A Homozygous Microdeletion within *ADAMTSL4* in Patients with Isolated Ectopia Lentis: Evidence of a Founder Mutation

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Purpose. The purpose of the study was to look for *ADAMTSL4* mutations in a cohort of German patients with isolated ectopia lentis from nonconsanguineous families.

Methods. Mutation screening was performed by PCR amplification of the coding exons of *ADAMTSL4* and subsequent sequencing.

Results. An identical homozygous deletion of 20 bp of coding sequence within exon 6 (NM_019032.4:c.759_778del20) was identified in eight individuals from seven unrelated families. In a screen of 360 ethnically matched, unaffected individuals, two heterozygous mutation carriers were found. The mutation was always accompanied by the identical haplotype, suggestive of a founder mutation.

Conclusions. The results emphasize the association of *ADAMTSL4* null mutations with isolated ectopia lentis and the presence of a founder mutation in the European population. Screening of *ADAMTSL4* should be considered in all patients with isolated ectopia lentis, with or without family history. In patients from nonconsanguineous families, the authors propose a two-step diagnostic approach, starting with an examination of exon 6 before sequencing the entire coding region of *ADAMTSL4*. (*Invest Ophthalmol Vis Sci.* 2011;52:695–700) DOI:10.1167/iovs.10-5740

Ectopia lentis (EL) is characterized by a dislocation of the lens due to instability of the zonular fibers. Patients usually have significant loss of visual acuity. Its severity depends on the degree of the dislocation and on accompanying findings such as refractive errors.

If it is not due to a trauma, EL is frequently associated with systemic or syndromal diseases such as Marfan syndrome, Weill-Marchesani syndrome, or homocystinuria. Moreover, it can occur as an isolated condition. For isolated EL, both autosomal dominant and autosomal recessive inheritances have been described. In families with autosomal dominant EL, mutations in the *FBN1* gene (OMIM 134797) have been identified, which also cause Marfan syndrome.[1] Recently, Ahram et al.[2] reported on a large family with isolated autosomal recessive EL caused by a homozygous nonsense mutation in *ADAMTSL4* (OMIM 610113). In a second consanguineous family with two affected siblings, a homozygous splice site mutation was identified.[3] In two very recent reports,[4,5] more patients from nonconsanguineous families (eight European families) with EL due to *ADAMTSL4* mutations have been described. Interestingly, in five of those eight families, the identical homozygous mutation was identified (c.759_778del20), and in two other families, this mutation was found in a compound heterozygous state.

ADAMTSL4 is a member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs)-like gene family and encodes a large protein with seven thrombospondin type 1 repeats. The thrombospondin type 1 repeat domain is found in many proteins with diverse biological functions, including cellular adhesion, angiogenesis, and patterning of the developing nervous system. Although the component ADAM within its gene name implicates a protein degrading activity of ADAMTSL4, the latter has not been detected so far.[2] Thus, the true biological function and mode of action of this protein, especially in the context of eye development, remains elusive. Here, we describe seven unrelated families with isolated EL with the homozygous c.759_778del20 *ADAMTSL4* mutation. Haplotype analyses suggest a founder mutation.

Methods

The aliases of the individuals participating in this study refer to the pedigrees shown in Figure 1 and follow the nomenclature system "family_generation-person." According to this, the oldest son of family 1, for example, will be referred to as patient F1_II-1. Informed consent was obtained from all assessed patients or their parents. The research adhered to the tenets of the Declaration of Helsinki.

Family 1

The boys (F1_II-1 and F1_II-2) were the first and second child, respectively, of healthy, nonconsanguineous German parents (Fig. 1B). EL was diagnosed in both patients within the first year of life. In F1_II-1, the lens was dislocated to the temporal–inferior side in the left eye and the nasal side in the right; in F1_II-2, it was dislocated to the temporal–inferior side in the left and inferiorly in the right eye. Further ocular findings in both patients included spherophakia, increased bulbar length, and subsequent high myopia. Apart from the ophthalmic findings, the boys were healthy; however, both had a herniotomy in early childhood. F1_II-2 also had benign ventricular extrasystolia that required no treatment. The younger sister (F1_II-3) was healthy and without any ocular disease (regularly assessed for EL during early childhood).

