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Correlation between novel compound heterozygous *ADAMTSL4* variants and primary phenotypes of ectopia lentis et pupillae

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Running title

Novel compound heterozygous mutations in *ADAMTSL4* causing ELeP

ABSTRACT

Purpose: To investigate molecular pathogenesis of congenital ectopia lentis accompanied by various ophthalmic manifestations.

Methods: Three female siblings, their spouse and offspring underwent ophthalmic and general medical examinations. Genetic variants were screened with the whole exome sequencing and analyzed in either a dominant or recessive inheritance manner. Gene mutations were ascertained with the Sanger sequencing after the polymerase chain reaction.

Results: All three female siblings were diagnosed as the Ectopia lentis et pupillae (ELeP) through combination of clinical examination and genetic analysis. No characteristic pathological changes of skeletal, metabolic and cardiac abnormalities were observed. Thirteen genetic variants were selected out through analyzing in dominant or recessive inheritance manner, but they were not associated with EL. Among them, *ALOX15B* variant may explain the skin disease in this pedigree. After inspection the known genes related to EL, novel compound heterozygous mutations (p.Ser264LeufsX37/ p.Gly757ValfsX62) in *ADAMTSL4* were discreetly identified in this ELeP pedigree.

Conclusions: Novel compound heterozygous *ADAMTSL4* variants are responsible for ELeP in the current pedigree. Correlation between *ADAMTSL4* variants and ELeP was firstly established based on our 12 years follow-up studies and previous reports of ELeP and of *ADAMTSL4*–related eye disorders. The primary phenotypes caused by *ADAMTSL4* variants include EL, EP, poor pupillary dilation, and axial elongation. Highly varying phenotypes including glaucoma, high myopia retinapathy, and poor vision and so on may be the secondary impairments. All these secondary impairments may be improved if proper clinical interventions are implemented in time.

***Keywords*:** Ectopia lentis et pupillae, Whole exome sequencing, *ADAMTSL4*, Compound heterozygous mutation

Abbreviations

AL: axial length； CEL: congenital ectopia lentis; EL: ectopia lentis; ELeP: ectopia lentis et pupillae; EP: ectopia pupils; IOP: intraocular pressures; NLP: no light perception; PCR: polymerase chain reaction; VEP: variant effect predictor; WES: whole exome sequencing.

1. Introduction

The congenital ectopia lentis (CEL) is heterogeneous eye conditions in which the lens partial or complete dislocates from its anatomical location within the pupillary area (Chandra and Charteris, 2014). Ophthalmic findings related to CEL are accompanied by ectopia pupillae (EP), myopia, amblyopia, chronic vitritis, refractive error, iridodonesis, astigmatism, cataract, glaucoma, retinal detachment (Fuchs and Rosenberg, 1998). CEL condition can be divided into without systemic association (isolated ectopia lentis, IEL) and those with systemic association including ectopia lentis et pupillae (ELeP), Marfan syndrome, homocystinuria, sulfite oxidase deficiency, Weill-Marchesani syndrome and indeterminate etiology (Fuchs and Rosenberg, 1998; Chee et al,. 2021). A large proportion of CEL is associated with heterozygous and complication symptoms. CEL can be present at the birth or occur at any age and caused by multiple factors including trauma, infection, hypermature cataract, anterior uveal tumors, and genetic variants. Therefore, understanding the pathogenesis, relation of ocular symptoms, and its progression, is crucial for diagnosis and intervention.

In this study, we investigated the pathogenesis and progression of EL in a pedigree since 2010. Ophthalmic manifestations accompanied by EL were difficulty to distinguish. We conducted tracing ophthalmologic and systemic examinations, and analyzed the relationship of major characters, in order to make a clear diagnosis and understand its progression. We screened the molecular pathogenesis with the method of whole exome sequencing, and identified novel compound heterozygous mutation of *ADAMTSL4* (A Disintegrin-like And Metalloproteinase with ThromboSpondin type 1 repeats like 4) variants responsible for the ocular symptoms. *ADAMTSL4* mutations have been identified to cause ELeP in the 2010 (Aragon-Martin et al., 2010； Christensen, et al., 2010). *ADAMTSL4* encoding protein is a secreted glycoprotein, binding fibrillin-1 microfibrils and accelerating microfibrils biogenesis in the eye (Gabriel, et al., 2012). However, correlation between *ADAMTSL4* variants and ELeP phenotypes has not been established yet (Rødahl et al., 2012). We discussed the characters of ELeP, *ADAMTSL4*-related eye disorders, and dynamic alteration in this pedigree. Primary phenotypes caused by *ADAMTSL4* variants were suggested in this study. We hoped to shine some lights on the correlation between *ADAMTSL4* variants and ELeP phenotypes, and therefore help clinical diagnosis and management.

2. Patients and methods

2.1. Participants

In 2010, the fifty years old female proband (Ⅱ-5) was first referred to the ophthalmic section for outpatients in the Xi'an No.1 Hospital for pain and sight loss in her left eye without apparent reason and no accompanying red eyes, fear of light, epiphora, *etc*. Trauma and other diseases factors were excluded. She reported that she had poor vision since she was five years old but

without treatment, and that her two siblings were also affected with similar symptoms (Figure 1A). She requested a genetic counseling and therefore all the relevant family members (n=22, Figure 1A) were then recruited in this study. All participants signed full informed consent. The study was approved by the Ethical Committee of the Northwest University and adhered to the tenets of the Declaration of Helsinki.

2.2 Clinical examination

All participants were examined and diagnosed by the physicians at the Xi'an No.1 Hospital. The clinical data comprised the available medical records and family history, ophthalmologic findings, metabolic screening, cardiological evaluation, and physical examination (including dysmorphia features). The ophthalmologic examinations included corneal (curvature, thickness, diameter, and astigmatism), pupil size, pupillary dilation, iris , lens, vitreous, fundus, refractive error, intraocular pressures (IOP), axial length (AL), anterior chamber angle, anterior chamber depth, visual field, nerve fiber thickness, ganglion cell, optic disc, *etc*. Systematic examinations were performed in all participants, which included visual inspection (skull, face, jaw, tooth, breast bone, spinal column, wrist or thumb sign, and skin), auscultation and instrument inspection (height, arm span, value of arm span/height, measurement of echocardiogram, measurement of aortic diameter value, checking valve abnormalities, chest X-ray examination to observe the presence of pneumothorax, lumbosacral CT, observing whether the dura mater to expand the lumbosacral dural ectasia).

