

Homozygous Mutations in *ADAMTS10* and *ADAMTS17* Cause Lenticular Myopia, Ectopia Lentis, Glaucoma, Spherophakia, and Short Stature

Jose Morales,¹ Latifa Al-Sharif,² Dania S. Khalil,² Jameela M.A. Shinwari,² Prashant Bavi,³ Rahima A. Al-Mahrouqi,^{1,4} Ali Al-Rajhi,¹ Fowzan S. Alkuraya,^{2,5,6,7} Brian F. Meyer,² and Nada Al Tassan^{2,*}

Weill-Marchesani syndrome (WMS) is a well-characterized disorder in which patients develop eye and skeletal abnormalities. Autosomal-recessive and autosomal-dominant forms of WMS are caused by mutations in *ADAMTS10* and *FBN1* genes, respectively. Here we report on 13 patients from seven unrelated families from the Arabian Peninsula. These patients have a constellation of features that fall within the WMS spectrum and follow an autosomal-recessive mode of inheritance. Individuals who came from two families and met the diagnostic criteria for WMS were each found to have a different homozygous missense mutation in *ADAMTS10*. Linkage analysis and direct sequencing of candidate genes in another two families and a sporadic case with phenotypes best described as WMS-like led to the identification of three homozygous mutations in the closely related *ADAMTS17* gene. Our clinical and genetic findings suggest that *ADAMTS17* plays a role in crystalline lens zonules and connective tissue formation and that mutations in *ADAMTS17* are sufficient to produce some of the main features typically described in WMS.

Introduction

Weill-Marchesani syndrome (WMS; MIM 277600) is a rare connective-tissue disorder, mainly characterized in the eye by subluxation of microspherophakic lenses and, consequently, severe myopia and possible glaucoma.^{1,2} Other symptoms include short stature, brachydactyly, joint stiffness, and heart defects.^{1,3–6} Both autosomal-recessive (AR) and autosomal-dominant (AD) forms of the disease have been described.^{7–10} Three mutations in *ADAMTS10* (MIM *608990, ADAM metalloproteinase with thrombospondin type 1 motif, 10) were reported in two Middle Eastern families with an AR form of the disease and in a single sporadic case of WMS. These include a nonsense mutation (p.R237X) and two splice mutations (810+1 G > A and 1190+1 G > A). All three mutations were predicted to affect the catalytic domain of *ADAMTS10*, which plays a role in the remodeling of connective tissue.⁸ Compound heterozygous p.A25T and p.E318X mutations in *ADAMTS10* were also identified in a sporadic case of WMS.^{8,11} In a large family with AD WMS, an in-frame (24 bp) deletion in *FBN1* (MIM *134797, fibrillin1), which is implicated in Marfan syndrome^{12,13}, was identified, confirming the genetic heterogeneity of this condition.^{7,12}

The purpose of the present study was to describe the clinical and molecular characterization of 13 affected individuals (twelve Saudi and one Yemeni) representing seven unrelated families who presented with varying manifestations of AR WMS. Two homozygous missense mutations

were identified in *ADAMTS10* in four WMS patients from two different families. Additionally, we identified three different homozygous truncating mutations in *ADAMTS17* (MIM *607511, ADAM metalloproteinase with thrombospondin type 1 motif, 17), a gene that we mapped by using linkage analysis in four affected members of a consanguineous family. In contrast to the phenotype related to *ADAMTS10*, the *ADAMTS17*-related phenotype is best described as WMS-like because none of the patients harboring *ADAMTS17* mutations met the full clinical definition of WMS. To the best of our knowledge, this is the first report of *ADAMTS17* mutations in humans, and our characterization of this *ADAMTS* protein supports its role in the development of a connective-tissue disorder that resembles WMS.

Material and Methods

Subjects and Samples

Blood samples for molecular analysis were obtained from participants, including patients (13 individuals) and unaffected family members (seven participants), who provided approved written informed consent in adherence to institutional and international guidelines (RAC# 2070008).

