Novel *TMEM98* mutations in pedigrees with autosomal dominant nanophthalmos

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Purpose: Autosomal dominant nanophthalmos is an inherited eye disorder characterized by a structurally normal but smaller eye. Patients with nanophthalmos have high hyperopia (far-sightedness), a greater incidence of angle-closure glaucoma, and increased risk of surgical complications. In this study, the clinical features and the genetic basis of nanophthalmos were investigated in two large autosomal dominant nanophthalmos pedigrees.
Methods: Fourteen members of a Caucasian pedigree from the United States and 15 members of a pedigree from the Mariana Islands enrolled in a genetic study of nanophthalmos and contributed DNA samples. Twenty of 29 family members underwent eye examinations that included measurement of axial eye length and/or refractive error. The genetic basis of nanophthalmos in the pedigrees was studied with linkage analysis, whole exome sequencing, and candidate gene (i.e., *TMEM98*) sequencing to identify the nanophthalmos-causing gene.
Results: Nine members of the pedigree from the United States and 11 members of the pedigree from the Mariana Islands were diagnosed with nanophthalmos that is transmitted as an autosomal dominant trait. The patients with nanophthalmos had abnormally short axial eye lengths, which ranged from 15.9 to 18.4 mm. Linkage analysis of the nanophthalmos pedigree from the United States identified nine large regions of the genome (greater than 10 Mbp) that were coinherited with disease in this family. Genes within these "linked regions" were examined for disease-causing mutations using exome sequencing, and a His196Pro mutation was detected in the *TMEM98* gene, which was recently reported to be a nanophthalmos gene. Sanger sequencing subsequently showed that all other members of this pedigree with nanophthalmos also carry the His196Pro *TMEM98* mutation. Testing the Mariana Islands pedigree for *TMEM98* mutations identified a 34 bp heterozygous deletion that spans the 3′ end of exon 4 in all affected family members. Neither *TMEM98* mutation was detected in public exome sequence databases.
Conclusions: A recent report identified a single *TMEM98* missense mutation in a nanophthalmos pedigree. Our discovery of two additional *TMEM98* mutations confirms the important role of the gene in the pathogenesis of autosomal dominant nanophthalmos.

A spectrum of eye disorders ranges from small eyes that are otherwise normal (nanophthalmos or simple microphthalmia) to small eyes that have a grossly disorganized and dysfunctional structure (microphthalmia). Clinical features of nanophthalmos include a short axial eye length (less than 20 mm), high hyperopia (spherical refraction greater than +6 diopters), and small corneal diameter [1-4]. These anatomic features of nanophthalmos result in a crowded anterior chamber and a high incidence of angle closure glaucoma. Nanophthalmic eyes also have thick and impermeable sclera, which has been associated with a high risk of developing choroidal effusions after any intraocular surgery [5]. Nanophthalmos may occur in isolation or as part of an ocular or systemic syndrome.

Nanophthalmos has a strong genetic basis, and autosomal dominant [3,4] and autosomal recessive [6] forms of inheritance have been reported. Mutations in the membrane-type frizzled-related protein (*MFRP*, OMIM 606227) have been detected in a large recessive nanophthalmos pedigree [7]. Similarly, a large autosomal dominant nanophthalmos pedigree was recently studied with linkage analysis, and a disease-causing gene was mapped to chromosome 17p12-q12 [8]. Subsequently, a mutation in the transmembrane protein 98 (*TMEM98*, OMIM 615949) gene was detected that is coinherited with nanophthalmos in this pedigree [4].

In this study, we describe two additional families with autosomal dominant inherited nanophthalmos, a Caucasian family of German heritage living in the United States and a Pacific Islander family of Micronesian heritage living in the Mariana Islands. We confirm the identification of *TMEM98* as a gene for autosomal dominant nanophthalmos with the identification of novel disease-causing mutations in the coding sequence of the *TMEM98* gene.

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METHODS

Patient resources: Research was approved by local Internal Review Boards and all patients provided written consent in advance of the study. The study design met the tenets of the Declaration of Helsinki and Association for Research in Vision and Ophthalmology guidelines. Participants were examined by an ophthalmologist and underwent an eye examination that included measurement of visual acuity, refractive error, and intraocular pressure as well as a slit-lamp examination of the anterior chamber and fundus examination. Axial eye length was measured in some patients with A-scan ultrasound (Alcon, Fort Worth, TX). Patients were diagnosed with nanophthalmos when short axial eye length (<20 mm) or significant hyperopia (spherical equivalent > +6.00) were observed in the absence of other ocular abnormalities. The control subjects showed no evidence of nanophthalmos. Some members of both pedigrees contributed DNA samples but were unavailable for examination, and medical records could not be obtained. These subjects are indicated on the pedigree drawing with stars (Figure 1), and their affection status was determined according to the family history report.

