Expanding the Phenotypic and Genotypic Landscape of Nonsyndromic High Myopia: A Cross-Sectional Study in 731 Chinese Patients

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Citation: Cai X-B, Zheng Y-H, Chen D-F, et al. Expanding the phenotypic and genotypic landscape of nonsyndromic HM across different study populations, including ZNF644, SCO2, CCDC111 (encoding the PRIMPOL protein), LRBP1, SLC39A5, LEPRELI, PHA2, and BSG. Expanding the phenotypic and genotypic landscape of nonsyndromic HM across different study populations, including ZNF644, SCO2, CCDC111 (encoding the PRIMPOL protein), LRBP1, SLC39A5, LEPRELI, PHA2, and BSG.18–32 Female-limited nonsyndromic HM has been associated with two X-linked genes, OPNL1W and ARR3.

MYOPIA. High myopia (HM) is defined as a refractive error worse than –6.00 diopter (D). This study aims to update the phenotypic and genotypic landscape of nonsyndromic HM and to establish a biological link between the phenotypic traits and genetic deficiencies.

METHODS. A cross-sectional study involving 731 participants varying in refractive error, axial length (AL), age, myopic retinopathy, and visual impairment. The phenotypic traits were analyzed by four ophthalmologists while mutational screening was performed in eight autosomal causative genes. Finally, we assessed the clinical relevance of identified mutations under the guidance of the American College of Medical Genetics and Genomics.

RESULTS. The relationship between refractive error and AL varied in four different age groups ranging from 3- to 85-years old. In adult groups older than 21 years, 1-mm increase in AL conferred 10.84% higher risk of pathologic retinopathy (Category ≥2) as well as 7.55% higher risk of low vision (best-corrected visual acuities <0.3) with P values < 0.001. The prevalence rates of pathologic retinopathy and low vision both showed a nonlinear positive correlation with age. Forty-five patients were confirmed to harbor pathogenic mutations, including 20 novel mutations. These mutations enriched the mutational pool of nonsyndromic HM to 1.5 times its previous size and enabled a statistically significant analysis of the genotype-phenotype correlation. Finally, SLC39A5, CCDC111, BSG, and PHA2 were more relevant to eye elongation, while ZNF644, SCO2, and LEPRELI appeared more relevant to refracting media.

CONCLUSIONS. Our findings shed light on how multiple HM-related phenotypes are associated with each other and their link with gene variants.

Keywords: high myopia, gene, mutation spectrum, genotype-phenotype correlation
with HM that has been both phenotypically and genetically studied.

**METHODS**

**Study Subjects**

The study was in line with the tenets of the Declaration of Helsinki and approved by Ethics Committee of the Eye Hospital of Wenzhou Medical University. A group of 731 Chinese individuals diagnosed with nonsyndromic HM and 1082 healthy controls were recruited for this study. Informed consent was obtained from each participant. The examinations of refractive error and BCVA were carried out by objective refractometer (Nidek, Aichi, Japan) and subjective refractometer. Spherical equivalent refractive errors (SE) were calculated as the total of the spherical power plus half of the cylindrical power. Patients with hyperopia or severe astigmatism (<−1.00 D) were excluded from this study. AL measurements were completed using IOL-Master (Carl Zeiss Meditec AG, Jena, Germany). Patients with extreme HM and poor BCVA were further sent for and verified by retinoscopy optometry. Myopic fundus photos (CR-2 Retinal Camera; Canon, Saitama, Japan) were graded by the same group of ophthalmologists as follows: normal fundus (Category 0); tessellated fundus only (Category 1); diffuse chorioretinal atrophy (Category 2); patchy chorioretinal atrophy (Category 3); and macular atrophy (Category 4). “Plus” signs were defined as the presence of the following additional features: (1) lacquer cracks, (2) choroidal neovascularization, and (3) Fuchs spot. Inconsistency of grading results by the ophthalmologists were addressed by further examinations of B-ultrasonography (AVISO; Quantel Medical, Couron d’Auvergne, France) and optical coherence tomography (RTVue-100; Optovue, Fremont, CA, USA).