Family 2

The patient (F2_II-2) was the second child of healthy, unrelated German parents (Fig. 1B). His EL was diagnosed during early childhood. The lens was dislocated to the nasal–superior side in both eyes. Other ocular features were spherophakia and high anisometropia. No other findings indicative of Marfan syndrome or other medical problems

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Submitted for publication April 21, 2010; revised August 24, 2010; accepted September 16, 2010.

Disclosure: **T.M. Neuhann**, None; **J. Artelt**, None; **T.F. Neuhann**, None; **S. Tinschert**, None; **A. Rump**, None

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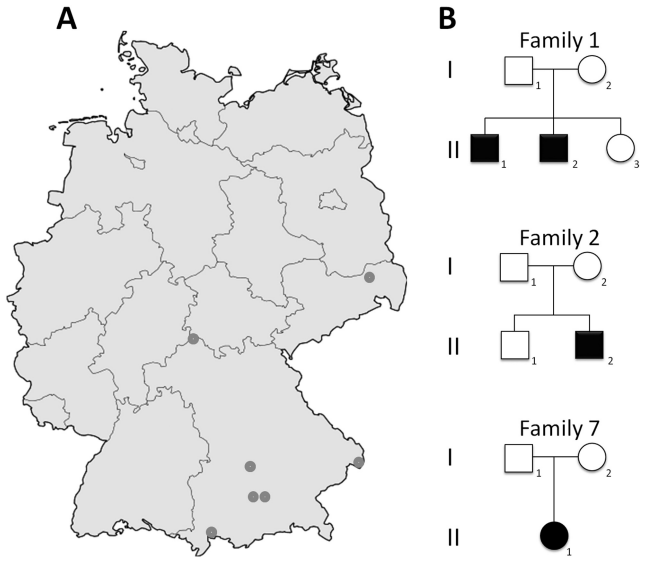

FIGURE 1. Provenience of patients and pedigrees of families participating in this study. (**A**) A map of Germany with *filled circles* indicating the provenience of the assessed families. (**B**) Pedigrees of families 1, 2, and 7. *Symbols* follow the recommendations of the National Society of Genetic Counselors.[20] *Filled symbols*: affected individuals (with EL); *open symbols*: unaffected individuals. *Roman numerals*: refer to generations; *Arabic numerals*: order of the individuals within each generation according to decreasing age.

were present. The elder brother (F2_II-1) and either parent (F2_I-1 and F2_I-2) were healthy and without any ophthalmic condition.

Family 3

Patient F3_II-3 was the third child of healthy, unrelated parents. In F3_II-3, bilateral EL was diagnosed at birth. The lens was dislocated superiorly in the left eye and to the temporal–superior side in the right eye. Additional ocular findings included iridodonesis and myopia. At the age of 25 years, she underwent surgery for an umbilical hernia.

Family 4

Patient F4_II-3 was the youngest of three siblings. There is no evidence of consanguinity in the family. He was diagnosed with bilateral EL in early childhood. Lens extraction was performed in the right eye at the age of 4 years; in the left eye, he had inferonasal EL. He also had an inguinal hernia. Apart from these findings, he was healthy. However, he reported having had mild cardiac arrhythmias in childhood. There was no follow-up regarding the arrhythmia.

Family 5

Patient F5_II-1 was the first of two children of healthy, unrelated parents. The younger sister was reported to have high myopia. The patient was diagnosed with bilateral EL in early childhood, and lens extraction was performed in the right eye at the age of 10 years. The lens in the left eye was discretely dislocated inferiorly. In addition, he had bilateral iridodonesis and a persistent pupillary membrane in the left eye. No extraocular disease was reported.

Family 6

Patient F6_II-2 was the second child of healthy parents. EL was diagnosed at the age of 15 months, and surgery was performed at the age of 18 months. From the age of 8 years, she had increased intraocular pressure (IOP). She had no extraocular disease or medical condition.

Family 7

Patient F7_II-1 was the only child of healthy, nonrelated parents (Fig. 1B), and had EL diagnosed at the age of 2 years. The girl also had spherophakia, high myopia, and iridodonesis. At the age of 2 years, she underwent lensectomy. Because of an increased IOP after surgery, iridectomy was performed. She had no other known extraocular disease.

None of the patients fulfilled the diagnostic criteria of Marfan syndrome according to the revised Ghent Nosology.[6] The patients' families originated from different parts of Germany (Fig. 1A).

