A Genotype-Phenotype Comparison of *ADAMTSL4* and *FBN1* in Isolated Ectopia Lentis

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Purpose. To describe the genotype-phenotype relationship of a cohort of consecutive patients with isolated ectopia lentis (EL) secondary to *ADAMTSL4* and *FBN1* mutations.

Methods. Patients underwent detailed ocular, cardiovascular, and skeletal examination. This was correlated with Sanger sequencing of *ADAMTSL4* and *FBN1* genes.

Results. Seventeen patients were examined, including one with ectopia lentis et pupillae. Echocardiography and skeletal examination revealed no sign of systemic disorders associated with EL, in particular Marfan syndrome (MFS). Nine patients (52.9%) were found to have mutations in *ADAMTSL4*, including four novel nonsense mutations. Four patients (25%) were found to have novel *FBN1* mutations, not previously reported as causing classical Marfan syndrome. One additional patient was found to have an *FBN1* mutation previously reported in classical MFS. Four patients (25%) were found to have no mutations in either gene. Median age of diagnosis of EL was 35 years in patients with *FBN1* mutations and 2 years in patients with *ADAMTSL4* mutations ($P < 0.01$). Mean axial length was 22.74 mm (95% confidence interval [CI]: 21.3–24.2) (*FBN1*) and 27.54 mm (95% CI: 24.2–30.9) (*ADAMTSL4*) ($P < 0.01$). Other ophthalmic features, including corneal thickness and power, foveal thickness, visual acuity, and direction of lens displacement, were similar for both groups.

Conclusions. *ADAMTSL4* is the most important known causative gene in isolated EL. Mutations in *ADAMTSL4* appear to cause earlier manifestation of EL and are associated with increased axial length as compared to *FBN1*. We suggest that *ADAMTSL4* be screened in all patients with isolated EL and that physicians be vigilant for the more severe ocular phenotype associated with mutations in this gene. (*Invest Ophthalmol Vis Sci.* 2012;53:4889–4896) DOI:10.1167/iovs.12-9874

Inherited ectopia lentis (EL) was first described in three generations by Horner[1] in the 19th century. Since Antoine Marfan first described the condition in 1896 that later bore his name (Marfan syndrome: MFS OMIM 154700), the association between EL and MFS has been established. MFS is a multisystem condition, diagnosed according to the defined Ghent criteria.[2] In 1991, the fibrillin-1 gene (*FBN1*) was linked with MFS,[3] and since then, more than 800 mutations in this gene have been identified.[4] In addition to MFS, other inherited causes of EL include isolated EL (OMIM 129600),[5] homocystinuria (OMIM 236200)[6] (cystathionine β-synthase gene), Weill-Marchesani syndrome (OMIM 277600)[7] (*FBN1* and *ADAMTS10* genes), Weill-Marchesani–like syndrome (OMIM 613195)[8] (*ADAMTS17* gene), Knobloch syndrome 1 (OMIM 267750)[9] (*COL18A1* gene), Knobloch syndrome 2 (OMIM 608454)[10] (*ADAMTS18* gene), and mutations in latent transforming growth factor-β–binding protein gene (*LTBP2*) (OMIM: 602091).[11] Isolated ectopia lentis is the most frequent of the alternative hereditary causes of EL. It can be inherited in an autosomal dominant manner (OMIM: 129600), most commonly caused by novel mutations in *FBN1* not described in patients with classical MFS.[12] Isolated EL can also be inherited in an autosomal recessive pattern (OMIM 225100). In 2009, Ahram and colleagues[13] first described a homozygous nonsense mutation in *ADAMTSL4* (OMIM: 610113) in a consanguineous family. Mutations in this gene have been reported across Europe in cases with isolated EL[14–16] and isolated ectopia lentis et pupillae (ELetP: OMIM 225200).[17] Phenotypic differences between patients with isolated EL caused by *FBN1* and *ADAMTSL4* mutations have not yet been established. We investigated a consecutive cohort of patients with isolated EL to document the relationship of their genotype to clinical phenotype.

Methods

Ethical approval was obtained from the regional ethics committee (protocol number 10/H0311/39) and the study conformed to the Declaration of Helsinki. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study.

Consecutive patients presenting between January 2011 and December 2011 and diagnosed with isolated EL (present or previously

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operated upon), attending clinics at Moorfields Eye Hospital, The Royal London Hospital, Ninewells Hospital, and Guy's Hospital, were identified and invited to participate in the study. Isolated EL was diagnosed according to current Ghent criteria.[2] Ophthalmic examination included visual acuity measurement, slit lamp examination, Goldman applanation tonometry for intraocular pressure measurement, gonioscopy, dilated examination of lens and fundus, fundal photography, spectral domain optical coherence tomography, corneal pachymetry and topography analysis (Pentacam high-resolution rotating Scheimpflug imaging system [Pentacam HR, Oculus, Wetzlar, Germany]), and axial length measurement (IOL Master [Carl Zeiss Meditex, Jena, Germany]). Statistical analysis (descriptive analysis and Mann–Whitney *U* test where appropriate) was performed by using SPSS for Windows version 19.0 (SPSS Inc., Chicago, IL).

