NA
HM483	*RIMS1*	c.[1591A>T];[=]	M	6	0.4	0.4	−7.00	−6.50	NA	NA
HM674	*TSPAN12*	c.[231del];[=]	M	22	0.1	HM	−19.00	NA	NA	NA
HM564	*USH2A*	c.[4679C>A];[9854del]	F	36	0.4	0.5	−20.00	−20.00	NA	NA
HM758	*USH2A*	c.[5051C>T];[5375G>A]	M	41	0.3	0.3	−11.25	−10.75	27.15	26.93
HM680	*AHI1*	c.[1483C>T];[1580C>T]	M	32	0.3	0.3	−10.50	−10.50	NA	NA
HM608	*CC2D2A*	c.[332C>T];[3242C>T]	M	3	0.8	1.0	−8.00	−10.00	NA	NA
HM482	*PDE6C*	c.[2005A>G];[2005A>G]	M	5	0.2	0.2	−12.50	−12.00	NA	NA
HM623	*CACNA1F*	c.[245G>A];[0]	M	4	NA	NA	−7.00	7.50	NA	NA
HM698	*CACNA1F*	c.[283G>A];[0]	M	3	0.6	0.5	−7.00	−8.25	25.79	26.21
HM308	*CACNA1F*	c.[926G>A];[0]	M	4	0.2	0.2	−7.00	−6.00	NA	NA
HM776	*CACNA1F*	c.[2722G>T];[0]	M	5	0.1	0.1	−11.50	−11.50	28.20	28.20
HM565	*CACNA1F*	c.[4519-1G>A];[0]	M	5	0.5	0.4	−4.25	−4.00	NA	NA
HM865	*CACNA1F*	c.[4699del];[0]	M	30	0.4	0.5	−16.62	−13.50	30.26	29.93
HM860	*RPGR*	c.[139_140insTCTGC];[=]	F	5	0.3	0.5	−7.25	−6.50	24.91	24.51
HM370	*RPGR*	c.[1282C>G];[=]	F	34	0.9	0.9	−10.25	−10.00	26.93	27.44
HM566	*RPGR*	c.[1967A>T];[0]	M	2	NA	NA	−10.00	−10.00	NA	NA

Table. Continued

Patient ID	Gene	Mutations	Sex	Age, Years at Exam	Best Visual Acuity		Refraction, D		Axial Length, mm	
					OD	OS	OD	OS	OD	OS
HM825	*RPGR*	c.[2135A>G];[0]	M	12	0.2	0.2	−11.75	−11.00	NA	NA
HM485	*RPGR*	c.[2200G>A];[=]	F	49	0.7	0.9	−19.50	−21.00	NA	NA
HM697	*RPGR*	c.[3240del];[0]	M	5	0.3	0.3	−6.00	−5.50	NA	NA
HM877	*RPGR*	c.[3361del];[0]	M	5	0.5	0.6	−11.00	−10.50	26.70	26.41
HM656*	*COL11A1*	c.[3494G>C];[=]	M	16	0.1	0.1	−20.00	−20.00	NA	NA
	JAG1	c.[1829G>A];[=]								
HM364*	*PRPH2*	c.[533A>G];[=]	F	33	0.2	0.5	−8.00	−7.25	NA	NA
	ROM1	c.[334G>A];[=]								
HM704*	*CACNA1F*	c.[3269C>T];[0]	M	3	0.2	0.1	−7.50	−7.25	25.80	25.63
	EFEMP1	c.[379G>C];[=]								
HM866*	*PAX2*	c.[1172C>T];[=]	F	4	0.5	0.6	−6.50	−7.00	24.79	25.07
	TREX1	c.[16C>T];[=]								
HM654*	*TOPORS*	c.[23G>C];[=]	F	24	0.5	0.7	−8.00	−9.50	NA	NA
	CEP290	c.[367C>T];[c.2470A>G]								

OD, right eye; OS, left eye; F, female; M, male; NA, not available; HM, hand movement.
* Patients with mutations in two genes.