Clinical Assessment

Medical and family history, particularly with regard to consanguinity and ocular and cardiac involvement, was obtained. Ocular history specifically addressed the need for spectacle correction and the age at which any correction occurred, medical or surgical

¹King Khaled Eye Specialist Hospital, Riyadh 11462, Saudi Arabia; ²Department of Genetics, ³Biological Repository Section, King Faisal Specialist Hospital and Research Centre, Riyadh 11211, Saudi Arabia; ⁴Ophthalmology Department, Al-Nahda Hospital, Muscat 134, Oman; ⁵Division of Genetics and Metabolism, Department of Medicine, Children's Hospital Boston and Harvard Medical School, Boston, MA 02115, USA; ⁶Department of Pediatrics, King Khalid University Hospital and College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia; ⁷Department of Anatomy and Cell Biology, College of Medicine, Alfaisal University, Riyadh 11533, Saudi Arabia

*Correspondence: naltassan@kfshrc.edu.sa

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treatment of glaucoma, and crystalline lens extraction. Physical examination of all affected individuals and available unaffected family members included assessment of height, weight, joint flexibility, and the presence of brachydactyly. Ophthalmologic evaluation included assessment of visual acuity, ocular motility status, slit lamp biomicroscopy, and gonioscopy, intraocular pressure measurement by Goldmann applanation tonometry, and dilated examination of the lens, retina, and optic nerve. Biometric ultrasound measurements of axial length, lens thickness, and depth of the anterior chamber were obtained for affected individuals. Goldmann manual kinetic perimetry (Haag Streit International, Koniz-Bern, Switzerland) or automated Humphrey perimetry (Humphrey Field Analyzer II; Carl Zeiss Meditec, Dublin, CA) was obtained in patients with elevated intraocular pressure. Shallow anterior chambers, microspherophakia, and lens subluxation were documented whenever possible by anterior segment photography (Carl Zeiss Meditec) and rotating Scheimpflug camera measurements (Pentacam HR, Oculus, Wetzlar, Germany).

Definitions of Clinical Abnormalities

Lenticular Myopia

High myopia (spherical equivalent greater than or equal to -5.00 Diopters in both eyes) without significant (>24.5 mm) enlargement of globe axial length.

Ectopia Lentis

Presence of crystalline lens subluxation either axially or laterally as determined by slit lamp biomicroscopy.

Glaucoma

Glaucomatous damage, as evidenced by characteristic glaucomatous optic nerve cupping of more than 0.6 vertically and/or glaucomatous changes in the visual field as determined by Humphrey automated perimetry or Goldmann static manual perimetry.

Spherophakia

Rounded shape of crystalline lens as evidenced by slit-lamp biomicroscopy exam and an increased antero-posterior diameter (>4 mm) documented by ultrasound.

Short Stature

Abnormally short stature was defined as that in the third percentile or less as determined by growth charts, recently studied and validated, for average height in the region.¹⁴

Brachydactyly

Presence of short, stubby fingers in comparison to normal-appearing hands in unaffected family members.

Stiffness of Hand Joints

Inability to flex hand completely, i.e., to make a full fist.

Congenital Heart Abnormalities

History of clinical heart problems detected since childhood.

Automated Sequencing

Purified PCR products covering genes of interest were sequenced with an ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Kit as instructed by the manufacturer. Results were exported in one of several formats for visualization, and sequences were analyzed with SeqMan 6.1 (Lasergene 6 software package).

Screening for Variants in *ADAMTS10* and *FBN1*

PCR products covering all coding exons, along with primers as previously described⁸, were used for sequencing the entire ORF of *ADAMTS10* (NM_030957.2) in patients. Primers covering the 66 coding exons of *FBN1* (NM_000138.3) were designed, and amplified products from affected individuals were sequenced (Table S1).

Linkage Analysis

SNP genotyping was performed with Affymetrix GeneChip Human Mapping 10K Arrays. SNP genotypes were called with Affymetrix GCOS 1.4 software and an overall SNP call rate of 95%–99%. Multipoint LOD score calculations were performed with the Allegro module of the Easy Linkage software package¹⁵ under the assumption of an autosomal-recessive mode of inheritance with 100% penetrance and disease allele frequency of 0.01%.

