RESULTS.

Medical examination. Molecular genetic studies included homozygosity mapping with SNP markers, DNA sequencing, and RT-PCR analysis.

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Ocular signs in affected persons were increased median corneal thickness and astigmatism, angle malformation with prominent iris processes, displacement of the pupil and lens, lens coloboma, spherophakia, loss of zonular threads, early cataract development, glaucoma, and retinal detachment. No cardiac or metabolic abnormalities known to be associated with ectopia lentis were detected. Affected persons shared a 0.67 cm region of homozygosity on chromosome 1. DNA sequencing revealed a novel mutation in ADAMTSL4, c.767_786del20. This deletion of 20 base pairs (bp) results in a frameshift and an introduction of a stop codon 113 bp downstream, predicting a C-terminal truncation of the ADAMTSL4 protein (p.Gln256ProfsX38). Expression of truncated ADAMTSL4 mRNA was confirmed by RT-PCR analysis. Three of 190 local blood donors were carriers of this mutation.

CONCLUSIONS.

Ectopia lentis et pupillae is associated with a number of malformations primarily in the anterior segment of the eye. The causative mutation, which is the first to be described in ectopia lentis et pupillae, disrupts the same gene function previously shown to cause isolated ectopia lentis. The mutation is ancient and may, therefore, be spread to a much larger population than the investigated one. (Invest Ophtalmol Vis Sci. 2010;51:6369–6373) DOI:10.1167/iovs.10-5597

Ectopia lentis et pupillae (MIM 225200) is a rare disorder that, in most cases, has been reported to be transmitted as an autosomal recessive trait.1,2 The molecular genetic cause has thus far not been reported. In a Danish national survey of 396 cases of congenital ectopia lentis, a nosologic classification was possible in 274 cases, of which 68.2% were associated with Marfan syndrome (MIM 154700), 21.2% with ectopia lentis et pupillae, 8% simple dominant ectopia lentis (MIM 129600), 1.1% homocystinuria (MIM 236200), 0.7% Weill-Marchesani syndrome (MIM 277600), and 0.7% sulfite oxidase deficiency (sulfocystinuria, MIM 272300).3 Both ectopia lentis (MIM 225100) and ectopia lentis et pupillae have been observed in the same family.4,5

In persons with ectopia lentis et pupillae, malformations are restricted to the eye. Typically, the lens and pupil are displaced in opposite directions. Additional signs include enlarged corneal diameter, increased corneal astigmatism, increased anterior chamber depth, thinning and flattening of the iris with loss of crypts, angle malformation caused by enlarged iris processes, persistent pupillary membrane, loss of zonular fibers, tilted disc, and increased axial length.6,7–9 Optic nerve hypoplasia has also been reported.7 Secondary manifestations include refractive errors (in some persons with anisometropic amblyopia), glaucoma, early cataract development, and retinal detachment.7 Membrane formation on the posterior aspect of the iris has been observed both in histologic sections10–12 and on ultrasound biomicroscopy.13

Several genes have been identified that may be involved in the development of nonsyndromic ectopia lentis. Variable expressivity of fibrillin (FBNI) mutations has been associated with autosomal dominant forms. Recently, mutations in ADAMTSL4 have been reported in two families with isolated autosomal recessive ectopia lentis.14,15 In the present study, we have examined five families with ectopia lentis et pupillae. We show that this condition is also caused by homozygosity for a mutation in ADAMTSL4, extending the phenotypic presentation of disturbed function of this gene.

MATERIALS AND METHODS

Patients and Clinical Examination

Patients with ectopia lentis et pupillae were ascertained through departmental records, and they and their unaffected close family members were invited to participate in the study. Each signed an informed letter of consent. The study was approved by the Regional Committee for Medical and Health Research Ethics, Western Norway (IRB #00001872), and adhered to the tenets of the Declaration of Helsinki. The parents, siblings, and offspring of the affected family members underwent general medical and ophthalmologic examination. The latter included slit-lamp examination, gonioscopy, Goldmann and Icare (Espoo, Finland) tonometry, corneal topography (Pentacam; Oculus, Wetzlar, Germany), and axial length measurements (IOL Master [Zeiss, Jena, Germany] or CineScan [Quantel Medical, Clermont-Ferrand, France]). Information on accompanying disorders was corroborated from hospital records.