14 (19.7%, 14/71) with hemizygous or heterozygous mutations in the XL genes.

The variants were further subdivided by the pathogenicity of the variants and associated phenotypes of genes, as follows: (1) The 80 variants in 34 genes were divided into three groups with reference to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines[44] (Supplementary Table S1). The 29 variants in 30 (10.1%, 30/298) patients in the strong group were considered as pathogenic mutations, while variants in the moderate and supporting groups need further functional study to confirm their pathogenicity. (2) Of the 34 genes, 11 genes have been reported to cause phenotypes including myopia, including *COL2A1*,[45] *COL11A1*,[25] *PRPH2*,[46] *FBN1*,[47] *GNAT1*,[48] *OPA1*,[49] *PAX2*,[50] *GUCY2D*,[51] *TSPAN12*,[52] *CACNA1F*,[53] and *RPGR*.[19] Variants of the 11 genes were identified in 44 (14.8%) patients (Fig. 1).

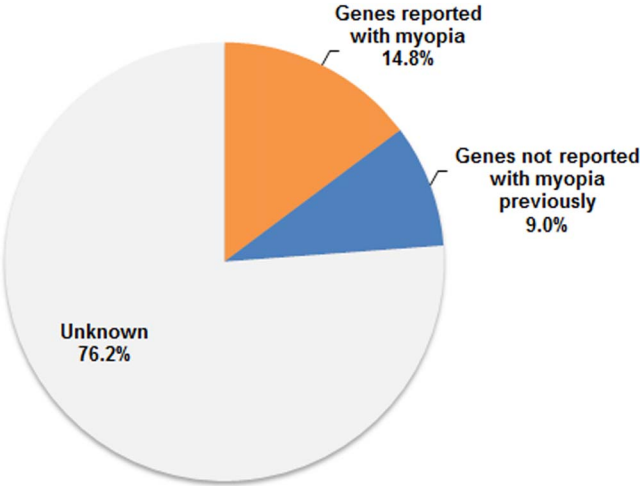

Figure 1. Mutation proportions grouped by the genes reported to cause myopia or not. In this study, 14.8% of patients (44/298) with eoHM harbored mutations in 11 genes that were previously reported to cause phenotypes including myopia, and 9.0% of patients (27/298) harbored mutations in 23 other genes not previously reported in myopia.

With reference to our previous studies on mutation investigation of RetNet genes in patients with RP, mutations in patients with eoHM in the current study showed differences from mutations in patients with RP (Fig. 2).[54–57] In the eoHM cohort, missense mutations account for approximately 72.5% while truncation mutations (including frameshift, stop gain, and splicing change mutations) account for 27.5%. In the RP cohort, missense and truncation mutations account for 50.0% and 50.0%, respectively. On the other hand, mutations in AD genes are the most common in patients with eoHM, while in the RP cohort, mutations in AR genes are the most common (Fig. 2).

The results from VEP annotation are shown in Supplementary Table S2. Twenty-seven variants were consistent with our original results. The other 53 variants showed variable variations because of different transcripts, but at least one annotation was the same as in our results.

Totally, 4407 variants in the 34 genes were identified in at least one of the 4300 East Asian samples from ExAC. After filtering, potential pathogenic variants in the 34 genes were identified in approximately 6.2% (265/4300) East Asian individuals, which is significantly lower than the 23.8% in patients with eoHM in the current study ($P < 9.38 \times 10^{-30}$).