2.3 Genetic analysis

Three female siblings (Ⅱ-1, -3, -5) complained of poor vision in early childhood. Two of them (Ⅱ-1, and -3) were diagnosed as EL at the age of 12 and 6 separately, and the patient (Ⅱ-5) was diagnosed as EL at the age of 50 (Table 1). Because non-apparent pathogeny was complained by these three patients which was further confirmed by the physicians, EL in this pedigree might be congenital. To explore the possible molecular pathogenesis, make an accurate diagnosis and genetic counseling, we used the whole exome sequencing (WES) to identify genetic variants in this family as previously reported (Yang et al., 2021). Genomic DNA of three patients and two female offspring of the patient (Ⅱ-3) was extracted from the leukocyte using the standard phenol-chloroform method, and sequenced by the Novel Bioinformatic Company (Shanghai, China). Quality of all DNA samples was controlled with Qubit 2.0 (ThermoFisher, MA, USA). Qualified samples were sequenced on an Illumina Hiseq 2000 machine (Temecula, CA, USA) after purifying with Agilent SureSelect. Raw data were evaluated by Fast-QC (http: //www.bioinformatics. babraham.ac.uk/projects/fastqc/). Using Burrows-Wheeler Aligner, the clean data were aligned to human reference genome GRCH37, Ensemble175. The Genome Analysis Toolkit was used for single-nucleotide polymorphism calling and somatic mutation.

According to impact information and variant frequency, the high risk (moderate and high) of mutant loci were selected out. The synonymous changes and SNPs which minor allele frequency (MAF) higher than 5% were removed. The potential pathogenic impacts were filtered using SIFT Human Protein DB (http://www.sift.jcvi.org/) and PolyPhen-2 (http://www.genetics.bwh.harvard.edu/pph2/) online software. The SIFT score was interpreted as deleterious from 0.0 to 0.05, and as tolerated from 0.05 to 1.0. The Polyphen-2 score was interpreted as confidently damaging from 0.85 to 1.0, as possibly damaging from 0.15 to 1.0, and as benign from 0.0 to 0.15. Pathogenicity predictions were conducted to evaluate damage impact of truncating mutation with Ensembl Variant Effect Predictor (VEP) (http://asia.ensembl.org/info/docs/tools/vep/index.html) as previously reported (Sundaram et al., 2018). The variants evaluated as "damaging" and "probably damaging" by the SIFT, or "possibly damaging" with the Polyphen-2, or the stop_gained variants predicted as high or moderate impact by the VEP, were considered to be furthermore analyzed. The selected variants were then analyzed in either dominant or recessive manner firstly, in order to find pathogenicity variants. The candidate mutations were analyzed in the DisGeNET database (https://www.disgenet.org/browser/1/1/3/54507/).

The target variants were validated with polymerase chain reaction (PCR) combining the Sanger sequencing. The primers were designed according to the results of WES and NCBI information. Genomic DNA was amplified by PCR using standard procedures. The primer sequence information of ultimate target variants was listed below: *ADAMTSL4*- rs765800065, F: 5'-CTGTCTGTCCACACCCCATC-3'; R: 5'-GGGACCGAAGGAAAAGGATC-3'. *ADAMTSL4*-rs747160538, F: 5'-CTCATCTATGTCTCCGCCTCTTC-3'; R: 5'-TTGTTCACCTGTGCCCATTC-3'. The PCR reaction system contained 10 μL Taq Mix, 0.8μL primers, about 4.0μL DNA sample, and up to 20.0μL total system with ddH_2O. The target sequences were amplified with an initial 5 min denaturation at 95℃, followed by 30 cycles of 94℃ for 30 s, 55℃ for 30 s to amplify rs765800065, or 57℃ for 30 s to amplify rs747160538, 72 ℃ for 30 s, and final extension at 72℃ for 5 min. The PCR products were consequently identified with Sanger sequencing.

3. Results

3.1. Clinical reports

In 2010, three female siblings (Ⅱ-1, -3, -5) were examined at the hospital. Pupils in the 4/6 eyes were dislocated to nasal direction (Figure 2A), and 2/6 eyes could not be determined because of pupil deformation after operation. 5/6 pupillary dilation was in the poor condition (Figure 2A). All lenses in 6 eyes were dislocated (Figure 2B). AL in the 5/6 eyes was longer than average (22-25 mm) (Figure 2C), and axial length of 4/6 eye was longer than 26.5 mm (Table 1), which may be related to high myopia. 4/6 eyes were characterized with astigmatism (>1D). All patients were checked and confirmed as without traumatic and any other apparent pathogeny causes by the physicians. According to the revised Ghent Nosology, three female patients (Ⅱ-1, 3, and 5) were

not fulfilled the Marfan syndrome diagnostic criteria. Three female patients were thus preliminarily diagnosed as congenital ELeP.

The left eye (LE) of proband (Ⅱ-5) had no light perception (NLP), higher IOP (50 mmHg), corneal edema, deep anterior chamber, open angle, and was diagnosed as secondary open-angle glaucoma at the absolute stage. IOP of the LE was kept normal after the vitrectomy combined with crystal excision. The patient Ⅱ-1 wore 20D degree eyeglass because of high myopia diagnosed at the age of 8 years old. Several years later, she felt better without eyeglass. In 2011, she was persuaded to be treated for poor vision. Her LE (vision 0.1, normal IOP) was successfully treated with vitrectomy combined with crystal excision and silicone oil injection surgery, resulting a vision 0.1 and normal IOP after the surgery. In 2018, we observed that NLP, higher IOP (45 mmHg), atropic optic nerve and glaucoma in her LE. Normal IOP was kept after treatment with silicone oil removal surgery. The patient (Ⅱ-3) reported the poor vision acuity when she was six years old. After diagnose, cataract surgery was thus conducted in the LE. EL in the right eye (RE) was diagnosed and the lens was removed at sixteen years old. Pupil in the RE was cut open, and the shape was changed because of operation. Poor vision and normal IOP were kept during our follow-up study.