Mutation Screening

Genomic DNA was extracted from whole blood by standard procedures. All *ADAMTSL4* exons, including splice sites and flanking intronic sequences were amplified with the primers and PCR conditions that have been published[2] (for the genomic localization of the amplicons, see Fig. 2). Sequence analysis of the PCR products was performed with dye termination (BigDye terminator; 3130xl genetic analyzer; Applied Biosystems, Inc. [ABI], Foster City, CA) cycle sequencing and subsequent electrophoresis.

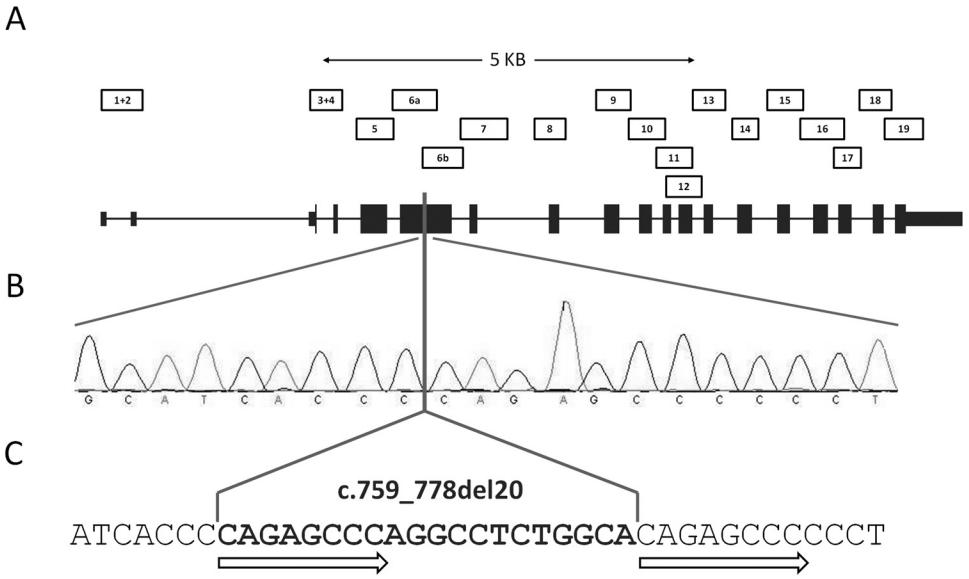

FIGURE 2. The *ADAMTSL4* gene: intron–exon structure, amplicons, and position of the c.759_778del20 deletion. (**A**) The complete *ADAMTSL4* gene is shown as a *horizontal line* (big boxes: exons; flat boxes: 5′- and 3′ untranslated regions; *thin line*: introns). The amplicons for mutation analysis are depicted as boxes above the gene model, with numbers indicating the exons that are covered by the corresponding PCR products. The c.759_778del20 deletion is pointed out by a *vertical line*. (**B**) Electropherogram of an individual with a homozygous deletion of a 20-bp coding sequence (c.759_778del20). (**C**) Reference sequence for part of exon 6; the sequence missing in our patients with EL is shown in *bold*. The deletion leads to "deduplication"[21] of a direct CAGAGCCC octamer repeat (*vertical arrows*).

Deletion–Detection PCR and Screening of Healthy Individuals

The oligonucleotides Adam_del_F (CATCACCCCAGAGCCCCC) and Adam_del_R (GGTGAGGGGGGCTCTGGG) were designed to flank the c.759_778del20 deletion (Fig. 2). In standard PCR conditions (annealing at 54°C), a 134-bp product was obtained from the wild-type allele and a 114-bp fragment was produced from the mutant allele (if present).

SNP-Genotyping

Variant-specific PCR primers were designed with the web-based, allele-specific primer (WasP) tool. Allele specificity was verified by genotyping the HapMap individuals NA12878, NA12891, NA12892, and NA19240, for which the genotype is known from complete genomic sequencing. The SNP rs41317515 was genotyped with the allele-specific forward primers rs41317515_CF (GATCTCTTCTAGAGGGGAAGACC) and rs41317515_GF (GATCTCTTCTAGAGGGGAAGACG) and the published reverse primer 6a-R.[2] All other SNPs used were genotyped as byproducts of the mutation screening process, since the primers used for PCR amplification typically flanked the exons at a distance of approximately 70 to 100 bp and thus included several SNPs.