The clinical data of the 71 patients carrying mutations are shown in the Table. Two probands (HM873 and HM565) showed a myopia greater than –6.00 D before 5 years of age, but it is suspected that they would have high myopia at 7 years of age, for the two had a family history of eoHM. None of the 71 patients were initially recorded with recognized signs of original diseases other than high myopia. Three patients with *CACNA1F* mutations agreed to revisit to undergo an electroretinograph (ERG) testing, and all three patients showed abnormal results. Patient HM565 (with c.4519-1G>A mutation) revealed a severe decrease in the amplitude of waves related to both rod and cone responses. Patient HM865 (with c.4699del mutation) revealed a moderate decrease in the amplitude of the waves related to the rod response and a severe decrease in amplitude of the waves related to the cone response. Patient HM776 (with c.2722G>T mutation) has no identifiable cone

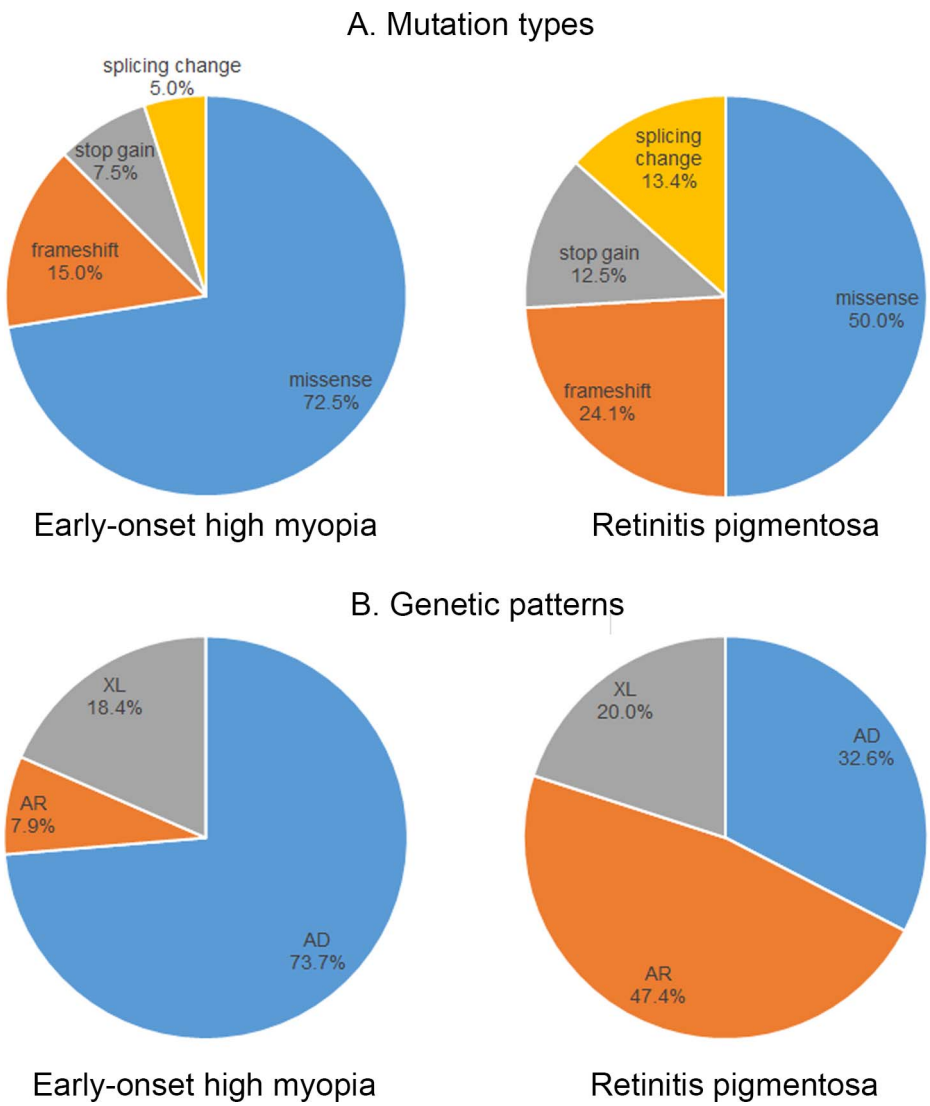

Figure 2. **(A)** Comparison of mutation types in RetNet genes detected in patients with eoHM and patients with retinitis pigmentosa in our previous reports. The proportion of four mutation types in the eoHM data is on the *upper left*, and the proportion of four mutation types of retinitis pigmentosa data is on the *upper right*. **(B)** Comparison of the genetic patterns of genes with identified mutations in patients with early-onset high myopia and genes in patients with retinitis pigmentosa in our previous reports. The proportions of genes associated with autosomal dominant, autosomal recessive, and X-linked traits in the early-onset high myopia data is on the *lower left* and in retinitis pigmentosa data on the *lower right*.

responses, but showed a severe decrease in the amplitude of the waves related to the rod response.