In July 2022, we re-investigated their conditions. We found 'Aphakic' in the area of the pupil in the 6/6 eyes, EP (Figure 2A and C), and poor pupillary dilation in the 5/6 eyes (Figure 2A). The lens in RE of patient (Ⅱ-1) was dislocated in vitreous chamber (Figure 2B). AL of 5/6 eyes was longer and slightly changed (Figure 2B and C). For example, AL in the RE of patient Ⅱ-1 increased 0.27 mm (Figure 2B). We also noticed some new characters occurred, such as open angle, mild higher IOP, tilted optic disc, tigroid fundus, myopic conus (Figure 2D), and lamellar macular hole in the RE of patient (Ⅱ-1) (Figure 2E), abnormal visual field (Figure 2F), and increased disc/cup ratio (0.79).

3.2. Genetic testing

The three patients (Ⅱ-1, -3, -5) were burdened with EL accompanied by varying ocular symptoms, and their spouses, young brother, their children, and grandchildren were currently free of EL (Figure 1). Three patients confirmed that their parents were free from the EL and they were in no consanguineous marriage (Figure 1). Because of varying ophthalmologic characters, we cannot determine which type of the disease was initially. To make an assistant diagnosis, the WES was used to screen the genetic variants. Two daughters of patient (Ⅱ-3) did not experience vision loss and EL, and were set as the control. Three female patients were at one generation, we thus analyzed variant results either in dominant or recessive inheritance manner. In the dominant manner, we identified the mutant genes, comprising *ALOX15B*, *AQP12A*, *INTS9*, *MUC12*, *MUC16*, *PABPC3*, *PAPPA-AS1*, *TEKT1*, *TRBV3-1*, and *TRIOBP*. In a recessive manner, we identified the mutant genes, including *OR10H5*, *NUDT18*, and *DHRSX*. All of these genes are not associated with EL or EP.

To avoid any possible chance of losing important information, we submitted and analyzed the mutant genes of the patient Ⅱ-3 in both the DisGeNet and the Metascape (https://metascape.org/). We did not gain the information about other known mutant genes related to EL, and only mutant *ADAMTSL4* was identified. The novel compound heterozygous mutations were further confirmed with PCR combining with Sanger sequencing (Figure 1B). Two frameshift_variants of *ADAMTSL4* have been excluded with the SIFT or Polyphen-2 because of truncate mutations. The mutant types included ENST00000271643.4:c.789dup （ENSP00000271643.4:p.Ser264LeufsX37, and ENST00000271643.4:c.2270del （ENSP00000271643.4:p.Gly757ValfsX62） (Figure 1B). These novel compound heterozygous mutations were evaluated as high impact, which occurred among three patients and were inherited by two offspring separately. The offspring (Ⅲ-4) carried the mutant type of ENST00000271643.4:c.2270del（NSP00000271643.4:p.Gly757ValfsX62）and the offspring (Ⅲ-6) carried the mutant type of ENST00000271643.4:c.789dup （ENSP00000271643.4:p.Ser264LeufsX37）(Figure 1A and B).

4 Discussions

Based on ophthalmologic and general examinations and genetic analysis, we concluded that three female patients were affected with ELeP and they were caused by novel compound heterozygous mutations of *ADAMTSL4* (p.Ser264LeufsX37/ p.Gly757ValfsX62). Combing our 12 years follow-up studies with previous reports about ELeP and *ADAMTSL4*-related eye disorders, for the first time, we suggest that *ADAMTSL4* variants are correlated to primary phenotypes including EL, EP, axial elongation, and poor pupillary dilation.

Because of family aggregation, we screened genetic variants with WES. Only three female siblings were affected by EL accompanied by varying ocular symptoms at one generation, we thus cannot determine the disease type and its inheritance manner in the first place. To identify genetic variants responsible for the disorders, we analyzed in either a dominant or recessive inheritance manner initially. Thirteen genes were selected out and analyzed their associations between variants and diseases in the DisGeNET database. All of them are not related to either EL or EP. Three genes including *ALOX15B*, *TRBV3-1*, and *NUDT18*, were related to skin and connective tissue disorders. We therefore re-investigated the relative traits. Three patients reported that they suffered from the skin disease at various levels. The patient (Ⅱ-1) reported that she suffered from facial desquamation at 8 years old, and the symptom was eliminated after two years, and was cleaned up now. The patient (Ⅱ-3) reported suffering from the skin pruritus on the whole body at 41 years old, and the symptom was not relieved when it was treated as psoriasis in the hospital. The color of scalp was gray. The skin color of chest, upper limb, and lower limbs was light red. The traits of lepidic and patchy pattern were also observed. The patient (Ⅱ-5) also reported suffering from the skin disease at 17 years old. Her symptom was similar to the patient (Ⅱ-3) (Supplementary figure 1). *ALOX15B* variant may be

responsible for the skin problem in this pedigree as previously reported (Clements et al., 2012; Lee DJ, et al., 2012; Setsu N, et al., 2006; Krieg and Fürstenberger, 2016).