Systemic examination included measurement of arm span, upper and lower segment height, skeletal examination (palate, scoliosis, pectus deformity, and acromegaly), and Beighton score analysis of joint hypermobility.[18] If not previously done, echocardiography was performed at St George's Hospital, London. Two-dimensional echocardiography was performed with either the Philips iE33 or Vivid 7 (GE Medical Systems, Milwaukee, WI). Standard cardiac views were obtained and analyzed according to protocols specified by the European Society of Echocardiography[19] and the American Society of Echocardiography.[20] Left ventricular (LV) wall thickness, left atrial diameter, LV diameter, LV mass, transverse aortic root dimension, and diastolic function were measured.

Genomic DNA was extracted from 2-mL peripheral blood by using the Flexigene DNA extraction kit (Qiagen, Crawley, UK). Genomic DNA was subjected to PCR by using a set of 65 oligonucleotide primer pairs (Sigma-Genosys, Cambridge, UK) to amplify all 65 exons and intron/exon boundaries of the *FBN1* gene as described.[21] Detailed protocols are available on request. PCR amplification of *ADAMTSL4* was carried out by using 18 oligonucleotide primer pairs to amplify all 19 exons of *ADAMTSL4* as previously described.[13]

Single-strand confirmation analysis and denaturing high-performance liquid chromatography and direct sequencing were carried out for *FBN1* analysis.[22] One hundred sixty chromosomes from unrelated control individuals, with the same ethnic distribution as the study group, were tested for identical mutations in *FBN1* and *ADAMTSL4* to establish if the mutations could be considered as polymorphisms, and to confirm their association with EL.

Results

Eighteen unrelated consecutive patients diagnosed with isolated EL were recruited. One patient was found to have an *FBN1* mutation, which has been previously reported in classical MFS (see below). Although no cardiac and skeletal features of MFS were present, this patient was rediagnosed as having MFS according to the revised Ghent criteria.[2] A further patient, on ophthalmic examination, was clarified to have ELetP. Detailed ocular phenotyping was therefore undertaken in 16 unrelated patients with isolated EL, and one patient with ELetP (Fig. 1A). Two patients were bilaterally phakic (Fig. 1B), and one patient was aphakic secondary to posterior lens dislocations. All other patients had had ocular surgery in the past for ectopia lentis: two via an anterior segment approach, and the remainder via a posterior approach.

Genetics

FBN1 cDNA sequence according to GenBank (RefSeq NM_000,138.3, provided in the public domain by http://www.ncbi.nlm.nih.gov/gene) was used, with the A of the ATG translation initiation codon as nucleotide +1. The initiation codon is identified as codon 1.

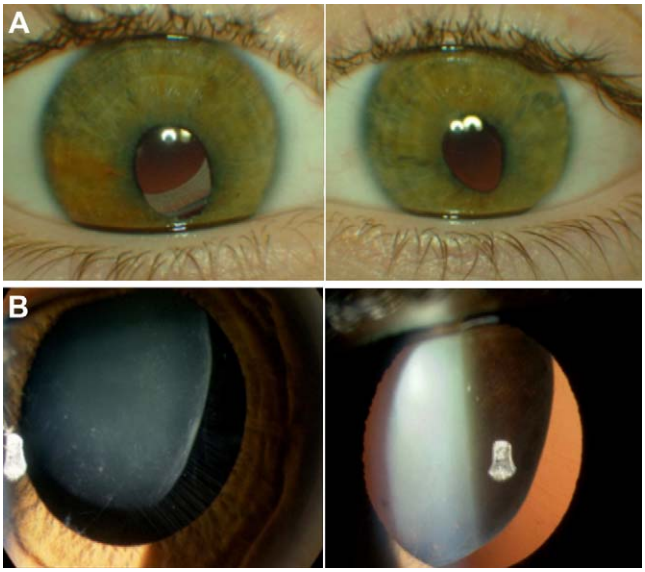

Figure 1. (**A**) Patient with ELetP. (**B**) Isolated EL in a patient with a homozygous c.767_786del20 (p.Gln256Profs*38) mutation in *ADAMTSL4*.

ADAMTSL4 cDNA sequence according to GenBank (RefSeq NM_019,032.4) was used, with the A of the ATG translation initiation codon as nucleotide +1. The initiation codon is identified as codon 1.