Discussion

In this study, whole exome sequencing was used to investigate mutations in genes associated with retinal dystrophies and high myopia-related systemic syndromes in 298 probands with eoHM. Potential pathogenic mutations were identified in 71 patients (23.8%, 71/298), of whom 62.0% (44/71) had mutations in genes responsible for ocular diseases accompanied by myopia. Looking back at previous studies, the findings are not surprising.

The association of eoHM with ocular and systemic diseases was previously investigated in several studies. For example, Logan et al.[58] investigated 112 children with high myopia before 10 years of age and found that only 8% of these children had "simple high myopia" without any ocular or systemic abnormalities, while the others had ocular or systemic disorders, including 14% with retinal dystrophies and 13% diagnosed as Stickler syndrome or Marfan syndrome. Another study found that 56% of children with high myopia had simple high myopia, while 25% had ocular abnormalities and 19% had systemic disorders, predominantly Stickler syndrome and Marfan syndrome.[59] These researchers proposed that eoHM might be the first indication of these other diseases.[59]

In the present study, mutations in *COL2A1* and *COL11A1* were detected in 5.0% (15 of 298) of our cohort with eoHM. These are two common causative genes of Stickler syndrome,[60] in which high myopia is the most common clinical feature and is the most common cause of inherited retinal detachment.[61] More than 90% of patients with Stickler syndrome have high myopia.[62,63] Some mutations in *COL2A1* cause solely ocular phenotypes, including high myopia and its associated complications (retinal detachment, vitreous degeneration, and cataract).[61,64,65] Therefore, separation of simple high myopia and ocular-predominant Stickler syndrome is difficult. Investigating mutations in *COL2A1* and *COL11A1* thus becomes important in patients with eoHM, especially for early diagnosis and early

intervention in Stickler syndrome, because prophylactic cryotherapy in patients with Stickler syndrome could reduce the risk of retinal detachment.[66]

Besides *COL2A1* and *COL11A1*, mutations in *CACNA1F* and *RPGR* were also predominantly identified in patients with eoHM in the current study. Hemizygous mutations of *CACNA1F* were identified in seven males of the 298 patients with eoHM. Mutations in *CACNA1F* mainly cause CSNB, with high myopia as one of their most common features.[67] To date, 98 mutations have been identified in *CACNA1F* (listed in HGMD), including 91 mutations causing CSNB, while the remaining 7 causing other retinal disorders. Of the 91 causative mutations of CSNB, 29 (31.9%) are missense mutations while others are truncation mutations. Of the other 7 mutations, 4 are missense and 3 are truncation mutations. In the present study, 5 of 7 mutations in *CACNA1F* are missense. Later ERG tests of three available patients revealed retinal dystrophies affecting both rod and cone responses. Mutations in *RPGR* are the most common cause of X-linked retinitis pigmentosa (XLRP).[68] Previous studies have described high myopia as a common sign of RP in patients with RPGR mutations, especially in carrier females who had high myopia without obvious signs of RP.[19] In the present study, mutations in *RPGR* were identified in 7 of 298 patients with eoHM; 3 were female with heterozygous mutations, and the other 4 were male with hemizygous mutations.

