Detection of Clinically Relevant Genetic Variants in Chinese Patients With Nanophthalmos by Trio-Based Whole-Genome Sequencing Study

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PURPOSE. Nanophthalmos is a rare genetic disorder commonly characterized by a short axial length (AL) and severe hyperopia. Mutations that have been identified through Mendelian genetic analysis can only explain a fraction of nanophthalmic cases. We investigate the clinically relevant genetic variants in nanophthalmos by whole-genome sequencing (WGS), including de novo mutations (DNMs) and inherited mutations.

METHODS. Clinically relevant genetic variants of 11 trios (11 nanophthalmic probands and their unaffected parents) from the Zhongshan Ophthalmic Center, China, were analyzed by WGS. We further screened three trios and 10 sporadic cases to identify the MYRF mutations.

RESULTS. In two of 11 trios, without evidence of the presence of deleterious inherited autosomal variants, two DNMs of MYRF (c.789delC, p.S264fs and c.789dupC, p.S264fs) were identified in the probands. These loss-of-function DNMs were predicted to result in premature stop codons and protein structure damage in both probands. In addition, deleterious inherited genetic variants in PRSS56 and MFRP were found in eight probands of the other nine trios. Expanded screening found an additional MYRF DNM (c.1435G>C, p.R478P) in one trio and a stop-gain MYRF mutation (c.2956C>T, p.R986X) in one sporadic case, suggesting the recurrence of MYRF mutations in nanophthalmic patients.

CONCLUSIONS. This is the first trio-based WGS study for nanophthalmos, revealing the potential role of DNMs in MYRF and rare inherited genetic variants in PRSS56 and MFRP. The underlying mechanism of MYRF in the development of nanophthalmos needs to be further investigated.

Keywords: de novo mutation, MYRF, nanophthalmos, trio analysis, whole-genome sequencing

Nanophthalmos and posterior microphthalmos are two subtypes of microphthalmos, which are bilateral small eyes derived from developmental defects.1 The estimated birth prevalence for microphthalmos is 0.002% to 0.017% worldwide and 0.009% in China.2,3 Nanophthalmos is a relatively rare condition characterized by a short axial length (AL; commonly <20 mm) due to small anterior and posterior segments, a thick choroid and sclera, and marked hyperopia.4,5 Due to their specific anatomic features, nanophthalmic patients are prone to develop a crowded anterior chamber and eventually angle-closure glaucoma.6 If not diagnosed and treated, angle-closure glaucoma can progress to optic nerve damage and blindness.

Nanophthalmos is a consequence of the developmental arrest of eyes in the early stages of embryogenesis. It is thought to have a strong genetic basis. With the availability of next-generation sequencing technology, genetic testing for nanophthalmos has been improved substantially.8 Recent sequencing studies of whole exomes and candidate genes have revealed some of the molecular mechanisms responsible for certain patients with nanophthalmos.6,8,9 Nanophthalmos 1 (NNO1, Online Mendelian Inheritance in Man [OMIM] 600165) at 11p was the first human locus identified to be associated with nanophthalmos.10 Subsequently, studies in a series of nanophthalmos pedigrees have detected transmembrane protein 98 (TMEM98, OMIM 615949) at 17q11.6,8,9 Membrane-type frizzled-related protein (MFRP, OMIM 606227) at 11q23.11 Serine protease 56 (PRSS56, OMIM 613858) at 19q13.12,13 Crumbs homolog 1 (CRB1, OMIM 604210) at 1q31.14,15 Bestrophin 1
Al established myelin regulatory factor (MYRF, OMIM 608329) as a novel nanophthalmos gene in the Caucasian population. Nanophthalmos may present as a sporadic or familial condition, with autosomal-recessive or -dominant inheritance. According to the OMIM database, autosomal-dominant genes include NNO1, OMIM 127830, NNO2, OMIM 607854, NNO3, OMIM 611897, NNO4, OMIM 611346, BEST1, and MYRF, and autosomal-recessive genes include PRSS56, CRB1, and MFRP (NNO2). However, taken together, these genetic mutations identified through classic Mendelian genetic analysis can only explain a fraction of nanophthalmos cases.

