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**Role of ADAMTSL4 Mutations in FBN1  
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# Role of *ADAMTSL4* Mutations in *FBN1*

## Mutation-Negative Ectopia Lentis Patients.



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Short Title: *FBN1* and *ADAMTSL4* mutations in ectopia lentis.

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**ABSTRACT:** Ectopia lentis (EL) is genetically heterogeneous with both autosomal-dominant and -recessive forms. The dominant disorder can be caused by mutations in *FBN1*, at the milder end of the type-1 fibrillinopathies spectrum. Recently in a consanguineous Jordanian family, recessive EL was mapped to locus 1q21 containing the *ADAMTSL4* gene and a nonsense mutation was found in exon 11 (c.1785T>G, p.Y595X). In this study, 36 consecutive probands with EL who did not fulfill the Ghent criteria for MFS were screened for mutations in *FBN1* and *ADAMTSL4*. Causative *FBN1* mutations were identified in 23/36 (64%) of probands while homozygous or compound heterozygous *ADAMTSL4* mutations were identified in 6/12 (50%) of the remaining probands. Where available, familial screening of these families confirmed the mutation co-segregated with the EL phenotype. This study confirms that homozygous frameshift mutations in *ADAMTSL4* are associated with autosomal-recessive EL in British families. Furthermore; the first compound heterozygous mutation is described resulting in a PTC and a missense mutation in the PLAC (protease and lacunin) domain. The identification of a causative mutation in *ADAMTSL4* may allow the exclusion of Marfan syndrome in these families and guide the clinical management, of particular relevance in young children affected by EL. ©2010 Wiley-Liss, Inc.

**KEY WORDS:** Ectopia lentis, Marfan syndrome, *FBN1*, *ADAMTSL4*

### INTRODUCTION

Marfan syndrome (MFS, MIM 154700, <http://www.ncbi.nlm.nih.gov/Omim/>) is an autosomal dominant inherited connective tissue disorder with a prevalence of about 1 in 5-10,000 (Pyeritz, 1993). MFS is characterised by major features in two of three systems (cardiovascular, ocular and skeletal systems) and major

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5 or minor features in the skin, lungs and dura. The Ghent nosology lay down clinical requirements for a  
6 diagnosis of MFS(De Paepe, et al., 1996).

7 MFS shows a high degree of variability in severity, age of onset, and clinical manifestations. Mutations in the  
8 *FBNI* gene (MIM 134797) encoding the fibrillin-1 protein account for about 91% of classic MFS  
9 cases(Comeglio, et al., 2007). Mutations in *FBNI* are also found to cause related, overlapping syndromes  
10 termed type 1 fibrillinopathies including isolated ectopia lentis (EL), isolated skeletal features and ascending  
11 aortic aneurysm in patients who do not fulfil the Ghent criteria for classic MFS, although the success rate for  
12 identifying mutations in *FBNI* can be as low as 27% in some of these patient sub-groups(Comeglio, et al.,  
13 2007).

14 EL is identified as a clinical feature of MFS affecting approximately 65% of patients(Faivre, et al., 2008).  
15 Furthermore, isolated EL has been characterised as an autosomal dominantly inherited disorder (MIM 129600)  
16 also caused by mutations in *FBNI*, on the milder end of the type 1 fibrillinopathies spectrum(Edwards, et al.,  
17 1994). Previously we reported a *FBNI* mutation detection rate of 50% in the EL patient population(Comeglio, et  
18 al., 2007). This lower detection rate compared to the MFS patient group suggests that EL is a genetically  
19 heterogeneous condition with further genes responsible.

20 Clinically, EL is heterogeneous, occurring for example in MFS, homocystinuria, ectopia lentis et pupillae  
21 and Weill-Marchesani syndrome with associated syndromic features. A genetically distinct recessive form of  
22 the disease (MIM 225100) has been linked to a locus on chromosome 1q21(Ahram, et al., 2009; al-Salem,  
23 1990). Recently a homozygous mutation in *ADAMTSL4* (MIM 610113) has been identified in an inbred  
24 Jordanian family with 15 affected individuals from 4 related sibships with autosomal-recessive isolated  
25 EL(Ahram, et al., 2009). This family showed variable degrees of lens displacement among affected members  
26 with deprivation amblyopia, retinal detachment and cataract but no manifestations in any other system. A  
27 second study has identified a distinct *ADAMTSL4* mutation in a consanguineous Turkish family affected by  
28 isolated EL (Greene, et al., 2010). The mutation abolishes the splice acceptor site in intron 4 leading to deletion  
29 of c.79 to c.344 of exon 5 and introduction of a PTC due to a frameshift.

30 *ADAMTSL4* encodes ADAMTS-like 4, part of the ADAMTS (A Disintegrin-like And Metalloproteinase with  
31 Thrombospondin type 1 repeats)-like family of proteases (Apte, 2009). The protein comprises seven  
32 thrombospondin type 1 repeat (TSR) domains, a cysteine-rich module, an ADAMTS-spacer 1 domain and a  
33 PLAC (Protease and LACunin) domain (Ahram, et al., 2009; Buchner and Meisler, 2003,  
34 <http://www.ncbi.nlm.nih.gov/cdd>). The ADAMTS family proteins are important in connective tissue  
35 organisation, anti-angiogenesis and cell migration and attachment. Mutations in ADAMTS proteins have been  
36 associated with syndromes including Ehlers-Danlos type VIIC(Colige, et al., 2004), Weill-Marchesani  
37 syndrome(Dagoneau, et al., 2004; Kutz, et al., 2008; Morales, et al., 2009) and geleophysic dysplasia(Le Goff,  
38 et al., 2008).

39 It is important to differentiate between patients affected by EL associated with *FBNI* mutations and those  
40 with isolated EL caused by homozygous mutations in *ADAMTSL4*, for genetic counselling and prenatal  
41 diagnostic screening purposes. In patients with apparently isolated EL it is important to rule out MFS, in  
42 particular in young children who may go on to develop cardiovascular features of MFS(Comeglio, et al., 2007).  
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## 45 MATERIAL AND METHODS

46 We investigated 36 consecutive probands (19M:17F, mean age 29±16.7years) including 2 children under the  
47 age of 5 affected by EL and referred to us for clinical genetic assessment, echocardiogram and *FBNI* mutation  
48 screening. Several clinical geneticists were involved in initial diagnosis and the clinical data were entered onto  
49 standardised forms obtained from the curators of the UMD *FBNI* mutation database. The returned forms were  
50 evaluated strictly by the author clinical geneticist (A.C).  
51

52 In each case a clinical diagnosis of MFS was excluded as probands did not fulfill the Ghent criteria. All  
53 probands had EL (major ocular involvement) and only minor or no involvement in the skeletal and skin systems  
54 and no cardiac system involvement.  
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56 In 11 individuals a causative mutation had been detected by single stranded conformational analysis (SSCA),  
57 a further 25 probands (including those found negative by SSCA historically) were analysed by denaturing High  
58 Pressure Liquid Chromatography (dHPLC). When an abnormally migrating band (SSCA) or peak (dHPLC) was  
59 observed, the corresponding exon fragment was sequenced to characterise the mutation.  
60

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*FBNI* cDNA sequence according to GenBank (RefSeq NM\_000138.3, <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.

*ADAMTSL4* cDNA sequence according to GenBank (RefSeq NM\_019032.4) was used, with the A of the ATG translation initiation codon as nucleotide +1. The initiation codon is identified as codon 1.

Peripheral blood samples were collected with the appropriate signed informed consent. Genomic DNA was extracted from 2ml peripheral blood using the Flexigene DNA extraction kit (Qiagen, Crawley, UK).

Genomic DNA was subjected to PCR using a set of 65 oligonucleotide primer pairs (Sigma-Genosys, Cambridge, UK) to amplify all 65 exons and intron/exon boundaries of the *FBNI* gene as described (Comeglio, et al., 2001). Detailed protocols are available on request. PCR amplification of *ADAMTSL4* was carried out using 18 oligonucleotide primer pairs to amplify all 19 exons of *ADAMTSL4* according to the method of Ahrm et al (Ahrm, et al., 2009). *ADAMTSL4* exons were sequenced using standard protocols as previously described (Comeglio, et al., 2007).

SSCA and dHPLC and direct sequencing were carried out as previously described (Comeglio, et al., 2001; Comeglio, et al., 2007). One hundred and sixty chromosomes (*FBNI*) and one hundred and forty six chromosomes (*ADAMTSL4*) from unrelated control individuals, with the same ethnic distribution as the study group were tested for identical mutations to establish if they could be considered either recurrent mutations or polymorphisms, and to confirm their association with the pathological condition under study. The *FBNI* mutations were not observed in the control group. The newly identified *ADAMTSL4* mutations were not identified in the control group.