Genetic studies: Venous blood samples were obtained from the participants for the study. Genomic DNA was extracted from peripheral whole blood using the QIAamp Blood Maxi Kit (Qiagen, Valencia, CA). Pedigree 991-F was analyzed with a genome-wide linkage analysis using Illumina 6K human mapping microarrays (Illumina, Santa Clara, CA), which examine 6,020 single nucleotide polymorphisms (SNPs). Sample processing and labeling were performed following the manufacturer's instructions. Genomic regions consistent with linkage were identified, and multipoint non-parametric linkage (NPL) scores were calculated using the MERLIN software package [9]. Penetrance and disease gene frequency were set to 99% and 0.001%, respectively.

Exome capture was performed on one member of pedigree 991-F (IV:3, Figure 1) with SureSelect Human All Exon v2 (Agilent, Santa Clara, CA) using the manufacturer's protocol. Paired-end, 50 nucleotide reads were obtained using a HiSeq2000 (Illumina, San Diego, CA) that achieved average exon coverage of greater than 50X.

The entire coding region of TMEM98 (NM_15544.2) was amplified from patient and control DNA using overlapping primer pairs in PCR reactions containing 12.5 ng of each patient's DNA, 1.25 µl of 10× buffer (100 mM Tris − HCL, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 300 µM of each dCTP, dATP, dGTP and dTTP, 1 pmol of each primer, and 0.25 U Biolase polymerase (Biolase) in a 8.35 µl of PCR solution. Primer sequences are listed in Appendix 1. Amplified DNA was scanned for mutations with bidirectional DNA sequencing with an Applied Biosystems model 3730 automated sequencer as previously described [10]. The mutations that result in amino acid substitutions were evaluated using the BLOSUM62 matrix [11], SIFT [12], and PolyPhen2 [13].
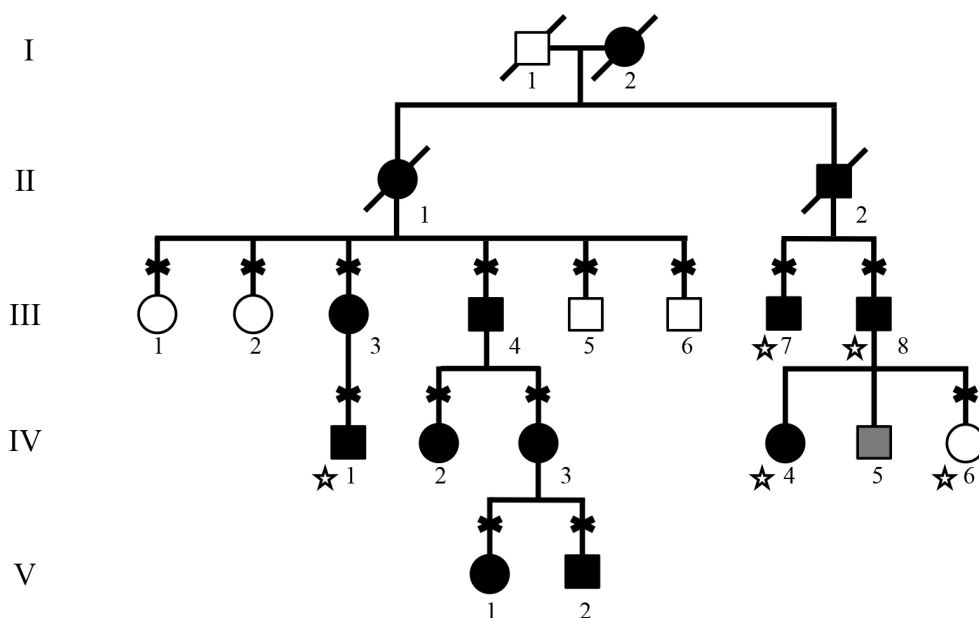