Mutation Detection in *ADAMTS17*

Genomic DNA (10–50 ng) was amplified by PCR in 25 μ l reactions via intronic primers that flank each of the 22 exons of *ADAMTS17* (NM_139057.2) (Table S2). Standard PCR conditions were as follows: 94°C for 12 min, 30 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 45 s, followed by an extension step at 72°C for 10 min. Mutations and variants in *ADAMTS17* were identified after PCR products were sequenced. Sequencing new PCR products in both directions confirmed mutations identified in each patient, and the segregation pattern was checked whenever applicable. Sequencing 300 ethnically matched normal controls further confirmed the mutations.

Characterization of Splicing Pattern for c.1721 +1 G > A in *ADAMTS17*

To ascertain the effect of the c.1721 +1 G > A splice mutation in lymphoblasts from patient WMS-D1, we amplified exons 11–13 and 11–14 from cDNA by using the common forward primer *ADAMTS17*-x11Frna, 5'-GACACATCCTGCAAGACCAA-3' and reverse primers *ADAMTS17*-x13Rrna, 5'-GAAGCTGGGCAGACCC TTG-3' and *ADAMTS17*-x14Rrna, 5'-AGGCTTATCGTCAACCAC-3' to generate wild-type amplicons of 300 bp and 384 bp, respectively.

Expression Analysis of *ADAMTS17*

First-strand cDNA libraries from multiple human adult and fetal tissues were obtained commercially (Genemed Synthesis, South San Francisco, CA and Capital Biosciences, Rockville, MD, USA). The following primers specific for the two reported *ADAMTS17* transcripts were designed: *ADAMTS17*-x17Frna, 5'-CCGCAGGA CTCTTGTTTCAT-3' and *ADAMTS17*-x19Rrna, 5'-ACTCAGACGCC TCCCAGAT-3' (used for generation of a 400 bp product specific for transcript ENST00000268070); and *ADAMTS17*-x14F, 5'-GCCATG TGAAGTCTACTGCTC-3' and *ADAMTS17*-x16Rrna, 5'-TCCACCA CACGGATCCTC-3' (used for generation of a 300 bp product from transcript ENST00000378898). A 100 bp β -actin fragment was used as a positive control for gene expression. Representative bands were excised from the gel, purified, and sequenced so that the origin of each isoform could be confirmed.

Immunoblot

ADAMTS17 mouse monoclonal antibody that recognizes amino acids 543–651 was obtained commercially (ab58099, Abcam, Cambridge, MA, USA). To assess the *ADAMTS17* antibody, we purified proteins from cultures of the following cell lines: normal breast cell line MCF-10 and breast cancer cell lines MCF-7 and T47D. In brief, cells were washed with PBS, then lysed in RIPA lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, and 0.1% SDS) containing complete protease inhibitor cocktail (Roche, Indianapolis, IN, USA). The protein concentration was analyzed by Bradford assay. Subsequently, for immunoblotting, 30 μ g of each cell lysate was separated on an 8% SDS-PAGE gel and then transferred onto polyvinylidene

difluoride membrane (PVDF). The membrane was incubated overnight with ADAMTS17 monoclonal antibody at a 1:500 dilution. The secondary horseradish peroxidase-conjugated rabbit anti-mouse antibody was visualized with a chemiluminescence detection procedure (Amersham Pharmacia Biotech [GE Healthcare Life Science], Piscataway, NJ, USA) according to the manufacturer's protocol, and by exposure to Kodak biomax MR film (Sigma Aldrich, St Louis, MO, USA).

Construction of Tissue Microarray

Tissue microarrays (TMA) were constructed as described previously.¹⁶ Two tissue cylinders with a diameter of 0.6 mm were punched from archival paraffin blocks and transferred into a recipient paraffin block via a semi-automatic precision instrument (Beecher Instruments, Silver Spring, MD, USA).