## RESULTS

We describe the findings in 36 probands, and their associated family members who were referred for *FBNI* screening in the Sonalee laboratory. EL patients were classified as patients that presented with major involvement of the ocular system, and only minor involvement of other systems.

### Mutations in *FBNI*

In patients affected by predominant EL where homocystinuria was excluded, the observed *FBNI* mutation yield was 23/36 or 64%. This yield is higher than our previous published data due to the re-analysis by dHPLC of 13 patients in whom no mutation was found previously using only SSCA (Comeglio, et al., 2007). This re-analysis yielded 6 further mutations. The *FBNI* mutation findings are summarised in Table 1. Familial segregation of the mutation with the EL phenotype was observed in 15/23 (65%) families. The remaining probands were spontaneous EL cases or no familial screening was possible.

Fourteen out of 23 mutations (61%) in *FBNI* involve a cysteine residue, 6/23 (26%) result in other missense mutations. Two out of 23 (9%) result in possible splice site alterations, one of which is also a missense mutation affecting the final codon in exon 27. Only 1/23 (4%) results in a premature termination codon (PTC). Fourteen out of 23 mutations (61%) were found in the 5' end of *FBNI* (exons 1-21 inclusive), 8 of which (35%) occur in the first 15 exons.

Table 1. Causative FBN1 mutations identified in the present study.

Case	Age	Diagnosis	SS	OS	CS	S	F	Exon	Nucleotide	Amino acid	Domain	References
1	16	EL	m	M	-	m	3	2	c.188A>G	p.Tyr63Cys	Cys-rich Region	(Comeglio, et al., 2007)
2	12	EL	m	M	-	m	-	2	c.203G>C	p.Cys68Ser	Cys-rich Region	(Comeglio, et al., 2007)
3	53	EL	-	M	-	m	9	6	c.718C>T	p.Arg240Cys	Hybrid motif #01	(Collod-Beroud, et al., 2003; Comeglio, et al., 2002)
4	36	EL	-	M	-	-	1	9	c.1095C>G	p.Cys365Trp	TB #01	(Comeglio, et al., 2007)
5	36	EL	m	M	-	m	-	13	c.1633C>T	p.Arg545Cys	cbEGF-like #04	(Collod-Beroud, et al., 2003; Comeglio, et al., 2002)
6	32	EL	-	M	-	-	5	13	c.1633C>T	p.Arg545Cys	cbEGF-like #04	(Collod-Beroud, et al., 2003)
7	3	EL	-	M	-	-	-	14	c.1786T>C	p.Cys596Arg	cbEGF-like #05	(Comeglio, et al., 2007)
8	7	EL	m	M	-	-	2	15	c.1900T>C	p.Ser634Pro	cbEGF-like #06	(Comeglio, et al., 2002)
9	16	EL	m	M	-	m	1	18	c.2210G>T	p.Gly737Val	cbEGF-like #07	This study
10	49	EL	m	M	-	m	5	18	c.2180G>A	p.Cys727Tyr	cbEGF-like #07	(Comeglio, et al., 2007)
11	38	EL	-	M	-	-	4	19	c.2415T>G	p.Cys805Trp	cbEGF-like #08	This study
12	28	EL	-	M	-	-	2	19	c.2306G>A	p.Cys769Tyr	cbEGF-like #08	This study
13	22	EL	m	M	-	-	2	21	c.2670C>G	p.Cys890Trp	Hybrid Motif #02	This study
14	34	EL	m	M	-	m	-	21	c.2645C>T	p.Ala882Val	Hybrid Motif #02	(Comeglio, et al., 2007)
4*	36	EL	-	M	-	-	1	24	c.2951T>A	p.Val984Asp	TB #03	This study
15	37	EL	m	M	-	m	2	26	c.3258T>A	p.Cys1086X	cbEGF-like #12	(Comeglio, et al., 2007)
16	56	EL	-	M	-	-	4	27	c.3463G>A**	p.Asp1155Asn	cbEGF-like #13/14	(Biggin, et al., 2004; Comeglio, et al., 2007; Milewicz, et al., 1996)
17	16	EL	-	M	-	-	1	37	c.4588C>T	p.Arg1530Cys	TB #04	This study
18	7	EL	m	M	-	-	-	41	c.5074_5706del	p.Arg1692Del	TB #05	(Comeglio, et al., 2007)
19	44	EL	-	M	-	m	3	IVS 47	c.5918-1G>A	Splice site	Intronic	This study
20	40	EL	m	M	-	m	3	55	c.6815A>G	p.Tyr2272Cys	cbEGF-like #35	(Comeglio, et al., 2007)
21	30	EL	m	M	-	-	-	55	c.6749A>G	p.Glu2250Gly	cbEGF-like #35	(Comeglio, et al., 2007)
22	32	EL	m	M	-	m	-	59	c.7339G>A	p.Glu2447Lys	cbEGF-like #38	(Comeglio, et al., 2007; Lonqvist, et al., 1994)
23	24	EL	m	M	-	m	3	59	c.7342T>C	p.Cys2448Arg	cbEGF-like #38	(Comeglio, et al., 2007)

Legend: SS=Skeletal system; OS=Ocular system; CS=Cardiovascular system; S=Skin system; F=Family history; M=Major involvement; m=minor involvement.

\*Second mutation identified in case 4; \*\*mutation affects the exon 27 splice site.

FBN1 cDNA sequence according to GenBank (RefSeq NM\_000138.3) was used, with the A of the ATG translation initiation codon as nucleotide +1. The initiation codon is identified as codon 1.

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### Mutations in *ADAMTSL4*

The remaining 12 probands were screened for mutations in *ADAMTSL4* by direct sequencing of all exons including the 5' untranslated exons. Homozygous or compound heterozygous mutations were detected in 6/12 (50%) of the remaining probands (one proband was excluded due to insufficient DNA available) suggesting recessive inheritance in these cases. The *ADAMTSL4* mutation findings are summarised in Table 2. Familial screening was carried out where available and the mutant genotype was present in a second affected family member in 2/6 families. In 4/6 families unaffected parents were shown to be heterozygous for the changes. The remaining families were unavailable for screening

#### *Homozygous mutations*

A homozygous deletion of 20 nucleotides (c.767\_786del AGGCCTCTGGCACAGAGCCC, p.Gln256ProfsX38) in exon 6 leading to a PTC was detected in case 26, a 15 year old male affected by isolated EL.

A homozygous nucleotide change in exon 12 (c.2008C>T, p.Arg670X) was identified in a consanguineous Caucasian family (Figure 1). Of four offspring, III:2 (Case 28) and III:4 are affected by EL and carry the homozygous mutation in exon 12 of *ADAMTSL4*.

A homozygous missense mutation was identified in exon 12 (c.1960C>T, p.Pro654Ser) in case 27, a 41 year old male from a consanguineous Afghani family with EL who demonstrated a slim marfanoid body build with arm span to height ratio greater than 1.05, arachnodactyly and somewhat hyperextensible skin. His echocardiogram was normal but he had a mild functional mitral systolic murmur. No family members were available for screening.

#### *Compound heterozygous mutations*

A compound heterozygous mutation was identified in two unrelated probands (cases 24 and 25) comprising the c.767\_786del AGGCCTCTGGCACAGAGCCC, (p.Gln256ProfsX38) in combination with a second heterozygous deletion of 11 nucleotides (c.826\_836del CGTGCATCCCC, p.Arg276SerfsX21) leading to a PTC at position 300. Case 24, from a British Caucasian family with two affected members, and case 25 from a Swedish family with one affected individual. Both of these probands are young children (6-year old female, 8-year old male) who have EL in the absence of any other system involvement according to the Ghent nosology. Interestingly one of these cases was affected by EL et pupillae. It is possible that these cases may go on to develop other clinical features in adulthood.

Three heterozygous mutations were identified in case 29, a 4-year old female. c.926G>A (p.Arg309Gln) in exon 6, c.3161A>G (p.Tyr1054Cys) in exon 19, and c.3153C>A (p.Tyr1051X) in exon 19. *In silico* analysis identified that p.Tyr1054Cys is in the PLAC domain of *ADAMTSL4* and affects one of the highly conserved tyrosine residues (Figure 1). Familial screening showed the unaffected mother and sibling carried the p.Tyr1051X mutation and the unaffected father carried the p.Tyr1054Cys mutation, suggesting that this case is in fact the result of compound heterozygosity producing two defective alleles of the gene in the proband. The mutation identified in exon 6 (p.Arg309Gln) was shown to be on the same allele as the p.Tyr1051X mutation and occurs in a region of the gene encoding no known structural or functional domain.



Table 2. Causative ADAMTSL4 mutations identified in the present study.