FBN1. Four patients (25%) were discovered to have heterozygous mutations in *FBN1* (Table 1), which have not previously been reported in MFS. Three of these, c.4259G>A (p.Cys1420Tyr), c.3464A>G (p.Asp1155Gly), and c.2473C>T (p.Pro825Ser), were missense mutations. The first affected a consensus amino acid in a calcium binding (cb) epidermal growth factor-like (EGF) (cbEGF-like) domain, whilst the second resulted in a change of a cysteine residue within a cbEGF-like domain. In silico analysis (SIFT,[23] PolyPhen[24]) revealed these mutations to be pathogenic. The third affected a nonconsensus amino acid in a cbEGF-like domain of fibrillin-1 and has previously been reported in a patient with a fibrillinopathy not fulfilling the Ghent criteria.[25] The fourth was an intronic mutation (c.1327+1 G>A) in IVS10 predicted to abolish a splice donor site.[26] A further patient (a female diagnosed with isolated EL at 46 years of age with no cardiovascular features of MFS) was found to have a missense mutation in *FBN1* (c.3344A>G (p.Asp1115Gly)), which has previously been reported in classical MFS.[27] Her diagnosis was therefore altered to MFS and she was not included for analysis in this study. The final study group thus consisted of 17 patients.

ADAMTSL4. Nine patients (53%) were found to have mutations in *ADAMTSL4*, which were thought to be causative. Six (66.7%) were homozygous for a nonsense 20-bp deletion (c.767_786del20 (p.Gln256Profs*38)). This mutation resulted in a frameshift leading to a premature termination codon (PTC) after 38 codons of altered reading frame.

The remaining three patients were presumed compound heterozygotes for *ADAMTSL4* mutations. These included four novel mutations (Tables 2 and 3) in exons 5 and 6 for isolated EL, and exon 14 for ELetP (Fig. 2). The patient with ELetP was presumed compound heterozygous for two mutations: the 20-bp deletion (above) and a novel mutation: c.2270dupG (p.Gly758Trpfs*59). Segregation analysis was not possible for the presumed compound heterozygous mutations. All mutations were nonsense, thus resulting in a PTC. One additional

Table 1. Genetic Information of Patients with Isolated EL and *FBN1* Mutations

				Genetic Mutation			
Patient	**FH**	**Consanguinity**	**Origin**	**Nucleotide**	**Amino Acid**	**Exon**	**Zygosity**
1	No	No	White British	c.2473C>T	p.Pro825Ser	20	Heterozygous
2	No	No	White British	c.3464A>G	p.Asp1155Gly	28	Heterozygous
3	No	No	White Polish	c.4259G>A	p.Cys1420Tyr	34	Heterozygous
4	No	No	White British	c.1327+1 G>A	Splice site mutation	10	Heterozygous

FH, family history.

patient (patient 8) was found to have only a heterozygous c.767_786del20 mutation. No other mutations were found in *ADAMTSL4* or *FBN1*. This mutation does not cause EL in heterozygous carriers. Familial segregation analysis was not possible. This patient was thus placed in the "unknown cause" group.

Unknown. Four patients (25%) were not found to have any causative mutations in *FBN1* or *ADAMTSL4*, including the patient described above with a heterozygous *ADAMTSL4* mutation. Two patients had affected family members. Case 5 (male) has reportedly two affected brothers. Case 6 has an affected maternal aunt. These suggest nonautosomal dominant inheritance. Family members were not available for analysis.

The *FBN1* and *ADAMTSL4* mutations described here were not observed in the control group. Furthermore, they are not reported in the Genbank dbSNP library, 1000 Genomes, or the Exome Variant Server.

Cardiovascular Findings

All patients were normotensive (<140/90 mm Hg) and none were found to have abnormal indices of LV or atrial dimensions, aortic root dimension, or LV function. There were no differences between groups.

Musculoskeletal Findings

Two patients had normal range Beighton joint hypermobility scores of 2/9. All others scored 0. No patients had any skeletal features of connective tissue disorder or MFS.

Ophthalmologic Phenotype

Sixteen patients with isolated EL and one with ELetP (Fig. 1B) were examined. Ophthalmic parameters were measured for each individual eye, and a mean was calculated per patient (Table 4). The mean values for all patients were then used in statistical analysis.