Immunohistochemistry

To validate the ADAMTS17 antibody, we used ADAMTS17 recombinant protein (Q01) specific for ADAMTS17 (NP_620688.2, amino acids 543–650) (Abnova, Taiwan) on MCF-7 breast cancer cell line and a control tissue microarray comprising various adult human normal tissues (colon, liver, kidney, pancreas, thyroid, lymph node, spleen, heart, lung, smooth muscle, ovary, and testis). Human TMA slides and mouse E14.5 embryo slides were incubated overnight in a 1:6000 dilution of anti-ADAMTS17 (ab58099, Abcam), and the Dako Envision Plus System kit was used as the secondary detection system with DAB as chromogen. Endogenous peroxidase activity was quenched with 3% hydrogen peroxidase in methanol. Endogenous biotin was blocked, and all slides were counterstained with hematoxylin, dehydrated, cleared, and coverslipped with premount. Cell line blocks of MCF-7 and T47D and tumor tissue samples of ovarian and colon cancers served as a positive control. Finally, two types of negative controls were used: the first was the exclusion of the primary antibody, and the second was a peptide competition assay. For the latter, ADAMTS17 antibody was preincubated with the ADAMTS17 recombinant protein (Q01) at a 1:2 ratio (w/w) for 1 hr at room temperature prior to staining. Tissues were scored on a four-tier system: 0, absence of staining; and 1, 2, and 3, weak, moderate, and strong staining, respectively.

Results

Clinical Manifestations

A total of 13 patients from seven unrelated families, including three with single affected individuals, were investigated. There were five cases (two families and one sporadic case) with complete features of AR WMS (lenticular myopia, ectopia lentis, spherophakia, short stature, brachydactyly, and decreased joint flexibility) and eight cases (two families and two sporadic cases) who had the previously mentioned WMS manifestations except for brachydactyly and decreased joint flexibility and who were labeled as having an AR WMS-like condition (Figure 1). A summary of the main ophthalmological and clinical features is presented in Table 1.

Regarding height, two patients were at or below the 25th percentile, two were at or below the fifth percentile, and the rest were at or below the third percentile according to

growth tables recently established for the Saudi population.¹⁴

Mean globe axial length, antero-posterior lens diameter, and anterior chamber depth for both eyes were 21.9 mm (range 20.1–23.0 mm), 5.1 mm (range 4.2–7.6 mm), and 2.1 mm (range 1.1–3.1), respectively.

Glaucomatous damage to the optic nerve and impairment of the visual field were documented in five patients, elevated intraocular pressures were documented in 11 patients, and clinically shallow anterior chambers were documented in all except one patient.

Mutations and Variants Identified in *ADAMTS10* and *FBN1*

Two missense homozygous mutations in the *ADAMTS10* gene, c.1553 G > A (p.G518D) and c.2098 G > T (p.G700C) (accession NP_112219.2), were identified in patients from families B and E, respectively, and were absent in 240 normal controls. Moreover, a number of previously reported coding and noncoding variants were identified by sequencing. These include c.401 C > G (p.T134S, rs7255721), c.2283 T > C (p.P761, rs4476282), c.2423 T > G (p.L808R, rs10420313), c.2584 G > T (p.A862S, rs10418929), c.435+24 A > G (rs7260282), and c.1085-47 A > G (rs7247944) in the *ADAMTS10* gene. In the *FBN1* gene, the following noncoding variants were identified; c.1960+76 G > A (rs17361868), c.4748-77 T > G (rs55694948), c.6037+54 T > A (rs2303502), and 6997+17 C > G (rs363832) (accession NP_000129.2).

Mutations in *ADAMTS17* in Patients with Partial WMS

Multipoint linkage analysis of family A, including unaffected parents, all four affected siblings, and the unaffected maternal half sibling, identified a disease locus on chromosome 15q26.3 (Ensembl cytogenetic band) with a maximum logarithm of odds (LOD) score of 3 (Figure 2). The linkage region spanned 2.05 Mb, and *IGF1R* (NM_000875), *SYNM* (NM_145728), *TTC23* (NM_022905), *MEF2A* (NM_001130926), *LYSMD4* (NM_152449), and *ADAMTS17* (NM_139057.2) from within this interval were selected as plausible candidates. A homozygous 1 bp insertion in exon 18, c.2458_2459insG (p.E820GfsX23; accession NP_620688.2) of *ADAMTS17* was identified. This out-of-frame insertion fully segregated with the phenotype in the family used for linkage analysis and was consistent with an autosomal-recessive mode of inheritance (Figure 2).