Case	Age	Diagnosis	SS	OS	CS	S	F	Exon	Nucleotide	Amino acid	Domain	Zygoty
24	6	EL	-	M	-	-	1	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
								6	c.826_836del	p.Arg276SerfsX21	-	
25	8	EL*	-	M	-	-	-	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
								6	c.826_836del	p.Arg276SerfsX21	-	
26	15	EL	-	M	-	-	-	6	c.767_786del	p.Gln256ProfsX38	-	Homozygous
27	41	EL	m	M	-	-	-	12	c.1960C>T	p.Pro654Ser	-	Homozygous
28	34	EL	-	M	-	-	1	12	c.2008C>T	p.Arg670X	ADAM TSR-1	Homozygous
29	4	EL	m	M	-	-	-	19	c.3153C>A	p.Tyr1051X	PLAC domain	Compound heterozygous
								19	c.3161A>G	p.Tyr1054Cys	PLAC domain	
								6	c.926G>A	p.Arg309Gln	-	

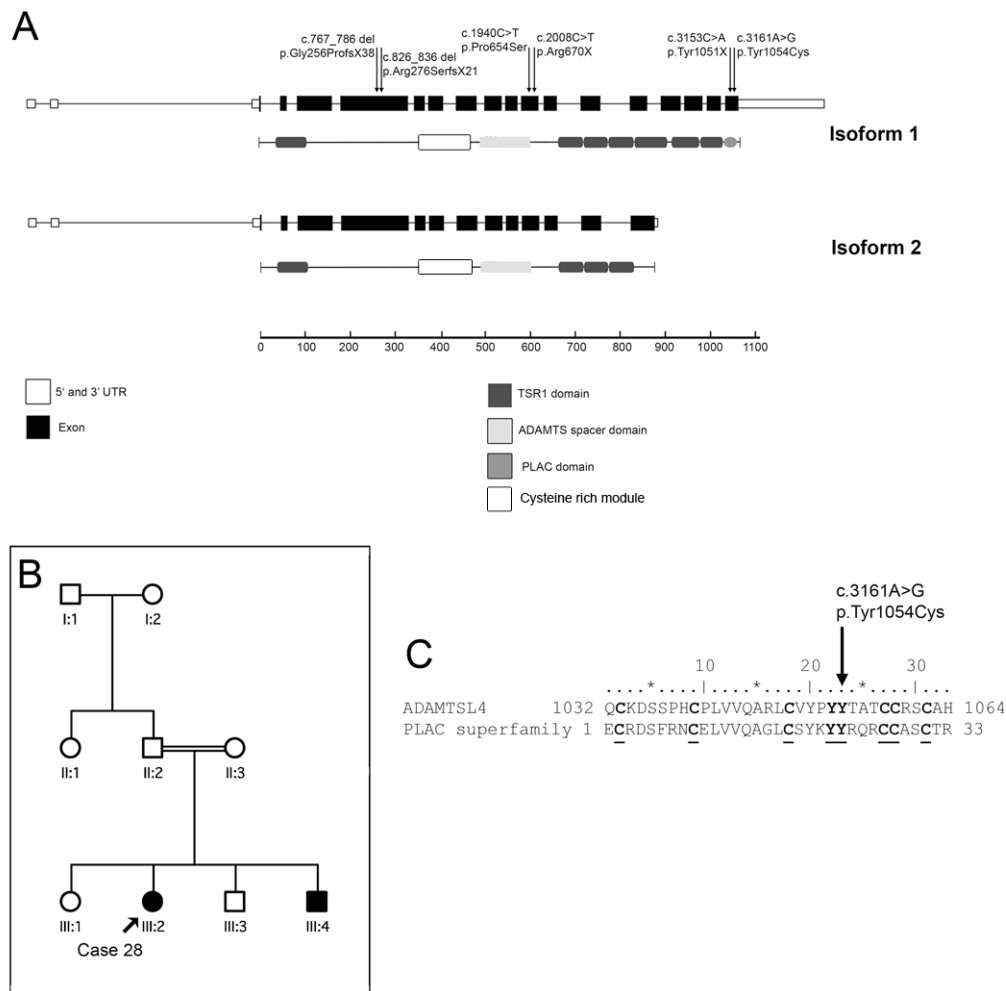
**Legend:** SS=Skeletal system; OS=Ocular system; CS=Cardiovascular system; S=Skin system; F=Family history; M=Major involvement; m=minor involvement.

\*Ectopia lentis et pupillae

ADAMTSL4 cDNA sequence according to GenBank (RefSeq NM\_019032.4) was used, with the A of the ATG translation initiation codon as nucleotide +1.

The initiation codon is identified as codon 1.

8 &lt;First author name, et al.&gt;



**Figure 1.** Panel A shows the schematic representation of *ADAMTSL4*. The exons and protein domains are shown for each isoform 1 (full length) and 2. Isoform 2 lacking three TSR1 domains and the PLAC domain. Arrows above the diagram indicate the approximate mutation positions. Panel B shows the pedigree of case 28 (III:2). Panel C shows the position of the c.3161A>G (p.Tyr1054Cys) mutation in the conserved PLAC domain of ADAMTS-like 4. The mutation affects one of the conserved tyrosine residues of the PLAC superfamily.

## DISCUSSION

This study describes the mutation findings in a group of 36 predominant EL patients referred for *FBNI* screening in the Sonalee laboratory. We believe this is the largest group of EL patients studied to date. The mutation detection rate observed in *FBNI* was 23/36 (64%), which has improved since our last publication (50%) due to rescreening of 13 patients, previously found mutation-negative by SSCA, using the more sensitive dHPLC detection method. Mutations were identified more often in the 5' region of the gene (61%), as previously observed (Comeglio, et al., 2007; Faivre, et al., 2009b). Mutations in the neonatal region (exons 24-32) were underrepresented (3/23, 13%).

Several studies have attempted to characterise mutation types and location in *FBNI* (Collod-Beroud, et al., 2003; Faivre, et al., 2009a; Faivre, et al., 2007; Rommel, et al., 2005). It is widely accepted that mutations in exons 24-32 are associated with severe neonatal MFS (Faivre, et al., 2009c). According to recent findings an association between predominant EL and the 5' end of the *FBNI* gene is apparent (Comeglio, et al., 2007; Faivre, et al., 2007). Here we report 14/23 (61%) of mutations in the first 21 exons, compared with 29% of total mutations in all patient sub-groups in the latest largest study (Faivre, et al., 2007). Recently, a large study

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5 suggested that mutations resulting in PTC are associated with major skeletal findings, and mutations affecting  
6 cysteines have been strongly associated with the presence of EL (Faivre, et al., 2007). Thirteen out of 23 (56%)  
7 probands had missense mutations involving cysteine compared to only one mutation resulting in a PTC.

8 In type 1 fibrillinopathies associated with mutations in the transforming growth factor-beta receptor  
9 (TGFBR) complex, it is important to note that EL is rarely present (Loeys, et al., 2006) suggesting that the role  
10 of fibrillin-1 in the TGF- $\beta$  pathway is of less importance in EL and thus mutations causing EL in *FBN1* are less  
11 common in domains associated with TGF- $\beta$  binding. Faivre *et al* suggest that correct cysteine orientation and  
12 disulfide bonding is important in structural integrity of suspensory ligaments and PTC mutations are associated  
13 with severe skeletal and skin features (Faivre, et al., 2007). Furthermore, TGFBR-related fibrillinopathies  
14 include severe skeletal and skin features suggesting that these features in both patients with a *FBN1* PTC  
15 mutation and patients with TGFBR-related disorders are associated with haploinsufficiency in the TGF- $\beta$   
16 signalling pathway.

17 *ADAMTSL4* encodes ADAMTS-like 4, and contains 7 predicted thrombospondin type 1 repeats (TSR1) that  
18 are believed to anchor the protein to the extracellular matrix (ECM) (Cal, et al., 2002). ADAMTS-like 4 is a  
19 protein resembling the ADAMTS ancillary domain lacking the proteolytic capability of ADAMTS proteases  
20 (Apte, 2009). ADAMTS proteases are thought to function in ocular physiology and members of the ADAMTS  
21 family have been implicated in ocular diseases, with autosomal-recessive mutations found in *ADAMTS10* in  
22 Weill-Marchesani syndrome (WMS) (Dagoneau, et al., 2004; Kutz, et al., 2008; Morales, et al., 2009),  
23 homozygous mutations in *ADAMTS2* associated with Ehlers-Danlos syndrome type VIIC (Colige, et al., 2004),  
24 and autosomal-recessive mutations in *ADAMTSL2* in geleophysic dysplasia (Le Goff, et al., 2008). Recently,  
25 *ADAMTSL7* has been implicated in incomplete WMS (Morales, et al., 2009).