MS-Genotyping

Microsatellites were genotyped with two panels of a linkage mapping set (panels 1 and 2 of the Prism Linkage Mapping Set, ver. 2.5; ABI). In combination, both panels provide labeled primer pairs for 31 microsatellites on human chromosome 1 (Prism Linkage Mapping Set, ver. 2.5 Panel Guide' ABI). Since the nearest ABI probe (D1S498) maps at a distance of 770 kb upstream of *ADAMTSL4*, we established our own microsatellite marker (TSL4CA), which maps 50 kb downstream of *ADAMTSL4*. The microsatellite consists of 12 to 18 CA-repeats and is amplified with the primers TSL4CAF (AAGAGCCAGAGTGGGACTGA) and TSL4CAR (CAAACAATGAGAACCCCACA). To allow versatile labeling, the forward primer was extended at its 5′-end by an 18 nt M13 tag (TGTAAAACGACGGCCAGT), and labeling was performed as published.[7]

Web Resources

The URLs for information presented herein are as follows (all are provided in the public domain, unless otherwise noted): Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (National Center for Biotechnology Information [NCBI]; Bethesda, MD); UCSC Genome Browser, http://genome.ucsc.edu/cgi-bin/hgGateway/ (University of California at Santa Cruz); design of allele-specific primers, http://bioinfo.biotec.or.th/wasp/ (The Genome Institute, BIOTEC, Pathumthani, Thailand); The 1000 Genomes Project, http://browser.1000genomes.org/index.html/ developed by a consortium; SNP database NCBI, http://www.ncbi.nlm.nih.gov/projects/SNP/ (NCBI); and Human Gene Mutation Database (HGMD) www.hgmd.org/ (freely available to registered academic/nonprofit users; Institute of Medical Genetics, University of Wales College of Medicine, Cardiff, Wales, UK).

Data Analyses

After PCR, analysis of the samples was performed by capillary electrophoresis (Prism 3130xl Genetic Analyzer; ABI), using a polymer (POP7; ABI). Data were visualized, allele sizes were scored (GeneMapper software, ver. 4.0; ABI), and results were exported to a data-analysis program (Excel; Microsoft, Redmond, WA) for further analysis and comparison.

Results

Mutation Detection

By sequencing 18 PCR-products (Fig. 2A) in patient F1_II-1, we detected a homozygous deletion of 20 bp (NM_019032.4: c.759_778del20) within exon 6 of *ADAMTSL4* (Figs. 2B, 2C). Additional mutations have not been found in this patient. Subsequent mutation analysis of exon 6 in the remaining members of the family revealed that the affected brother, F1_II-2, carried the same deletion on both alleles. The healthy parents as well as the unaffected sister were heterozygous carriers of the c.759_778del20 mutation. Not expecting that the affected child of family 2 would have the very same deletion, we sequenced all PCR products in this patient. Again, we found the homozygous deletion c.759_778del20, and no additional mutation was detected. Consistent with the recessive nature of the c.759_778del20 deletion, the parents of F2_II-2 were both heterozygous carriers of the mutation. The unaffected brother was not examined.

Since the c.759_778del20 deletion had been identified repeatedly in German EL patients, we took a two-step approach and started our mutation screening by sequencing exon 6 in the patients from families 3, 4, 5, 6, and 7. All five individuals carried the c.759_778del20 deletion homozygously.

Heterozygous Carriers

We screened 360 ethnically matched anonymous individuals by PCR. In two cases (DNA samples P14829 and P8732) a band smaller than the expected product of 134 bp was identified in agarose gel electrophoresis. In both cases, the normal band was present as well, indicating heterozygosity for these putative deletions. Subsequent sequence analysis revealed that each deletion was indeed present on one allele only and, furthermore, both individuals carried the very same c.759_778del20 mutation we had observed in our families.

ADAMTSL4 Haplotypes

Since the amplicons designed for mutation analysis covered not only the corresponding exons but also included 50 to 100 bp of flanking intronic sequence (Fig. 2A), several SNPs were genotyped as a byproduct of the mutation analysis. In our cohort of German patients and controls, we detected sequence variation at four SNP loci: rs41317515, rs9659061, rs12124948, and rs10687239 (Fig. 3). However, the c.759_778del20 deletion always, without any exception, was accompanied by the haplotype C-A-T-ins4 at these SNPs (Fig. 3A). To determine the frequency of the deletion-associated SNP alleles in our normal population, we genotyped 20 to 40 unrelated, healthy German individuals, either by allele-specific PCR (rs41317515) or by sequencing (rs9659061, rs12124948, and rs10687239). On average, the deletion-associated SNP alleles had a frequency of approximately 50% in the control cohort (Fig. 3B). Since we did not have access to relatives of these controls, we could not construct haplotypes by segregation analysis. In our EL families, however, we detected various different haplotypes by segregation analysis. Thus, the deletion-associated haplotype C-A-T-ins4 is just one among several others that strongly suggest a founder for the c.759_778del20 deletion.