Screening the entire ORF of the *ADAMTS17* gene in similar WMS-like individuals identified a homozygous truncating mutation, c.760 C > T (p.Q254X), in the two affected members of family F (both parents were heterozygous), and a third homozygous mutation, c.1721+1 G > A, was identified in a sporadic case (WMS-D1). These mutations were absent in 300 ethnically matched normal controls (Figure 2).

mRNA analysis with two primer sets flanking exon 12 indicated the presence of three aberrant transcripts in patient WMS-D1 lymphoblasts as a result of the

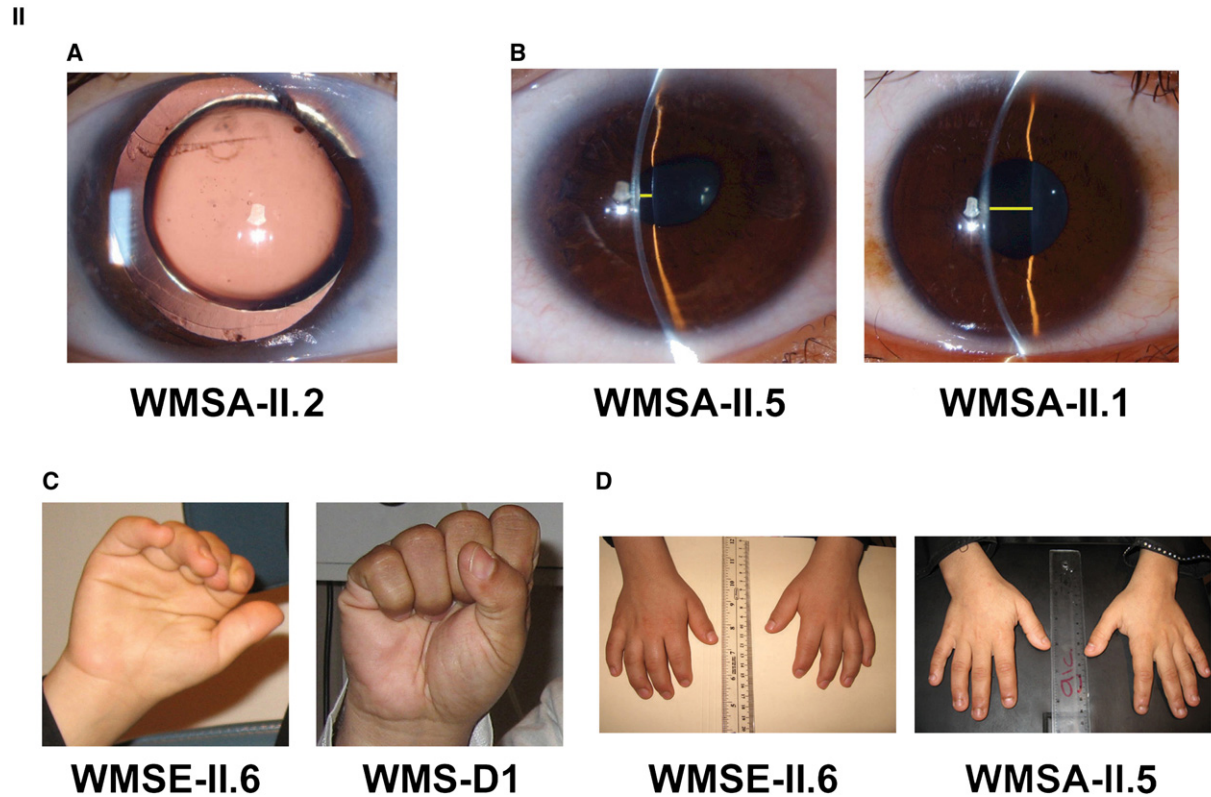
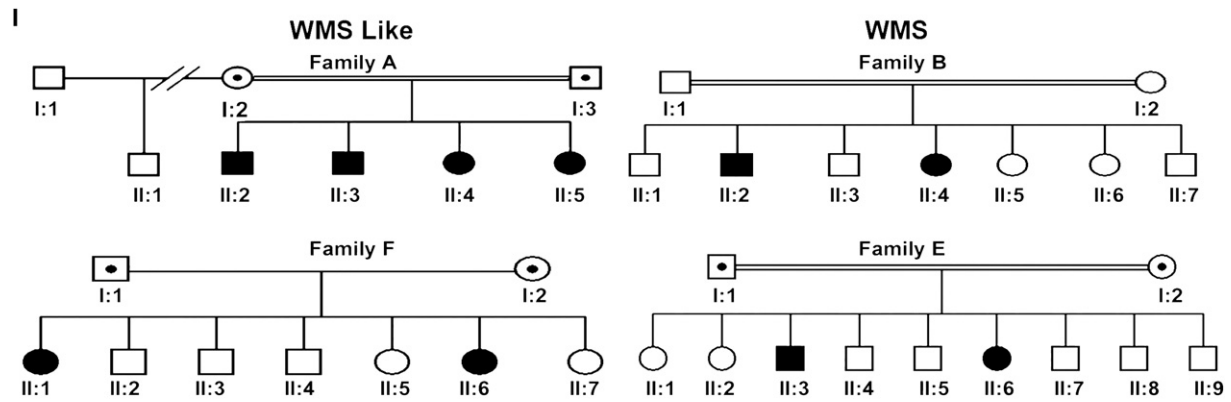