26 Interestingly, the previously identified *ADAMTSL4* mutation leading to a PTC in exon 11 results in only  
27 isolated EL (Ahrum, et al., 2009), and similarly the mutations described here leading to either homozygous or  
28 compound heterozygous PTCs (found in exon 6 and exon 12, see Table 2) are associated with EL in the absence  
29 of any other system involvement.

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31 The PTC mutations in exon 6 and exon 12 may result in a truncated protein of 300 amino acid residues and  
32 670 respectively and may abolish the C-terminal TSR1 domains and therefore the anchoring to the ECM (see  
33 Figure 1), if a protein is translated at all and the mRNA does not undergo a nonsense-mediated decay (NMD)  
34 process. This however is not possible to predict without analysis of the protein product in these instances. Since  
35 ADAMTS-like 4 does not carry a catalytic domain found in other ADAMTS proteins, it is suggested the  
36 pathogenic role of *ADAMTSL4* mutations may be due to the structural role of the protein in the assembly or  
37 integrity of the ECM.

38 The mutations identified in exon 19 both occur in the PLAC domain at the c-terminal end (see Figure 1). The  
39 PLAC domain contains a 6-cysteine conserved repeat, first described in lacunin, an extracellular matrix protein  
40 originally identified in *Manduca sexta*. The role of this domain is not known but it is thought to be important in  
41 matrix proteases and proprotein convertases. The p.Tyr1051X mutation identified in exon 19 abolishes this  
42 domain. Furthermore, the p.Tyr1054Cys affects one of the conserved amino acids in the PLAC domain (see  
43 Figure 1) and introduces an extra cysteine residue into the domain that is predicted to be disulfide bonded which  
44 could lead to disruption of the domain structure. This domain is however not present in isoform 2 of ADAMTS-  
45 like 4. Hence, the phenotypic effects seen in this patient may be due to disruption of the PLAC domain function  
46 or overall protein function of ADAMTS-like 4 isoform 1.

47 Although there is some evidence that *ADAMTSL4* is expressed in the eye (<http://smd.stanford.edu>), in order  
48 to determine the importance of the protein in the zonular filaments and confirm the significance of mutations in  
49 *ADAMTSL4* in EL it would be of great interest to localize the protein or mRNA within zonular filament tissue.

50 All available clinical and surgical details were gathered retrospectively for each patient. Because of the time  
51 range of blood sample collection, many details were no longer available. It was therefore not possible to  
52 establish clear differences in phenotype between the subgroups, namely those with *FBN1*, *ADAMTSL4* and no  
53 demonstrable mutation. Detailed genotype-phenotype analysis will be performed prospectively on a larger  
54 group of EL patients.  
55

## 56 CONCLUSIONS

57  
58 The *FBN1* mutation yield in patients referred for screening in the Sonalee Laboratory with EL and only  
59 minor involvement of any other system described here is 64%, increased from our previous findings of 50%.  
60 This yield is however artificially suppressed due to the inclusion of patients with recessively inherited EL due to

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5 mutations in *ADAMTSL4*. We believe *FBN1* mutations are therefore responsible for a higher proportion of  
6 dominantly inherited EL and some of the sporadic cases are in fact cases of recessive EL either in  
7 consanguineous or non-consanguineous families.

8 We believe these findings confirm the importance of *ADAMTSL4* in autosomal recessive EL and suggest that  
9 certain mutations are recurrent in different families either as homozygous mutations or compound heterozygous  
10 mutations in non-consanguineous families. This study also demonstrates that *ADAMTSL4* is an important cause  
11 of EL outside of the original Jordanian family where it was first identified. In all cases of *ADAMTSL4*  
12 mutations, the findings were compatible with autosomal-recessive inheritance. It is therefore suggested that  
13 even in children of non-consanguineous families with apparent *de-novo* EL, screening for mutations in  
14 *ADAMTSL4* should be undertaken in *FBN1* mutation negative cases, to eliminate cardiac risk and guide genetic  
15 counselling and long-term management.

16 In this consecutive series of probands there remains 20% of EL with no proven genetic basis in which new  
17 dominant or recessive genetic determinants are predicted to be found. At least one, probably more genes remain  
18 to be described and candidates should include other members of the ADAMTS family of proteins.

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Table 1. Causative FBN1 mutations identified in the present study.

Case	Age	Diagnosis	SS	OS	CS	S	F	Exon	Nucleotide	Amino acid	Domain	References
1	16	EL	m	M	-	m	3	2	c.188A>G	p.Tyr63Cys	Cys-rich Region	(Comeglio, et al., 2007)
2	12	EL	m	M	-	m	-	2	c.203G>C	p.Cys68Ser	Cys-rich Region	(Comeglio, et al., 2007)
3	53	EL	-	M	-	m	9	6	c.718C>T	p.Arg240Cys	Hybrid motif #01	(Collod-Beroud, et al., 2003; Comeglio, et al., 2002)
4	36	EL	-	M	-	-	1	9	c.1095C>G	p.Cys365Trp	TB #01	(Comeglio, et al., 2007)
5	36	EL	m	M	-	m	-	13	c.1633C>T	p.Arg545Cys	cbEGF-like #04	(Collod-Beroud, et al., 2003; Comeglio, et al., 2002)
6	32	EL	-	M	-	-	5	13	c.1633C>T	p.Arg545Cys	cbEGF-like #04	(Collod-Beroud, et al., 2003)
7	3	EL	-	M	-	-	-	14	c.1786T>C	p.Cys596Arg	cbEGF-like #05	(Comeglio, et al., 2007)
8	7	EL	m	M	-	-	2	15	c.1900T>C	p.Ser634Pro	cbEGF-like #06	(Comeglio, et al., 2002)
9	16	EL	m	M	-	m	1	18	c.2210G>T	p.Gly737Val	cbEGF-like #07	This study
10	49	EL	m	M	-	m	5	18	c.2180G>A	p.Cys727Tyr	cbEGF-like #07	(Comeglio, et al., 2007)
11	38	EL	-	M	-	-	4	19	c.2415T>G	p.Cys805Trp	cbEGF-like #08	This study
12	28	EL	-	M	-	-	2	19	c.2306G>A	p.Cys769Tyr	cbEGF-like #08	This study
13	22	EL	m	M	-	-	2	21	c.2670C>G	p.Cys890Trp	Hybrid Motif #02	This study
14	34	EL	m	M	-	m	-	21	c.2645C>T	p.Ala882Val	Hybrid Motif #02	(Comeglio, et al., 2007)
4*	36	EL	-	M	-	-	1	24	c.2951T>A	p.Val984Asp	TB #03	This study
15	37	EL	m	M	-	m	2	26	c.3258T>A	p.Cys1086X	cbEGF-like #12	(Comeglio, et al., 2007)
16	56	EL	-	M	-	-	4	27	c.3463G>A**	p.Asp1155Asn	cbEGF-like #13/14	(Biggin, et al., 2004; Comeglio, et al., 2007; Milewicz, et al., 1996)
17	16	EL	-	M	-	-	1	37	c.4588C>T	p.Arg1530Cys	TB #04	This study
18	7	EL	m	M	-	-	-	41	c.5074_5706del	p.Arg1692Del	TB #05	(Comeglio, et al., 2007)
19	44	EL	-	M	-	m	3	IVS 47	c.5918-1G>A	Splice site	Intronic	This study
20	40	EL	m	M	-	m	3	55	c.6815A>G	p.Tyr2272Cys	cbEGF-like #35	(Comeglio, et al., 2007)
21	30	EL	m	M	-	-	-	55	c.6749A>G	p.Glu2250Gly	cbEGF-like #35	(Comeglio, et al., 2007)
22	32	EL	m	M	-	m	-	59	c.7339G>A	p.Glu2447Lys	cbEGF-like #38	(Comeglio, et al., 2007; Lonqvist, et al., 1994)
23	24	EL	m	M	-	m	3	59	c.7342T>C	p.Cys2448Arg	cbEGF-like #38	(Comeglio, et al., 2007)

Legend: SS=Skeletal system; OS=Ocular system; CS=Cardiovascular system; S=Skin system; F=Family history; M=Major involvement; m=minor involvement.  
 \*Second mutation identified in case 4; \*\*mutation affects the exon 27 splice site.

Table 2. Causative ADAMTSL4 mutations identified in the present study.

