

# Exome sequencing identified null mutations in *LOXL3* associated with early-onset high myopia

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**Purpose:** To identify null mutations in novel genes associated with early-onset high myopia using whole exome sequencing.

**Methods:** Null mutations, including homozygous and compound heterozygous truncations, were selected from whole exome sequencing data for 298 probands with early-onset high myopia. These data were compared with those of 507 probands with other forms of eye diseases. Null mutations specific to early-onset high myopia were considered potential candidates. Candidate mutations were confirmed with Sanger sequencing and were subsequently evaluated in available family members and 480 healthy controls.

**Results:** A homozygous frameshift mutation (c.39dup; p.L14Afs\*21) and a compound heterozygous frameshift mutation (c.39dup; p.L14Afs\*21 and c.594delG; p.Q199Kfs\*35) in *LOXL3* were separately identified in two of the 298 probands with early-onset high myopia. These mutations were confirmed with Sanger sequencing and were not detected in 1,974 alleles of the controls from the same region (507 individuals with other conditions and 480 healthy control individuals). These two probands were singleton cases, and their parents had only heterozygous mutations. A homozygous missense mutation in *LOXL3* was recently reported in a consanguineous family with Stickler syndrome.

**Conclusions:** Our results suggest that null mutations in *LOXL3* are likely associated with autosomal recessive early-onset high myopia. *LOXL3* is a potential candidate gene for high myopia, but this possibility should be confirmed in additional studies. *LOXL3* null mutations in human beings are not lethal, providing a phenotype contrary to that in mice.

In the human genome, null mutations in protein-coding genes can lead to a wide range of phenotypic effects, ranging from invisible to severe phenotypes [1]. Null mutations are typically responsible for recessive diseases such as Duchenne muscular dystrophy [2] and erythropoietic protoporphyria [3] in which approximately 70% to 80% of the detected mutations are null mutations. Analyzing null mutations specific to certain diseases among the millions of genomic variants captured by whole exome sequencing is crucial for the identification of novel disease genes.

We previously analyzed several known myopia genes and myopia-associated genes based on whole exome sequencing data obtained from samples of 298 probands with early-onset high myopia (eoHM) [4,5]. However, we identified one null mutation in one proband that was associated with high myopia as well as other variants in a small proportion of probands, which had undetermined pathogenicity [4,5]. The cause of the remaining majority of this cohort is unknown. These findings suggest that variants in novel genes might cause this disease.

Therefore, in the current study, our aim was to identify null mutations in novel genes associated with eoHM using whole exome sequencing.

## METHODS

**Subjects:** This study is part of a project established to investigate genetic defects associated with eoHM. Our aim was to identify novel genes responsible for eoHM using the same eoHM cohort that was used in our previous study [5]. Briefly, probands were recruited from the clinic at the Zhongshan Ophthalmic Center according to the following inclusion criteria: 1) spherical refraction in each meridian of  $\leq -6.00$  D in both eyes, 2) development of high myopia before the age of 7 years, and 3) no other known ocular or related systemic diseases. The 507 controls were unrelated probands with genetic eye diseases other than myopia, including retinal degeneration and glaucoma. The 480 healthy controls had bilateral refraction of between  $-0.50$  and  $+1.0$  D spherical equivalents without a family history of high myopia and had a best unaided visual acuity of 1.0 or better without another known eye or systemic disease. Written informed consent was obtained from the participants or their guardians following the tenets of the Declaration of Helsinki. This

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study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center.

**Sequencing analysis:** Whole exome sequencing was performed with an Agilent SureSelect Human All Exon Enrichment Kit V4 array (51,189,318 bp; Agilent, Santa Clara, CA) that covered more than 20,000 genes (approximately 334,000 exons). DNA fragments were sequenced using an Illumina HiSeq 2000 system (Illumina, San Diego, CA). The average sequencing depth was 125-fold. Reads were mapped against UCSC hg19 ([GenomeUCSC](http://genome.ucsc.edu)) using Burrows-Wheeler Aligner ([BWA](http://bwa-bio.sourceforge.net)). The parameters used for whole exome sequencing have been previously described [5].

