

Brief Report

Expansion of Ocular Phenotypic Features Associated With Mutations in *ADAMTS18*

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IMPORTANCE We describe novel ocular phenotypic features caused by mutations in *ADAMTS18*. The exact role of *ADAMTS18* in ocular disease is unclear, and our work further contributes to the understanding of this gene and its protein.

OBJECTIVE To expand the phenotypic characterization in patients with homozygous mutations in *ADAMTS18* and report novel mutational data.

DESIGN, SETTING, AND PARTICIPANTS A case series with genetic investigations was conducted at tertiary referral clinical and university settings. Three families participated.

MAIN OUTCOME MEASURES Phenotype and genotype description of 3 families.

RESULTS Four affected patients from 3 families with an unusual ocular phenotype had full ophthalmic and systemic examination. A single affected individual in the first family had bilateral microcornea, ectopic pupils, and cone-rod dystrophy. In a second family, 2 brothers showed bilateral microcornea, childhood cataract, ectopia lentis, rhegmatogenous retinal detachment, and cone-rod dystrophy. In the third family, a single affected individual had the same features as those in family 2, without ectopia lentis. Causative mutations were sought using homozygosity mapping, Sanger sequencing, and massively parallel sequencing of the whole exome. Novel homozygous mutations in *ADAMTS18* were identified, consisting of c.1067T>A [p.L356*] in the first proband, c.2159G>C [p.C720S] in the 2 affected brothers, and c.1952G>A [p.R651Q] in the third proband. All 3 mutations are predicted to be pathogenic.

CONCLUSIONS AND RELEVANCE Mutations in *ADAMTS18* are associated with ocular developmental abnormalities including microcornea, ectopia lentis, and early onset of cone-rod dystrophy. This report provides further evidence that *ADAMTS18* plays a key role in ocular development. Physicians should consider screening *ADAMTS18* in patients with microcornea and cone-rod dystrophy.

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Six families with disease caused by mutation in *ADAMTS18* (OMIM *607512) have been described; one had early-onset retinal dystrophy,¹ and the others had features of a recently described group of abnormalities including myopic chorioretinal atrophy, myopia, and telecanthus.²

A 23-exon gene, *ADAMTS18* is on chromosome 16q23.1. It encodes ADAMTS18, one of the 19 ADAM metalloproteinases with thrombospondin type 1 motif proteins. These zinc-dependent proteases play a role in cell migration, coagulation, angiogenesis, and extracellular matrix regulation.³ The present report describes 4 newly identified patients with mutations in *ADAMTS18* and presents novel clinical and mutational data.

Methods

Institutional review board approval was acquired from local committees (Moorfields Eye Hospital, London, England; Edinburgh, Scotland; and Giessen, Germany), and written informed consent was obtained from each patient. Three male probands (family 1, 2, and 3) (Figure 1A, C, and E, respectively) and 1 male sibling (family 2) (Figure 1C) underwent detailed clinical assessment. This included visual acuity, slit-lamp examination, fundus examination, refraction, and axial length measurement (IOL Master; Carl Zeiss Meditec). Full-field and pattern electroretinography were performed incorporating the International Society for Clinical Electrophysiology of Vision standards.⁴ Genomic DNA was extracted from peripheral blood using standard procedures.

Trio-based whole exome sequencing using genomic DNA from the affected son (IV:1) and unaffected parents (III:1 and III:2) (Figure 1A) was performed in family 1 at the Wellcome Trust Sanger Institute as part of the UK10K project (<http://www.uk10k.org.uk>) as previously described.⁵ In summary, sheared fragments of genomic DNA enriched for targeted sequences (SureSelect All Exon 50Mb; Agilent Technologies) were sequenced with 75 base pair paired-end reads (HiSeq platform; Illumina). Quality filtering, alignment, and variant calling were performed.⁵ The exome variant was stringently filtered to enrich for unique variants by excluding those present in dbSNP 137 (<http://www.ncbi.nlm.nih.gov/projects/SNP>), 1000 Genomes Project (<http://www.1000genomes.org>), and NHLBI Exome Sequencing Project (<http://exome.gs.washington.edu>) polymorphism databases with a minor allele frequency greater than 0.005. Assuming autosomal recessive inheritance and identity by descent, homozygous nonsense and nonsynonymous variants in the affected proband were reviewed. The DNA from proband II:1 in family 2 (Figure 1C) was hybridized (Genome-Wide Human SNP Array 6.0; Affymetrix) to investigate regions of homozygosity. Bidirectional Sanger sequencing of *ADAMTS18* exon 7 was undertaken in family 1 for confirmation of next generation sequencing. Bidirectional sequencing of all 23 exons was undertaken in family 2 and, based on the ocular phenotype, the proband (II:1) and unaffected father (I:1) in family 3 (Figure 1E). Primers and conditions are available on request.