Case	Age	Diagnosis	SS	OS	CS	S	F	Exon	Nucleotide	Amino acid	Domain	Zygoty
24	6	EL	-	M	-	-	-	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
								6	c.826_836del	p.Arg276SerfsX21	-	
25	8	EL*	-	M	-	-	1	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
								6	c.826_836del	p.Arg276SerfsX21	-	
26	15	EL	-	M	-	-	-	6	c.826_836del	p.Arg276SerfsX21	-	Homozygous
27	41	EL	m	M	-	m	-	12	c.1960C>T	p.Pro654Ser	-	Homozygous
28	34	EL	-	M	-	-	1	12	c.2008C>T	p.Arg670X	ADAM TSR-1	Homozygous
29	4	EL	m	M	-	-	-	19	c.3153C>A	p.Tyr1051X	PLAC domain	Compound heterozygous
								19	c.3161A>G	p.Tyr1054Cys	PLAC domain	
								6	c.926G>A	p.Arg309Gln	-	

**Legend:** SS=Skeletal system; OS=Ocular system; CS=Cardiovascular system; S=Skin system; F=Family history; M=Major involvement; m=minor involvement.

\*Ectopia lentis et pupillae



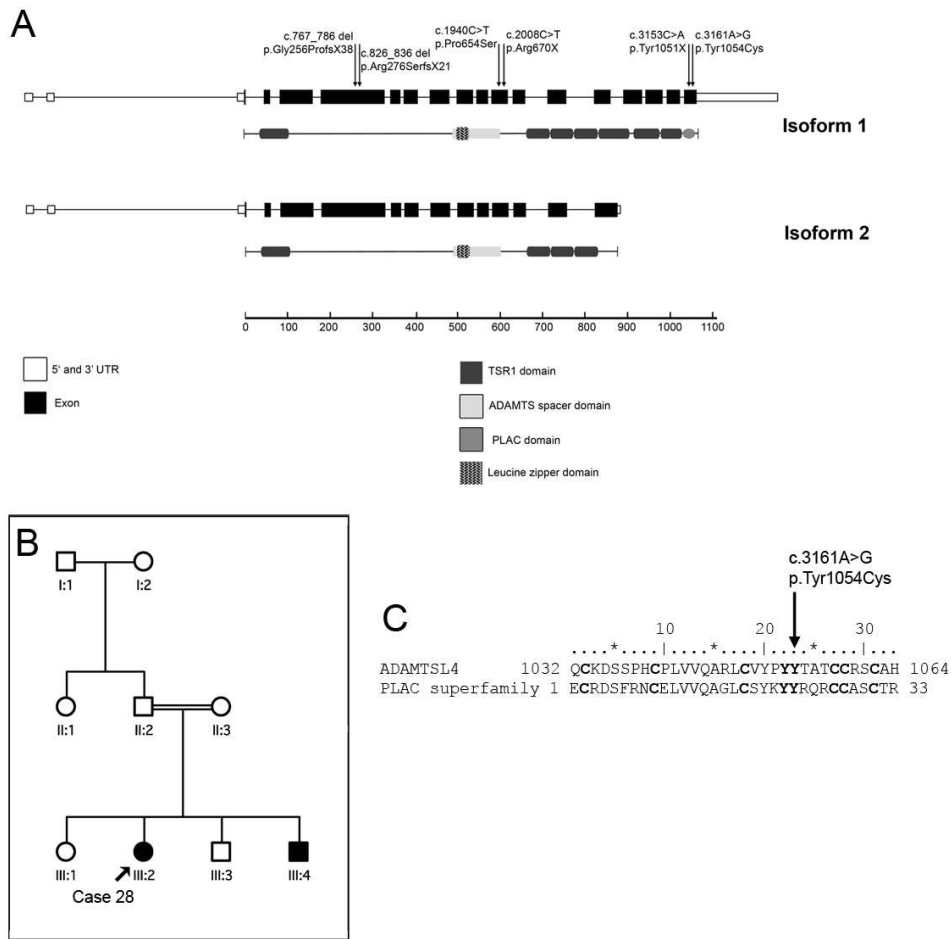


Figure 1. Panel A shows the schematic representation of the ADAMTSL4. The exons and protein domains are shown for each isoform 1 (full length) and 2. Isoform 2 lacking three TSR1 domains and the PLAC domain. Arrows above the diagram indicate the approximate mutation positions. Panel B shows the pedigree of case 28 (III:2). Panel C shows the position of the c.3161A>G (p.Tyr1054Cys) mutation in the conserved PLAC domain of ADAMTS-like 4. The mutation affects one of the conserved tyrosine residues of the PLAC superfamily.

149x150mm (200 x 200 DPI)

MUTATION IN BRIEF

HUMAN MUTATION

For Peer Review

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# Role of *ADAMTSL4* Mutations in *FBN1*

## Mutation-Negative Ectopia Lentis Patients.



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Short Title: *FBN1* and *ADAMTSL4* mutations in ectopia lentis.

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**ABSTRACT:** Ectopia lentis (EL) is genetically heterogeneous with both autosomal-dominant and -recessive forms. The dominant disorder can be caused by mutations in *FBN1*, at the milder end of the type-1 fibrillinopathies spectrum. Recently in a consanguineous Jordanian family, recessive EL was mapped to locus 1q21 containing the *ADAMTSL4* gene and a nonsense mutation was found in exon 11 (c.1785T>G, p.Y595X). In this study, 36 consecutive probands with EL who did not fulfill the Ghent criteria for MFS were screened for mutations in *FBN1* and *ADAMTSL4*. Causative *FBN1* mutations were identified in 23/36 (64%) of probands while homozygous or compound heterozygous *ADAMTSL4* mutations were identified in 6/12 (50%) of the remaining probands. Where available, familial screening of these families confirmed the mutation co-segregated with the EL phenotype. This study confirms that homozygous frameshift mutations in *ADAMTSL4* are associated with autosomal-recessive EL in British families. Furthermore; the first compound heterozygous mutation is described resulting in a PTC and a missense mutation in the PLAC (protease and lacunin) domain. The identification of a causative mutation in *ADAMTSL4* may allow the exclusion of Marfan syndrome in these families and guide the clinical management, of particular relevance in young children affected by EL. ©2010 Wiley-Liss, Inc.

**KEY WORDS:** Ectopia lentis, Marfan syndrome, *FBN1*, *ADAMTSL4*

### INTRODUCTION

Marfan syndrome (MFS, MIM 154700, <http://www.ncbi.nlm.nih.gov/Omim/>) is an autosomal dominant inherited connective tissue disorder with a prevalence of about 1 in 5-10,000 (Pyeritz, 1993). MFS is characterised by major features in two of three systems (cardiovascular, ocular and skeletal systems) and major

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5 or minor features in the skin, lungs and dura. The Ghent nosology lay down clinical requirements for a  
6 diagnosis of MFS(De Paepe, et al., 1996).

7 MFS shows a high degree of variability in severity, age of onset, and clinical manifestations. Mutations in the  
8 *FBNI* gene (MIM 134797) encoding the fibrillin-1 protein account for about 91% of classic MFS  
9 cases(Comeglio, et al., 2007). Mutations in *FBNI* are also found to cause related, overlapping syndromes  
10 termed type 1 fibrillinopathies including isolated ectopia lentis (EL), isolated skeletal features and ascending  
11 aortic aneurysm in patients who do not fulfil the Ghent criteria for classic MFS, although the success rate for  
12 identifying mutations in *FBNI* can be as low as 27% in some of these patient sub-groups(Comeglio, et al.,  
13 2007).

14 EL is identified as a clinical feature of MFS affecting approximately 65% of patients(Faivre, et al., 2008).  
15 Furthermore, isolated EL has been characterised as an autosomal dominantly inherited disorder (MIM 129600)  
16 also caused by mutations in *FBNI*, on the milder end of the type 1 fibrillinopathies spectrum(Edwards, et al.,  
17 1994). Previously we reported a *FBNI* mutation detection rate of 50% in the EL patient population(Comeglio, et  
18 al., 2007). This lower detection rate compared to the MFS patient group suggests that EL is a genetically  
19 heterogeneous condition with further genes responsible.

20 Clinically, EL is heterogeneous, occurring for example in MFS, homocystinuria, ectopia lentis et pupillae  
21 and Weill-Marchesani syndrome with associated syndromic features. A genetically distinct recessive form of  
22 the disease (MIM 225100) has been linked to a locus on chromosome 1q21(Ahram, et al., 2009; al-Salem,  
23 1990). Recently a homozygous mutation in *ADAMTSL4* (MIM 610113) has been identified in an inbred  
24 Jordanian family with 15 affected individuals from 4 related sibships with autosomal-recessive isolated  
25 EL(Ahram, et al., 2009). This family showed variable degrees of lens displacement among affected members  
26 with deprivation amblyopia, retinal detachment and cataract but no manifestations in any other system. A  
27 second study has identified a distinct *ADAMTSL4* mutation in a consanguineous Turkish family affected by  
28 isolated EL (Greene, et al., 2010). The mutation abolishes the splice acceptor site in intron 4 leading to deletion  
29 of c.79 to c.344 of exon 5 and introduction of a PTC due to a frameshift.