Apart from this study, high myopia has been reported in a number of patients with mutations in other genes, and some researchers have considered that high myopia might be a risk factor for RP.[19] However, all these previous studies were performed in patients who had manifested obvious fundus changes for retinal dystrophies accompanied by high myopia. The current study is the first systematic investigation on mutations of genes responsible for retinal dystrophies in patients with eoHM. Pathogenic mutations were identified in one-fourth of the patients. None of these patients were found to have symptoms or obvious fundus changes of retinal dystrophies. Therefore, a possible hypothesis is that high myopia may be an earlier feature than fundus changes from mutations in these genes. This was supported by a recent study showing an early occurrence of high myopia, before significant fundus changes, in four patients with *RBP3* mutations.[69] However, further evidence is required to confirm this conclusion, especially further specific clinical examinations. Unfortunately, detailed ocular and systemic examinations were lacking in the 298 participants with eoHM. We could not determine whether the patients had other, albeit minor, changes when they were examined or monitor how they had progressed. A clearer conclusion would be obtained if the genetic results could be combined with detailed clinical examinations, especially long-term follow-up assessment for the 71 patients with mutations. In any case, we suggest that genes associated with retinal diseases should be borne in mind when genetic studies are performed in patients with eoHM. Our study can be viewed as the beginning of that process.

In conclusion, in this study, systematic mutation analysis of RetNet genes was performed using whole exome sequencing data of patients with eoHM, and potential pathogenic mutations were detected in 23.8% of patients. The high mutation frequency of RetNet genes in these patients can provide clues for genetic screening and further specific clinical examination of high myopia to promote long-term follow-up assessment and prompt treatment of some diseases.

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References

1. Percival SP. Redefinition of high myopia: the relationship of axial length measurement to myopic pathology and its relevance to cataract surgery. *Dev Ophthalmol*. 1987;14:42–46.
2. Young TL, Metlapally R, Shay AE. Complex trait genetics of refractive error. *Arch Ophthalmol*. 2007;125:38–48.
3. Saw SM, Gazzard G, Shih-Yen EC, Chua WH. Myopia and associated pathological complications. *Ophthalmic Physiol Opt*. 2005;25:381–391.
4. Tsai MY, Lin LL, Lee V, Chen CJ, Shih YF. Estimation of heritability in myopic twin studies. *Jpn J Ophthalmol*. 2009; 53:615–622.
5. Lam DS, Tam PO, Fan DS, Baum L, Leung YF, Pang CP. Familial high myopia linkage to chromosome 18p. *Ophthalmologica*. 2003;217:115–118.
6. Yang Z, Xiao X, Li S, Zhang Q. Clinical and linkage study on a consanguineous Chinese family with autosomal recessive high myopia. *Mol Vis*. 2009;15:312–318.
7. Schwartz M, Haim M, Skarsholm D. X-linked myopia: Bornholm eye disease. Linkage to DNA markers on the distal part of Xq. *Clin Genet*. 1990;38:281–286.
8. Tran-Viet KN, Powell C, Barathi VA, et al. Mutations in SCO2 are associated with autosomal-dominant high-grade myopia. *Am J Hum Genet*. 2013;92:820–826.
9. Shi Y, Li Y, Zhang D, et al. Exome sequencing identifies ZNF644 mutations in high myopia. *PLoS Genet*. 2011;7: e1002084.
10. Aldahmesh MA, Khan AO, Alkuraya H, et al. Mutations in LRPAP1 are associated with severe myopia in humans. *Am J Hum Genet*. 2013;93:313–320.
11. Guo H, Jin X, Zhu T, et al. SLC39A5 mutations interfering with the BMP/TGF-beta pathway in non-syndromic high myopia. *J Med Genet*. 2014;51:518–525.
12. Mordechai S, Gradstein L, Pasanen A, et al. High myopia caused by a mutation in LEPREL1, encoding prolyl 3-hydroxylase 2. *Am J Hum Genet*. 2011;89:438–445.
13. Guo H, Tong P, Peng Y, et al. Homozygous loss-of-function mutation of the LEPREL1 gene causes severe non-syndromic high myopia with early-onset cataract. *Clin Genet*. 2014;86: 575–579.
14. Jiang D, Li J, Xiao X, et al. Detection of mutations in LRPAP1, CTSH, LEPREL1, ZNF644, SLC39A5, and SCO2 in 298 families with early-onset high myopia by exome sequencing. *Invest Ophthalmol Vis Sci*. 2015;56:339–345.
15. Li J, Jiang D, Xiao X, et al. Evaluation of 12 myopia-associated genes in Chinese patients with high myopia. *Invest Ophthalmol Vis Sci*. 2015;56:722–729.
16. Flaxel CJ, Jay M, Thiselton DL, et al. Difference between RP2 and RP3 phenotypes in X linked retinitis pigmentosa. *Br J Ophthalmol*. 1999;83:1144–1148.
17. Jayasundera T, Branham KE, Othman M, et al. RP2 phenotype and pathogenetic correlations in X-linked retinitis pigmentosa. *Arch Ophthalmol*. 2010;128:915–923.
18. Yokoyama A, Maruiwa F, Hayakawa M, et al. Three novel mutations of the RPGR gene exon ORF15 in three Japanese families with X-linked retinitis pigmentosa. *Am J Med Genet*. 2001;104:232–238.