In terms of whole-exome sequencing (WES), it depends on different exon-capture kits/platforms, which might miss some exon variants. In addition, the exon-capture kits were designed based on the available human gene/exon annotation database, which has been updating all the times. Whole-genome sequencing (WGS) will not have these issues. By comparison with WES, WGS encompasses a greater number of annotated exons with coverage sufficient for variant calling. In this study, to investigate the novel genetic mechanism potentially explaining other cases of nanophthalmos, we conducted a trio-based WGS study for nanophthalmos, representing, to the best of our knowledge, the first report with this specific purpose.

**METHODS**

**Description of the Participants**

This study was approved by the Human Research Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-Sen University (Guangzhou, China) and adhered to the tenets of the Declaration of Helsinki. All subjects provided written informed consent. In the discovery stage, we recruited 11 trios with a nanophthalmos proband and unaffected parents. The unrelated probands consisted of seven males and four females, and the mean age at recruitment was 30.09 ± 12.14 (11–48)-years old. In the validation stage, three additional trio families and 10 sporadic cases with mean age of 42.15 ± 13.47 (15–65)-years old were recruited for expanded screening of MYRF gene. The validation cohort consisted of six males and seven females. The diagnosis of nanophthalmos was made by a short AL (<20 mm) and the absence of morphologic malformation. All eligible individuals underwent the following ophthalmic evaluations: best-corrected visual acuity (BCVA), slit-lamp examination, IOP measurement (Goldmann applanation tonometry), and fundus examination. The AL was measured by A-scan ultrasonography (Quantel Medical, Cournon d’Auvergne Cedex, France). Anterior chamber depth (ACD) and anterior chamber angle were examined with an ultrasound biomicroscope (UBM; Paradigm Medical Industries, Salt Lake City, UT, USA). The angle grading of all probands were IV by gonioscopy. Subjects with uncontrolled ocular infection, severe systemic diseases, or lens nucleus of grade 4 or harder according to the Emery-Little classification, or who declined to participate were excluded.

**Trio-Based Whole-Genome Sequencing**

Peripheral blood samples were obtained from all subjects, and genomic DNA was extracted using an isolation kit (OSR-M104-T1; Tiangen Biotech, Beijing, China) according to the manufacturer’s instructions. The DNA was sheared into fragments of 350 bp using a Covaris LE220 Focused Ultrasonicator (Covaris, Woburn, MA, USA). The NEXTflex Rapid DNA-Seq Kit (BIOO Scientific, Austin, TX, USA) was used to prepare the WGS library, followed by enzymatic fragmentation with end-repair/A-tailing and ligation. Adapter-ligated DNA was purified using AMPure XP beads, and the resultant fragments were amplified for eight cycles of PCR. The final DNA library was purified with AMPure XP beads, and then the library concentration was quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The libraries were then sequenced using the Novaseq 6000 sequencer (Illumina, San Diego, CA, USA) at KingMed Diagnostics (Guangzhou, China). The sequencing depth in subjects of the discovery stage ranged from 44.81× to 71.06× (proportion of sequencing depth >10× per sample ranged from 91.48% to 92.28%). The Q20 (1 error in 100) read quality score ranged from 93.77% to 95.87%, and the Q30 (1 error in 1000) ranged from 86.00% to 92.11%. The mapping rate of clear data ranged from 99.44% to 99.88%, and the genome coverage ranged from 91.73% to 92.38% (Supplementary Table S1).