To estimate the time when the founder mutation took place, we genotyped microsatellite markers on chromosome 1 (Table 1) in our EL patients as well as in a cohort of unrelated, healthy German individuals. For the nearest marker (TSL4CA, 50-kb distance to *ADAMTSL4*) 100% of our EL patients carried the 209 allele (Fig. 4, left), in which a CA-dinucleotide unit is repeated 14 times. In the control cohort, this allele had a frequency of only 37% (Fig. 4, left), again strongly suggesting a founder of the c.759_778del20 deletion. Concerning the second closest marker (D1S498, 770-kb distance to *ADAMTSL4*), the EL patient group showed some sequence variety (Table 1, Fig. 4, right), which is probably due to recombination events and suggests that the founder mutation is very old. However, there is still a predominant allele, which occurred in 75% of the

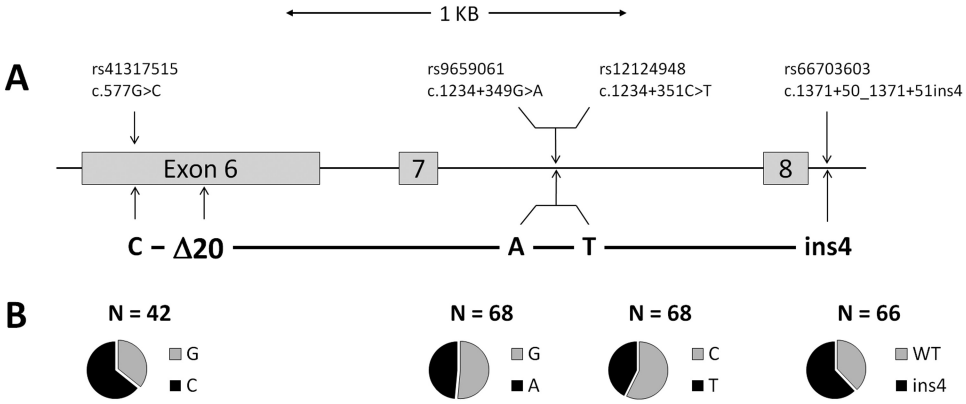

FIGURE 3. SNP haplotype associated with the c.759_778del20. (**A**) The genotype at four SNP positions (shown above the *ADAMTSL4* gene model) was determined for all members of our EL families. Familial segregation allowed the identification of a deletion-associated haplotype: in all individuals tested, the deletion comes along with the haplotype shown as a *bold line*. (**B**) For each of the SNPs, normal German probands were genotyped. The observed allele frequency is illustrated by a pie chart for each SNP. n = number of haplotypes investigated for each SNP.

EL cases but in only 20% of the control cohort (Fig. 4, right). In conclusion, there is strong evidence of a founder mutation and the founder event appears to have occurred more than 100 generations ago.

DISCUSSION

This series is the largest one of families with isolated EL due to an *ADAMTSL4* mutation described so far. Whereas the previous mutations reported by Ahram et al.[2] and Greene et al.[3] are confined to a single, consanguineous family, the mutation described here has occurred in eight patients from seven unrelated nonconsanguineous families with EL. It has been described simultaneously by two other groups from Norway and the United Kingdom that assessed patients with EL for *ADAMTSL4* mutations.[4,5] Including this report, 14 nonconsanguineous families have now been identified with the c.759_778del20 deletion (12 homozygous, 2 compound heterozygous) from different countries in Europe. In addition, it was observed twice in a random sample of 360 healthy German individuals plus in samples from 190 Norwegian blood donors, indicating that the mutation is widespread.

All the patients described herein shared the identical SNP haplotype and were homozygous for the 209 allele of microsatellite marker TSL4CA. Neither the SNP-haplotype nor the 209 allele was overrepresented in the control subjects (Figs. 3, 4), and so we assume that the frequency of the mutation is due to a common founder. The founder must reach several generations back, since there is some allele variation due to recombination at marker D1S498 (with the 202 allele being clearly overrepresented; Fig. 4), and the families are neither related nor do they all come from the same region. A founder was also proposed by Christensen et al.,[5] who assumed that the common ancestor to their collective lived ~4000 years ago. If this assumption is correct and considering that this mutation has been identified in 14 of the 15 nonconsanguineous families with EL described thus far, it is very probable that there is one European founder for this mutation.