19. Jin ZB, Liu XQ, Hayakawa M, Murakami A, Nao-i N. Mutational analysis of RPGR and RP2 genes in Japanese patients with retinitis pigmentosa: identification of four mutations. *Mol Vis*. 2006;12:1167–1174.
20. Xiao X, Jia X, Guo X, Li S, Yang Z, Zhang Q. CSNB1 in Chinese families associated with novel mutations in NYX. *J Hum Genet*. 2006;51:634–640.
21. Hemara-Wahanui A, Berjukow S, Hope CI, et al. A CACNA1F mutation identified in an X-linked retinal disorder shifts the voltage dependence of Cav1.4 channel activation. *Proc Natl Acad Sci U S A*. 2005;102:7553–7558.
22. Xu X, Li S, Xiao X, Wang P, Guo X, Zhang Q. Sequence variations of GRM6 in patients with high myopia. *Mol Vis*. 2009;15:2094–2100.
23. Zeitz C, Jacobson SG, Hamel CP, et al. Whole-exome sequencing identifies LRIT3 mutations as a cause of autosomal-recessive complete congenital stationary night blindness. *Am J Hum Genet*. 2013;92:67–75.
24. Ahmad NN, McDonald-McGinn DM, Zackai EH, et al. A second mutation in the type II procollagen gene (COL2AI) causing stickler syndrome (arthro-ophthalmopathy) is also a premature termination codon. *Am J Hum Genet*. 1993;52:39–45.
25. Annunen S, Korkko J, Czarny M, et al. Splicing mutations of 54-bp exons in the COL11A1 gene cause Marshall syndrome, but other mutations cause overlapping Marshall/Stickler phenotypes. *Am J Hum Genet*. 1999;65:974–983.
26. Van Camp G, Snoeckx RL, Hilgert N, et al. A new autosomal recessive form of Stickler syndrome is caused by a mutation in the COL9A1 gene. *Am J Hum Genet*. 2006;79:449–457.
27. Baker S, Booth C, Fillman C, et al. A loss of function mutation in the COL9A2 gene causes autosomal recessive Stickler syndrome. *Am J Med Genet A*. 2011;155A:1668–1672.
28. Dietz HC, McIntosh I, Sakai LY, et al. Four novel FBN1 mutations: significance for mutant transcript level and EGF-like domain calcium binding in the pathogenesis of Marfan syndrome. *Genomics*. 1993;17:468–475.
29. Montgomery RA, Geraghty MT, Bull E, et al. Multiple molecular mechanisms underlying subdiagnostic variants of Marfan syndrome. *Am J Hum Genet*. 1998;63:1703–1711.
30. Suzuki OT, Sertie AL, Der Kaloustian VM, et al. Molecular analysis of collagen XVIII reveals novel mutations, presence of a third isoform, and possible genetic heterogeneity in Knobloch syndrome. *Am J Hum Genet*. 2002;71:1320–1329.
31. Zhang Q, Xiao X, Li S, et al. Mutations in NYX of individuals with high myopia, but without night blindness. *Mol Vis*. 2007;13:330–336.
32. Yip SP, Li CC, Yiu WC, et al. A novel missense mutation in the NYX gene associated with high myopia. *Ophthalmic Physiol Opt*. 2013;33:346–353.
33. Wang Q, Wang P, Li S, et al. Mitochondrial DNA haplogroup distribution in Chaoshanese with and without myopia. *Mol Vis*. 2010;16:303–309.
34. Thumann G. Prospectives for gene therapy of retinal degenerations. *Curr Genomics*. 2012;13:350–362.
35. Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. *J Comput Biol*. 1997;4:311–323.
36. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–249.
37. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4:1073–1081.
38. Hanein S, Perrault I, Gerber S, et al. Leber congenital amaurosis: comprehensive survey of the genetic heterogeneity, refinement of the clinical definition, and genotype-phenotype correlations as a strategy for molecular diagnosis. *Hum Mutat*. 2004;23:306–317.