Null mutations, including homozygous and compound heterozygous truncation variants, were selected from whole exome sequencing data on the 298 probands with eoHM. These data were compared with those of the 507 probands with other forms of eye disease. Null mutations specific to eoHM were considered potential candidates. The minor allele frequency of each variant was obtained from public databases, including [dbSNP](http://www.ncbi.nlm.nih.gov/snp), [1000 Genomes](http://www.1000genomes.org), [Exome Variation Server](http://www.broadinstitute.org/exome_variation_server), and Exome Aggregation Consortium ([EXAC](http://www.broadinstitute.org/exome_aggregation_consor_tium)). Null variants with a minor allele frequency of  $>0.01$  were excluded, and the remaining variants were further confirmed using Sanger sequencing and subsequently validated in available family members and 480 healthy controls. Primers were designed using the [Primer3](http://primer3.sourceforge.net) online tool and are listed in Appendix 1. The methods used to perform Sanger sequencing, including amplification, sequencing, and analysis of the target fragments, have been previously described [4]. The variants are described according to the Human Genome Variation Society ([HGVS](http://www.hgvs.org)).

## RESULTS

Evaluation of the whole exome sequencing data on 298 probands with eoHM revealed the presence of millions of variants targeting approximately 20,000 genes and null mutations in a few genes, *LRPAPI* (Gene ID 4043; OMIM 104225) and *LOXL3* (Gene ID 84695; OMIM 607163), that appeared to associate with high myopia after a series of bioinformatic filters. Null mutations in *LRPAPI* have been associated with high myopia in humans [6]. Previously, we identified an additional null mutation (c.199delC) in *LRPAPI* in a consanguineous family that has been reported in our previous study of known myopia genes [4]. Here, the null mutations detected in *LOXL3* included a homozygous frameshift mutation (c.39dup; p.L14Afs\*21) and a compound heterozygous frameshift variant (c.39dup; p.L14Afs\*21 and c.594delG; p.Q199Kfs\*35), which were identified in two of the 298 probands with eoHM (Table 1, Figure 1A). These

mutations in *LOXL3* were confirmed with Sanger sequencing and were absent in 1,974 alleles of ethnicity-matched controls from the same region (507 individuals with other conditions and 480 healthy control individuals; Table 1). These null mutations were also not present in the 1000 Genomes, Exome Variant Server, and Exome Aggregation Consortium databases. The two probands were singleton cases, and their parents carried only heterozygous mutations (Figure 1A). These null mutations in *LOXL3* were predicted to result in degradation of the transcript by nonsense-mediated mRNA decay [7,8]. The mutation frequencies and spectra in different types of variants of *LOXL3* are shown in Appendix 2. Other less likely pathogenic heterozygous variants in *LOXL3* are listed in Appendix 3.

The two probands with a *LOXL3* mutation developed high myopia before reaching 7 years of age. One proband had a refractive error of  $-18.50$  DS for the right eye and  $-18.00$  DS for the left eye, and the other had a refractive error of  $-23.00$  DS for the left eye and retinal detachment in the right eye (Table 2). Examination with an ophthalmoscope revealed myopic fundus with crescent and tigroid forms in the two probands (Figure 1B-D).

## DISCUSSION

In the current study, we revealed homozygous frameshift (c.39dup, p.L14Afs\*21) and compound heterozygous frameshift (c.39dup, p.L14Afs\*21; c.594delG, p.Q199Kfs\*35) mutations in *LOXL3* in two of the 298 probands with eoHM. These null mutations cosegregated with high myopia and were absent in the 1,974 alleles of the controls.

High myopia is a leading cause of visual impairment worldwide. Several lines of evidence indicated that excess elongation of eye size and axial length is due to abnormal extracellular matrix (ECM) remodeling in the sclera mediated by the transforming growth factor (TGF)-beta pathway [9-14]. *LOXL3*, a member of the lysyl oxidase gene family, encodes an extracellular copper-dependent amine oxidase. *Loxl3* expression is enriched in the retina and the central nervous system [15,16]. The encoded protein is induced through the TGF-beta pathway [17,18] and plays a critical role in the covalent cross-linking of collagen and elastin in the ECM, which is essential for ECM integrity in connective tissues [15,16,19-21].

Abnormal *LOXL3* function has been reported to be the cause of multiple types of defects in humans as well as in animals. A recent study identified a homozygous missense mutation (c.2027G>A, p.C676Y) in exon 12 of the *LOXL3* gene as the cause of autosomal recessive Stickler syndrome in a consanguineous family [22], with high myopia a

TABLE 1. LOXL3 MUTATIONS IDENTIFIED IN FAMILIES WITH EARLY-ONSET HIGH MYOPIA.