Cause of the c.759_778del20 Deletion

The most striking feature concerning the sequence environment of this deletion is the occurrence of two identical copies of an octamer sequence, spaced in a distance of 12 bp (Fig. 2C). The deletion of this spacer, together with one repeat unit results in a loss of 20 bp of coding sequence. Supposedly, the octamer sequence is not only affected by the deletion but may have also been the very cause of it. This assumption is strongly supported by a recent survey[8] of 8399 microdeletions, which resulted in the observation that direct repeats and symmetric elements appear to be predominantly involved in mediating both microdeletions and microinsertions, probably by strand slippage during replication.[9]

Consequences on Posttranscriptional Level

The c.759_778del20 deletion results in a frameshift and, therefore, leads to a premature stop of translation. This alteration can have two consequences: On failure to strip off all exon–junction complexes, the mRNA is recognized as erroneous and will be subjected to nonsense mediated decay (NMD). Alternatively, a truncated ADAMTSL4 protein (p.Q256PfsX38) may be produced. Based on the current data, a distinction between these two scenarios cannot be made.

However, among the few different *ADAMTSL4* mutations that have been reported, protein-truncating mutations appear to be overrepresented so far. This may indicate that it is the

TABLE 1. Microsatellite Genotypes of EL Patients in This Study

	TSL4CA Distance −50 kb		D1S498 Distance +770 kb		D1S484 Distance +10.2 Mb		D1S2878 Distance +14.8 Mb		D1S196 Distance +17.1 Mb	
	Allele 1	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 1**	**Allele 1**	**Allele 2**
F1_II-1	**209**	**209**	200	**202**	278	278	156	156	332	332
F1_II-2	**209**	**209**	200	**202**	278	278	156	156	332	334
F2_II-2	**209**	**209**	**202**	**202**	278	278	174	174	330	332
F3_II-3	**209**	**209**	**202**	**202**	278	280	170	170	324	330
F4_II-3	**209**	**209**	198	**202**	280	282	156	156	328	330
F5_II-1	**209**	**209**	**202**	**202**	278	278	156	156	324	334
F6_II-2	**209**	**209**	**202**	**202**	276	278	156	156	322	322
F7_II-1	**209**	**209**	200	**202**	276	280	158	158	322	328

Data are expressed in base pairs. Numbers in bold indicate most frequent alleles in EL patients. Distance, the distance of the marker from the *ADAMTSL4*-gene on the long arm of chromosome 1; negative values, proximal location (i.e. located closer to the centromere and upstream of *ADAMTSL4*); positive values, distal (downstream) location.