The sequencing data were processed with Burrows-Wheeler Aligner (BWA) for alignment on a reference sequence of hg19 and the GATK-HaplotypeCaller package (GATK version 3.5-0-g36282e; Broad Institute, Cambridge, MA, USA) for local realignment, quality recalibration, and variant calling. Then, the Variant Call Format (VCF) files were analyzed using the ANNOVAR tools. The number of single nucleotide variants (SNVs) and small insertions/deletions (indels) per subject is summarized in Supplementary Table S1. Potential Mendelian violations were identified if there was a heterozygous genotype in the proband and homozygous wild-type genotype in the unaffected parents. After eliminating the low-quality variants by GATK’s filters, Mendelian violations ranged from 51,151 to 151,542 in 11 trios (Supplementary Table S1). Then these variants were filtered using the following criteria: (1) sequence coverage more than 10×; (2) frequency in 1000 Genome Project (1000G), dbSNP137 and Exome Aggregation Consortium (ExAC) less than 1%; (3) nonsense mutation, frameshift mutation, splice site, or predicted damaging missense mutation; and (4) 35% to 65% of reads were the reference call. Finally, a total of 15 de novo mutations (DNMs) were detected in 11 probands (1.36 DNMs per proband), which were consistent with published papers. Of these variants, eight were splice sites, four were missense mutations, two were frameshift mutations, and one was a synonymous variant (Supplementary Table S2 and S3). The analysis workflow is shown in Figure 1.

We determined the inherited autosomal variants by comparing the genotypes of probands and parents. Rare variants (frequency <0.1%) were filtered to maintain only deleterious variants, including nonsense mutations, frameshift mutations, splice sites, or predicted damaging missense mutations (based on predictive programs, such as SIFT, PolyPhen-2, MutationTaster, VEST3, MCAP, CADD, Eigen, FATHMM, and GenoCanyon; Supplementary Table S4). Additionally, the American College of Medical Genetics and Genomics (ACMG) guidelines (Table 1) and minor allele frequency of each variant in the Genome Aggregation Database (GnomAD) (Table 2) were employed.

**Expanded screening to Identify MYRF Mutations in Trios and Sporadic Cases**

Additional three trio families and 10 sporadic nanophthalmos patients were recruited for expanded screening of MYRF gene. WGS was also performed in the additional trios. The sequencing depth in these nine samples ranged from 47.44× to 66.21× (proportion of sequencing depth ≥10× ranged from 91.55%–92.27%). The Q20 ranged from 93.89% to 94.92%, and the Q30 ranged from 86.25% to 92.30%. The mapping rate of clear data ranged from 99.55% to 99.72%, and the genome
coverage ranged from 91.76% to 92.38% (Supplementary Table S1). WES at an average sequencing depth of 106.39× (range 92.41× to 122.19×) was performed in the further 10 sporadic cases by using Novaseq 6000 sequencer (Illumina). The detailed statistical information of the WES data was shown in Supplementary Table S5.

Sanger Sequencing of Target Regions for Validation

Amplification using PCR followed by Sanger sequencing was used to confirm the potential DNMs of MYRF in each family member of Trio numbers 8, 10, and V1 (NM_001127392: c.789delC, c.789dupC and c.1433G>C) and the stop-gain MYRF mutation in sporadic patient number 2 (NM_001127392: c.2956C>T). Sequencing was performed on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). The reference genes were obtained from the Ensembl genome browser (in the public domain, http://www.ensembl.org). The primers used for PCR amplification are listed in Supplementary Table S6.

### TABLE 1. Clinical Characteristics of Patients With MYRF Variants

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Change</th>
<th>Mutation Type</th>
<th>ACMG Category</th>
<th>Sex</th>
<th>Age, y</th>
<th>AL, mm</th>
<th>ACD, mm</th>
<th>BCVA</th>
<th>IOP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (Trio number 8)</td>
<td>c.789delC, p.S264fs</td>
<td>Frameshift deletion</td>
<td>Pathogenic</td>
<td>F</td>
<td>16</td>
<td>18.09</td>
<td>18.07</td>
<td>2.68</td>
<td>2.37</td>
</tr>
<tr>
<td>10 (Trio number 10)</td>
<td>c.789dupC, p.S264fs</td>
<td>Frameshift duplication</td>
<td>Pathogenic</td>
<td>F</td>
<td>30</td>
<td>17.58</td>
<td>17.35</td>
<td>2.76</td>
<td>2.49</td>
</tr>
<tr>
<td>V1 (Trio number V1)</td>
<td>c.1433G&gt;C, p.R478P</td>
<td>Missense</td>
<td>Likely pathogenic</td>
<td>M</td>
<td>35</td>
<td>17.70</td>
<td>18.02</td>
<td>0.5CT</td>
<td>0.10</td>
</tr>
<tr>
<td>Sporadic case number 2</td>
<td>c.2956C&gt;T, p.R986X</td>
<td>Stop-gain</td>
<td>Likely pathogenic</td>
<td>M</td>
<td>32</td>
<td>17.00</td>
<td>17.00</td>
<td>3CT</td>
<td>0.63</td>
</tr>
</tbody>
</table>

CT, corneal thickness; del, deletion; dup, duplication; fs, frameshift; F, female; M, male; NLP, no light perception.