Figure 1. Nanophthalmos pedigree 991-F from the United States. Subjects with nanophthalmos are indicated with black symbols, while healthy subjects are indicated with white symbols. Gray symbols indicate unknown affection status. Diagnosis was made with a clinical exam or a review of the medical records for all available family members. Diagnosis of family members indicated with stars was determined by family history. The subjects who contributed a DNA sample for the study are indicated with an "X."
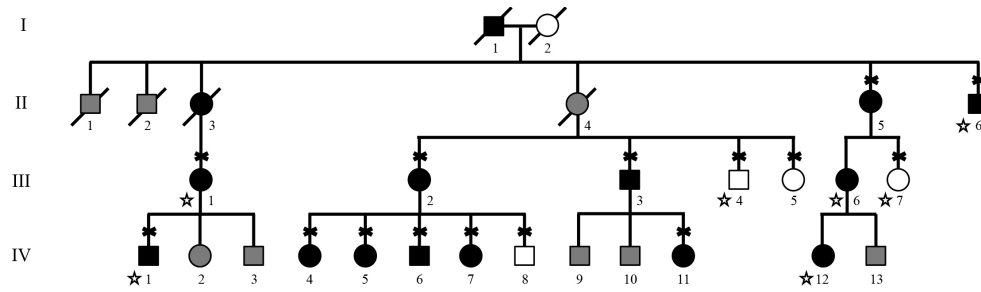
Figure 2. Nanophthalmos pedigree 951-N from the Marianas Islands. Subjects with nanophthalmos are indicated with black symbols, while healthy subjects are indicated with white symbols. Gray symbols indicate unknown affection status. Diagnosis was made with a clinical exam or a review of the medical records for all available family members. Diagnosis of family members indicated with stars was determined by family history. The subjects who contributed a DNA sample for the study are indicated with an "X."

RESULTS

Clinical features of nanophthalmos: Ophthalmic examinations were performed on 20 members of two large nanophthalmos pedigrees (pedigree 991-F from the United States, Figure 1; pedigree 951-N from the Marianas Islands, Figure 2). Both pedigrees exhibit an autosomal dominant inheritance pattern with high penetrance that spans at least four generations. Six of ten examined members of pedigree 991-F were diagnosed with nanophthalmos while an additional three members had nanophthalmos according to the family history report. Mean axial eye lengths were 17.1 mm±0.31 mm for the available patients. Affected family members also had a high degree of hyperopia with spherical equivalent refractions of +13.0±0.89 diopters (Table 1). Unaffected family members had much less hyperopia (+0.71±0.81 diopters).

Eight of ten examined members of Pacific Islander pedigree 951-N had nanophthalmos and an average axial eye length of 17.4 mm±0.94 mm, while unaffected family members had more normal eye length (22.7±0.38 mm; Table 2). Along with short axial eye lengths, family members with nanophthalmos also had hyperopia with spherical equivalent refractions of +3.93±1.57 diopters. Three additional family members had nanophthalmos according to the family history report; however, they were not available for examinations, and no medical records could be obtained.

Genetic studies of nanophthalmos pedigrees: DNA from 20 affected family members of pedigrees 991-F and 951-N was genotyped at 6,020 SNPs. The genotypes were evaluated for coinheritance with nanophthalmos using linkage analysis. In pedigree 991-F, a 20.2 Mbp segment of chromosome 17p12-q12, bounded by SNPs rs530664 and rs16523, was transmitted with nanophthalmos in all members of the family. An

				Spherical Equivalent OD (diopters)	Spherical Equivalent OS (diopters)
Individual	**Diagnosis**	**AEL OD (mm)**	**AEL OS (mm)**		
III-3	Nanophthalmia	NA	NA	12.75	13
III-4	Nanophthalmia	NA	NA	14.125	13.375
IV-2	Nanophthalmia	16.7	17.1	12.875	13.125
IV-3	Nanophthalmia	17.4	17.3	11.125	12.25
V-1	Nanophthalmia	NA	NA	13.75	
V-2	Nanophthalmia	NA	NA	13.375	
Nanophthalmia mean		17.1±0.31 mm			
III-1	Normal	NA	NA		
III-2	Normal	NA	NA		
III-5	Normal	NA	NA		
III-6	Normal	NA	NA		
Normal Mean		NA			

TABLE 1. CLINICAL FEATURES OF PEDIGREE 991-F.