Figure 1. Family Pedigrees and Clinical Signs

(I) The pedigrees of the four families with more than one affected individual. Families A and F show autosomal-recessive inheritance and a WMS-like condition, and families B and E are classical autosomal-recessive WMS families.

(II) (A) Slit-lamp photograph of the right eye of patient WMSA-II.2 displaying clear microspherophakia and showing the equator of an apparently smaller-than-normal crystalline lens within a dilated pupil. There is evidence of prior glaucoma surgery, including a peripheral iridectomy and some peripheral iris synechiae. (B) Comparison of a very shallow anterior chamber highlighted by a yellow line outlining the space between the lens and the inner surface of the cornea on patient WMSA-II.5 and a normal deep anterior chamber of her unaffected half-sibling, WMSA-II.1. (C) Stiffness of joints is illustrated by the inability of patient WMSE-II.6 to make a fist; in contrast, patient WMS-D1 (not displayed on pedigrees, but corresponding to patient number five in Table 1) is making a normal full fist. (D) The hands of patient WMSE-II.6 with short, stubby fingers (brachydactyly) in comparison with the normal hands of patient WMSA-II.5.

c.1721+1 G > A mutation. The first transcript resulted from exon 12 skipping. The second and third transcripts retained exon 12 but also included different segments of intron 12 (Figure 3). All three transcripts harbored a frame-shift and are predicted to produce truncated proteins of 579 and 574 amino acids, respectively.

Variants Identified in *ADAMTS17*

A number of coding and noncoding variants were identified in *ADAMTS17*. These include 19 intronic variants, 7 synonymous and 3 non-synonymous variants (Table S3). Some of these variants are likely to represent previously undescribed SNPs.

Table 1. Summary of Clinical Features of Affected Individuals with WMS and WMS-like Syndrome