Homozygosity Mapping

Genomic DNA was isolated from whole blood (QIAsymphony; Qiagen, Hilden, Germany). A genomewide single nucleotide polymorphism (SNP) scan was performed using a gene chip array (250K SNP Chip; Affymetrix, Santa Clara, CA), and a search for regions of homozygosity...
was performed using the PLINK program. Haplotype constructions and analyses were performed manually.

DNA Sequencing and Mutation Detection

PCR primers for amplification of exons and flanking intron sequences of the ADAMTSL4 were designed by the OLIGO software (National Bioscience, Plymouth, MN). DNA was amplified by PCR using standard procedures. After PCR amplification, the PCR products were treated with SAP/exonuclease I (Amersham, Chalfont St. Giles, UK) and were sequenced (PRISM BigDye Terminator kit, v1.1; Applied Biosystems, Foster City, CA) and analyzed (ABI 3730 Genetic Analyzer; Applied Biosystems). DNA sequences were subjected to reference-based analysis (SeqScape software; Applied Biosystems). DNA from 190 healthy local blood donors was used to estimate the local population frequency of heterozygotes.

RNA Analysis

Blood was collected in blood RNA tubes (Tempus; Applied Biosystems), and total RNA was purified (ABI 6100 system; Applied Biosystems). The quality of RNA was analyzed (Experion system; Bio-Rad, Hercules, CA). cDNA synthesis was performed using a reverse transcription kit (TaqMan; Applied Biosystems). PCR amplification of the cDNA was performed using ADAMTSL4 c.350 forward 5’-AGTCTCGGGGAAGGGGTGGC-3’ and ADAMTSL4 c.1061 reverse 5’-AGGCTCCAGAGGGAGCTGGC-3’ primers. PCR products were sequenced using the ADAMTSL4 c.610 forward 5’-TTTCGG-CAACGGCAGCCC-3’ and ADAMTSL4 c.1061 reverse 5’-AGGCTCCAGAGGGAGCTGGC-3’ primers.

RESULTS

Ocular Involvement

Ten affected persons and 11 of their first-degree relatives of five families (Fig. 1) in Hordaland County in Western Norway were examined. In affected persons, the median central corneal thickness was 589 μm (range, 528–650 μm); the median corneal astigmatism in unoperated eyes was 3.1 D (range, 0.4–6.8 D), and the median white-to-white distance was 11.8 mm (range, 10.1–14.7 mm).

In unoperated eyes, the median depth of the anterior chamber was 3.75 mm (range, 3.00–4.10 mm). The median axial length of affected eyes was 22.79 mm (range, 21.98–26.80 mm). In family members C-II-1 and B-III-4, increased intraocular pressure was detected in one eye at the age of 29 and 30 years, respectively. Before treatment, the intraocular pressure was 35 mm Hg in the right eye of C-II-1 and 28 mm Hg in the left eye of B-III-4. C-II-1 was treated with trabeculotomy resulting in hypotony and loss of visual function, whereas B-III-4 has maintained adequate control with local treatment with a β-blocker. By gonioscopy, a fine iris-like tissue was seen extending from the iris root to the cornea.

Prominent dislocation of the pupil was seen in nine eyes of five persons (Fig. 2), seven upward (A-III-3, B-II-4, B-III-4, C-II-1) and two downward (D-II-1). In family members B-II-4 and E-II-5, a dislocated pupil was seen primarily in one eye. Family members A-III-6, C-II-3, and E-II-2 showed only mild pupil dislocation. In family members A-III-6 and E-II-2, this also occurred primarily in one eye. A-III-2 had centrally positioned pupils. Transillumination of the iris was seen in A-III-3, A-III-6, B-II-4, B-III-4, and C-II-1. These eyes also responded poorly to mydriatics. A fibrotic ring around the pupil was seen in A-III-3, A-III-6, B-II-4, B-III-4, and C-II-1.