Family members are identified by their pedigree symbols from Figure 1. NA - Not available.
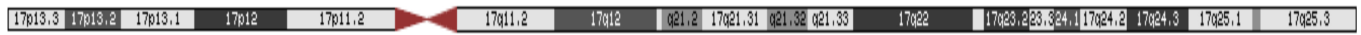
Figure 3. Diagram of the overlapping regions of chromosome 17 that are coinherited with nanophthalmos in the pedigrees (991-F and 951-N) and in the Awadalla and coworkers' pedigree [4].

overlapping 19.7 Mb section of chromosome 17, bounded by SNPs rs917541 and rs16523, was similarly coinherited with nanophthalmos in pedigree 951-N. These data suggest that the gene that causes autosomal dominant nanophthalmos in pedigrees 991-F and 951-N lies between SNPs rs917541 and rs16523 on chromosome 17 (Figure 3). This pericentric region of chromosome 17 has previously been linked to autosomal dominant nanophthalmos in a Chinese pedigree [8].

Exome sequencing was performed for patient IV:3 in pedigree 991-F (Figure 1) to identify the DNA sequence variants contained within the linked genomic regions. Within the linked region on chromosome 17, four non-synonymous DNA sequence variations were detected in four genes, *ZNF286A*, *ZNF287*, *SLC47A1* (OMIM 609832), and *TMEM98* (Figure 3). Each variant was evaluated as a potential nanophthalmos-causing mutation by testing the rest of pedigree 991-F for coinheritance with disease and evaluating the pathogenicity of the

mutation using sequence analysis programs, including SIFT, PolyPhen, and BLOSUM62. Of the four variants, only the His196Pro mutation in *TMEM98* was coinherited with nanophthalmos, absent from healthy family members, and had pathogenic scores on all three sequence analysis systems (Table 3). The *TMEM98* missense mutation in pedigree 991-F, His196Pro, produced a BLOSUM62 score of −2, a PolyPhen score of 0.998 (probably damaging), and a SIFT score of 0 (damaging). All indicate that this mutation is likely pathogenic (Table 3). As data from this experiment were being collected, another research group reported the discovery of an autosomal dominant nanophthalmos gene, trans membrane 98 gene (*TMEM98*), that was located within the same chromosome 17 locus linked with disease in our pedigree [4] (Figure 3). Consequently, the *TMEM98* gene has become the top candidate gene for causing glaucoma in our pedigrees.

Pedigree 951-N was subsequently analyzed for *TMEM98* mutations. Eleven members with nanophthalmos and four

TABLE 2. CLINICAL FEATURES OF PEDIGREE 951-N.

Individual	Diagnosis	AEL OD (mm)	AEL OS (mm)	Spherical Equivalent OD (diopters)	Spherical Equivalent OS (diopters)
II-5	Nanophthalmia	NA	15.86	NA	7.00
III-2	Nanophthalmia	18	18	6	NA
III-3	Nanophthalmia	16.81	16.62	NA	NA
IV-4	Nanophthalmia	18.43	18.21	4	4
IV-5	Nanophthalmia	17.08	17.19	2.5	4
IV-6	Nanophthalmia	17.78	18.35	5	6
IV-7	Nanophthalmia	18.38	18.27	2	1.88
IV-11	Nanophthalmia	15.87	16.19	NA	NA
Nanophthalmia Mean		17.4±0.94 mm		+3.93±1.57 diopters	
III-5	Normal	22.36	22.35	0	0
IV-8	Normal	23.01	23.02	0	0
Normal Mean		22.7±0.38 mm		0.00 diopters	

Family members are identified by their pedigree symbols from Figure 2. NA - Not available.

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Mutation in pedigree 991-F                                      H196P