**Comprehensive Mutational Screening**

Peripheral blood sample was collected from each participant and genomic DNA was extracted. Pairs of primers specific for coding sequences and intron/exon junctions of eight causative genes were designed. PCR was conducted and amplified products were subjected to 1.5% agarose gel electrophoresis to confirm the fragment size, and subsequently sequenced. After direct Sanger sequencing, the results were analyzed and compared with the University of California Santa Cruz Genome Browser database.

**Pathogenicity Evaluation**

To evaluate the pathogenicity of candidate mutations, we explored the 1000 Genomes database (1000G), dbSNP135, the HapMap database, the Exome Aggregation Consortium (ExAC), and in-house data. Sequence variants were standardized on the basis of Human Genome Variant Society variation nomenclature (in the public domain, http://www.hgvs.org/mutnomen/), checked by the Mutalyzer program (in the public domain, http://www.lovdl.nl/mutalyzer/), and assessed under the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG). Insertions or deletions (InDels) were analyzed through the BWA (in the public domain, http://bio-bwa.sourceforge.net/) and GATK programs (in the public domain, https://www.broadinstitute.org/gatk/). Missense variants were evaluated with bioinformatics tools, and functional predictive scores were annotated through Wannovar (in the public domain, http://www.wannovar.wglab.org/). Converted rank-scores were calculated using minimum-maximum normalization (Supplementary Text S1).

**Statistical Analysis**

One eye with higher myopic refractive error from per subject was chosen for statistical analysis. However, when the eye has retinal detachment, in which case the measurement of AL would be inaccurate through IOL-Master, we would choose the other eye of the same subject. We performed multivariate linear regression and multivariate logistic regression to determine the association between (1) AL and SE; (2) myopic retinopathy or visual impairment and AL or age; and (3) genetic findings and the clinical grading of AL and SE. The relationships were depicted using the R language program (The R Project for Statistical Computing, Vienna, Austria) and assessed with the $\chi^2$ test. Two-tailed $P$ values of $<0.05$ were considered statistically significant. SPSS version 22.0 (IBM, Corp., Armonk, NY, USA) was used for all statistical analyses.

**RESULTS**

**Clinical Findings**

The study cohort included 731 nonsyndromic HM patients ranging from 3- to 85-years old. The value of SE ranged from $-6.00$ to $-38.00$ D, while the value of AL ranged from 25.01 to 36.73 mm. One eye with a higher myopic refractive error from each subject was chosen for further study of the phenotypic spectrum (Table 1). The fundus changes were recorded by the same group of ophthalmologists. Among them, 333 adult patients older than 21 years were identified to have gradable fundus photographs that were taken by the same panel of ophthalmic technicians (Fig. 1). Typical “plus” signs and staphyloma are shown in Supplementary Figure S1.

**Relationship Between Refractive Error and Axial Length for Varying Ages**

A statistically significant trend of a $-1.95$ D ($P < 0.001$, $R^2 = 0.64$) increase in refractive error per 1-mm growth in AL ($\Delta$SE/$\Delta$AL) was observed. In this specific cohort of 731 high-myopic patients, a 1-mm difference in AL equated with $-2.21$ D ($P < 0.001$, $R^2 = 0.65$) of refractive error difference for the 3- to 21-year olds, $-2.12$ D ($P < 0.001$, $R^2 = 0.67$) for the 22- to 40-year olds, $-1.92$ D ($P < 0.001$, $R^2 = 0.60$) for the 41- to 59-year olds, and $-1.69$ D ($P < 0.001$, $R^2 = 0.55$) for the group no younger than 60 years (Fig. 2A). Linear regression adjusted for age showed that a 0.030 reduction ($P < 0.001$, $R^2 = 0.98$) in the
FIGURE 1. Typical fundus photographs based on the international classification and grading system for myopic maculopathy. Category 0 is characterized by no myopic retinal changes; Category 1 is characterized by tessellated fundus with easily visible outline of choroidal vessels around the arcade vessels; Category 2 is characterized by diffuse chorioretinal atrophy, showing a yellowish-white appearance of the posterior pole; Category 3 is characterized by patchy chorioretinal atrophy, which appears as well-defined, grayish-white lesions around the centermost macular region; Category 4 is characterized macular atrophy, appearing as a widespread chorioretinal atrophic lesion affecting the foveal region.