Results

Family 1

Clinical

The proband (IV:1) is the son of first cousins (Figure 1A) of Turkish origin. He had a history of reduced vision from age 6 months. Anterior-segment examination revealed bilateral microcornea (horizontal corneal diameter 8.5 mm in each eye at age 4 years) and ectopia pupillae. Fundus examination showed peripheral retinal pigmentation. There was no significant refractive error. The axial length was 23.04 mm in each eye when measured at age 3 years. At that age best-corrected visual acuity (BCVA) was 2.25 logMAR (<20/500 Snellen) OD and 1.43 logMAR (20/500) OS. Electroretinography performed at age 4 years showed a cone-rod dystrophy (CRD) pattern of abnormality. Systemic examination was unremarkable; there was no evidence of any occipital defect. Other family members were unaffected.

Genetic

An initial filter for homozygous nonsense mutations in the whole-exome sequencing data revealed c.1067T>A [p.L356*] in exon 7 of *ADAMTS18*. Bidirectional Sanger sequencing confirmed this mutation to be homozygous in the proband and heterozygous in both unaffected parents (Figure 1B).

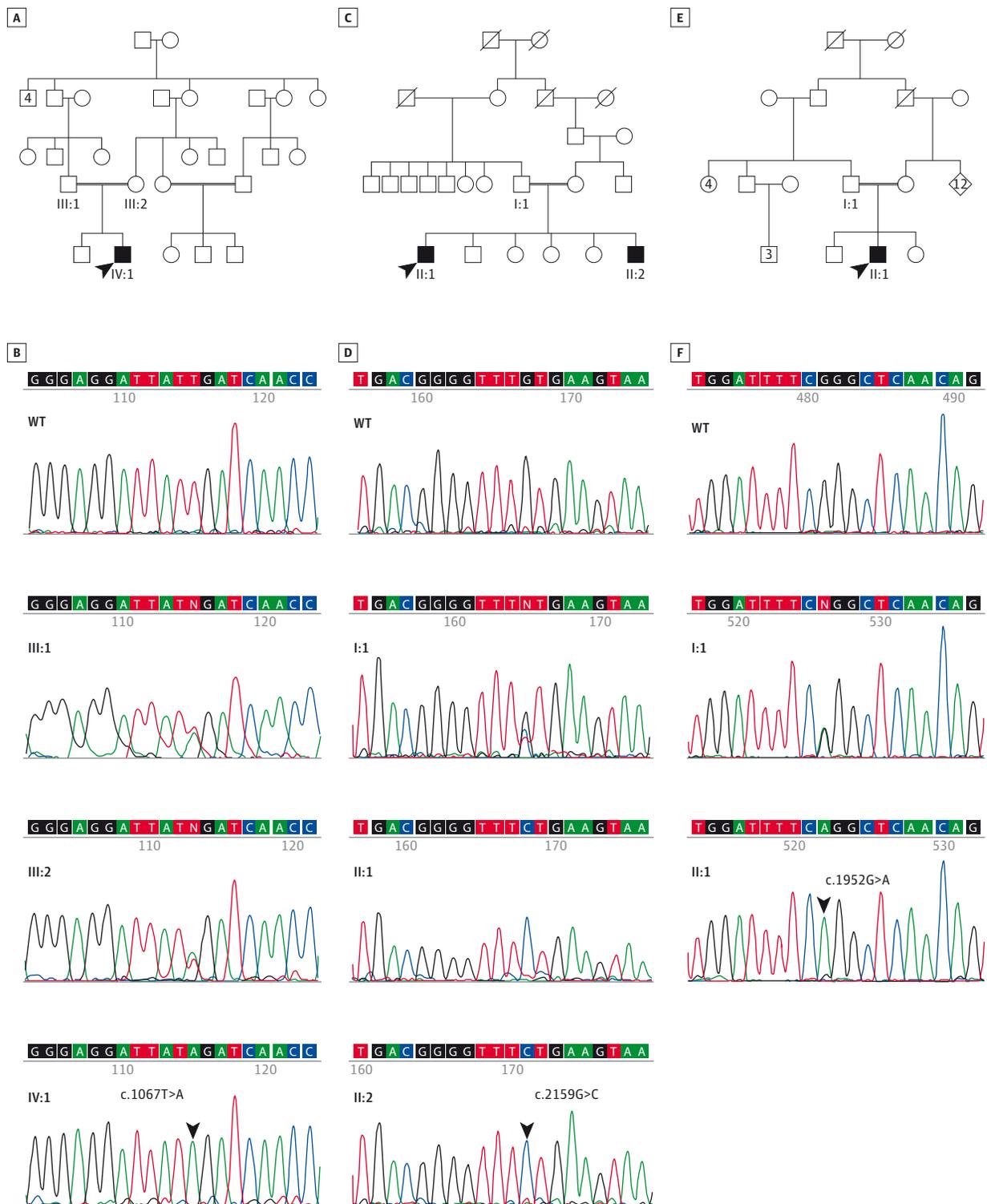
Family 2 (GC14463)