Number	Family ID	Patient ID	Age ^a	Gender	Myopia Level ^b	Highest IOP in mmHg ^c	Glaucomatous Nerve Damage	Height in cm	Percentile ^d	Brachydactyly	Joint Stiffness	Congenital Heart Abnormalities ^e	Average Axial Length ^f	Shallow Anterior Chamber	Peripheral Iris Synechiae	Gene/Mutation
WMS-like																
1	Family A	II.2	16	M	−7.8	NA	Yes	160	0.25	No	No	No	23.0	Yes	Yes	<i>ADAMTS17</i> / c.2458_2459insG
2	Family A	II.3	15	M	−12.0	21	No	150	<0.25	No	No	No	22.8	No	No	<i>ADAMTS17</i> / c.2458_2459insG
3	Family A	II.4	12	F	−11.6	42	Yes	127	<0.03	No	No	No	22.5	Yes	Yes	<i>ADAMTS17</i> / c.2458_2459insG
4	Family A	II.5	10	F	−10.5	23	No	118	<0.03	No	No	No	21.5	Yes	Yes	<i>ADAMTS17</i> / c.2458_2459insG
5	Family D	WMS-D1	33	M	−11.9	33	Yes	155	0.03	No	No	No	21.3	Yes	No	<i>ADAMTS17</i> / c.1721+1G > A
6	Family F	II.1	30	F	−8.6	41	Yes	145	0.05	No	No	No	21.7	Yes	Yes	<i>ADAMTS17</i> / c.760 C > T
7	Family F	II.6	11	F	−5.5	40	No	128	<0.05	No	No	No	20.7	Yes	Yes	<i>ADAMTS17</i> / c.760 C > T
8	Family G	WMS-G1	36	F	−9.0	60	No	134	<0.03	No	No	Yes	21.9	Yes	Yes	No mutations in <i>ADAMTS10</i> , <i>ADAMTS17</i> and <i>FBN1</i>
WMS																
9	Family B	II.2	17	M	−14.4	35	No	142	<0.03	Yes	Yes	No	22.4	Yes	No	<i>ADAMTS10</i> / c.1553 G > A
10	Family B	II.4	14	F	−8.9	19	No	133	<0.03	Yes	Yes	No	21.4	Yes	No	<i>ADAMTS10</i> / c.1553 G > A
11	Family E	II.3	22	M	−19.1	23	No	148	<0.03	Yes	Yes	No	23.7	Yes	No	<i>ADAMTS10</i> / c.2098 G > T
12	Family E	II.6	13	F	−11.0	27	No	132	<0.03	Yes	Yes	Yes	20.1	Yes	No	<i>ADAMTS10</i> / c.2098 G > T
13	Family C	II.1	70	M	13.8	30	Yes	142	<0.03	Yes	Yes	No	21.4	Yes	No	No mutations in <i>ADAMTS10</i> , <i>ADAMTS17</i> and <i>FBN1</i>

Family and Patient ID: family and patient identification as per pedigree (all families displayed autosomal-recessive inheritance).

^a Age: in years at the time of the study. M, male; F, female.

^b Average spherical equivalent in diopters for both eyes.

^c Maximal IOP in mmHg in either eye.

^d Percentile as per Saudi growth charts.¹⁴

^e Patients WMEII.6 and WMS-G1 had congenital cardiac valvular abnormalities as per clinical history; they required catheterism and valvuloplasty at ages 4 and 6, respectively.

^f Average eye globe axial length from both eyes, measured by ultrasound.