### FIGURE 1. Flow chart of the discovery of the de novo mutations in nanophthalmos by WGS. The numbers in parentheses represent the range of the number of variants at each stage in 11 trios in the discovery stage. A Mendelian violation was identified if there was a heterozygous genotype in the proband and homozygous wild-type genotype in both parents; and low-quality variants were eliminated with GATK’s recommended filters. Deleterious variants included nonsense mutations, frameshift mutations, splice sites, or predicted damaging missense mutations. Zero DNMs from one trio, one DNM from seven trios, two DNMs from two trios, and four DNMs from one trio.
A Trio-Based WGS Study for Nanophthalmos

RESULTS

The analysis workflow of the discovery of the DNMs in the WGS is shown in Figure 1. Among the total of 11 trios in the discovery stage, by comparing probands with their unaffected parents, two DNMs in MYRF at 11q12.2 were identified in two probands. Patient number 8 (trio number 8) was a 16-year-old girl from Henan Province, China with bilaterally short AL (OD: 18.09 mm; OS: 18.07 mm). She had poor eyesight from an early age and was diagnosed with amblyopia at the age of 7 years. When she was 15-years old, her vision gradually diminished. Ophthalmic examinations showed that her BCVAs were 0.1 (−8.75 diopters [D]) OD and 0.2 (−11.00 D) OS. Her IOP was 27-mm Hg OD and 24-mm Hg OS. UBM showed that her AC was 2.68-mm OD and 2.37-mm OS. Patient number 10 (trio number 10) was a 30-year-old woman from Guizhou Province, China with bilaterally short AL (OD: 17.58 mm; OS: 17.33 mm). She had blurred eyes for more than 20 years and had experienced a rapid decline in vision in her right eye when she was 30-years old. Her IOP was 0.86 mm Hg OD and 0.47 mm Hg OS. BCCs were 0.1 (−11.00 D) OD and 0.5 (−11.75 D) OS. UBM showed that her AC was 2.76 mm OD and 2.49 mm OS. Both patients were diagnosed with secondary angle-closure glaucoma when they came to our department. The clinical characteristics of the two patients are shown in Table 1.

Two novel DNMs in MYRF (NM_001127392) were confirmed by Sanger sequencing (c.789delC [p.S264fs] in trio number 8 and c.789dupC [p.S264fs] in trio number 10, see Figs. 2A–2B). The sequencing depth for the two mutations in the probands is 93× and 64×, with 51.6% and 54.7% reads were mutation calls, respectively. By referring to the standards and guidelines from the ACMG, those two DNMs were classified as “pathogenic,” based on PVS1 (frameshift) and PS2 (de novo). Biological relationships between parents and probands were confirmed (Supplementary Tables S9 and S10). Both of these DNMs are loss-of-function variants (frameshift). None of the DNMs were found in the following publicly available databases, including GnomAD (Table 2), Exome Sequencing Project 6500 (ESP6500siiv2), and 1000G. Although the DNMs in MYRF have been a given dbSNP reference SNP ID (rs118679980 and rs5769724302; and rs774654800 was merged into rs769274302, Build 151), they occur at very low frequencies (4.05 × 10−5 and 1.62 × 10−4, respectively) in

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### Table 2. The Minor Allele Frequencies in the GnomAD Database for the Variants Found in Nanophthalmic Patients