30 *ADAMTSL4* encodes ADAMTS-like 4, part of the ADAMTS (A Disintegrin-like And Metalloproteinase with  
31 Thrombospondin type 1 repeats)-like family of proteases (Apte, 2009). The protein comprises seven  
32 thrombospondin type 1 repeat (TSR) domains, a cysteine-rich module, an ADAMTS-spacer 1 domain and a  
33 PLAC (Protease and LACunin) domain (Ahram, et al., 2009; Buchner and Meisler, 2003,  
34 <http://www.ncbi.nlm.nih.gov/cdd>). The ADAMTS family proteins are important in connective tissue  
35 organisation, anti-angiogenesis and cell migration and attachment. Mutations in ADAMTS proteins have been  
36 associated with syndromes including Ehlers-Danlos type VIIC(Colige, et al., 2004), Weill-Marchesani  
37 syndrome(Dagoneau, et al., 2004; Kutz, et al., 2008; Morales, et al., 2009) and geleophysic dysplasia(Le Goff,  
38 et al., 2008).

39 It is important to differentiate between patients affected by EL associated with *FBNI* mutations and those  
40 with isolated EL caused by homozygous mutations in *ADAMTSL4*, for genetic counselling and prenatal  
41 diagnostic screening purposes. In patients with apparently isolated EL it is important to rule out MFS, in  
42 particular in young children who may go on to develop cardiovascular features of MFS(Comeglio, et al., 2007).  
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## 45 MATERIAL AND METHODS

46 We investigated 36 consecutive probands (19M:17F, mean age 29±16.7years) including 2 children under the  
47 age of 5 affected by EL and referred to us for clinical genetic assessment, echocardiogram and *FBNI* mutation  
48 screening. Several clinical geneticists were involved in initial diagnosis and the clinical data were entered onto  
49 standardised forms obtained from the curators of the UMD *FBNI* mutation database. The returned forms were  
50 evaluated strictly by the author clinical geneticist (A.C).

51 In each case a clinical diagnosis of MFS was excluded as probands did not fulfill the Ghent criteria. All  
52 probands had EL (major ocular involvement) and only minor or no involvement in the skeletal and skin systems  
53 and no cardiac system involvement.

54 In 11 individuals a causative mutation had been detected by single stranded conformational analysis (SSCA),  
55 a further 25 probands (including those found negative by SSCA historically) were analysed by denaturing High  
56 Pressure Liquid Chromatography (dHPLC). When an abnormally migrating band (SSCA) or peak (dHPLC) was  
57 observed, the corresponding exon fragment was sequenced to characterise the mutation.  
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*FBNI* cDNA sequence according to GenBank (RefSeq NM\_000138.3, <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.

*ADAMTSL4* cDNA sequence according to GenBank (RefSeq NM\_019032.4) was used, with the A of the ATG translation initiation codon as nucleotide +1. The initiation codon is identified as codon 1.

Peripheral blood samples were collected with the appropriate signed informed consent. Genomic DNA was extracted from 2ml peripheral blood using the Flexigene DNA extraction kit (Qiagen, Crawley, UK).

Genomic DNA was subjected to PCR using a set of 65 oligonucleotide primer pairs (Sigma-Genosys, Cambridge, UK) to amplify all 65 exons and intron/exon boundaries of the *FBNI* gene as described (Comeglio, et al., 2001). Detailed protocols are available on request. PCR amplification of *ADAMTSL4* was carried out using 18 oligonucleotide primer pairs to amplify all 19 exons of *ADAMTSL4* according to the method of Ahrm et al (Ahrm, et al., 2009). *ADAMTSL4* exons were sequenced using standard protocols as previously described (Comeglio, et al., 2007).

SSCA and dHPLC and direct sequencing were carried out as previously described (Comeglio, et al., 2001; Comeglio, et al., 2007). One hundred and sixty chromosomes (*FBNI*) and one hundred and forty six chromosomes (*ADAMTSL4*) from unrelated control individuals, with the same ethnic distribution as the study group were tested for identical mutations to establish if they could be considered either recurrent mutations or polymorphisms, and to confirm their association with the pathological condition under study. The *FBNI* mutations were not observed in the control group. The newly identified *ADAMTSL4* mutations were not identified in the control group.

## RESULTS

We describe the findings in 36 probands, and their associated family members who were referred for *FBNI* screening in the Sonalee laboratory. EL patients were classified as patients that presented with major involvement of the ocular system, and only minor involvement of other systems.

### Mutations in *FBNI*

In patients affected by predominant EL where homocystinuria was excluded, the observed *FBNI* mutation yield was 23/36 or 64%. This yield is higher than our previous published data due to the re-analysis by dHPLC of 13 patients in whom no mutation was found previously using only SSCA (Comeglio, et al., 2007). This re-analysis yielded 6 further mutations. The *FBNI* mutation findings are summarised in Table 1. Familial segregation of the mutation with the EL phenotype was observed in 15/23 (65%) families. The remaining probands were spontaneous EL cases or no familial screening was possible.

Fourteen out of 23 mutations (61%) in *FBNI* involve a cysteine residue, 6/23 (26%) result in other missense mutations. Two out of 23 (9%) result in possible splice site alterations, one of which is also a missense mutation affecting the final codon in exon 27. Only 1/23 (4%) results in a premature termination codon (PTC). Fourteen out of 23 mutations (61%) were found in the 5' end of *FBNI* (exons 1-21 inclusive), 8 of which (35%) occur in the first 15 exons.

Table 1. Causative FBN1 mutations identified in the present study.

Case	Age	Diagnosis	SS	OS	CS	S	F	Exon	Nucleotide	Amino acid	Domain	References
1	16	EL	m	M	-	m	3	2	c.188A>G	p.Tyr63Cys	Cys-rich Region	(Comeglio, et al., 2007)
2	12	EL	m	M	-	m	-	2	c.203G>C	p.Cys68Ser	Cys-rich Region	(Comeglio, et al., 2007)
3	53	EL	-	M	-	m	9	6	c.718C>T	p.Arg240Cys	Hybrid motif #01	(Collod-Beroud, et al., 2003; Comeglio, et al., 2002)
4	36	EL	-	M	-	-	1	9	c.1095C>G	p.Cys365Trp	TB #01	(Comeglio, et al., 2007)
5	36	EL	m	M	-	m	-	13	c.1633C>T	p.Arg545Cys	cbEGF-like #04	(Collod-Beroud, et al., 2003; Comeglio, et al., 2002)
6	32	EL	-	M	-	-	5	13	c.1633C>T	p.Arg545Cys	cbEGF-like #04	(Collod-Beroud, et al., 2003)
7	3	EL	-	M	-	-	-	14	c.1786T>C	p.Cys596Arg	cbEGF-like #05	(Comeglio, et al., 2007)
8	7	EL	m	M	-	-	2	15	c.1900T>C	p.Ser634Pro	cbEGF-like #06	(Comeglio, et al., 2002)
9	16	EL	m	M	-	m	1	18	c.2210G>T	p.Gly737Val	cbEGF-like #07	This study
10	49	EL	m	M	-	m	5	18	c.2180G>A	p.Cys727Tyr	cbEGF-like #07	(Comeglio, et al., 2007)
11	38	EL	-	M	-	-	4	19	c.2415T>G	p.Cys805Trp	cbEGF-like #08	This study
12	28	EL	-	M	-	-	2	19	c.2306G>A	p.Cys769Tyr	cbEGF-like #08	This study
13	22	EL	m	M	-	-	2	21	c.2670C>G	p.Cys890Trp	Hybrid Motif #02	This study
14	34	EL	m	M	-	m	-	21	c.2645C>T	p.Ala882Val	Hybrid Motif #02	(Comeglio, et al., 2007)
4*	36	EL	-	M	-	-	1	24	c.2951T>A	p.Val984Asp	TB #03	This study
15	37	EL	m	M	-	m	2	26	c.3258T>A	p.Cys1086X	cbEGF-like #12	(Comeglio, et al., 2007)
16	56	EL	-	M	-	-	4	27	c.3463G>A**	p.Asp1155Asn	cbEGF-like #13/14	(Biggin, et al., 2004; Comeglio, et al., 2007; Milewicz, et al., 1996)
17	16	EL	-	M	-	-	1	37	c.4588C>T	p.Arg1530Cys	TB #04	This study
18	7	EL	m	M	-	-	-	41	c.5074_5706del	p.Arg1692Del	TB #05	(Comeglio, et al., 2007)
19	44	EL	-	M	-	m	3	IVS 47	c.5918-1G>A	Splice site	Intronic	This study
20	40	EL	m	M	-	m	3	55	c.6815A>G	p.Tyr2272Cys	cbEGF-like #35	(Comeglio, et al., 2007)
21	30	EL	m	M	-	-	-	55	c.6749A>G	p.Glu2250Gly	cbEGF-like #35	(Comeglio, et al., 2007)
22	32	EL	m	M	-	m	-	59	c.7339G>A	p.Glu2447Lys	cbEGF-like #38	(Comeglio, et al., 2007; Lonqvist, et al., 1994)
23	24	EL	m	M	-	m	3	59	c.7342T>C	p.Cys2448Arg	cbEGF-like #38	(Comeglio, et al., 2007)

**Legend:** SS=Skeletal system; OS=Ocular system; CS=Cardiovascular system; S=Skin system; F=Family history; M=Major involvement; m=minor involvement.