To identify the molecular etiology related to EL, we searched the variants in the patient Ⅱ-3, setting the control of her two daughters. None of other known mutant genes was reported to cause EL, including *FBN1*, *CBS*, *ADAMTSL4*, *ADAMTS10*, *ADAMTS17*, *COL18A1*, *PAX6*, *LTBP2*, and *VSX2* (Chandra and Charteris, 2014). We however finally identified novel compound heterozygous mutations of *ADAMTSL4*. The variants were once excluded when we analyzed in the dominant or recessive inheritance manner. Their scores of both SIFT and Polyphen-2 could not be evaluated because of truncating mutation. Then the pathogenicity impact was evaluated as high with VEP, and as causal mutation (Variant-disease association score, 0.700) in the DisGeNET. The *ADAMTSL4* mutation of ENST00000271643.4:c.789dup localizes in the region (77-342 aa), which is described to be related with disorders (Aragon-Martin et al., 2010; Christensen et al., 2010; Neuhann et al., 2011). Therefore it may be a hot mutant spot. The second mutant site (ENST00000271643.4:c.2270del) is in the encoding region of the thromobospondin type 1 repeat (TSR). Both mutations can cause the truncating mutation of *ADAMTSL4* encoding protein (Figure 1), and thus the loss of the last three of four TSRs, which anchored the ADAMTSL4 and assembled the extracellular matrix (Ahram et al., 2009). The ophthalmologic findings in this pedigree are similar to the characters of ELeP reported previously (Cruysberg and Pinckers, 1995; Goldberg, 1988). Based upon ophthalmologic findings and result of genetic variants, we thus confirmed that compound heterozygous mutations (p.Ser264LeufsX37/ p.Gly757ValfsX62) of *ADAMTSL4* were responsible for ELeP in autosomal recessive manner in this pedigree.

It is still a thorny issue for diagnose on ELeP, a rare genetic syndrome, which is with variable signs including EL, EP, abnormal development of corneal, iris, and visual axial, *etc.* Its phenotypes vary from simple EL and EP to miscellaneous clinical characters, such as increased corneal thickness, enlarged corneal diameters, alteration of pupil shape, poor pupillary dilatation, axial myopia, abnormalities of the iris, cataract, and retinal detachment (Cruysberg and Pinckers, 1995; Goldberg, 1988). The ocular symptoms along with EL vary among different patients in a pedigree, even between both eyes of the same individual. In addition, although it is considered as non-systemic disorder, Cleft lip and palate (Sha'ban and Asfour, 2003), craniosynostosis (Chandra et al., 2013a), and patchy depigmentation of the skin, hair and lashes (Manitto et al., 1998), have been reported previously. As for molecular pathogensis, *ADAMTSL4* variants have been identified broadly in EL-related eye disorders, including isolated EL (OMIM 225100) (Ahram et al., 2009; Aragon-Martin et al., 2010; Chandra et al., 2012; Chandra et al., 2013a; Dollfus et al., 2010; Neuhann et al., 2015; Overwater et al., 2017; Reinstein et al., 2016; van Bysterveldt et al., 2017; Guo. Et al., 2022), autosomal recessive ELeP (OMIM 225200)(Christensen et al., 2010; Sharifi et al., 2013;Overwater et al., 2017; Safi et al., 2019), and EL with craniosynostosis (OMIM 603595)(Chandra et al., 2013a; Gustafson, et al., 2022). About the inheritance manner of *ADAMTSL4*-related eye disorders,

previous studies exhibit major in recessive manner (Ahram et al., 2009; Aragon-Martin et al., 2010; Dollfus et al., 2010; Christensen et al., 2010; Chandra et al., 2012; Reinstein et al., 2016; van Bysterveldt et al., 2017; Gustafson, et al., 2022), and other manners including pseudodominant and dominant styles (Sharifi, et al, 2013; Scanga and Nischal, 2022; Chen TH, et al., 2022). Therefore, exploring the genotype-phenotype correlation will be extremely important for a better diagnosis, management, treatment and prognosis (Reich, et al, 2021).

To establish the genotype-phenotype correlation between *ADAMTSL4* variants and ELeP phenotypes, firstly, we analyzed the ocular phenotypes in ELeP pedigrees previously reported. Major clinical findings were concentrated on characters including EL, poor vision, poor pupillary dilation, EP, abnormalities of the iris, cataract, and retinal detachment (Cruysberg and Pinckers, 1995; Goldberg, 1988). Secondly, we collected and analyzed the data previously reported about *ADAMTSL4* variants. Genotype-phenotype analysis was performed prospectively and summarized in the supplementary table 1 and 2. Most of variants cause isolated EL or ELeP, even for the same variant such as homozygous mutations of p.Gln256ProfsX38 and p.Q752X (Supplementary table 1), compound heterozygous mutations of p.Gln256ProfsX38 and p.Arg276SerfsX21 (Supplementary table 2). Particularly, ELeP caused by *ADAMTSL4* mutations exhibits the major characters, such as poor vision, increased IOP, poor pupillary dilation, long AL, and glaucoma (Supplementary table 1 and 2). Therefore, *ADAMTSL4*-related eye disorders form a continuum starting with minor eye anomalies to typical eye findings (Rødahl et al., 2012). Thirdly, we may conclude the correlation between *ADAMTSL4* variants and ocular phenotypes in the ELeP, when combine the results above-mentioned with ADAMTSL4 distribution. ADAMTSL4 is widely distributed in ciliary body, lens capsule, iris, and choroidal tissue of the human eye, and promotes fibrillin-1 depositing into the lens zonule and maintains the zonule (Gabriel et al., 2010; Gabriel et al., 2012; Chandra , et al., 2013b). ADAMTSL4 is required for the stable anchorage of zonule fibers into the lens capsule and seeding microfibrils formation (Collin et al., 2015; Hubmacher and Apte, 2015). The results above-mentioned are related to EL, which is decisive symptom exhibited totally in all previous reports when *ADAMTSL4* mutations happened. With the mouse model, Collin et al (2015) suggested that mutant *ADAMTSL4* influence stable anchorage of zonule fibers to the lens capsule and AL, and inconformity of pupil location or shape was observed (Collin et al., 2015). Although EP is varying, it is distinctive feature distinguished from isolated EL. In the case of ELeP, pupils may be dislocated in an opposite direction to the EL, and its shape is also reported to be changed. ADAMTSL4 distribution in iris and choroidal tissue, the ciliary body, and ciliary processes of the human eye, supports that *ADAMTSL4* mutations may increase axial myopia (Chandra et al., 2013a). Mean AL in the *ADAMTSL4* mutation groups has been suggested to be longer (Chandra et al., 2012). Axial elongation has been confirmed to be associated with myopia and myopic progression (Hou et al., 2018; Xu et al., 2022). AL increased rapidly before 12 years old and then slowed and stabilized (Zhang et al., 2017). Axial elongation may also be considered as a significant feature in the ELeP cases. In this study, we recapitulated the major phenotypes including EL (4/6