Age of Diagnosis. For the purpose of analysis, patients who reported the diagnosis of congenital EL were allocated the age of onset of 0.5 years. The median age of diagnosis of ectopia lentis was 35 years (range, 15–46 years) in the *FBN1* group, 8.5 years (range, 3–47 years) in the unknown group, and 2 years (range, 0.5–46 years) in the *ADAMTSL4* group. Nine of 10 in the *ADAMTSL4* group were diagnosed in childhood. One patient (patient 13, Table 4) was diagnosed at 46 years of age. Two explanations may account for this late diagnosis. Firstly, the patient was found to have a novel mutation in exon 5, and it is possible that this mutation somehow protects from an early manifestation of EL. However, she admitted to having poor vision most of her life, and at diagnosis was found to have significant EL. It is more likely that her vision was affected by EL at an earlier age, but was not diagnosed. Excluding this outlier, the median age of diagnosis of EL in the *ADAMTSL4* group was 2 years (range, 0.5–9 years). Comparing the mutation groups revealed that patients with *ADAMTSL4* mutations were affected by isolated EL at a significantly younger age than those with *FBN1* mutations (Fig. 3A) (2 years vs. 35 years, $P < 0.01$).

Axial Length. Patient 16 and 17 (ELetP) were examined at 8 years and 11 years of age. All others were assessed as adults (>18 years old). We excluded axial length (AL) of the children in the analysis, as AL in this age range is not comparable to that

Table 2. Genetic Information of Patients with EL and *ADAMTSL4* Mutations

				Genetic Mutation			
Patient	**FH**	**Consanguinity**	**Origin**	**Nucleotide**	**Amino Acid**	**Exon**	**Zygosity**
8†	No	No	White British	c.767_786del20	p.Gln256Profs*38	6	Heterozygous
9	No	No	White British	c.767_786del20	p.Gln256Profs*38	6	Homozygous
10	No	No	White British	c.767_786del20	p.Gln256Profs*38	6	Homozygous
11	No	No	White British	c.767_786del20	p.Gln256Profs*38	6	Homozygous
12	No	No	White British	c.293delG	p.Gly99Alafs*34	5	Presumed compound
				c.925C>T	p.Arg309*	6	heterozygous
13	No	No	White British	c.237delC	p.Pro80Argfs*53	5	Presumed compound
				c.767_786del20	p.Gln256Profs*38	6	heterozygous
14	No	No	White British	c.767_786del20	p.Gln256Profs*38	6	Homozygous
15	Affected brother	No	White British	c.767_786del20	p.Gln256Profs*38	6	Homozygous
16	No	No	White British	c.767_786del20	p.Gln256Profs*38	6	Homozygous
17	No	No	White British	c.767_786del20	p.Gln256Profs*38	6	Presumed compound
				c.2270dupG	p.Gly758Trpfs*59	14	heterozygous

† Patient 8: Heterozygous mutation not thought to be causative.

Table 3. Published Mutations in *ADAMTSL4* Causing EL

Exon/Intron	Mutation	Reference
Exon 11	c.1785T/G	Ahram et al.[13] (2009)
Intron 4	IVS4-1G>A	Greene et al.[14] (2010)
Exon 5	c.293delG	This study
	c.237delC	This study
Exon 6	c.767_786del	Aragon-Martin et al.[15] (2010) Neuhann et al.[16] (2011) Christensen et al.[17] (2010)
	c.826_836del	Aragon-Martin et al.[15] (2010)
	c.926G>A	Aragon-Martin et al.[15] (2010)
	c.925C>T	This study
Exon 12	c.2008C>T	Aragon-Martin et al.[15] (2010)
	c.1960C>T	Aragon Martin et al.[15] (2010)
Exon 14	c.2270dupG	This study
Exon 19	c.3153C>A	Aragon-Martin et al.[15] (2010)
Exon 19	c.3161A>G	Aragon Martin et al.[15] (2010)

in adults. Mean AL was 22.74 mm (95% confidence interval [CI]: 21.3–24.2) for the *FBN1* group, 27.54 mm (95% CI: 24.2–30.9) in the *ADAMTSL4* group, and 24.55 mm (95% CI: 18.8–30.3) for the unknown group. Comparing the two mutation groups (Fig. 3B) revealed that patients with *ADAMTSL4* mutations had significantly longer ALs ($P < 0.01$). If the AL of the children with isolated EL are included, analysis still reveals the difference to be significant ($P = 0.01$).

Visual Acuity. Mean ETDRS letters score was 59 (95% CI: 17–101) for the *FBN1* group, 58 (95% CI: 44–73) for the *ADAMTSL4* group, and 72 (95% CI: 60–83) for the unknown group. The differences were not significant.

Corneal Thickness. Mean corneal thickness was 410.2 μm (95% CI: −26 to 846) for the *FBN1* group, 566.1 (95% CI: 515.3–616.8) for the *ADAMTSL4* group, and 561.1 μm (95% CI: 552.4–569.8) in the unknown group (not significant).

Corneal Power. Mean corneal refractive power was 40.9 diopters (D) (95% CI: 40.0–41.8) in the *FBN1* group, 41.5 D (95% CI: 39.7–43.3) in the *ADAMTSL4* group, and 41.7 D (95% CI: 39.6–43.8) in the unknown group (not significant).