39. Kelsell RE, Gregory-Evans K, Payne AM, et al. Mutations in the retinal guanylate cyclase (RETGC-1) gene in dominant cone-rod dystrophy. *Hum Mol Genet*. 1998;7:1179–1184.
40. Smith M, Whittock N, Searle A, Croft M, Brewer C, Cole M. Phenotype of autosomal dominant cone-rod dystrophy due to the R838C mutation of the GUCY2D gene encoding retinal guanylate cyclase-1. *Eye (Lond)*. 2007;21:1220–1225.
41. Payne AM, Morris AG, Downes SM, et al. Clustering and frequency of mutations in the retinal guanylate cyclase (GUCY2D) gene in patients with dominant cone-rod dystrophies. *J Med Genet*. 2001;38:611–614.
42. Roberts RG, Gardner RJ, Bobrow M. Searching for the 1 in 2,400,000: a review of dystrophin gene point mutations. *Hum Mutat*. 1994;4:1–11.
43. Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. *Nat Genet*. 2015;47:717–726.
44. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424.
45. Brown DM, Vandenburgh K, Kimura AE, Weingeist TA, Sheffield VC, Stone EM. Novel frameshift mutations in the procollagen 2 gene (COL2A1) associated with Stickler syndrome (hereditary arthro-ophthalmopathy). *Hum Mol Genet*. 1995;4:141–142.
46. Khan AO, Al Rashaed S, Neuhaus C, Bergmann C, Bolz HJ. Peripherin mutations cause a distinct form of recessive Leber congenital amaurosis and dominant phenotypes in asymptomatic parents heterozygous for the mutation [published online ahead of print June 10, 2014]. *Br J Ophthalmol*. doi:10.1136/bjophthalmol-2015-306844.
47. Schrijver I, Liu W, Brenn T, Furthmayr H, Francke U. Cysteine substitutions in epidermal growth factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes. *Am J Hum Genet*. 1999;65:1007–1020.
48. Naeem MA, Chavali VR, Ali S, et al. GNAT1 associated with autosomal recessive congenital stationary night blindness. *Invest Ophthalmol Vis Sci*. 2012;53:1353–1361.
49. Chen S, Zhang Y, Wang Y, et al. A novel OPA1 mutation responsible for autosomal dominant optic atrophy with high frequency hearing loss in a Chinese family. *Am J Ophthalmol*. 2007;143:186–188.
50. Miyazawa T, Nakano M, Takemura Y, et al. A case of renal-coloboma syndrome associated with mental developmental delay exhibiting a novel PAX2 gene mutation. *Clin Nephrol*. 2009;72:497–500.
51. Gregory-Evans K, Kelsell RE, Gregory-Evans CY, et al. Autosomal dominant cone-rod retinal dystrophy (CORD6) from heterozygous mutation of GUCY2D, which encodes retinal guanylate cyclase. *Ophthalmology*. 2000;107:55–61.
52. Poulter JA, Davidson AE, Ali M, et al. Recessive mutations in TSPAN12 cause retinal dysplasia and severe familial exudative vitreoretinopathy (FEVR). *Invest Ophthalmol Vis Sci*. 2012;53:2873–2879.
53. Bech-Hansen NT, Naylor MJ, Maybaum TA, et al. Loss-of-function mutations in a calcium-channel alpha1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. *Nat Genet*. 1998;19:264–267.
54. Xu Y, Guan L, Shen T, et al. Mutations of 60 known causative genes in 157 families with retinitis pigmentosa based on exome sequencing. *Hum Genet*. 2014;133:1255–1271.
55. Li S, Guan L, Fang S, et al. Exome sequencing reveals CHM mutations in six families with atypical choroideremia initially diagnosed as retinitis pigmentosa. *Int J Mol Med*. 2014;34:573–577.