Family	Exon	Position	DNA change	Protein change	Status	Co-segregation	Note	Allele frequency		
								Normal control	Others <sup>§</sup>	Databases <sup>#</sup>
HM293	E2	74779723	c.39dup	p.L14Afs*21	Homo	Yes	Novel	0/960	0/1014	None
HM407	E2	74779723	c.39dup	p.L14Afs*21	Hetero	Yes	Novel	0/960	0/1014	None
HM407	E4	74776594	c.594delG	p.Q199Kfs*35	Hetero	Yes	Novel	0/960	0/1014	None

Note: Homo, homozygous; Hetero, heterozygous. §, Samples from patients with other eye diseases, including glaucoma and retinal degeneration. #, Databases including 1000Genomes, Exome Variant Server, dbSNP, and Exome Aggregation Consortium.

constant feature in this family [22]. This missense mutation was located in an evolutionarily conserved region and was predicted to be pathogenic [22]. In animal studies, knock-down of *lox3b* in zebrafish led to craniofacial abnormalities [21], and *Loxl3*<sup>-/-</sup> mice demonstrated craniofacial and spinal defects and smaller lungs at the embryonic stage (E18.5) [23]. The structure and axial length of the eyes in *Loxl3*-knockout mice were hard to determine, as all the knockout mice showed perinatal lethality [23]. We have generated a heterozygous *Loxl3*-knockout mouse model but we were

unable to get any homozygous *Loxl3*-knockout mouse (unpublished data), also suggesting embryonic lethal in mice on complete absence of *Loxl3*. In the current study, the two unrelated patients with null mutations in *LOXL3* exhibited high myopia without other known ocular or related systemic diseases, representing milder phenotypes than observed in previous studies in zebrafish or mouse [21,23]. Although the mechanism by which different *LOXL3* mutations cause variable phenotypes is unclear, high myopia is a common symptom present in syndromic diseases such as congenital