eyes), poor vision (6/6 eyes), EP (5/6 eyes), poor pupillary dilation (5/6 eyes), axial elongation (5/6 eyes), and astigmatism (4/6 eyes) (Table 1). We observed abnormal shape and dislocation in the vitreous chamber in the RE of patient Ⅱ-1 (Figure 2D). Abnormal condition of lens may cause a refractive error, which subsequently leads to astigmatism, myopia, or hyperopia (Patient Ⅱ-1 RE, +3D; Patient Ⅱ-5 RE,+9D).When lens moves in the vitreous, inflammatory processes, secondary glaucoma, hemorrhages, retinal changes, and other ocular symptoms may be triggered. Abnormal changes of pupils are related to poor vision and limited visual field. Axial elongation may be associated with high myopia retinapathy (In both eyes of patient Ⅱ-1, RE of patient Ⅱ-3, and LE of patient Ⅱ-5) (Table 1), exhibiting tilted optic disc, tigroid fundus, myopic conus, and posterior staphyloma (Figure 2E and F). 2/6 eyes (LE of patients Ⅱ-1 and Ⅱ-5) are NLP, which were diagnosed as secondary glaucoma. Both high myopia retinapathy and refractive error are major factors causing poor vision or low vision. If secondary glaucoma is overlaid, three symptoms lead to blindness. Although ELeP has strongly heterozygous and complicated symptoms, primary phenotypes related to *ADAMTSL4* mutations may be concluded as EL, EP, axial elongation, and poor pupillary dilation, based upon the previous reports and our studies. The other phenotypes, including alteration of pupil shape, abnormalities of the iris, cataract, and retinal detachment, may be secondary injury. Different individual option and clinical interventions achieved different outcomes. The lenses of the patient Ⅱ-3 and Ⅱ-5 were surgically removed in time, glaucoma did not occur. Because the patient Ⅱ-1 chose not to be treated timely, her ocular symptoms became worse, including blindness in the left eye, and worse visual field and potential blindness in the right eye (Figure 2 D, E, and F).

Overall, we conclude that novel compound heterozygous mutation of *ADAMTSL4* is responsible for congenital ELeP in this study, conforming to autosomal-recessive inheritance manner. If the patients suffer from other clinical issues, such as skin disease in this study, relative symptoms may be caused by other variants. Primary phenotypes of *ADAMTSL4*-related eye disorders are concentrated on EL, EP, axial elongation and poor pupillary dilation. A variety of problems including cataract, glaucoma, uveitis, and evaluated IOP may be secondary complications. It is reasonable to conclude that an early precise diagnosis and a proper timely intervention could improve secondary complications.

Authors' contributions

Junhong Zhao and Jing Zhang diagnosed the participants, collected the samples, and completed the clinical part of the manuscript. You Zhou and Kejin Zhang extracted the DNA samples and confirmed the mutations with method of PCR combing with Sanger sequencing. Lijun Shang and Junlin Li completed the genetic analysis and the manuscript.

Declaration of interest

The authors declare no conflict of interest.

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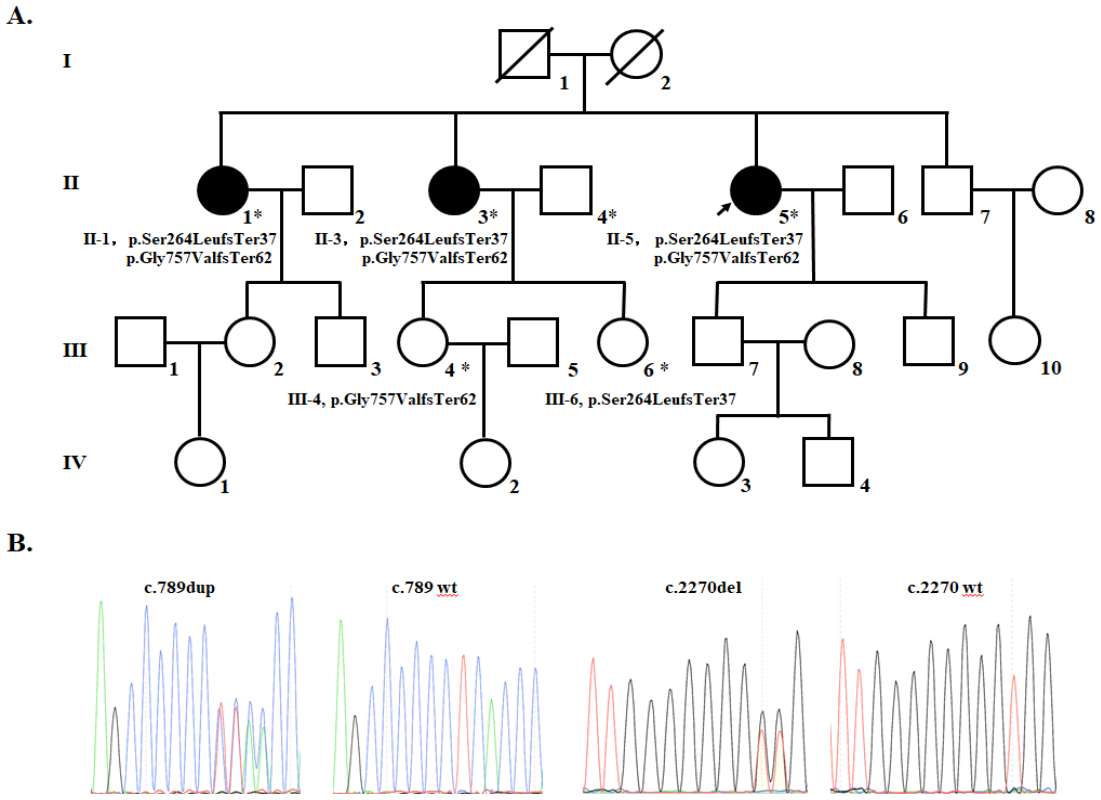


Figure 1. Novel compound heterozygous mutations in *ADAMTSL4* identified in three patients.