Foveal Thickness. Mean foveal thickness was 352.5 μm (95% CI: −73.2 to 778.2) for the *FBN1* group, 302.4 μm (95% CI: 251.8–353.0) for the *ADAMTSL4* group, and 359.5 μm (95% CI: 306.5–412.5) in the unknown group (not significant).

Intraocular Pressure (IOP). Mean IOP was 16.9 mm Hg (95% CI: 13.7–20.1) in the *FBN1* group, 16.6 mm Hg (95% CI: 13.7–19.6) in the *ADAMTSL4* group, and 18 mm Hg (95% CI: 12.9–23.1) in the unknown group (not significant).

Optic Disc Features. Mean cup to disc ratio was 0.25 (95% CI: 0.02–0.48) in the *FBN1* group, 0.2 (95% CI: 0.1–0.3) in the *ADAMTSL4* group, and 0.36 (95% CI: 0.16–0.56) in the unknown group (not significant).

No difference was observed in any features of the optic nerve head.

Other Ophthalmic Features. No pattern was noted with regard to the direction of lens subluxation or dislocation. Furthermore, gonioscopy did not reveal any unusual angle strands. All angles were open (>45°) at the time of examination. No significant pattern was observed for other associated ophthalmic conditions.

Discussion

Non-traumatic EL is most commonly associated with MFS. In the most extensive multinational study of 1013 patients with MFS,[4] EL was found in 54% of patients with MFS. Although more than 800 mutations in *FBN1* have been identified,[4] analysis suggests that a significantly higher proportion of

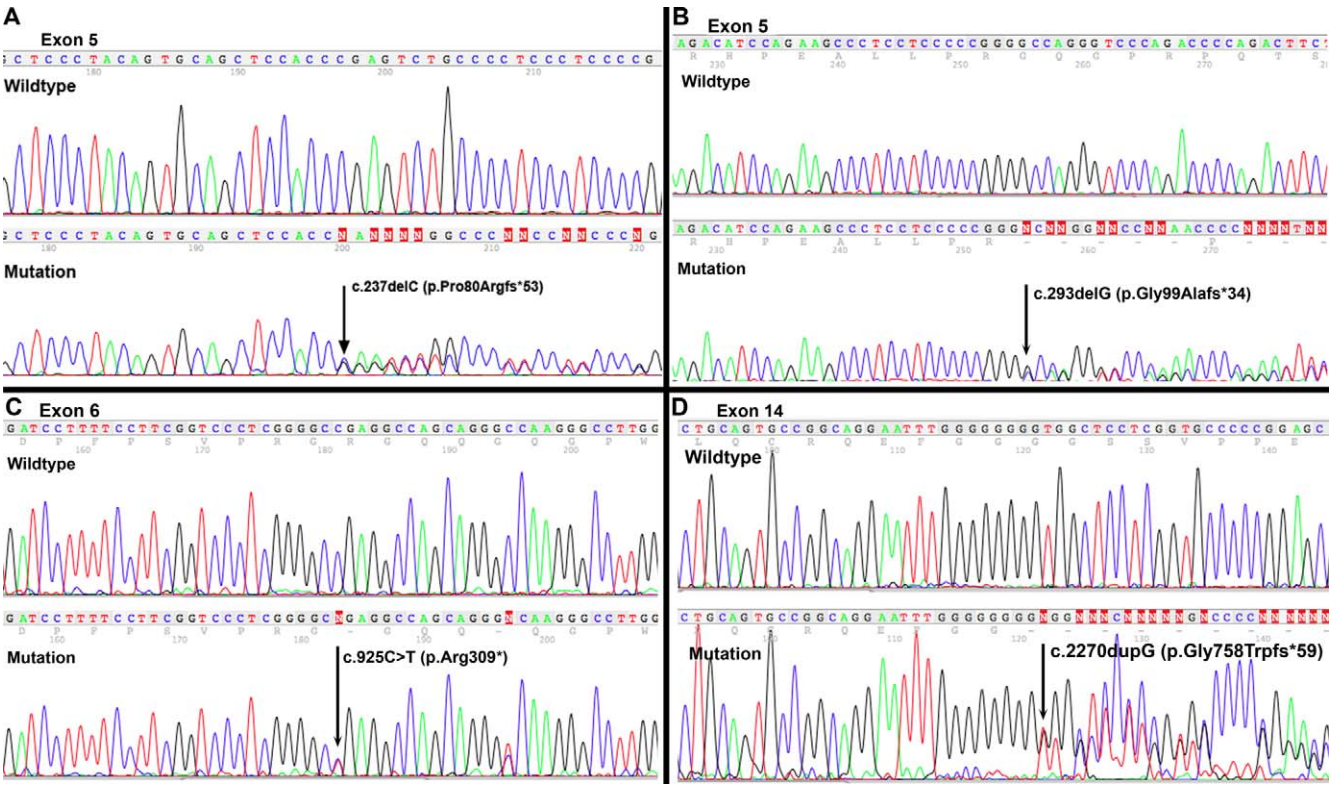

Figure 2. Sequence chromatographs showing novel mutations in *ADAMTSL4*.