FIGURE 2. The relationship between refractive error and AL for varying ages. (A) SE plotted as a function of AL for the whole high-myopic group as well as for each age group. The black line is the fitted line for the whole group, while the blue line is for the 3- to 21-year olds; the purple line is for the 22- to 40-year olds; the reddish-orange line is for the 41- to 59-year olds; the green line is for the group older than 60 years. The length of the horizontal column in different colors represents the specific value of ΔSE/ΔAL for each age group. (B) SE:AL ratio plotted as a function of SE for different age groups. The SE:AL ratio decreases with increasing SE at distinct rates, ranging from 0.030/ to 0.027/0 C1 D, for varying ages. (C) SE:AL ratio plotted as a function of AL for different age groups. The SE:AL ratio reduces fastest with increasing AL (0.059/mm) in the 3 to 21-year olds, followed by 0.055/mm in the 22- to 40-year olds, 0.046/mm in the 41- to 59-year olds, and 0.037/mm in the group older than 60 years.
SE:AL ratio was observed per −1-D increase in refractive error for the 3- to 21-year olds, as well as a 0.029/−1-D reduction (P < 0.001, R² = 0.98) for the 22- to 40-year olds, a 0.028/−1-D reduction (P < 0.001, R² = 0.97) for the 41- to 59-year olds, and a 0.027/−1-D reduction for the group no younger than 60 years (P < 0.001, R² = 0.95) (Fig 2B). Additionally, we observed a 0.059 reduction (P < 0.001, R² = 0.51) in the SE:AL ratio per 1-mm increase in AL for the 3- to 21-year olds, as well as a 0.055/mm reduction (P < 0.001, R² = 0.54) for the 22- to 40-year olds, a 0.046/mm reduction (P < 0.001, R² = 0.43) for the 41- to 59-year olds, and a 0.037/mm reduction (P < 0.001, R² = 0.32) for the group older than 60 years (Fig. 2C).

**Increasing Axial Length and Aging Confer Risks of Pathologic Retinopathy and Low Vision**

Within the 333 patients with gradable fundus photographs, there was no statistically significant correlation between the value of AL and age (P = 0.59). The value of AL ranged from 25.01 to 36.73 mm and was equally divided into 13 groups. To enable comparison between all the AL groups, three groups with the longest AL but a small number of affected patients were classified into the following five stages: BCVA < 0.5 (V1); 0.7 ≤ BCVA < 1.0 (V2); 0.5 ≤ BCVA < 0.7 (V3); 0.3 ≤ BCVA < 0.5 (V4); and BCVA < 0.3 (V5) (Table 2). Likewise, the visual impairment also showed a positive correlation with the elongation of axial length (Fig. 3B). Notably, 1-mm increase in AL conferred 10.84% higher risk of pathologic retinopathy as well as a lower risk (18/135, 13.35%) of low vision.

**Mutations Identified in the HM Cohort**

We comprehensively sequenced the 8 genes in 731 non-syndromic HM patients and identified 29 pathogenic mutations in 45 index cases (Fig. 4A). Thereinto, 20 novel mutations had neither been reported previously nor been found in the 1000G database (Table 3). We considered a variation as “pathogenic” according to the following standards: (1) splice-site variations fulfilled the GT-AT rules, (2) nonsense/frameshift variants were present, and (3) nonframeshift InDels/missense mutations were predicted to be damaging or probably damaging by the standards and guidelines from ACMG and 11 bioinformatics tools (Supplementary Table S1). Within the list, one was a nonsense mutation, and one was a frameshift mutation that both severely affected protein function; one was a nonframeshift InDel, and 26 were missense mutations that were all predicted to have deleterious effects on protein function through bioinformatics analyses (Fig 4B). All of these mutations were absent from the control group, and their minor allele frequencies (MAF) were less than 0.05.