A. the pedigree shows the participating members and their immediate relatives. The mutant types are listed to exhibit the novel compound heterozygous inheritance manner. Filled symbols: affected people. The proband is shown with thin arrow. Asterisks indicate people examined in the present study.

B. mutant types of *ADAMTSL4* in the patient Ⅱ-3 family, including ENST00000271643.4:c.789dup（ENSP00000271643.4:p.Ser264LeufsX37） and ENST00000271643.4:c.2270del（ENSP00000271643.4:p.Gly757ValfsX62）, which were confirmed with PCR combining with Sanger sequencing. Mutant type and wild type (wt) are shown in the figure 1B.

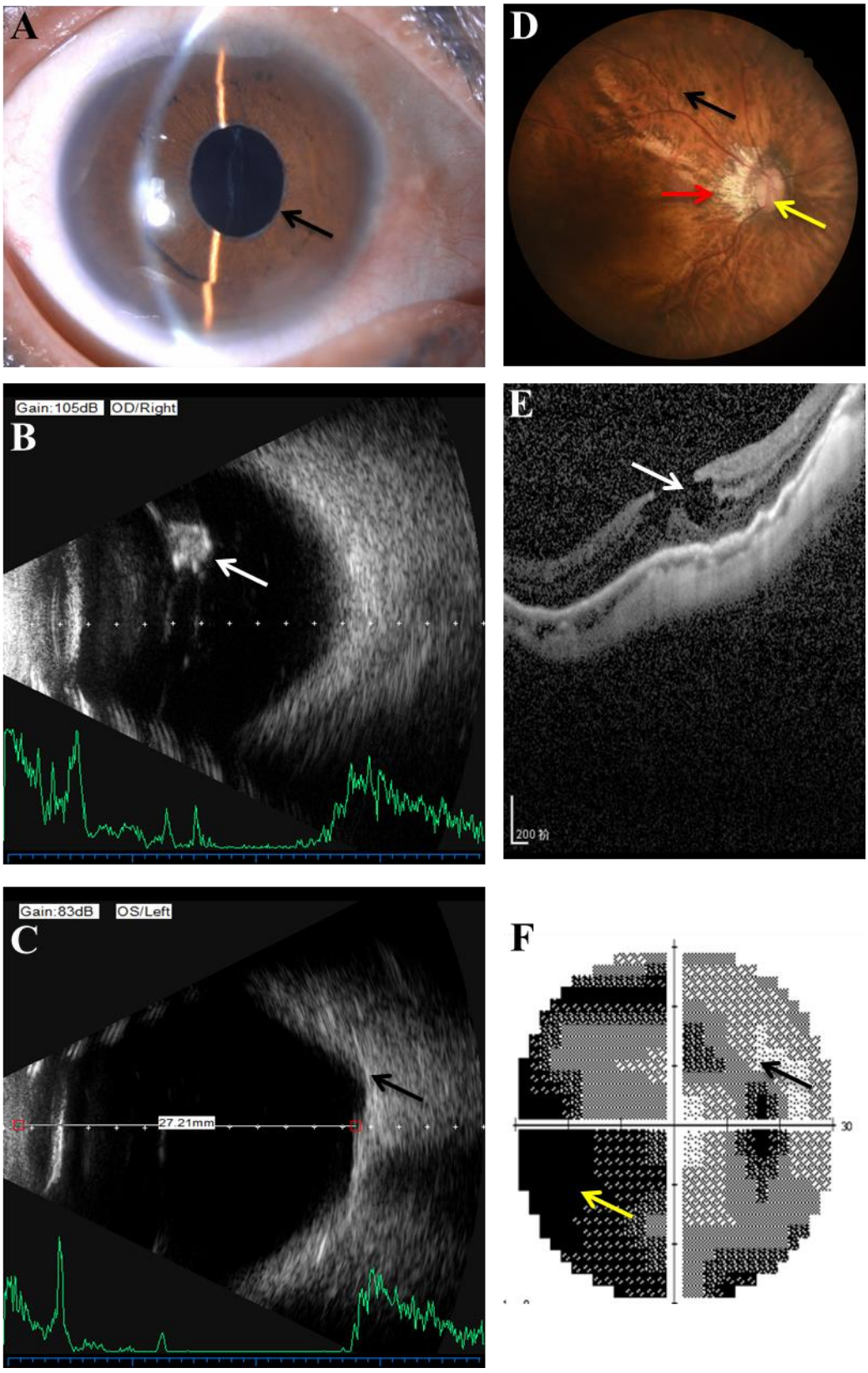


Figure 2. Ophthalmic examination in ELeP patients.

A, Anterior segment photograph of right eye of patient Ⅱ-1, shows no lens in the pupil area, and poor pupillary dilation (Black arrow).

B, B-ultrasound examination of patient Ⅱ-1, shows the lens in the right eye completely dislocated

in the vitreous cavity, irregular and contracture section (White arrow).

C, B-ultrasound examination of left eye of patient Ⅱ-3, shows Posterior sclera staphyloma (Black arrow), which is related to higher myopia.

D, Fundus photograph of right eye of Ⅱ-1,shows tilted optic disc (yellow arrow), conus (red arrow), and tigroid fundus (black arrow), which are related to high myopic retinopathy.