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56. Xu Y, Guan L, Xiao X, et al. Mutation analysis in 129 genes associated with other forms of retinal dystrophy in 157 families with retinitis pigmentosa based on exome sequencing. *Mol Vis*. 2015;21:477–486.
57. Shen T, Guan L, Li S, et al. Mutation analysis of Leber congenital amaurosis-associated genes in patients with retinitis pigmentosa. *Mol Med Rep*. 2015;11:1827–1832.
58. Logan NS, Gilmartin B, Marr JE, Stevenson MR, Ainsworth JR. Community-based study of the association of high myopia in children with ocular and systemic disease. *Optom Vis Sci*. 2004;81:11–13.
59. Marr JE, Halliwell-Ewen J, Fisher B, Soler L, Ainsworth JR. Associations of high myopia in childhood. *Eye (Lond)*. 2001; 15:70–74.
60. Richards AJ, McNinch A, Martin H, et al. Stickler syndrome and the vitreous phenotype: mutations in COL2A1 and COL11A1. *Hum Mutat*. 2010;31:E1461–E1471.
61. Snead MP, McNinch AM, Poulson AV, et al. Stickler syndrome, ocular-only variants and a key diagnostic role for the ophthalmologist. *Eye (Lond)*. 2011;25:1389–1400.
62. Stickler GB, Hughes W, Houchin P. Clinical features of hereditary progressive arthro-ophthalmopathy (Stickler syndrome): a survey. *Genet Med*. 2001;3:192–196.
63. Liberfarb RM, Levy HP, Rose PS, et al. The Stickler syndrome: genotype/phenotype correlation in 10 families with Stickler syndrome resulting from seven mutations in the type II collagen gene locus COL2A1. *Genet Med*. 2003;5:21–27.
64. McAlinden A, Majava M, Bishop PN, et al. Missense and nonsense mutations in the alternatively-spliced exon 2 of COL2A1 cause the ocular variant of Stickler syndrome. *Hum Mutat*. 2008;29:83–90.
65. Richards AJ, Baguley DM, Yates JR, et al. Variation in the vitreous phenotype of Stickler syndrome can be caused by different amino acid substitutions in the X position of the type II collagen Gly-X-Y triple helix. *Am J Hum Genet*. 2000;67: 1083–1094.
66. Fincham GS, Pasea L, Carroll C, et al. Prevention of retinal detachment in Stickler syndrome: the Cambridge prophylactic cryotherapy protocol. *Ophthalmology*. 2014;121:1588–1597.
67. Wutz K, Sauer C, Zrenner E, et al. Thirty distinct CACNA1F mutations in 33 families with incomplete type of XLCSNB and Cacna1f expression profiling in mouse retina. *Eur J Hum Genet*. 2002;10:449–456.
68. Prokisch H, Hartig M, Hellinger R, Meitinger T, Rosenberg T. A population-based epidemiological and genetic study of X-linked retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2007; 48:4012–4018.
69. Arno G, Hull S, Robson AG, et al. Lack of interphotoreceptor retinoid binding protein, caused by homozygous mutation of RBP3, is associated with high myopia and retinal dystrophy. *Invest Ophthalmol Vis Sci*. 2015;56:2358–2365.

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