\*Second mutation identified in case 4; \*\*mutation affects the exon 27 splice site.

FBN1 cDNA sequence according to GenBank (RefSeq NM\_000138.3) was used, with the A of the ATG translation initiation codon as nucleotide +1. The initiation codon is identified as codon 1.

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**Mutations in *ADAMTSL4***

Twelve of the 13 remaining probands in whom no *FBN1* mutation had been identified (one proband was excluded due to insufficient DNA available) were screened for mutations in *ADAMTSL4* by direct sequencing of all exons including the 5' untranslated exons. Of these, 6/12 were the only affected in the family but of these families 1 was consanguineous; in 6/12 families there were affected siblings and of these families 1 was consanguineous. Homozygous or compound heterozygous mutations were detected in 6/12 (50%) of the remaining probands suggesting recessive inheritance in these cases. The *ADAMTSL4* mutation findings are summarised in Table 2. Familial screening was carried out where available and the mutant genotype was present in a second affected family member in 2/6 families. In 4/6 families unaffected parents were shown to be heterozygous for the changes. The remaining families were unavailable for screening. Three of 6 of the families with no known identified mutation in *FBN1* or *ADAMTSL4* indicated dominant inheritance. No screening for large rearrangements or deletions in *FBN1* or *ADAMTSL4* was carried out in these families.

**Homozygous mutations**

A homozygous deletion of 20 nucleotides (c.767\_786del AGGCCTCTGGCACAGAGCCC, p.Gln256ProfsX38) in exon 6 leading to a PTC was detected in case 26, a 15 year old male affected by isolated EL.

A homozygous nucleotide change in exon 12 (c.2008C>T, p.Arg670X) was identified in a consanguineous Caucasian family (Figure 1). Of four offspring, III:2 (Case 28) and III:4 are affected by EL and carry the homozygous mutation in exon 12 of *ADAMTSL4*.

A homozygous missense mutation was identified in exon 12 (c.1960C>T, p.Pro654Ser) in case 27, a 41 year old male from a consanguineous Afghani family with EL who demonstrated a slim marfanoid body build with arm span to height ratio greater than 1.05, arachnodactyly and somewhat hyperextensible skin. His echocardiogram was normal but he had a mild functional mitral systolic murmur. No family members were available for screening.

**Compound heterozygous mutations**

A compound heterozygous mutation was identified in two unrelated probands (cases 24 and 25) comprising the c.767\_786del AGGCCTCTGGCACAGAGCCC, (p.Gln256ProfsX38) in combination with a second heterozygous deletion of 11 nucleotides (c.826\_836del CGTGCATCCCC, p.Arg276SerfsX21) leading to a PTC at position 300. Case 24, from a British Caucasian family with two affected members, and case 25 from a Swedish family with one affected individual. Both of these probands are young children (6-year old female, 8-year old male) who have EL in the absence of any other system involvement according to the Ghent nosology. Interestingly one of these cases was affected by EL et pupillae. It is possible that these cases may go on to develop other clinical features in adulthood.

Three heterozygous mutations were identified in case 29, a 4-year old female. c.926G>A (p.Arg309Gln) in exon 6, c.3161A>G (p.Tyr1054Cys) in exon 19, and c.3153C>A (p.Tyr1051X) in exon 19. *In silico* analysis identified that p.Tyr1054Cys is in the PLAC domain of *ADAMTSL4* and affects one of the highly conserved tyrosine residues (Figure 1). Familial screening showed the unaffected mother and sibling carried the p.Tyr1051X mutation and the unaffected father carried the p.Tyr1054Cys mutation, suggesting that this case is in fact the result of compound heterozygosity producing two defective alleles of the gene in the proband. The mutation identified in exon 6 (p.Arg309Gln) was shown to be on the same allele as the p.Tyr1051X mutation and occurs in a region of the gene encoding no known structural or functional domain.

Table 2. Causative ADAMTSL4 mutations identified in the present study.

Case	Age	Diagnosis	SS	OS	CS	S	F	Exon	Nucleotide	Amino acid	Domain	Zygoty
24	6	EL	-	M	-	-	1	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
								6	c.826_836del	p.Arg276SerfsX21	-	
25	8	EL*	-	M	-	-	-	6	c.767_786del	p.Gln256ProfsX38	-	Compound heterozygous
								6	c.826_836del	p.Arg276SerfsX21	-	
26	15	EL	-	M	-	-	-	6	c.767_786del	p.Gln256ProfsX38	-	Homozygous
27	41	EL	m	M	-	-	-	12	c.1960C>T	p.Pro654Ser	-	Homozygous
28	34	EL	-	M	-	-	1	12	c.2008C>T	p.Arg670X	ADAM TSR-1	Homozygous
29	4	EL	m	M	-	-	-	19	c.3153C>A	p.Tyr1051X	PLAC domain	Compound heterozygous
								19	c.3161A>G	p.Tyr1054Cys	PLAC domain	
								6	c.926G>A	p.Arg309Gln	-	

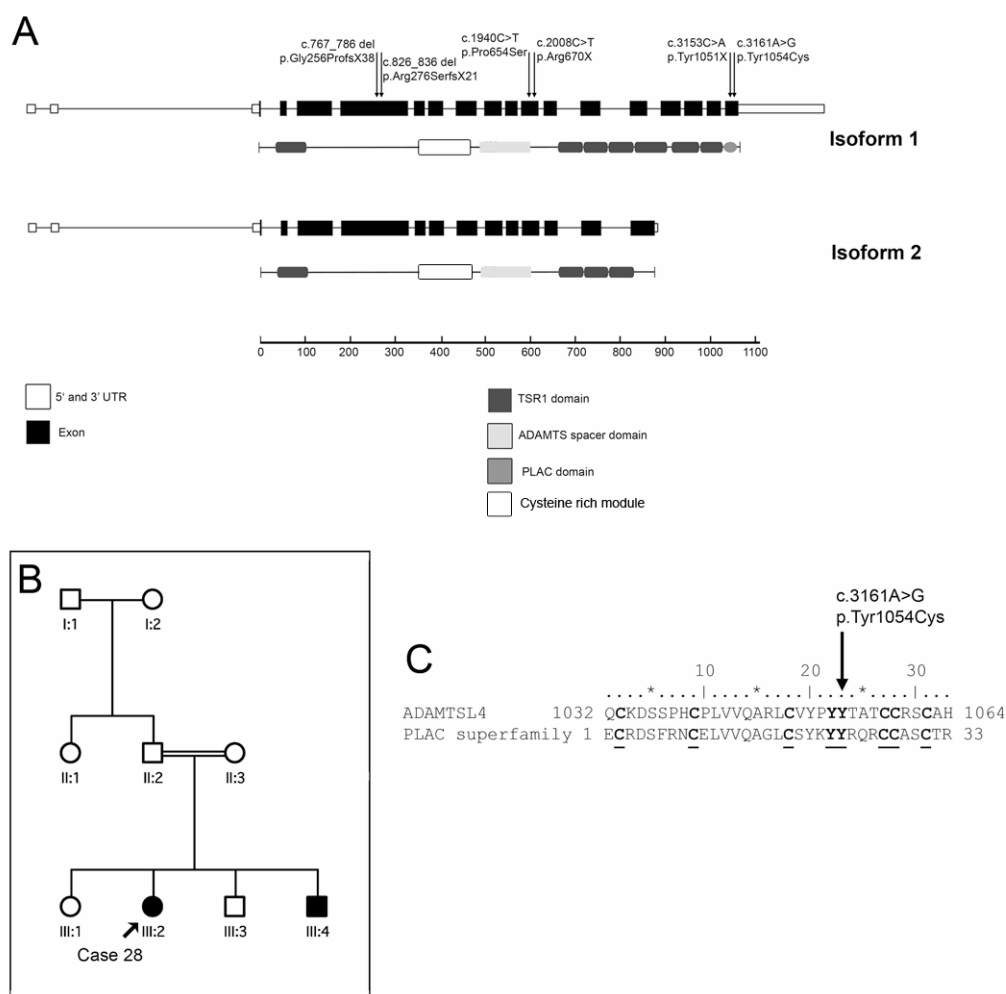
**Legend:** SS=Skeletal system; OS=Ocular system; CS=Cardiovascular system; S=Skin system; F=Family history; M=Major involvement; m=minor involvement.