Table 4. Ocular Phenotype of EL Patients

Patient	Age (y)	Sex	Genetic Mutation	Age of Diagnosis (y)	Axial Length (mm)	Visual Acuity (ETDRS Letters)	Direction of Subluxation	Surgery	Other Ophthalmic Disorders
1	55	M	*FBN1*	46	24.03	46.50	Nasal	PPV	Nil
2	70	M	*FBN1*	29	22.16	84.50	Inferior	PPV	Nil
3	18	F	*FBN1*	15	22.68	77.50	Temporal	Nil	Nil
4	64	F	*FBN1*	41	22.10	28.50	Inferior	PPV	Narrow angles
5	58	M	U	47	24.15	79.50	Nasal	PPV	Nil
6	27	M	U	3	29.16	68.00	Inferior	PPV	Exophoria
7	13	F	U	11	20.32	76.50	Inferior	PPV	Nil
8	57	M	*ADAMTSL4*	6	24.60	64.00	Temporal	PPV	Nil
9	45	F	*ADAMTSL4*	2	27.12	84.50	Temporal	PPV	Exophoria
10	62	F	*ADAMTSL4*	9	31.34	67.00	Posterior	Nil	Staphyloma
11	31	F	*ADAMTSL4*	C	24.53	54.50	Posterior	PPV	Exotropia
12	20	M	*ADAMTSL4*	C	25.89	68.50	Posterior	PPV	Retinal detachment lattice degeneration
13	46	F	*ADAMTSL4*	46	21.49	77.50	Nasal	Phaco	None
14	38	F	*ADAMTSL4*	1	31.68	41.50	Superior	PPV	Staphyloma
15	19	M	*ADAMTSL4*	4	24.69	46.00	Temporal	Nil	Nil
16	8	M	*ADAMTSL4*	2	22.73	45.00	Inferonasal	Nil	Nil
17	11	F	*ADAMTSL4*	2	20.99	71.50	Superior	Phaco	ELetP

C, congenital; Phaco, phacoemulsification; PPV, pars plana vitrectomy; U, no mutation found.

missense mutations involving cysteine residues and mutations at the 5′ end are causative in EL.[4,15,28]

After MFS, the most common clinical manifestation of inherited EL is isolated EL. Although the exact prevalence is unclear, a Danish national study suggested that a nosologic diagnosis could not be given in up to 31% of cases of congenital EL.[29] Up to 10% of cases of congenital EL in that series had autosomal recessive EL. Both *FBN1*[12] and *ADAMTSL4*[15] have been shown to be causative, primarily in autosomal dominant and autosomal recessive EL, respectively.

Gene mutations causing isolated EL are found in *FBN1* and *ADAMTSL4*. Patients with isolated EL and *FBN1* mutations have been reported to develop cardiovascular complications,[30] thus prompting close long-term systemic follow-up. These patients may be expected to have a similar phenotype to MFS. Indeed, if the mutations in *FBN1* become established in other patients with a confirmed diagnosis of MFS, that is enough to change the diagnosis for these patients to MFS.[2] The results of this study suggest that patients with *ADAMTSL4* mutations represent a distinct group from those with *FBN1* mutations, thus highlighting the importance of analyzing for this gene in isolated EL patients with no clear dominant family history.

Although Christensen et al.[17] did not comment on their patients' age of onset of ELetP, all patients reported by Neuhann et al.[16] and our group,[15] with *ADAMTSL4* mutations, were affected before the age of 15 years. In the cohort investigated in the current study, the median age of diagnosis for patients with *ADAMTSL4* mutations was significantly younger than for those with *FBN1* mutations. This fits well with the observation that the median age of EL diagnosed in MFS seems to be later.[4] Thus, mutations in *ADAMTSL4* may cause a more severe ocular phenotype than mutations in *FBN1*. Notably, of all patients diagnosed before the age of 10 years in the current cohort ($n = 9$), 8 (89%) had *ADAMTSL4* mutations. This suggests that a proband diagnosed with isolated EL as a child, with no features of MFS, should have *ADAMTSL4* screening as the primary candidate gene.