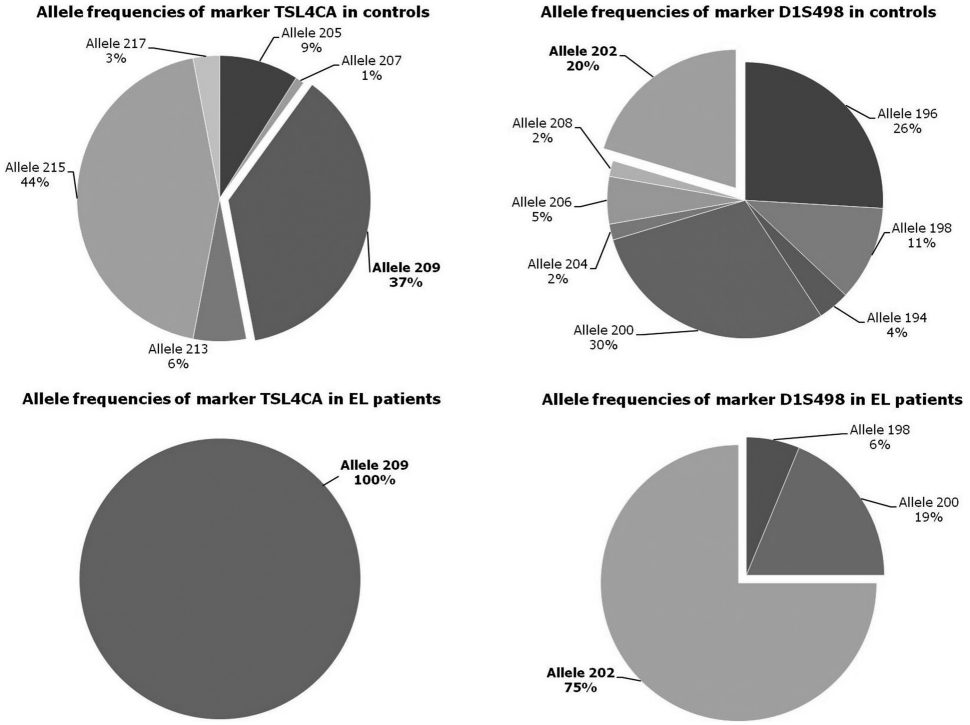

FIGURE 4. The percentage of allele frequencies of the two assessed markers closest to the *ADAMTSL4* gene. *Top left*: alleles of marker TSL4CA (50 kb proximal to *ADAMTSL4*) found in a screen of healthy control persons. *Bottom left*: alleles of marker TSL4CA found in all the patients with EL in this study. *Top right*: alleles of marker *D1S498* (770 kb distal to *ADAMTSL4*) found in a screen of healthy German control persons. *Bottom right*: Alleles of marker *D1S498* found in the patients with EL in this study.

shortening of the protein rather than its absence (or null function) that matters in the pathogenesis of EL. Such a scenario has been observed for the laminin gene family, in which nonsense and frameshift mutations clearly outnumber missense mutations.[10]

Consequences for the Phenotype

ADAMTSL4 is expressed in many adult human tissues, including different tissues of the eye.[2,11] Because of the absence of other organ manifestations in the patients' *ADAMTSL4* mutations reported so far, we assume that the ADAMTSL4 protein is a unique and vital component for zonular fiber integrity and that the loss of its function in other tissues can be compensated for by other proteins, such as other members of the ADAMTS superfamily. Although none of the affected persons had major extraocular symptoms, there was some evidence of mild connective tissue instability in the two brothers F1_II-1 and F1_II-2 as well as in F3_II-3 and F4_II-3 (inguinal hernia and umbilical hernia, respectively). Reports on more patients are needed to establish a convincing genotype–phenotype correlation.

The ADAMTS superfamily comprises 24 proteins taking part in various biological processes, among them connective tissue organization.[12,13] Mutations in other genes of the family have been associated with connective tissue disorders, such as autosomal recessive Weill-Marchesani syndrome (WMS) (*ADAMTS10*, *ADAMTS17*)[14,15] and geleophysic dysplasia (*ADAMTSL2*).[16] ADAMTSL2 has been shown to interact with a connective tissue pathway (interacts with TGF-β signaling)[16]; thus, the ADAMTS proteins seem to play an important role in connective tissue homeostasis. Since mutations in *FBN1* cause autosomal dominant syndromal (Marfan syndrome, WMS) and isolated EL[1,17] and fibrillin-1 is a major component of the zonular fibers, one could expect ADAMTSL4 to interact with fibrillin-1 and thereby cause EL when not functioning. However, the zonular fibers are complex structures,[18,19] and a role/target for ADAMTSL4 other than fibrillin-1 within the zonular fiber homeostasis or within a signaling pathway cannot be excluded.

Implications for Patient Care and Genetic Counseling

The families and results presented herein provide further evidence that so-called idiopathic EL exhibits an autosomal recessive inheritance pattern and is caused by mutations in *ADAMTSL4*. In patients with luxation or subluxation of the lens, usually only the well-known syndromes such as Marfan syndrome or homocystinuria are considered. However, EL caused by *ADAMTSL4* mutations has to be thought of as a major differential diagnosis. Thus, screening for mutations within *ADAMTSL4* should be performed in all patients with isolated EL or EL et pupillae, with or without family history and in young children, who do not (yet) show features of a systemic disorder such as Marfan syndrome. Because of our results in patients from nonconsanguineous (European) families, we propose a fast and economic two-step approach to diagnosis, starting with an examination of exon 6 before sequencing the entire coding region of *ADAMTSL4*.

Acknowledgments

The authors thank the family members for their gracious participation in this study, and Dolores Friebel, Department of Pediatric Neurology, Children's Hospital, Medical Faculty, Carl Gustav Carus University of Technology, Dresden, Germany, and Yasmin Mehraein, Institute of Human Genetics, University of Munich, for their support.

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