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Gene</th>
<th>Mutation</th>
<th>MAF in GnomAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Trio number 1)</td>
<td>PRSS56</td>
<td>c.1066dupC, p.Gln356fs (het)</td>
<td>58/150520</td>
</tr>
<tr>
<td>2 (Trio number 2)</td>
<td>PRSS56</td>
<td>c.1687delC, p.Arg563fs (het)</td>
<td>0</td>
</tr>
<tr>
<td>3 (Trio number 3)</td>
<td>PRSS56</td>
<td>c.343dupG, p.Ala115fs (het)</td>
<td>1/97248</td>
</tr>
<tr>
<td>4 (Trio number 4)</td>
<td>PRSS56</td>
<td>c.1508T&gt;A, p.Met503lys (het)</td>
<td>0</td>
</tr>
<tr>
<td>5 (Trio number 5)</td>
<td>MFRP</td>
<td>c.1066dupC, p.Gln356fs (hom)</td>
<td>58/150520</td>
</tr>
<tr>
<td>6 (Trio number 6)</td>
<td>MFRP</td>
<td>c.781G&gt;A, p.Gly261Arg (het)</td>
<td>2/12732</td>
</tr>
<tr>
<td>7 (Trio number 7)</td>
<td>MFRP</td>
<td>c.1183+3_1186+50del (het)</td>
<td>0</td>
</tr>
<tr>
<td>8 (Trio number 8)</td>
<td>MYRF</td>
<td>c.789delC, p.S264fs (het)</td>
<td>0</td>
</tr>
<tr>
<td>9 (Trio number 9)</td>
<td>MYRF</td>
<td>c.661C&gt;T, p.Pro221ser (het)</td>
<td>3/226800</td>
</tr>
<tr>
<td>10 (Trio number 10)</td>
<td>MYRF</td>
<td>c.1411G&gt;A, p.Val471Met (het)</td>
<td>4/243284</td>
</tr>
<tr>
<td>11 (Trio number 11)</td>
<td>MFRP</td>
<td>c.789dupC, p.S264fs (het)</td>
<td>0</td>
</tr>
<tr>
<td>V1 (Trio number V1)</td>
<td>MYRF</td>
<td>c.1453G&gt;C, p.R478P (het)</td>
<td>0</td>
</tr>
<tr>
<td>Sporadic case number 2</td>
<td>MYRF</td>
<td>c.2956C&gt;T, p.R986G (het)</td>
<td>0</td>
</tr>
</tbody>
</table>

MAF minor allele frequency; het, heterozygous; hom, homozygous.

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Paternity and Maternity Tests

Paternity and maternity tests were used to determine the biological relationships between parents and probands in Trio 8, 10, and V1. Genomic DNA isolation was carried out for each family member according to “Specifications for examination in forensic DNA laboratory” (GA/T 383-2014). 21Plex Short Tandem Repeat (STR) Fluorescence DNA Detection Kit (Jiangsu Superbio Biomedical Co., Ltd., Nanjing, China) was used to determine genetic characteristics. Twenty-one STR loci (D5S818, D8S1179, D12S391, D19S433, FGA, and Amelogenin) were analyzed. The products were separated and analyzed on ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Data were analyzed using GeneMapper ID software (Applied Biosystems). In parallel, appropriate positive and negative controls were performed and confirmed to give the expected and correct results for each sample submitted.

Bioinformatic Analyses

We performed a gene ontology (GO) analysis using the Database for Annotation, Visualization, and Integrated Discovery (David, in the public domain, https://david.ncifcrf.gov/) (Supplementary Table S7). The SWISS-MODEL (in the public domain, https://swissmodel.expasy.org/) was used to predict the impact of DNMs on the protein structure of MYRF. Evolutionary conservation analysis of altered amino acid residues was performed by comparisons across different species (in the public domain, https://www.ncbi.nlm.nih.gov/protein/MYRF).

Gene Coexpression and Genetic Interaction Network Analyses

To build the gene coexpression and genetic interaction networks, we reviewed the PubMed database to look for candidate genes related to nanophthalmos, microphthalmia, anophthalmia, and coloboma (MAC), high hyperopia, angle-closure glaucoma, and visualized by GeneMANIA in the Cytoscape program.
the Asian population according to the ExAC database. Thus, the theoretical probability of two DNMs in 11 individuals is as low as $7.20 \times 10^{-10}$. Through sequence alignment and protein structure prediction, we found that the de novo loss-of-function (frameshift) mutations in MYRF resulted in premature stop codons (patient number 8: codon 271; patient number 10: codon 337) and protein structure damage in both patients (Fig. 2E). Evolutionary conservation analysis showed that the impaired amino acid residues were mostly evolutionarily conserved, indicating that the mutation was likely pathogenetic (Fig. 3A).