**Genetic Landscape and Genotype–Phenotype Correlations From the Worldwide Perspective**

We retrospectively summarized all the reported mutations in the eight genes from worldwide HM populations (Table 4). A total of 40 mutations have been reported in 103 individuals from previous studies. In combination with our mutational findings, we achieved a clear picture of an expanding genetic landscape of HM from a worldwide perspective (Fig. 5A). The high frequency of mutations and should be given a priority during genetic screening of nonsyndromic HM patients. With the exception of SCO2, mutations in the other genes were mostly reported in Asia-Pacific populations. Homozygous mutations in LRPAP1 and LEPREL1 for autosomal-recessive
FIGURE 3. The association between myopic retinopathy or visual impairment and AL or age. (A) The positive correlation between myopic retinopathy and AL. The bar graph on the left shows the number of patients with different categories of myopic retinopathy in different AL groups, with the gray dots representing the distribution of age; the proportional graph in the middle shows the percentage of patients with different categories of myopic retinopathy in different AL groups, with the gray line representing the mean age of each group; the boxplot on the right shows how patients with different categories of myopic retinopathy distribute over different ranges of AL. (B) The positive correlation between visual impairment and AL. The bar graph on the left shows the number of patients with different stages of visual impairment in different AL groups, with the gray dots representing the distribution of age; the proportional graph in the middle shows the percentage of patients with different stages of visual impairment in different AL groups, with the gray line representing the mean age of each group; the boxplot on the right shows how patients with different stages of visual impairment distribute over different ranges of AL. (C) The positive correlation between myopic retinopathy and age. The bar graph on the left shows the number of patients with different categories of myopic retinopathy in different age groups, with the gray dots representing the distribution of AL; the proportional graph in the middle shows the percentage of patients with different categories of myopic retinopathy in different age groups, with the gray line representing the mean AL of each group; the boxplot on the right shows how patients with different categories of myopic retinopathy are distributed over different age ranges. (D) The positive correlation between visual impairment and age. The bar graph on the left shows the number of patients with different stages of visual impairment in different age groups, with
HM have been discovered as a result of consanguineous marriage, and mutations detected in LRPAP1 were all frameshift mutations, while those in other genes were mainly missense. The only splicing mutation was discovered in BSG. A total of 113 individuals harboring mutations in the eight genes, 68 from the previous studies and 45 from this study, were identified to have detailed records of refractive error, AL, and age. The eight individuals harboring LRPAP1 mutations were all at young ages. In the other seven gene groups, age was normally distributed ($P = 0.07$), and one-way ANOVA further confirmed that there was no significant intergroup difference in age ($P = 0.70$). All patients were categorized into three levels according to their ALs as follows: (1) Level 1: AL < 27 mm; (2) Level 2: 27 ≤ AL < 30 mm; (3) Level 3: AL ≥ 30 mm (extreme high axial myopia) (Fig 5B). According to the relationship between refractive error and AL for the general HM cohort in our study, we categorized the refractive errors of all the patients into three grades corresponding to the levels of their ALs as follows: (1) Grade 1: SE > -11.00 D; (2) Grade 2: -17.00 < SE ≤ -11.00 D; (3) Grade 3: SE ≤ -17.00 D (Fig. 5C). The patients with mutations in LRPAP1 showed severe abnormalities in refractive error and AL from an early age. The percentages of patients of different levels and grades were calculated for comparison in the other seven groups. Finally,

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**Figure 4.** Mutational spectrum of nonsyndromic high myopia. (A) Pie chart showing mutations identified in this Chinese high-myopia cohort. Mutations marked with asterisks and in red font are novel mutations. The percentages of patients with mutations in different genes are shown in different colors. (B) Comparative pathogenic analysis of the 26 missense mutations. The detailed functional predictive rank-scores are shown in the heat map, with deeper colors indicating higher pathogenicity. The gray block represents the lack of predictive information. All predictions were made based on the standards and guidelines of the American College of Medical Genetics and Genomics and a series of bioinformatics tools. The variant in the bottom right (SC02, p.R20P) is a previously reported nonpathogenic polymorphism that was used as a negative control.
In the present study, a comprehensive phenotypic network of nonsyndromic HM was constructed, within which the SE, AL, and refractive error were the nodes. The network was built using a combination of genetic and environmental factors, allowing for the identification of potential risk factors and pathways associated with HM. The network analysis revealed several key nodes, including SLC39A5 and CCDC111, which exhibited strong associations with HM. These findings suggest that these genes may play a significant role in the development of HM.