Clinical

The male proband (II:1) is 1 of 6 siblings of Pakistani origin born to parents who are first cousins once removed (Figure 1C). At age 1 year he had reduced vision, a divergent squint, and nystagmus. He was later found to have bilateral cataracts and underwent bilateral cataract surgery at age 2 years. Strabismus surgery was performed at the time of the second lens extraction. At age 10 years his BCVA was 1.0 logMAR (20/200 Snellen) OU. He had bilateral microcornea (8-mm horizontal diameter at age 31 years), peripapillary atrophy, and peripheral retinal pigmentation (Figure 2A and B). Axial lengths were 26.06 mm (right eye) and 25.86 mm (left eye). Electroretinography at age 21 years showed CRD.

The proband's brother (Figure 1C) (II:2) presented at age 2 years with reduced vision. He developed a rhegmatogenous retinal detachment in the right eye at age 11 years secondary to multiple small dialyses in the anterior retina. Surgery was unsuccessful and this eye eventually became phthisical. He developed a rhegmatogenous retinal detachment in the second eye at age 18 years, which was successfully repaired. When examined 1 year before the development of the retinal detachments, his BCVA was 0.5 logMAR (20/63 Snellen) OD and 0.33 logMAR (20/40) OS. He was significantly hypermetropic (+6.25 diopters spheres [DS] bilaterally). He had bilateral microcornea (8-mm horizontal diameter at age 24 years), ectopia lentis with mild lens opacities, and macular pigmentary abnormalities. Ultrasound A scan revealed an axial length of 21.7 mm in the right eye and 29.5 mm in the left eye (with a posterior staphyloma). At age 24 years his BCVA was 0.5 logMAR (20/63 Snellen) in the functioning left eye. Fundus examination re-