To further examine the relationships of MYRF with genes associated with nanophthalmos, MAC, AL, high hyperopia and angle-closure glaucoma (Supplementary Table S8), gene coexpression and genetic interaction network analyses were performed (Fig. 4). Among these networks, MYRF was coexpressed with TMEM98 (nanophthalmos-related gene), RSP01 (AL-related gene), BMP4 (MAC-related gene), and PITX3 (MAC-related gene). Genetic interaction network analysis indicated that MYRF interacted with GLIS3 (angle-closure glaucoma-related gene).

The inherited autosomal variants in the 11 trios are shown in Figure 5 and Table 3, and the description of probands is summarized in Supplementary Text S1. Given that all the parents were unaffected, the autosomal mutations were limited to the model of recessive inheritance, including PRSS56 and MFRP. We found that eight of 11 (72.7%) families carried rare mutations that segregated in an autosomal-recessive manner with deleterious effects. These low-frequency PRSS56 and MFRP mutations were predicted to be damaging in five and three families, respectively (Fig. 5; Tables 2, 3). The detailed locations of all variants in PRSS56 and MFRP are shown in Figure 6. Among them, p.Gln356fs in PRSS56 and p.Asn167fs in MFRP have been reported in nanophthalmos cases.

In the validation stage, we further screened the MYRF mutations in three trios and 10 sporadic cases. Interestingly, we also identified one MYRF DNM (c.1433G>C, p.R478P) in trio number V1, which was confirmed by Sanger sequencing (Fig. 2C). This DNM was classified as “likely pathogenic,” based on PS2, PM2, and PP3 following the ACMG guidelines. Biological relationships between parents and proband were confirmed (Supplementary Table S11). Multiple lines of computational evidence support a deleterious effect (Supplementary Table S4). Additionally, we also identified a MYRF stop-gain mutation (c.2956C>T, p.R986X) in a sporadic nanophthalmic patient, which was then confirmed by Sanger sequencing (Fig. 2D). Based on PVS1 and PM2 following the ACMG guidelines, this mutation was classified as “likely pathogenic.” Both of these MYRF mutations found in the trio and the sporadic case were absent from the GnomAD, ESP6500siv2, 1000G, and ExAC databases. Evolutionary conservation analysis showed that the mutations were mostly evolutionarily conserved (Figs. 3B, 3C). The clinical characteristics of the two patients are shown in Table 1. Taken together, the recurrence of the MYRF mutations in both trio and sporadic case supported the potential pathogenicity role for MYRF gene in Chinese nanophthalmos patients.

**DISCUSSION**

Our study presents the first trio-based WGS analysis of nanophthalmos, a rare genetic ocular disorder. Nanophthalmos (dwarf eye) can be regarded as an extreme developmental
spectrum of AL and chamber angle. The study of nanophthalmos in Chinese patients would have important implications for understanding the development of AL, shallow anterior chamber, narrow anterior chamber angle, and angle-closure glaucoma in Asian populations. Furthermore, the regulatory mechanism of AL would also likely be beneficial for the study of myopia, as the mechanism responsible for “short eye” likely colocalizes with pathways that are involved in longer eyes (myopia). In addition to these clinical features in the anterior segment, the choroid and sclera are thicker in nanophthalmic than healthy eyes. Thus, the underlying mechanism of nanophthalmos can be exploited for the study of both anterior and posterior segments. In the present study, we discovered two novel frameshift DNMs (c.789delC, p.S264fs and c.789dupC, p.S264fs) in the MYRF gene in two Chinese nanophthalmos probands, and identified one different MYRF DNM (c.1433G>C, p.R478P) and one MYRF stop-gain mutation (c.2956C>T, p.R986X) in the expanded screening of trios and sporadic nanophthalmic patients.