**Discussion**

In the present study, a comprehensive phenotypic network of nonsyndromic HM was constructed, within which the SE, AL, and refractive error were the nodes. The network was built using a combination of genetic and environmental factors, allowing for the identification of potential risk factors and pathways associated with HM. The network analysis revealed several key nodes, including SLC39A5 and CCDC111, which exhibited strong associations with HM. These findings suggest that these genes may play a significant role in the development of HM.
age increased was observed, with $R^2 > 0.50$ for each group. A previous study based on UK populations showed a similar finding of a decreasing value of $\Delta SE/\Delta AL$ along with an increasing age, but with lower coefficients of determination between mean SE and AL (6- to 7-year olds: increasing age, but with lower coefficients of determination $0.24$; and 18- to 25-year olds: $0.45)$. Notably, a composite graph of data for all three groups obtained an approximate value of $0.05/mm$ reduction for all ages and found an obviously decreasing slope of the reduction as age increased. Thus, we assumed that Cruickshank et al. obtained an approximate value of $0.05/mm$ reduction for all age groups due to the narrow age range in their study. Consequently, the wide age ranges in our study would help better understand the relationship between refractive error and AL. The older eye could not be considered to have the same mathematic relationship as a younger eye, probably due to the fluidity in the coordination of ocular components at different stages of eye development. For instance, the aging crystalline lens has been shown to undergo a flattening of curvatures, a decrease in the refractive index and other substantial age-related changes, which may partially account for the nonlinear and nonconstant change in the value of refractive error during the ocular elongation.

Optical calculations based on Gullstrand reduced model eye parameters predict a reduction in the SE/AL ratio as the AL increases. We discovered a 0.059 reduction in SE/AL ratio per 1-mm increase in AL for the 3- to 21-year olds, 0.055 for the 22- to 40-year olds, 0.046 for the 41- to 59-year olds, and 0.037 for the group no younger than 60 years. However, Cruickshank et al. observed a 0.05 reduction per 1-mm increase in AL for all age groups. We then carefully analyzed the plotted figures showing a reduction of 0.05/mm and the equations reflecting the relationship between refractive error and AL for varying ages and found an obviously decreasing slope of the reduction as age increased. Thus, we assumed that Cruickshank et al. obtained an approximate value of $0.05/mm$ reduction for all age groups due to the narrow age range in their study. Consequently, the wide age ranges in our study would help better understand the relationship between refractive error and AL. The older eye could not be considered to have the same mathematic relationship as a younger eye, probably due to the fluidity in the coordination of ocular components at different stages of eye development. For instance, the aging crystalline lens has been shown to undergo a flattening of curvatures, a decrease in the refractive index and other substantial age-related changes, which may partially account for the nonlinear and nonconstant change in the value of refractive error during the ocular elongation.

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### Table 4. Summary of Known Mutations in the Eight Autosomal Causative Genes for Nonsyndromic High Myopia

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide</th>
<th>Protein</th>
<th>Type</th>
<th>Ethnicity</th>
<th>Number of Affected Individuals</th>
<th>Reference</th>
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<td>1</td>
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<td>p.R319T</td>
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<td>Chinese</td>
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<td>23, 26</td>
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<td>p.D851H</td>
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<td>Chinese</td>
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<td>p.T401A</td>
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<td>p.L280fs'118</td>
<td>Frameshift</td>
<td>Saudi</td>
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<td>p.Q69X</td>
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<td>-</td>
<td>Splicing</td>
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<td>p.Q5X</td>
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relevant to the varying AL remains unclear. The wide range of AL and the similar distribution of age in different AL groups in our study allowed a reliable cross-sectional study on the association between the category of myopic retinopathy and the value of AL. To have such an explicit understanding of the implications of a progressively elongating AL to the visual system avoids obscuring the true nature of the myopic change and helps correctly estimate the efficacy of myopia control interventions. The age gaps between different categories showed that it would be a long-term process for the young populations with HM to develop retinopathy and they might well retain good vision before the pathologic changes occurred. Once the retinopathy was triggered, however, it would progress quickly in the following years.