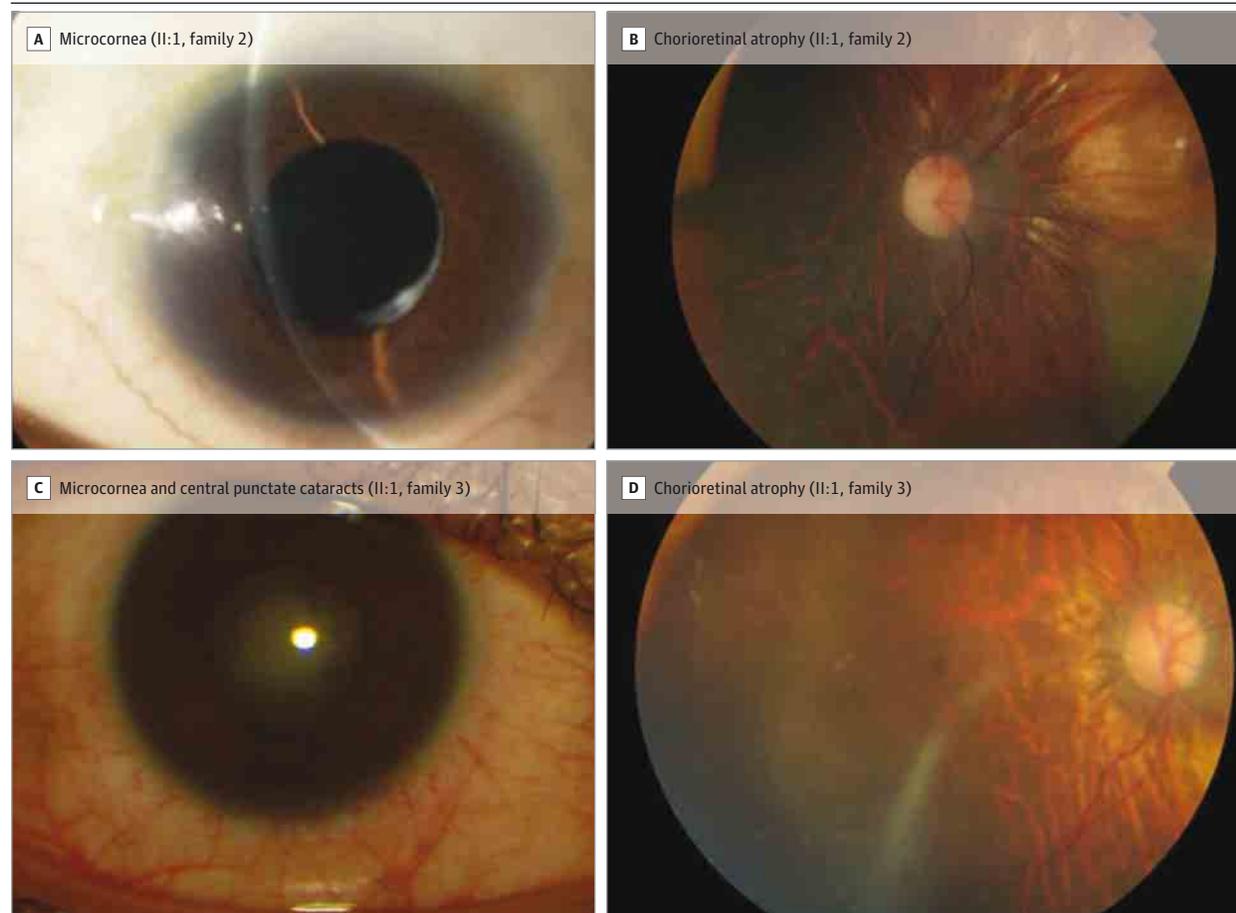
Figure 1. Pedigrees and Mutations



Pedigrees and sequence chromatographs demonstrating mutations in family 1 (c.1067T>A [p.L356*]) (A and B), family 2 (c.2159G>C [p.C720S]) (C and D), and family 3 (c.1952G>A [p.R651Q]) (E and F). In the pedigrees, square symbols indicate males; circles, females; diamond, either sex; slashes within the squares and circles, individuals who have died; and double connecting lines,

consanguinity. Black symbols indicate affected individuals and arrowheads indicate probands. The numbers within the circles, squares, and diamonds indicate the number of members of that sex. In the sequence chromatographs, arrowheads indicate mutations. WT indicates wild type.

Figure 2. Fundal and Anterior Segment Images of Proband in Family 2 and Family 3



A, Anterior segment of II:1 (family 2), illustrating microcornea. B, Fundus image of II:1 (family 2), illustrating chorioretinal atrophy. C, Anterior segment of II:1 (family 3), illustrating microcornea and central punctate cataracts. D, Fundus image of II:1 (family 3), illustrating chorioretinal atrophy.

vealed peripapillary choroidal atrophy. Systemic examination was unremarkable.

Genetic

The pedigree was consistent with AR inheritance (Figure 1C). Single-nucleotide polymorphism genotyping highlighted 8 regions of homozygosity covering 39.3 Mb. One homozygous region on 16q22.2-23.2 contained a candidate gene, *ADAMTS18*. Bidirectional Sanger sequencing revealed a missense mutation (c.2159G>C [p.C720S]) in exon 14 present in the homozygous state in both affected brothers and in the heterozygous state in their unaffected father (Figure 1D).

Family 3 (GC20482)

Clinical

The proband is the son of first-cousin parents of Pakistani origin. At age 6 years he had reduced vision (0.2 logMAR [20/32 Snellen] OD and 0.3 logMAR [20/40] OS), microcornea, and myopia (spherical equivalence -5 DS bilaterally). By age 8 years his myopia had increased (-8 DS bilaterally), with visual deterioration to 0.4 logMAR (20/50 Snellen). Electroretinography at this age revealed cone dysfunction but normal rod function. By age 13

years there was electroretinographic evidence of rod involvement and his refractive error had increased to -14 DS. There was microcornea (7.5 mm horizontal bilaterally), central punctate lens opacities, smooth irides, and chorioretinal atrophy, with macula pigmentary changes in each eye (Figure 2C and D). He developed a total rhegmatogenous retinal detachment in the right eye at age 15 years, secondary to a giant retinal tear. The left eye was similarly affected a year later; both were successfully repaired. Axial lengths were 27.7 mm (right eye) and 29.0 mm (left eye) at age 15 years. Magnetic resonance imaging revealed no occipital defect. No other family members were affected.