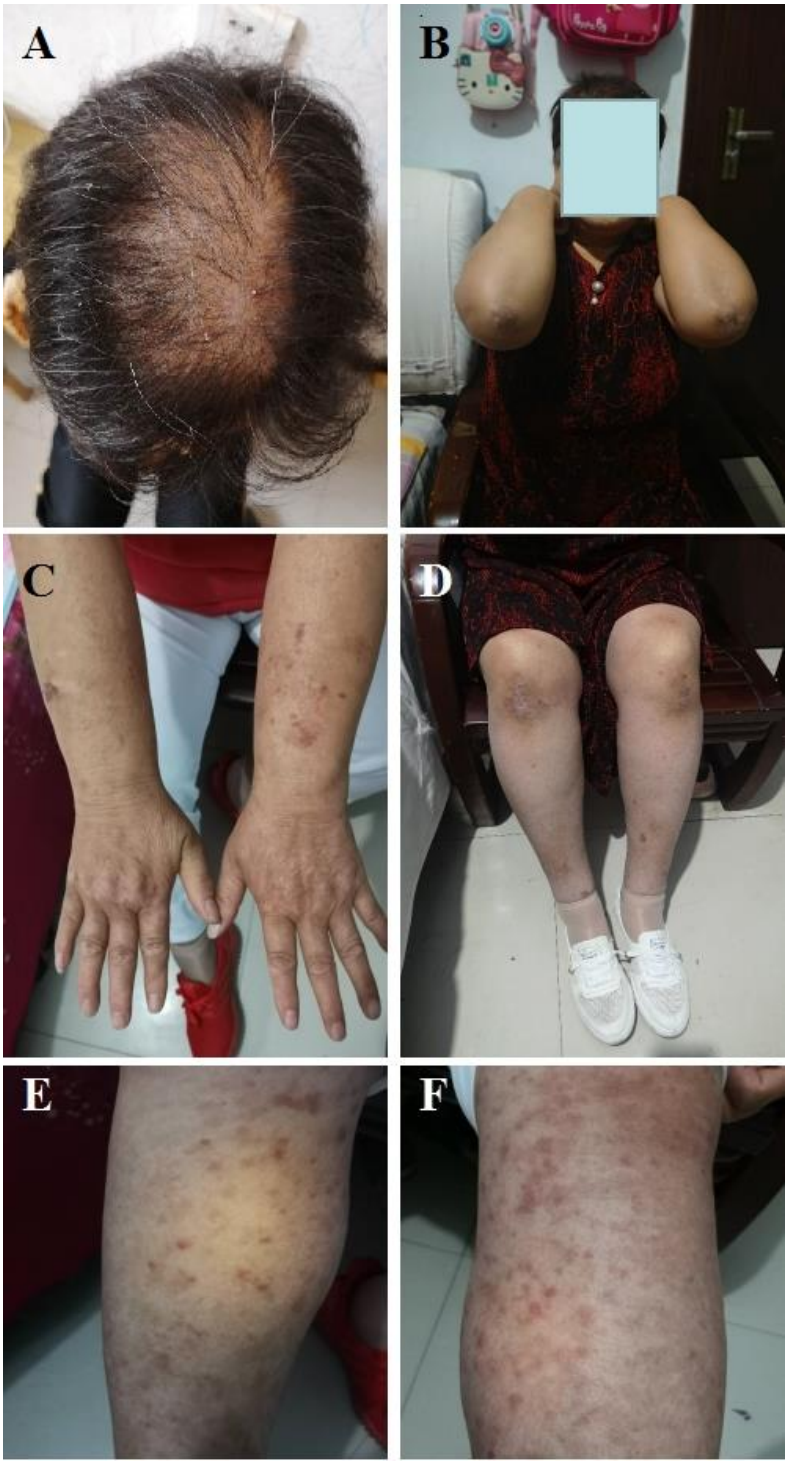
E, Fundus OCT scan in the right eye of patient Ⅱ-1, shows lamellar macular hole (White arrow).

F, Visual field photograph of right eye of patient Ⅱ-1,shows extension physiological blind spot (black arrow), and peripheral visual field impairment (yellow arrow), which is analogous to tubular visual field.

Table 1. Current ocular phenotypes of ELP patients in this pedigree

	II-1(LE/RE)	II-3(LE/RE)	II-5(LE/RE)	Normal range
Age (years in 2022)	68	65	62	
Age of first consultation (years)	12	6	50	
Best corrected visual acuity	NLP/0.5	0.25^{-1}/0.4	NLP/0.5	1.0/1.0
[a] Highest IOP (mm Hg)	45/22	19/20	50/21	10-21
[b] Axial elongation	29.95±0.008/30.33±0.007	[c]27.74±0.22/28.21±0.018	25.44±0.052/22.98±0.005	22.0-25.0
Diopter (5 m)	NA/ 0DS	+3DS/ +3DS+4DCx100^0	NA/ +9DS	0 D/0 D
[b] Astigmatism	1.72D@26^0/1.00D@14^0	[d]2.7D@59^0/4.59D@98	1.84D@19^0/0.96@164^0	<1.0 D
'Aphakic' in the area of the pupil	+/-	+/+	+/+	-/-
Direction of pupillae dislocation	Nasal/ Nasal	Surgical microcoria/surgical mydriasis	Nasal/ Nasal	no dislocation
Iridodonesis/Iris transmission	-/-	-/-	-/-	-/-
Poor pupillae dilatation	+/+	Surgical microcoria /surgical mydriasis	+/+	-/-
Anterior chamber angle	Open/Open	[e] PPS /[e] PPS	Open/Open	Open/Open
Visual field ([f] VFI,[g] MD)	NA/ 46%, -19.6dB	NA/88%, -10.15dB	NA/82%, -15.26dB	0%, 0 dB
Average thickness of [h] MGC (μm)	36/76	NA/81	47/83	>76
Perioptic nerve fiber thickness (μm)	69/ 63	NA/ 98	47/83	>76
Vertical cup/disc ratio (C/D)	0.98/0.79	NA/0.24	0.94/0.06	<0.6
High myopia fundus disease	+/+, [i] MLH	NA/+	+/-	-/-
Amblyopia (correct vision <0.6)	NA/+	+/+	NA/+	-/-
Glaucoma	+/-	-/-	+/-	-/-
Cataract	-/-	+/-	-/-	-/-

[a]IOP, intraocular pressure was measured with non-contact tonometer. [b]Axial elongation, corneal curvature and astigmatism were measured with LS900. [c]LE Axial elongation of II-3 was measured with B-ultrasonic. [d] Astigmatism of II-3 was measured with TOMEY; [e]PPS: partial peripheral iris anterior synechia.. [f]VFI, Visual field index. [g]MD:mean defect. [h]MGC, macular ganglion cells. [i]LMH: lamellar macular hole.