TMEM98 sequence alignment (AA's 186-206)
Homo Sapien (human)                               IDQSLSAAEEHLEVLREAALA
Macaca mulatta (rhesis monkey)                    IDQSLSAAEEHLEVLREAALA
Canis lupus familiaris (dog)                      IDQSLSAAEEHLEVLREAALA
Felis catus (cat)                                 IDQSLSAAEEHLEVLREAALA
Mus musculus (mouse)                              IDQSLSAAEEHLEVLREAALA
Cavia porcellus (guinea pig)                      IDQSLSAAEEHLEVLREAALA
Pelodiscus sinensis (turtle)                      IDQSLSAAEEHLAVLREAALA
Xenopus laevis (frog)                             IDQSLSAAEDHLAVLREAALA
Aligator sinensis (alligator)                     IDRSLSAAEEHMTVLREAALA
Danio Rerio (zebrafish)                           IDQSLHAAEDHMVVLREAALA
Zootermopsis nevadensis (termite)                 IDQALTDMDPHLFVLREAALA
Camponotus floridanus (carpenter ant)             IDQALNDMDTHLLVLRNAALA
Apis dorsata (honey bee)                          IDQALNDMDSHLSILRNAALA
Strongylocentrotus purpuratus (sea urchin)        IDGSIKQMESHLQVLRDAAIT
Trichinella spiralis (nematode)                   LRVALSDLEMHLQQLREA
Trichuris trichiura (whipworm)                    I---LSDLELHLKKLREAA
Serinus canaria (canary)                          VERSLAAAEEHMAVLRQAAMA
Cuculus canorus (cuckoo bird)                     VERSLAAAEERIRVFRKA
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Figure 4. TMEM98 sequence analysis. Alignment of protein sequences encoded by human *TMEM98* with orthologous genes spanning the His196Pro mutation detected in pedigree 991-F. The histidine amino acid at codon 196 indicated in blue letters is conserved across many species; the flanking amino acids that are not conserved are indicated in red.

healthy family members were tested for disease-causing mutations using Sanger DNA sequencing. A novel 34 bp heterozygous deletion was detected in all affected family members that spans the last 28 bp of exon 4 and the first 6 bp of intron 4 (c.694_721delAGAATGAAGACTGGATCGA AGATGCCTCgtaagg). This *TMEM98* deletion eliminates a splice site and results in a premature termination of translation. The mutation is coinherited with nanophthalmos in pedigree 951-F and is absent from all healthy family members.

Sequence analysis of both *TMEM98* mutations provided additional support for their pathogenicity. Neither the His196Pro mutation nor the 34 bp deletion was detected in public DNA sequence databases including the Exome Variation Server and the 1000 Genomes Project [14]. Both mutations span highly conserved sequences based on the UCSC Genome Browser and BlastP. The His196Pro mutation detected in pedigree 991-F alters a histidine amino acid that is highly conserved across many disparate species (Figure 4),

which provides additional support for the mutation's potential pathogenicity.

DISCUSSION

Some forms of nanophthalmos are inherited as a Mendelian trait and are caused primarily by a single gene. Several cases of recessive nanophthalmos have been attributed to mutations in the *MFRP* gene [7]. Recently, Awadalla and coworkers identified *TMEM98* as the first autosomal dominant nanophthalmos gene. A missense mutation in the *TMEM98* gene (A193P) was associated with autosomal dominant nanophthalmos in one large pedigree [4].

TMEM98 encodes a transmembrane protein that has ubiquitous expression in humans [4]. Furthermore, Awadalla and coworkers demonstrated that *TMEM98* is also produced within ocular tissues including the sclera, choroid, retinal pigment epithelium, and iris that may have central roles in the pathogenesis of nanophthalmos [4,15]. Little is known about

TABLE 3. NON-SYNONYMOUS MUTATIONS DETECTED IN NANOPHTHALMOS PATIENT IV-3 OF PEDIGREE 991-F.

Gene	Mutation	Co-inheritance with disease in pedigree	SIFT	Polyphen	Blosum62
ZNF286A	Pro87Ser	No	0.01 (damaging)	0.001 (benign)	−1
ZNF287	Cys48Arg	No	0.07 (tolerated)	0.993 (probably damaging)	−3
SLC47A1	Ile414Thr	Yes	0 (damaging)	0.45 (benign)	−1
TMEM98	His196Pro	Yes	0 (damaging)	0.998 (probably damaging)	−2

Exome sequencing identified four non-synonymous mutations in the chromosome 17 critical region. Mutations were evaluated for pathogenicity based on co-inheritance with nanophthalmos in pedigree 991-F and with three mutation analysis software packages.

the function of the protein encoded by *TMEM98*; however, its expression in the sclera suggest that mutations may contribute to its pathologic thickening in nanophthalmos. Moreover, *TMEM98* expression in tissues of the outflow pathway makes it plausible that *TMEM98* mutations might contribute to the development of secondary glaucoma in nanophthalmos. The cause of the abnormally small eyes in nanophthalmos is unknown. However, identification and future studies of *TMEM98* may provide new insights into the developmental mechanisms that determine eye size, scleral thickness, and risk for glaucoma.