\*Ectopia lentis et pupillae

ADAMTSL4 cDNA sequence according to GenBank (RefSeq NM\_019032.4) was used, with the A of the ATG translation initiation codon as nucleotide +1. The initiation codon is identified as codon 1.



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**Figure 1.** Panel A shows the schematic representation of *ADAMTSL4*. The exons and protein domains are shown for each isoform 1 (full length) and 2. Isoform 2 lacking three TSR1 domains and the PLAC domain. Arrows above the diagram indicate the approximate mutation positions. Panel B shows the pedigree of case 28 (III:2). Panel C shows the position of the c.3161A>G (p.Tyr1054Cys) mutation in the conserved PLAC domain of ADAMTS-like 4. The mutation affects one of the conserved tyrosine residues of the PLAC superfamily.

## DISCUSSION

This study describes the mutation findings in a group of 36 predominant EL patients referred for *FBNI* screening in the Sonalee laboratory. We believe this is the largest group of EL patients studied to date. The mutation detection rate observed in *FBNI* was 23/36 (64%), which has improved since our last publication (50%) due to rescreening of 13 patients, previously found mutation-negative by SSCA, using the more sensitive dHPLC detection method. Mutations were identified more often in the 5' region of the gene (61%), as previously observed (Comeglio, et al., 2007; Faivre, et al., 2009b). Mutations in the neonatal region (exons 24-32) were underrepresented (3/23, 13%).

Several studies have attempted to characterise mutation types and location in *FBNI* (Collod-Beroud, et al., 2003; Faivre, et al., 2009a; Faivre, et al., 2007; Rommel, et al., 2005). It is widely accepted that severe neonatal MFS is associated with mutations in exons 24-32 (Faivre, et al., 2009c). According to recent findings an association between predominant EL and the 5' end of the *FBNI* gene is apparent (Comeglio, et al., 2007; Faivre, et al., 2007). Here we report 14/23 (61%) of mutations in the first 21 exons, compared with 29% of total mutations in all patient sub-groups in the latest largest study (Faivre, et al., 2007). Recently, a large study

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5 suggested that mutations resulting in PTC are associated with major skeletal findings, and mutations affecting  
6 cysteines have been strongly associated with the presence of EL(Faivre, et al., 2007). Thirteen out of 23 (56%)  
7 probands had missense mutations involving cysteine compared to only one mutation resulting in a PTC.

8 In type 1 fibrillinopathies associated with mutations in the transforming growth factor-beta receptor  
9 (TGFBR) complex, it is important to note that EL is rarely present(Loeys, et al., 2006) suggesting that the role  
10 of fibrillin-1 in the TGF- $\beta$  pathway is of less importance in EL and thus mutations causing EL in *FBN1* are less  
11 common in domains associated with TGF- $\beta$  binding. Faivre *et al* suggest that correct cysteine orientation and  
12 disulfide bonding is important in structural integrity of suspensory ligaments and PTC mutations are associated  
13 with severe skeletal and skin features(Faivre, et al., 2007). Furthermore, TGFBR-related fibrillinopathies  
14 include severe skeletal and skin features suggesting that these features in both patients with a *FBN1* PTC  
15 mutation and patients with TGFBR-related disorders are associated with haploinsufficiency in the TGF- $\beta$   
16 signalling pathway.

17 *ADAMTSL4* encodes ADAMTS-like 4, and contains 7 predicted thrombospondin type 1 repeats (TSR1) that  
18 are believed to anchor the protein to the extracellular matrix (ECM)(Cal, et al., 2002). ADAMTS-like 4 is a  
19 protein resembling the ADAMTS ancillary domain lacking the proteolytic capability of ADAMTS proteases  
20 (Apte, 2009). ADAMTS proteases are thought to function in ocular physiology and members of the ADAMTS  
21 family have been implicated in ocular diseases, with autosomal-recessive mutations found in *ADAMTS10* in  
22 Weill-Marchesani syndrome (WMS)(Dagoneau, et al., 2004; Kutz, et al., 2008; Morales, et al., 2009),  
23 homozygous mutations in *ADAMTS2* associated with Ehlers-Danlos syndrome type VIIC(Colige, et al., 2004),  
24 and autosomal-recessive mutations in *ADAMTSL2* in geleophysic dysplasia(Le Goff, et al., 2008). Recently,  
25 *ADAMTSL7* has been implicated in incomplete WMS(Morales, et al., 2009).

26 Interestingly, the previously identified *ADAMTSL4* mutation leading to a PTC in exon 11 results in only  
27 isolated EL(Ahram, et al., 2009), and similarly the mutations described here leading to either homozygous or  
28 compound heterozygous PTCs (found in exon 6 and exon 12, see Table 2) are associated with EL in the absence  
29 of any other system involvement.

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31 The PTC mutations in exon 6 and exon 12 may result in a truncated protein of 300 amino acid residues and  
32 670 respectively and may abolish the C-terminal TSR1 domains and therefore the anchoring to the ECM (see  
33 Figure 1), if a protein is translated at all and the mRNA does not undergo a nonsense-mediated decay (NMD)  
34 process. This however is not possible to predict without analysis of the protein product in these instances. Since  
35 ADAMTS-like 4 does not carry a catalytic domain found in other ADAMTS proteins, it is suggested the  
36 pathogenic role of *ADAMTSL4* mutations may be due to the structural role of the protein in the assembly or  
37 integrity of the ECM.

38 The mutations identified in exon 19 both occur in the PLAC domain at the c-terminal end (see Figure 1). The  
39 PLAC domain contains a 6-cysteine conserved repeat, first described in lacunin, an extracellular matrix protein  
40 originally identified in *Manduca sexta*. The role of this domain is not known but it is thought to be important in  
41 matrix proteases and proprotein convertases. The p.Tyr1051X mutation identified in exon 19 abolishes this  
42 domain. Furthermore, the p.Tyr1054Cys affects one of the conserved amino acids in the PLAC domain (see  
43 Figure 1) and introduces an extra cysteine residue into the domain that is predicted to be disulfide bonded which  
44 could lead to disruption of the domain structure. This domain is however not present in isoform 2 of ADAMTS-  
45 like 4. Hence, the phenotypic effects seen in this patient may be due to disruption of the PLAC domain function  
46 or overall protein function of ADAMTS-like 4 isoform 1.

47 Although there is some evidence that *ADAMTSL4* is expressed in the eye (<http://smd.stanford.edu>), in order  
48 to determine the importance of the protein in the zonular filaments and confirm the significance of mutations in  
49 *ADAMTSL4* in EL it would be of great interest to localize the protein or mRNA within zonular filament tissue.

50 All available clinical and surgical details were gathered retrospectively for each patient. Because of the time  
51 range of blood sample collection, many details were no longer available. It was therefore not possible to  
52 establish clear differences in phenotype between the subgroups, namely those with *FBN1*, *ADAMTSL4* and no  
53 demonstrable mutation. Detailed genotype-phenotype analysis will be performed prospectively on a larger  
54 group of EL patients.  
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## 56 CONCLUSIONS

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58 The *FBN1* mutation yield in patients referred for screening in the Sonalee Laboratory with EL and only  
59 minor involvement of any other system described here is 64%, increased from our previous findings of 50%.  
60 This yield is however artificially suppressed due to the inclusion of patients with recessively inherited EL due to

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5 mutations in *ADAMTSL4*. We believe *FBN1* mutations are therefore responsible for a higher proportion of  
6 dominantly inherited EL and some of the sporadic cases are in fact cases of recessive EL either in  
7 consanguineous or non-consanguineous families.

8 We believe these findings confirm the importance of *ADAMTSL4* in autosomal recessive EL and suggest that  
9 certain mutations are recurrent in different families either as homozygous mutations or compound heterozygous  
10 mutations in non-consanguineous families. This study also demonstrates that *ADAMTSL4* is an important cause  
11 of EL outside of the original Jordanian family where it was first identified. In all cases of *ADAMTSL4*  
12 mutations, the findings were compatible with autosomal-recessive inheritance. It is therefore suggested that  
13 even in children of non-consanguineous families with apparent *de-novo* EL, screening for mutations in  
14 *ADAMTSL4* should be undertaken in *FBN1* mutation negative cases, to eliminate cardiac risk and guide genetic  
15 counselling and long-term management.

16 In this consecutive series of probands there remains 20% of EL with no proven genetic basis in which new  
17 dominant or recessive genetic determinants are predicted to be found. At least one, probably more genes remain  
18 to be described and candidates should include other members of the ADAMTS family of proteins.  
19

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