Myopia is the most common ocular manifestation of MFS,[2] and Maumenee[31] found a mean AL of 24.65 (±2.21) mm in MFS. Population studies suggest normal AL ranges between 23.4 and 23.9 mm.[32,33] One might therefore expect that patients with EL secondary to *FBN1* mutations would have longer ALs than normal and possibly than those of individuals with *ADAMTSL4* mutations. We, however, found the converse: AL was significantly longer in our group of adult patients with *ADAMTSL4* mutations. Although we excluded the AL of an 8-year-old child with isolated EL, analysis revealed that inclusion would not have altered the statistical significance greatly. Additionally, the mean AL in this proband was 22.73 mm. This child had progressive high myopia (–9 Dioptre Sphere [DS]), which, if not secondary to anterior lens displacement, would suggest that the AL as an adult will be greater. Although the median AL was lower in 10 patients with *ADAMTSL4* mutations and ELetP (median, 22.79 mm),[17] this may reflect a difference in the conditions of EL and ELetP. Indeed, the one patient in our cohort with ELetP had a lower AL, although she was assessed as a child and her AL may still increase. Both a primate study[34] and a recent clinical study[35] have suggested that peripheral refraction and blur may have a more significant effect than foveal blur on the development of axial myopia. Ectopic pupils may alter this phenomenon and thus account for the difference between ELetP and EL.

We suggest that the increased AL seen in our *ADAMTSL4* cohort, compared to the *FBN1* cohort, may be related to the young age of onset of the EL in the *ADAMTSL4* group. AL is known to increase at a faster rate in children younger than 10 years,[36] and AL growth has been suggested to be greater in children with EL than the normal population.[37] It is thought that retinal blur and change of focal plane can result in increased AL.[38,39] Reduced quality of retinal imaging during childhood triggers AL elongation, a phenomenon known as "form deprivation myopia" (FDM).[40,41] The phenomenon known as "lens-induced myopia" (LIM) is thought to be due to optical blur inducing AL increase.[41] It appears that FDM is controlled by local retinal mechanisms, whilst LIM is controlled by local and central mechanisms.[42] Indeed, such induced myopia can have a similar effect on the contralateral eye.[43] Although the critical age before which FDM has an impact is unclear, it is evident that the earlier the deprivation is initiated and the longer it is maintained, the greater is the degree of the relative myopia.[41] We therefore suggest that the younger age of onset of EL in our *ADAMTSL4* compared to *FBN1* cohorts may

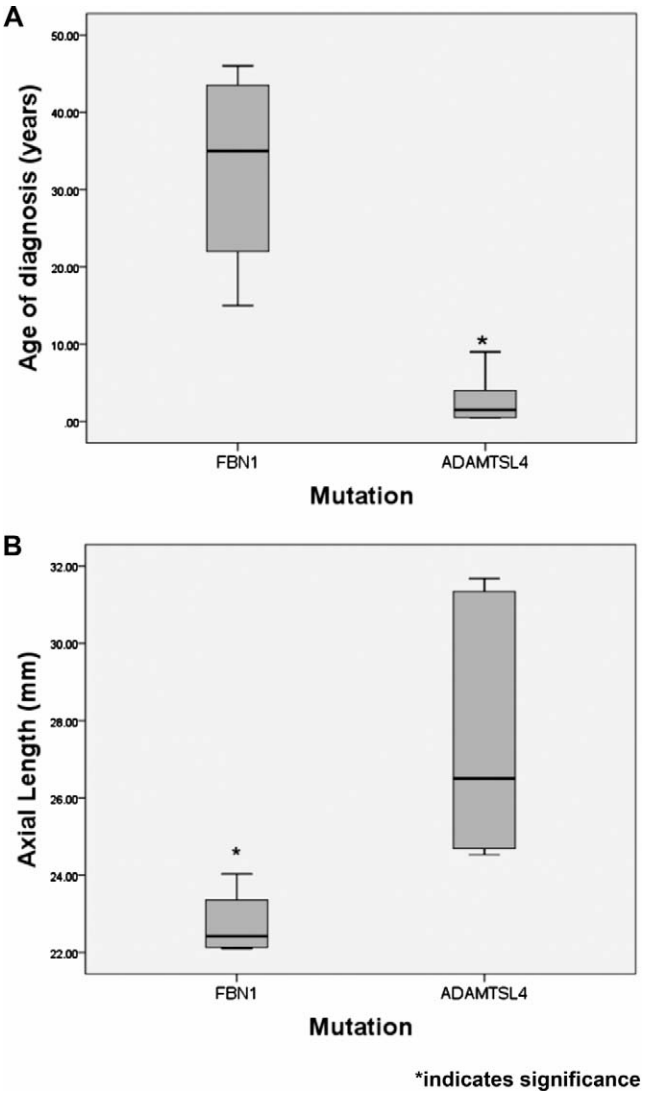

Figure 3. (**A**) Age of diagnosis (years) of isolated EL in patients found to have *FBN1* or *ADAMTSL4* mutations. (**B**) AL (mm) in patients diagnosed with isolated EL secondary to *FBN1* or *ADAMTSL4* mutations.

have resulted in increased AL. The relationship between the optics of EL and axial myopia has been previously suggested.[44] Conversely, one cannot exclude the possibility that this increased AL is secondary to effects of *ADAMTSL4* itself.