In this study, we confirmed the association between *TMEM98* mutations and nanophthalmos by reporting two novel *TMEM98* mutations (His196Pro and c.694_721delAG AATGAAGACTGGATCGAAGATGCCTCgtaagg) in autosomal dominant pedigrees. Both *TMEM98* mutations identified in our nanophthalmos pedigrees are coinherited with disease, absent from sequencing databases, alter conserved DNA sequences, and are judged likely to be pathogenic based on their destructive effects on the encoded TMEM98. Our contribution of two additional large nanophthalmos pedigrees with *TMEM98* variants that are coinherited with disease greatly strengthens Awadalla and coworkers' discovery and assertion that mutations in *TMEM98* cause nanophthalmos [4,16].

APPENDIX 1: OLIGO SEQUENCES FOR AMPLIFICATION OF TMEM98.

To access the data, click or select the words "Appendix 1."

ACKNOWLEDGMENTS

REFERENCES

1. Brockhurst RJ. Nanophthalmos with uveal effusion. A new clinical entity. Arch Ophthalmol 1975; 93:1989-99. [PMID: 1200903].

2. Singh OS, Simmons RJ, Brockhurst RJ, Trempe CL. Nanophthalmos: a perspective on identification and therapy. Ophthalmology 1982; 89:1006-12. [PMID: 7177565].

3. Vingolo EM, Steindl K, Forte R, Zompatori L, Iannaccone A, Sciarra A, Del Porto G, Pannarale MR. Autosomal dominant simple microphthalmos. J Med Genet 1994; 31:721-5. [PMID: 7815444].

4. Awadalla MS, Burdon KP, Souzeau E, Landers J, Hewitt AW, Sharma S, Craig JE. Mutation in TMEM98 in a large white kindred with autosomal dominant nanophthalmos linked to 17p12-q12. JAMA Ophthalmol 2014; 132:970-7. [PMID: 24852644].

5. Calhoun FP. The management of glaucoma in nanophthalmos. Trans Am Ophthalmol Soc 1975; 73:97-122. [PMID: 1246819].

6. Martorina M. Familial nanophthalmos. J Fr Ophtalmol 1988; 11:357-61. [PMID: 3171103].

7. Sundin OH, Leppert GS, Silva ED, Yang JM, Dharmaraj S, Maumenee IH, Santos LC, Parsa CF, Traboulsi EI, Broman KW, Dibernardo C, Sunness JS, Toy J, Weinberg EM. Extreme hyperopia is the result of null mutations in MFRP, which encodes a Frizzled-related protein. Proc Natl Acad Sci USA 2005; 102:9553-8. [PMID: 15976030].

8. Hu Z, Yu C, Li J, Wang Y, Liu D, Xiang X, Su W, Pan Q, Xie L, Xia K. A novel locus for congenital simple microphthalmia family mapping to 17p12-q12. Invest Ophthalmol Vis Sci 2011; 52:3425-9. [PMID: 21345985].

9. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin|[mdash]|rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 2002; 30:97-101. [PMID: 11731797].

10. Fingert JH, Robin AL, Ben R. Roos, Davis LK, Scheetz TE, Wassink TH, et alCopy number variations on chromosome 12q14 in patients with normal tension glaucoma. Hum Mol Genet 2011; 20:2482-94. [PMID: 21447600].

11. Eddy SR. Where did the BLOSUM62 alignment score matrix come from? Nat Biotechnol 2004; 22:1035-6. [PMID: 15286655].

12. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res. Cold Spring Harbor Lab 2001; 11:863-74. [PMID: 11337480].

13. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods 2010; 7:248-9. [PMID: 20354512].

14. 1000 Genomes Project Consortium, Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, Hurles ME, McVean GA. A map of human genome variation from population-scale sequencing. Nature 2010; 467:1061-73. [PMID: 20981092].

15. Wagner AH, Anand VN, Wang WH, Chatterton JE, Sun D, Shepard AR, Jacobson N, Pang IH, Deluca AP, Casavant TL, Scheetz TE, Mullins RF, Braun TA, Clark AF. Exon-level expression profiling of ocular tissues. Exp Eye Res 2013; 111:105-11. [PMID: 23500522].

16. Does the Association Between TMEM98 and Nanophthalmos Require Further Confirmation?-. Reply. JAMA Ophthalmol. 2015; 133:359-60. [PMID: 25429596].