The important role of genetic factors in the occurrence of HM has been implicated in several studies since the first effort was made to decipher the hereditary determinants in the 1960s. The largest cohort that has been genetically studied included 298 nonsyndromic HM patients, which only identified seven causative mutations in ZNF644, SCO2, LRPAP1, and SLC39A5, with no mutations discovered in LEPR1. Other studies only focused on one or two genes in a rather limited number of patients. In this work, we comprehensively sequenced the eight causative genes in a cohort of 731 HM patients. Mutations discovered in this cohort were analyzed by bioinformatics tools after confirming their low frequencies in authoritative databases and absence in the geographically matched control group. Ten mutations in SLC39A5 were identified in 13 index cases, including seven novel mutations. A loss-of-function in SLC39A5 interrupts the well-known pathway TGF-β/bone morphogenic protein, which regulates sclera metabolism through modulation of the extracellular matrix, and is thus assumed to be associated with HM. Two CCDC111 mutations were identified in 10 index cases, including one missense mutation (Y89D) previously reported in a four-generation Chinese family. Further study established
that the major DNA replication defect relevant to this mutant was likely to contribute, at the molecular and cellular levels, to the onset of HM. Another CCDC111 mutation (V131Gfs*6) is only present in Asian populations, indicating that it is probably a specific mutation for HM in Asian. Seven mutations in ZNF644 were identified in nine index cases, including four novel mutations. ZNF644 has been recognized as a zinc finger transcription factor widely expressed and is involved in eye development, potentially contributing to axial elongation. Four BG1 mutations were identified in seven cases, including three novel mutations. We discovered BG1 as a causative gene for the elongation of AL and the occurrence of early-onset HM. Three novel mutations in P4HA2, encoding prolyl 4-hydroxylase 2, were identified in three index cases. Mutations of P4HA2 were supposed to result in unstable collagens in the sclera, and the disrupted sclera were responsible for the longer AL of the eye as well as other HM phenotypes. Three novel mutations in SCO2 were identified in three index cases. SCO2 encodes a copper homeostasis protein whose deficiencies have been linked to photoreceptor loss and myopia with increased scleral wall elasticity. By adding these 20 novel mutations, we enriched the mutational pool of nonsyndromic HM to 1.5 times its previous size.

The eight genes were all hypothesized to play roles in scleral remodeling and ocular elongation. Although AL is imporantly a biologically refractive status is clinically a complex variable determined by the optical power of the cornea and the lens in addition to the AL. Whether the mutations in these genes influence the function of the dioptric media within the eye has rarely been investigated. We here supposed SLCA39A5, CCDC111, BG1, and P4HA2 were more relevant to ocular elongation, while LEPR, ZNF644, and SCO2 had a greater impact on the dioptric media. The first SCO2 study for HM successfully induced myopia in a mouse model by applying a −15.00-D lens over one eye and detected a significant decrease in messenger RNA levels of SCO2, suggesting that SCO2 was closely related to refractive conditions. Visual experience has been relevant to human myopia, because children with corneal opacities or eyelid ptosis can develop myopia. Patients with mutations in LEPR1 were found to have variable expressivity of early-onset cataracts, which might partially account for the development of HM. ZNF644 was supposed to follow the trend of other transcription factor genes pathogenic to ocular diseases and play an important role in both dioptric media and the ocular wall. Further studies are needed to elucidate the exact mechanisms of these genes in eye development.

In conclusion, our findings shed light on a network connecting multiple clinical features of HM, updating the current knowledge of how these features are associated with each other. Moreover, by adding 20 novel mutations to the 40 previously reported mutations from the worldwide perspective, we were able to expand the genetic landscape of nonsyndromic HM and further establish a biological link between the phenotypic traits and genetic deficiency.

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Reference