Dislocation of the lens was seen in all persons, though to a variable extent. Minimal displacement was seen in both eyes of B-III-4 and in one eye of E-II-2. In those with prominent displacement of the pupil, the lens was dislocated in the opposite direction. A-III-2, who had centrally positioned pupils, had a downward dislocation of the lens, primarily in his right eye. Except in D-II-1, zonular fibers were missing in the area between the displaced pupil and the edge of the dislocated lens. Spherophakia was seen in both eyes of A-III-6. A small coloboma of the dislocated lens was seen in A-III-6 and E-II-2. Cataract developed relatively early, and all affected persons

![Figure 1](https://example.com/figure1.png)
older than 45 had undergone cataract surgery with intracapsular cataract extraction (B-II-4 at 43, C-II-1 at 46, and C-II-3 at 50 years of age).

C-II-3 had a retinal detachment at the age of 53 (OD) and a vitreous hemorrhage at the age of 56 (OS). The retina was successfully reattached after scleral buckling with a circular band. Peripheral retinal degenerations were seen in the eye with the retinal detachment.

Median value for best-corrected visual acuity of the best eye was 6/6 (range, 6/5–6/12). The corresponding figure for the worst eye was 6/18 (range, 6/6–6/36), excluding the eye with no light perception after trabeculotomy in C-II-1. In A-III-3 and D-II-1, the lens was dislocated to such an extent that an aphakic visual axis was created. These persons were highly hyperopic and had spherical equivalents ranging from +7.75 D to +12.25 D. B-III-4 was nearly emmetropic in both eyes (OD, −1.5 D; OS, +0.25 D). A-III-6 was myopic (OD, −11.75 D; OS, −7 D).

The more affected eye in E-II-2 was also myopic (−14.75 D), whereas the other was nearly emmetropic (+0.25 D). The spherical equivalent of E-II-5 was emmetropic, but he had a large astigmatism in both eyes (−7.5 D and −5 D). The myopia seen in A-III-6 and E-II-2 did not correspond to an elongated axial length and was, therefore, most likely caused by spherophakia. Minor exophoria was seen in B-III-4, C-II-1, and C-II-3, whereas A-III-2 had hyperopia that measured 12 to 14 PD on far vision.

No ocular abnormalities were seen in obligate heterozygotes or in other healthy family members.

Nonocular Features
All affected persons were of normal stature. None presented any features typical of Marfan or Weill-Marchesani syndrome. Echocardiography was performed in two persons, and the results were normal. Plasma homocysteine levels were measured in two persons and were within the normal range. Other diseases seen among the 10 patients were as follows: surgically corrected craniosynostosis, 1; diabetes mellitus type 1 and autoimmune thrombocytopenia, 1; raised arterial blood pressure, 2; episode of venous thrombosis probably associated with homozygosity for the factor V Leiden mutation, 2; carcinoma of the prostate gland, 1; unilateral testis retention, 1; and spermatocoele, 1.

Genetic Analysis
Genealogical studies showed that families B and C shared several ancestors as recently as five generations ago, whereas no relationship was established between families B and C and the three other families. The only region of homozygosity shared by all families was located on the long arm of chromosome 1 (Fig. 3).17 In family D, genealogical studies showed numerous parental common ancestors (the first couple five generations earlier). However, the region of homozygosity in the affected boy in this family was only 2.9 cm (5.1 Mb), best fitting with a common ancestor 25 to 50 generations back.17 The mother in family E originated from Eastern Norway, and her affected children were homozygous for a minuscule segment of 0.85 cm (1.28 Mb) within the candidate region on chromosome 1. The maximal segment all patients had in common was 0.65 cm (1.0 Mb), ranging from SNP markers at 148,500,906 and 149,507,166 bp from chromosome 1pter (NCBI Build 36.3). Such a region on chromosome 1 could possibly have originated from a common ancestor for all affected persons 150 generations ago.17 This region harbored 39 genes, among them ADAMTSL4. DNA sequencing of ADAMTSL4 revealed an out-of-frame 20-bp deletion, c.767_786del20 [AGGCCTCTGGCACAGAGCCC] reference sequence NM_019032.4 (Fig. 4). RT-PCR analysis of ADAMTSL4 mRNA revealed a transcript shortened by 20 bp. The 20-bp deletion introduced a stop codon 113 bp downstream of the deletion (data not shown), predicting a truncation of the ADAMTSL4 protein (p.Gln256ProfsX38).

Screening of 190 local blood donors identified three heterozygotes. Assuming genetic equilibrium for this apparently ancient mutation, this corresponded to a prevalence of homozygous persons of approximately 1:16,000 in this population (with wide confidence intervals).