Other phenotypic characteristics we measured did not differ significantly between groups. Importantly, corneal refractive power was equal. It has been reported that patients with MFS have flatter corneas and it has been suggested that keratometry values of 42 D or less may be used as clinical diagnostic criteria.[45] The corneal curvatures in all our three groups were below this value.

Furthermore, central corneal thickness is suggested to be lower in Marfan syndrome than in controls.[45,46] The only article addressing this in *ADAMTSL4* mutations suggests that the mean corneal thickness in 10 patients with the 20-bp mutation and ELetP is 589 μm (range, 528–630 μm),[17] leading the authors to suggest that patients with this mutation may have thicker corneas. This was not replicated in our larger cohort and may again reflect a difference in the clinical phenotypes of EL and ELetP. The patient with ELetP in the present cohort had a mean corneal thickness of 560 nm. However, our data on corneal measurement must be interpreted with caution, as many of the patients had had intraocular surgery, which could affect the keratometry and pachymetry measurements.

ADAMTS-like 4 protein has recently been shown to be expressed throughout ocular tissue, and is thought to colocalize with fibrillin-1.[47] Additionally, Gabriel and colleagues[47] suggest that ADAMTS-like 4 is a fibrillin-1–binding protein that facilitates microfibril assembly. We suggest, however, that in view of the more severe phenotype found in our cohort with *ADAMTSL4* mutations, there may be additional independent roles for ADAMTS-like 4.

Four mutations in *FBN1* in our cohort have as yet to be reported in MFS. These patients, with no cardiac features of MFS, were therefore confirmed as having isolated EL. One of our initial cohorts, previously diagnosed with isolated EL, was found to have a mutation in *FBN1* (c.3344A>G (p.Asp1115Gly)) previously reported in MFS, thus altering her diagnosis. This patient' case is one of those in the 10% of discordance between the old and new Ghent criteria.[2] Careful systemic monitoring of patients with *FBN1* mutations and EL is recommended.

The four patients in our cohort with as yet unknown mutations provide an interesting group of EL. The pedigrees of the two probands with other affected members suggest nonautosomal dominant inheritance. Further screening of novel candidate genes for these and other such patients would be of interest.

The most common mutation reported in *ADAMTSL4* associated with EL is a 20-bp deletion (c.767_786del) described in 15 unrelated families across Europe.[15–17] This mutation is thought to have originated more than 4000 years ago[17] in a common ancestor.

The most common mutation we found in *ADAMTSL4* was this deletion. This confirms the importance of this mutation across Europe, and supports our suggestion to investigate exon 6 first. Of additional interest is patient 8. This patient had a heterozygous change in this mutation, with no other heterozygous changes found. It is difficult to envisage this to be causative, and the patient's parents were unavailable for segregation analysis. Such single heterozygous changes have been previously described in other autosomal recessive ocular conditions.[48]

We have discovered a further four nonsense mutations in exons 5, 6, and 14 of *ADAMTSL4*. Whether these or previously reported nonsense mutations result in truncated protein, or mRNA that undergoes nonsense-mediated degradation, is as yet unknown, although it has been proposed that the latter is more likely.[49]

This study brings the total of *ADAMTSL4* mutations causing autosomal recessive EL and ELetP to 13, including the four novel mutations described here (Table 3). The importance of this gene in isolated EL is becoming more apparent. We have previously suggested that *FBN1* mutations account for most isolated cases of EL.[15] This new cohort of unrelated individuals, however, suggests that *ADAMTSL4* mutations account for a greater proportion. This may reflect the more specific recruitment in this study from ophthalmic units. We suggest that in cases without a clear dominant inheritance, *ADAMTSL4* is the most commonly indicated gene, and exons 6 and 5 appear to be the most relevant to screen first.

Conclusions

Our study investigated the genotype-phenotype correlation of patients with isolated EL caused by *FBN1* and *ADAMTSL4* mutations. Distinguishing between these groups of patients is

clearly of importance. We showed that *ADAMTSL4* mutations are the most common cause of this condition and seem to produce a more severe ocular phenotype with earlier onset. This poses further questions as to the function of this protein. We also showed that most patients diagnosed with isolated EL in childhood are affected by *ADAMTSL4* mutations. Although patients with *ADAMTSL4* mutations carry a lower cardiovascular risk than those with *FBN1* mutations, their ocular comorbidity may be greater. Physicians need to thus be vigilant in such cases.

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