Genetic

The pedigree (Figure 1E) was consistent with AR inheritance. Bidirectional Sanger sequencing of *ADAMTS18* revealed a homozygous missense mutation (c.1952G>A [p.R651Q]) in exon 13, which was in the heterozygous state in the unaffected father (Figure 1F).

Genetic Summary

The mutations identified in all 3 families were absent from online databases (GenBank dbSNP library, 1000 Genomes,

Table. In Silico Pathogenicity Prediction of the Homozygous Mutations Identified in This Study

Family	Nucleotide	Amino Acid	SIFT Score ^a	PolyPhen Score ^b	MutationTaster Probability ^c
1	c.1067T>A	p.L356*	NA	NA	1.000
2	c.2159G>C	p.C720S	0	1	0.999
3	c.1952G>A	p.R651Q	0	0.998	0.999

Abbreviation: NA, not available.

^a SIFT scores are explained in Kumar et al.⁶

^b PolyPhen scores are explained in Adzhubei et al.⁷

^c MutationTaster probabilities are explained in Schwarz et al.⁸

and Exome Variant Server), and are predicted to be disease causing by in silico analysis (SIFT,⁶ PolyPhen,⁷ and MutationTaster⁸) (Table). The affected residues are conserved in vertebrates.

The mutation in family 1 results in premature termination of messenger RNA at codon 356. This transcript is predicted to undergo nonsense-mediated decay because it is truncated early in the transcript. Hence, this mutation is predicted to result in a null phenotype.

The missense mutations found in families 2 and 3 occur in the cysteine-rich region of the *ADAMTS18* protein spanning 644 to 749. The affected amino acid residues within the region show complete conservation throughout vertebrate orthologues, and the cysteine-rich region on the whole is highly conserved (Supplement [eFigure]), suggesting a possible structural role for this region of the protein.

Discussion

This report describes the detailed phenotype in 4 patients with novel homozygous mutations in *ADAMTS18*. Anterior-segment findings included microcornea, early-onset cataracts, ectopia lentis, and ectopia pupillae. Aldahmesh and colleagues² reported anterior segment features in all members of 5 families, regardless of which of the 4 mutations in *ADAMTS18* they described. Microcornea is a rare ocular phenotype usually associated with other signs of anterior-segment dysgenesis. Our data confirm the role of *ADAMTS18* mutations in the development of microcornea. Further characterization of anterior-segment features, including central corneal thickness, intraocular pressure, and angle structure, would be of interest in future evaluation of patients with mutations in this gene. All patients in the present series who underwent electroretinography had a CRD pattern of abnormality with

macular involvement. Of the previous reports of mutations in *ADAMTS18*, Peluso et al¹ described a patient with infantile-onset retinal dystrophy, and Aldahmesh and colleagues² suggested scotopic and photopic dysfunction in the only patient who had electroretinography. We suggest that retinal dystrophy is a key feature in patients with *ADAMTS18* mutations. There is some overlap of the phenotype associated with *ADAMTS18* mutations and Knobloch syndrome, a rare autosomal recessive disorder associated with mutations in the *COL18A1* gene.⁹ In Knobloch syndrome, there are several similar developmental abnormalities of the eye including retinal dystrophy and an increased risk of retinal detachment. One patient with Knobloch syndrome was described¹⁰ as having what appeared to be a causative mutation in *ADAMTS18*. This patient was later found to have a causative mutation in *COL18A1*.¹¹ However, a key feature of Knobloch syndrome is occipital skull defects. No patient in the present series had any occipital abnormalities and that diagnosis was excluded.

The patients in our series did not manifest the telecanthus or posteriorly rotated ears described by Aldahmesh and colleagues.² In addition, the finding of myopic chorioretinal atrophy was not a consistent feature in our patients.

Mutations in other members of the *ADAMTS* family cause early-onset cataract and ectopia lentis (*ADAMTSL4*,^{12,13} *ADAMTS10*,¹⁴ and *ADAMTS17*¹⁴), and ectopia pupillae (*ADAMTSL4*¹⁵). It is evident that this family of genes plays a significant role in ocular development.

Conclusions

This report describes the detailed ocular phenotype associated with mutations in *ADAMTS18*. Screening of this gene should be considered in patients presenting with microcornea and CRD.

ARTICLE INFORMATION

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full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Chandra, Arno, Moore. **Acquisition, analysis, or interpretation of data:** All authors.

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Critical revision of the manuscript for important intellectual content: Chandra, Arno, Sergiounotis, Charteris, Holder, Borman, Webster, FitzPatrick, Moore.

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Correction: This article was corrected on June 4, 2014, to fix the byline and also on June 12, 2014, to fix the byline and Author Contributions section.

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