Our trio-based WGS and two-stage screening support that MYRF might be a potentially nanophthalmos gene, which is consistent with the latest research using a linkage analysis and pooled sequencing approach by Garnai et al. MYRF is a constrained gene that is intolerant to loss of function mutations according to the ExAC database (pLI = 1), which may be caused by depletion of haploinsufficiency. Northern blot analysis revealed a high level of MYRF in a retinal pigment epithelial cell line. BioGPS (in the public domain, http://biogps.org) and SAGE (in the public domain, https://cgap.nci.nih.gov/) also indicated a high mRNA expression of MYRF gene in normal retinal tissues. The retina is important for normal emmetropization, which optimizes the image focus in response to visual experience by adjusting the axial dimensions. Whether MYRF participates in this postnatal process is unknown. It has previously been reported that MYRF is responsible for central nervous system (CNS) myelination and may regulate oligodendrocyte (OL) differentiation, which contains a DNA-binding domain (DBD), an intramolecular chaperone autoprocessing (ICA) domain, and a transmembrane domain. Followed by a unique self-cleavage in the ICA domain, the DBD domain is liberated from the endoplasmic reticulum (ER) and translocated to the nucleus to regulate myelin gene expression. Through coexpression and genetic interaction network analysis of MYRF, we reported shared genes among nanophthalmos and related diseases. Interestingly, coexpressed with MYRF, TMEM98, a known nanophthalmos gene, also encodes an ER-associated transmembrane protein.

Figure 3. Evolutionary conservation of amino acids affected by MYRF mutations. (A) p.Ser264fs; (B) p.R478P; and (C) p.R986X.

Figure 4. Gene coexpression and genetic interaction network analyses demonstrated that MYRF has correlations with validated pathogenic genes. MYRF was coexpressed with TMEM98 (nanophthalmos-related gene), RSPO1 (AL), BMP4 (MAC), and PITX3 (MAC). MYRF interacted with GLIS3 (angle-closure glaucoma).
**Fig. 5.** Pedigree charts of trios with nanophthalmos-relevant inherited-autosomal variants. The square represents male and circle represents female; black represents nanophthalmic patients and white represents unaffected parents. (A–I) Inherited-autosomal variants in each individual are labeled under each figure (square or circle).

**Table 3.** The Inherited Autosomal Variants in Nanophthalmic Patients

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Gene</th>
<th>Mutation</th>
<th>Frequency in ExAC</th>
<th>ACMG Category</th>
<th>ACMG Evidence</th>
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</thead>
<tbody>
<tr>
<td>1 (Trio number 1)</td>
<td>PRSS56</td>
<td>c.1066dupC, p.Gln356fs (het)</td>
<td>5/10538</td>
<td>Pathogenic</td>
<td>PVS1, PM2, PP5, PP4</td>
</tr>
<tr>
<td>2 (Trio number 2)</td>
<td>PRSS56</td>
<td>c.1687delC, p.Arg563fs (het)</td>
<td>0</td>
<td>Pathogenic</td>
<td>PVS1, PM2, PP4</td>
</tr>
<tr>
<td>4 (Trio number 4)</td>
<td>PRSS56</td>
<td>c.1508T&gt;A, p.Met503lys (het)</td>
<td>0</td>
<td>Pathogenic</td>
<td>PVS1, PM2, PP4</td>
</tr>
<tr>
<td>5 (Trio number 5)</td>
<td>MFRP</td>
<td>c.577_578delAG, p.Ser193fs (hom)</td>
<td>0</td>
<td>Pathogenic</td>
<td>PVS1, PM2, PP4</td>
</tr>
<tr>
<td>6 (Trio number 6)</td>
<td>PRSS56</td>
<td>c.1186G&gt;A, p.Glu396lys (het)</td>
<td>0</td>
<td>Uncertain significance</td>
<td>PVS1, PM2, PP4</td>
</tr>
<tr>
<td>7 (Trio number 7)</td>
<td>PRSS56</td>
<td>c.1186delC, p.Gln356fs (het)</td>
<td>5/10538</td>
<td>Pathogenic</td>
<td>PVS1, PM2, PP4</td>
</tr>
<tr>
<td>9 (Trio number 9)</td>
<td>MFRP</td>
<td>c.661C&gt;T, p.Pro221Ser (het)</td>
<td>1/8259</td>
<td>Uncertain significance</td>
<td>PVS1, PM2, PP4</td>
</tr>
<tr>
<td>11 (Trio number 11)</td>
<td>MFRP</td>
<td>c.498delC, p.Asn167fs (hom)</td>
<td>14/120550</td>
<td>Pathogenic</td>
<td>PVS1, PM2, PP4</td>
</tr>
</tbody>
</table>