DISCUSSION
In the present study we performed a detailed clinical and genetic examination of persons with autosomal recessive ectopia lentis et pupillae. Affected family members showed a...
Figure 3. Regions of homozygosity in the affected persons encompassing the ADAMTSL4 locus on chromosome 1. The regions of homozygosity are shown for all the affected persons we investigated with SNP arrays in families A to E, except for II-5 in family E, who has identical haplotypes with his sister. The maternal (M) and paternal (P) haplotypes could be determined for the affected in families A, D, and E and the son (III-4) in family B. Most breakpoints are unique, supported by the finding of different adjacent haplotypes (not shown). There were two families from the south of Bergen in Western Norway (families C, B) who shared parts of the same ancestral haplotype (red), and three from the north of Bergen (families A, D, E), who share another haplotype (blue), including only a tiny part (~1.3 Mb) of the ancestral (red) haplotype. The family of the mother in family E, however, originated from Eastern Norway, with no genealogical indication of ancestry in Western Norway. Her daughter’s maternal haplotype shares only 1.28 Mb of the ancestral red haplotype and also marks the centromeric boundary of the common homozygosity region at position 148.501 Mb from 1pter (C). The telomeric boundary of the common homozygosity region is determined by the breakpoint in the paternal haplotype in the affected boy in family D at 149.507 Mb from 1pter (T). ADAMTSL4 is located at 148.788 to 148.800 Mb from 1pter. This region of homozygosity for the same (red) haplotype contains only 35 SNPs (1 Mb, 0.67 cM). A homozygosity region of 0.67 cM corresponds to approximately 300 meioses (150 generations) back to the most recent common ancestor. The chromosome ideogram and physical distance from 1pter are given according to NCBI MapView version 36.3. The families are designated with their respective letters, given at the top of the figure.

Figure 4. Mutation analysis of the ADAMTSL4. Partial sequence chromatograms showing the wild-type (wt) DNA sequence of ADAMTSL4 of an unaffected person and the DNA sequence of an affected person homozygous for the ADAMTSL4 c.767_786del20 mutation. Lines indicate the position of the deletion.
generations or 4000 years ago).\(^{17}\) If this supposition is correct, this mutation may also be encountered in patients with this disorder in other countries.

In most families with ectopia lentis et pupillae, the mode of inheritance is autosomal recessive. Cruysberg and Pinckers\(^{2}\) described a kindred in which affected members were observed in three generations, and they suggested autosomal dominant inheritance with reduced penetrance. Others (http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=225200), however, have suggested that pseudodominance could explain this observation. In family B, affected persons are seen in two generations. Because the mode of inheritance in this family was shown to be autosomal recessive by molecular genetic studies, family B clearly represents an example of pseudodominance.

The \textit{ADAMTSL4} gene is widely expressed throughout the body, including in the eye.\(^{18}\) The precise function of \textit{ADAMTSL4} has yet to be determined. \textit{ADAMTS}-like (\textit{ADAMTS}) proteins lack the metalloproteinase domain found in \textit{ADAMTS} proteins (\textit{ADAM} [\textit{A} Disintegrin and \textit{M}etalloproteinase] with \textit{T}hrombospondin domains) and are, therefore, thought to be catalytically inactive. Both \textit{ADAMTS} and \textit{ADAMTSL} proteins function as secreted proteins, and they can be anchored to the extracellular matrix by one or more thrombospondin type I domains (see Ref.\(^{19}\) for review). Mutations in \textit{ADAMTS10} and \textit{ADAMTS17}\(^ {20}\) have been associated with Weill-Marchesani syndrome.\(^ {20}\) From the association of \textit{ADAMTSL4} mutation with isolated ectopia lentis, a role in the regulation of fibrillin assembly, architecture, or both has been suggested.\(^ {14}\)

In summary, in five seemingly unrelated Norwegian families with autosomal recessive ectopia lentis et pupillae, we found homozygosity for a novel mutation in \textit{ADAMTSL4}. The disruption of the same gene function in persons with isolated ectopia lentis and in those with ectopia lentis et pupillae suggests that these phenotypes can be facets of the same condition.

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\textbf{References}