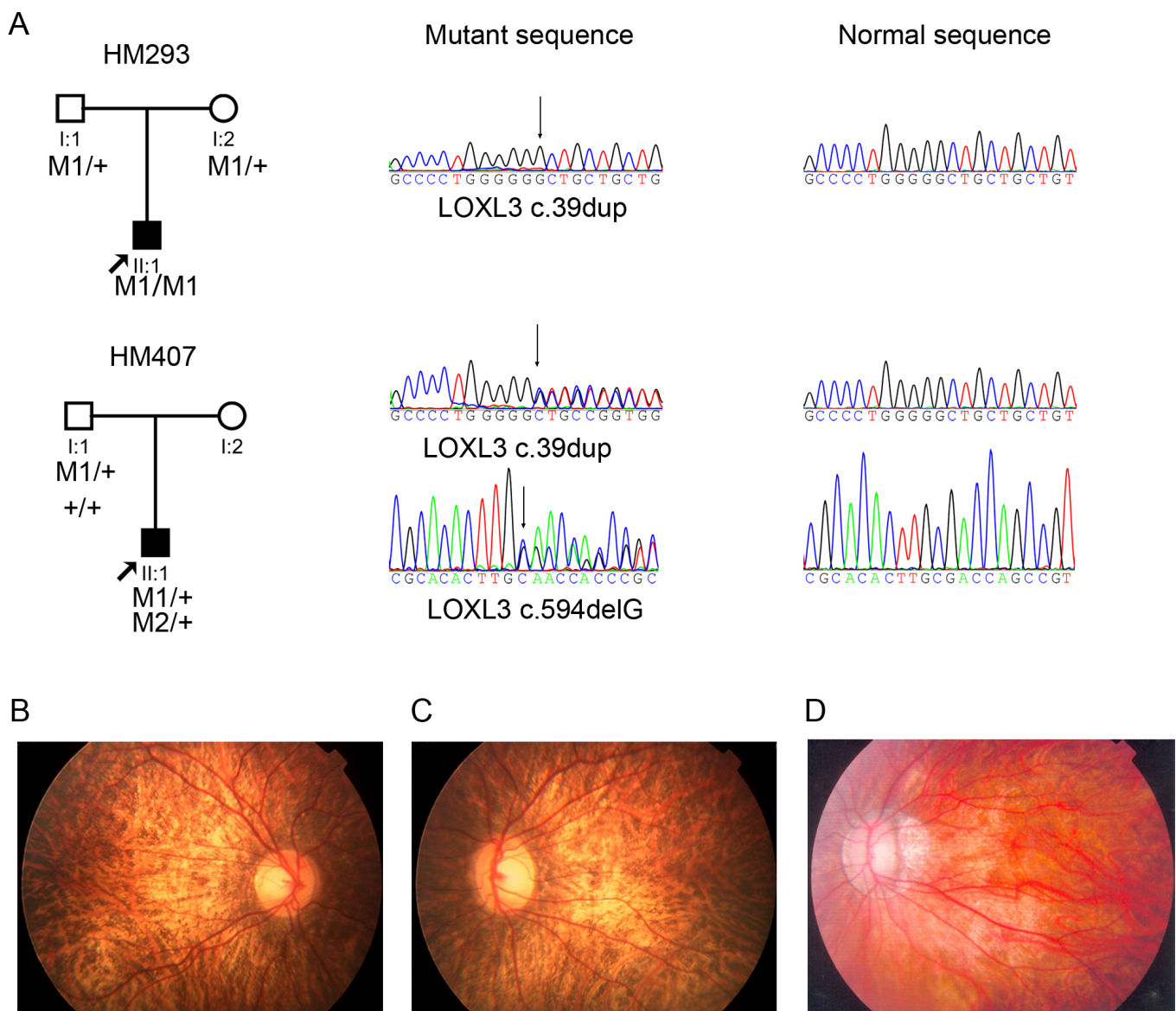


Figure 1. Null mutations in *LOXL3* identified in two probands with early-onset high myopia. **A**: Sequence chromatography and pedigrees of HM293 and HM407. Sequence changes detected in the patients with early-onset high myopia are presented in the left column, whereas healthy sequences appear in the right column. The sample from the mother in family HM407 was not available. M1, c.39dup; M2, c.594delG; +, wild-type. **B**, **C**, **D**: Fundus photos for both eyes of HM293III1 (**B**, **C**) and the left eye of HM427III1 (**D**) revealed myopic fundus with crescent and tigroid forms. The fundus photo for the right eye of HM427III1 is not available.

TABLE 2. CLINICAL INFORMATION FOR PATIENTS WITH LOXL3 MUTATIONS.

Patient	Gender	Age at exam (years)	First symptom	BCVA		Refraction		Axial length (mm)		Fundus
				right/left	right	left	right/left	right/left		
HM293III	Male	3	PV	NA/NA	-18.50DS-1.25DC	-18.00DS-2.00DC	NA/NA	NA/NA	Myopic/Myopic	
HM407III	Male	15	PV	HM/0.04	NA <sup>#</sup>	-23.00DS-3.50DC	27.15/33.58	RD/Myopic	RD/Myopic	

Note: PV, poor vision; NA, not available; HM, hand move; RD, retinal detachment; #, refraction was not available due to retinal detachment.

night blindness, caused by mutations in *NYX* [24], and Bornholm eye disease, caused by *OPNILW* [25]. Mutations in *NYX* and *OPNILW* have also been reported to cause high myopia alone due to mutations in different locations [26-28]. The null mutations in *LOXL3* were determined to be located in exons 2 and 4, which is distinct from the location of the previously reported mutation in Stickler syndrome. Because of the lack of follow-up visits to further confirm the phenotypic information, we can only assume that mutations located in different locations in *LOXL3* might have independent effects on patient phenotypes.

In conclusion, our results reveal the presence of null mutations in *LOXL3* in families with eoHM. Due to limited phenotypic information and a lack of functional studies, these findings only indicate that null mutations in *LOXL3* are likely to be associated with autosomal recessive eoHM. Meanwhile, our current approach may miss other types of variants if they are associated with high myopia. Our upcoming study will be designed to solve this issue by examining all other variants across the whole exome between cases and controls. The molecular mechanism underlying the role of *LOXL3* in high myopia, as well as in Stickler syndrome, will be the subject of further study.

#### APPENDIX 1. PRIMERS USED FOR POLYMERASE CHAIN REACTION

To access the data, click or select the words “[Appendix 1.](#)”

#### APPENDIX 2. THE VARIANT FREQUENCIES AND THE PROPORTION OF VARIANTS TYPES OF *LOXL3* IN THE 298 PATIENTS WITH EARLY-ONSET HIGH MYOPIA.

To access the data, click or select the words “[Appendix 2.](#)” In this study, 1.68% (5/298) of patients with eoHM harbored variants in *LOXL3*, in which 0.67% (2/298) of patients carried two null variants, 0.67% (2/298) of patients carried two heterozygous missense variants, and 0.34% (1/298) of patients carried one splicing change.

#### APPENDIX 3. RARE VARIANTS IDENTIFIED IN 298 PATIENTS WITH EOHM AND 507 CONTROLS

To access the data, click or select the words “[Appendix 3.](#)” Note: NA, not available; Hetero, heterozygous; §, Samples from patients with other eye diseases, including glaucoma and retinal degeneration; #, Databases including 1000Genomes, Exome Variant Server, dbSNP, and Exome Aggregation Consortium. †, Allele frequency found in Exome Aggregation Consortium database but not found in any other databases.

#### ACKNOWLEDGMENTS

The authors thank all of the patients and controls for their participation in this study. Supported by the National Natural Science Foundation of China (U1201221), the Natural Science Foundation of Guangdong (S2013030012978), and the Fundamental Research Funds of the State Key Laboratory of Ophthalmology.

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 20 February 2016. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.