**TMEM98** binds to the C-terminal of **MYRF** and inhibits the self-cleavage of **MYRF**, functioning as a negative feedback regulator of **MYRF** in OL differentiation and myelination. Conditional ablation of **MYRF** results in severe CNS dysmyelination, with stalling of oligodendrocytes at the premyelinating stage and exhibiting severe deficits in myelin gene expression. Emery et al. have confirmed the lack of myelin ensheathment in the optic nerves of conditional knockout mice by electron microscopy, whereas control optic nerves were actively myelinating. Considering the axonal and myelin damage in the optic nerves in chronic angle-closure glaucoma, **MYRF** may play a contributing role in the occurrence of chronic angle-closure glaucoma. Both the Human Protein Atlas database and Mouse ENCODE Project demonstrate that **MYRF** is highly expressed in stomach, intestine, lung, liver, and heart, supporting the role of **MYRF** beyond the CNS. Recent trio studies have reported DNMs in **MYRF** as candidates in congenital diaphragmatic hernia and congenital heart disease. Most of them were damaging missense mutations located in the conserved DBD and ICD. Additionally, **MYRF** was
identified during the course of constructing a transcript map of the region encompassing the BEST1 gene (OMIM 607854) and eye tissue-specific cDNA selection.56 BEST1 mutations have been reported to be associated with autosomal-dominant vitreoretinchoroidopathy (ADVIRC), autosomal-recessive bestrhopinopathy (ARB), and angle-closure glaucoma, all of which have been reported to be associated with nanophthalmos.57,58 In our study, the two DNMs (c.789delC, p.S264fs and c.789dupC, p.S264fs) cause premature stop codons of MYRF, damage the normal protein structure and thus cause serious harm to the function of MYRF; the additional DNM (c.1433G>T, p.R478P) located in the DNA binding domain of MYRF, leading to heterozygous arginine to proline substitution at amino acid position 478 (Supplementary Fig. S1); and the stop-gain mutation (c.2956C>T, p.R986X) led to the damage of Myelin gene regulatory factor-C-terminal domain 2. The detailed locations of all mutations in MYRF are shown in Fig. 2E. The recurrence of MYRF mutations in trios and sporadic case supported the potential pathogenicity role for MYRF gene in Chinese patients with nanophthalmos. Both PRSS56 and MFRP are known nanophthalmos genes. A significant number of recessive nanophthalmos cases have been detected with mutations in PRSS56 and MFRP.11,12 Nair et al.59 reported that PRSS56<sup>Grm4</sup> mutant mice had a shortened axial length and higher susceptibility to angle closure. MFRP seems to play an important role in ocular growth during childhood, functioning as a regulator of ocular size.53 Our results further confirmed the role of these two genes in the pathogenesis of nanophthalmos. Some limitations in this study should be considered. First, there is no direct evidence supporting the causative interaction between MYRF and nanophthalmos, yet the recurrence of MYRF mutations and overlapping coexpression and genetic interaction with confirmed nanophthalmos-candidate genes indicate that MYRF is likely to be responsible for the development of nanophthalmos. Second, the DNMs are heterogeneous variants found in probands with nanophthalmos, suggesting that their inheritance pattern is autosomal dominant. However, we should be cautious about this conclusion, and more family data are needed to make a final conclusion. Third, because we only included the Chinese population, further validation using an enlarged multicenter clinical collection of larger trios and sporadic patients and investigation of its molecular mechanisms are needed to ascertain the causative role of MYRF mutations in the development of nanophthalmos.

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