Supplementary figure 1. Major skin symptom in the proband (Ⅱ-5).

A, characters of thin hair, dark color and scaling symptoms in the head skin.

B-**F,** light red and swollen observed in the elbows (B), the upper limbs (C), the knees (D), the left leg (E), and the right leg (D).

Supplementary table 1. Summary of *ADAMTSL4* homozygous variants causing eye disease and major phenotypes.

[a]Variants	**p.Gln256ProfsX38**	**p.Arg309X**	**p.Ala388GlyfsX8**	**p.Tyr595X**	**p. Arg647AlafsX49**	**p.Pro654Ser**	**p.Q752X**
	c.759_778del20 or c.767_786del20	c.925C > T	c.1162dupG	c.1785T>G	c.1937dup	c.1960C>T	c.2254C>T
Eye disease and major symptom	[1] Case 1-8, **Isolated EL** diagnosed at early childhood. Spherophakia (case1, 3,5,). Iridodonesis (case 3,5,8). Myopia(case 3,8). Different direction of EL dislocation individually. [2] Case1-6, **isolated EL**. diagnosed at early childhood. Case 7, **ELeP.** Myopia(case 1-3,6). [3] Case1-5, **EL** diagnosed at early childhood. Case 6 diagnosed at <20ys. **ELeP** (case2,3,5,6). Iridodonesis (case 3-5). Increased IOP(case 2,5).	[4] Bilateral **EL** diagnosed at 4 years old. cataract, unilateral hearing loss, facial feature.	[5] Isolated **EL** diagnosed at 15 months and 6 months. Case 1 exhibits bilateral epicanthic folds, brachydactyly of the fifth finger. Case 2 exhibits high myopia, spherophakia, syndactyly toes, mildly microcephalic.	[6] Isolated **EL** diagnosed at 5-6 years old. Complications found in the affected family members include deprivation amblyopia, retinal detachment, and cataract.	[7] Bilateral **ELeP** diagnosed at 4 years old.	[8] **EL** diagnosed at 41 years old. arachnodactyly，slim marfanoid body，hyperextensible skin，mild functional mitral systolic murmur. minor involvement in skin system. major involvement in ocular system.	[9] Bilateral **ELeP** diagnosed at 46, 18, 6, 3, and 10 years old. poor pupillary dilation. poor vision. Iris transillumination (case 1 and 2). long AL (case 1 and 4).

[a]Variants included in the table are evaluated as pathogenicity predication, such as SIFT — damaging; Polyphen2: probably damaging; Mutationtaster — disease causing; **References:** [1.] Invest Ophthalmol Vis Sci. 2011, 52(2):695- 700. [2] Am J Med Genet A. 2015, 167A(10): 2376-81. [3] Invest Ophthalmol Vis Sci, 2010 , 51(12): 6369-73. [4]Eur J Med Genet. 2017. 60(9):465-473. [5] Am J Med Genet A. 2015, 167A(10): 2376-81. [6] Am J Hum Genet,. 2009, 84(2):274-8. [7] Eur J Med Genet. 2017. 60(9):465-473. [8] Human Mutation Mutation in Brief, 2010, 31: E1622-31. [9] Br J Ophthalmol, . 2013,97(5):583-7. References, only listed the published paper containing detail ocular examination.

Supplementary table 2. Summary of *ADAMTSL4* heterozygous mutations causing eye disease and major phenotypes.

[a]**Variants**	**p.Pro80ArgfsX53** **p.Gln256ProfsX38**	**p.Gly99AlafsX34** **p.Arg309X**	**p.Leu249TyrfsX21** **p.Gln256ProfsX38**	**p.Gln256ProfsX38** **p.Gln752X**	**p.Gln256ProfsX38** **p.Arg276SerfsX21**	**p.Leu606PhefsX38** **p.Arg865His**	**p.Q752X** **p.Q754fs**	**p.Ser264LeufsX37** **p.Gly757ValfsX62**
	c.237delC c.767_786del20	c.293delG c.925C>T	c.745del, c.767_786del	c.767_786del, c.2254C > T	c.767_786del c.826_836del	c.1783dupT c. 2594G>A	c.2254C>T c.2270dupG	c.789dup c.2270del
major symptom	Isolated **EL** diagnosed at 46 years old.	Congenital isolated **EL**, with retinal detachment, lattice degeneration.	Bilateral **EL** diagnosed at 9 years old, with pes planus, pectus carinatum.	Bilateral **EL** diagnosed at 24 and 10 years old separately. Case 1 exhibits cataract, iridodonesis, multiple skeletal and system features. And not belong to MFS. Case 2 exhibits translucent irides. Minimal mitral valve prolapse, pes planus.	Case 1, **EL** diagnosed at 6 years old. Case 2, **ELeP** diagnosed at 8 years old. They are unrelated.	Isolated **EL** diagnosed at 21 years old, with glaucoma and high intraocular, pressure in the left eye, secondary iris atrophy, axial elongation	Isolated **EL** diagnosed at 15 years old, with axial elongation	Three female patients in a pedigree, **ELeP** diagnosed at 12, 6, and 50 years old separately, with common features of poor vision. Case 1, glaucoma in the left eye, high myopia retinopathy, facial desquamation (8 years old). Case 2, cataract, high myopia retinopathy in the RE, gray scalp, lepidic and patchy pattern skin. Case 3, high myopia retinopathy in the LE, glaucoma, poor vision, gray scalp, lepidic and patchy pattern skin.
[b]**References**	Invest Ophthalmol Vis Sci. 2012, 53(8):4889-96.		Eur J Med Genet. 2017. 60(9):465-473.		Human Mutation Mutation in Brief, 2010, 31: E1622-31.	Acta Ophthalmol,. 2015, 93(1):e91-2.	Br J Ophthalmol, . 2013,97(5):583-7.	In this study.

[a] Variants included in the table are evaluated as pathogenicity predication, such as SIFT — damaging; Polyphen2: probably damaging; Mutation taster — disease causing. [b] References, only listed the published papers with detail information about ocular examination.