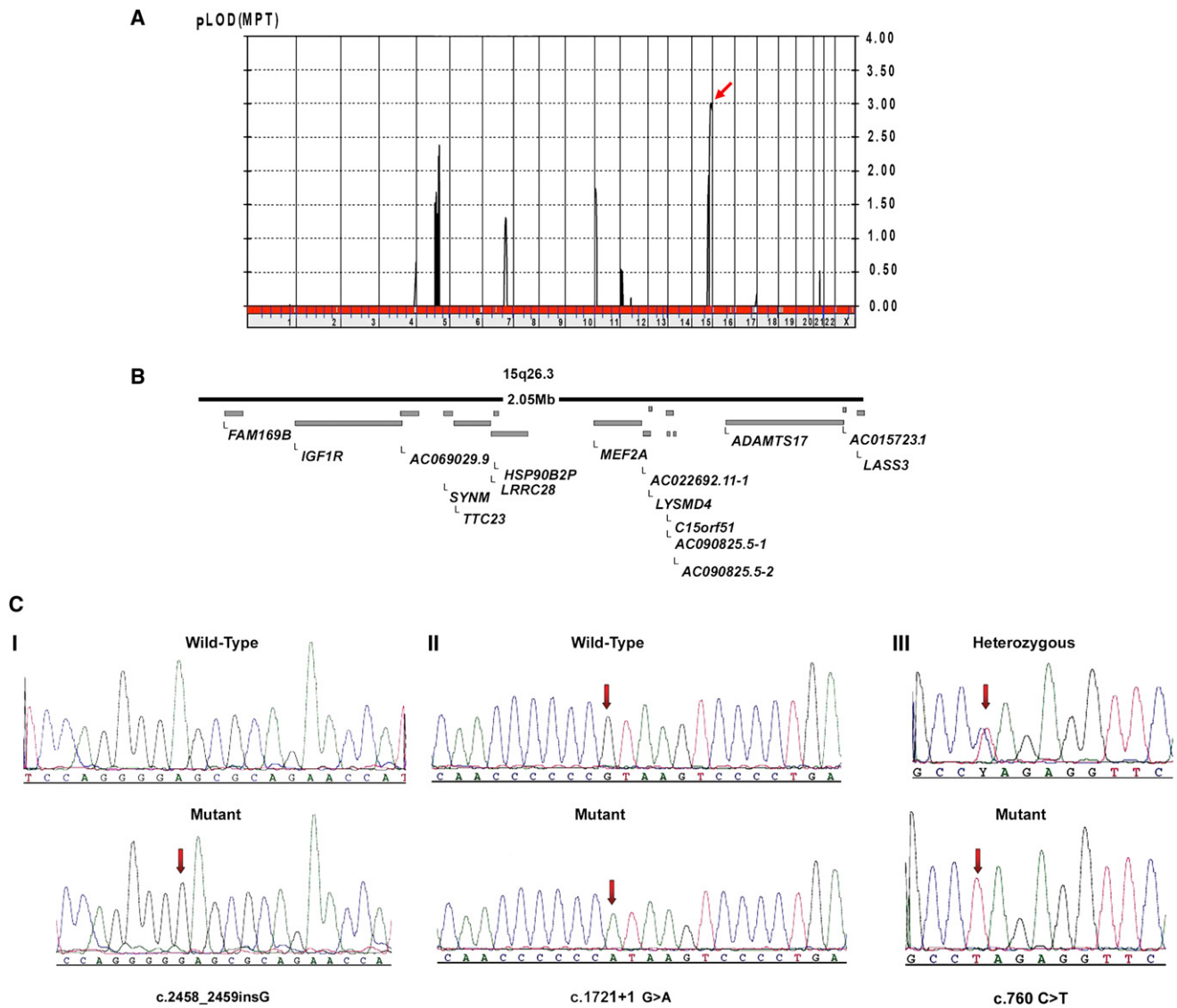


Figure 2. ADAMTS17 Mutations in Patients with Autosomal-Recessive WMS-like Syndrome

(A) Linkage analysis via the Allegro module reveals a LOD score of 3 on chromosome 15, as indicated by an arrow.

(B) Linkage interval of 2.05 Mb as annotated in the Ensembl genome database.

(C) Sequence chromatograms of homozygous mutations encountered in *ADAMTS17*. (I) c.2458_2459insG (p.E820GfsX23) in exon 18.

(II) c.1721+1 G > A splice mutation in intron 12. (III) c.760 C > T (Q254X) in exon 4. Arrows point to mutation sites.

Expression Analysis of *ADAMTS17*

There are two reported isoforms of *ADAMTS17* with different initiation codons: a transcript that spans 22 exons and codes for 1095 amino acids and a shorter transcript of 16 exons that codes for 502 amino acids. These will be designated in the text as isoforms “a” and “b,” respectively. Expression of the two transcripts was investigated in cDNA libraries from different adult and fetal tissues (Figure 4). The lung, brain, whole eye, and retina showed high expression of both transcripts. Isoform “a” showed weaker expression in the heart, kidney, and skeletal muscle, whereas bone marrow lacked expression of this transcript. Expression of isoform “b” in the kidney, bone marrow, and skeletal muscle was weak. Both transcripts demonstrated high expression of *ADAMTS17* in the fetal brain,

heart, kidney, and whole eye, whereas expression was weak in the liver.

Our analysis using primers specific for transcript “b” uncovered a previously uncharacterized third transcript where there was a 69 bp inclusion from intron 15; this was confirmed by sequencing of the PCR band. This exon might represent an alternatively spliced exon originating from transcript “b.” This transcript showed high expression in adult liver and lungs and weak expression in the remaining adult and fetal tissues studied and could represent one of the *ADAMTS17* minor isoforms.

Tissue Distribution of *ADAMTS17*

ADAMTS17 is a member of the secreted metalloproteinase family of proteins that are believed to bind to the extra

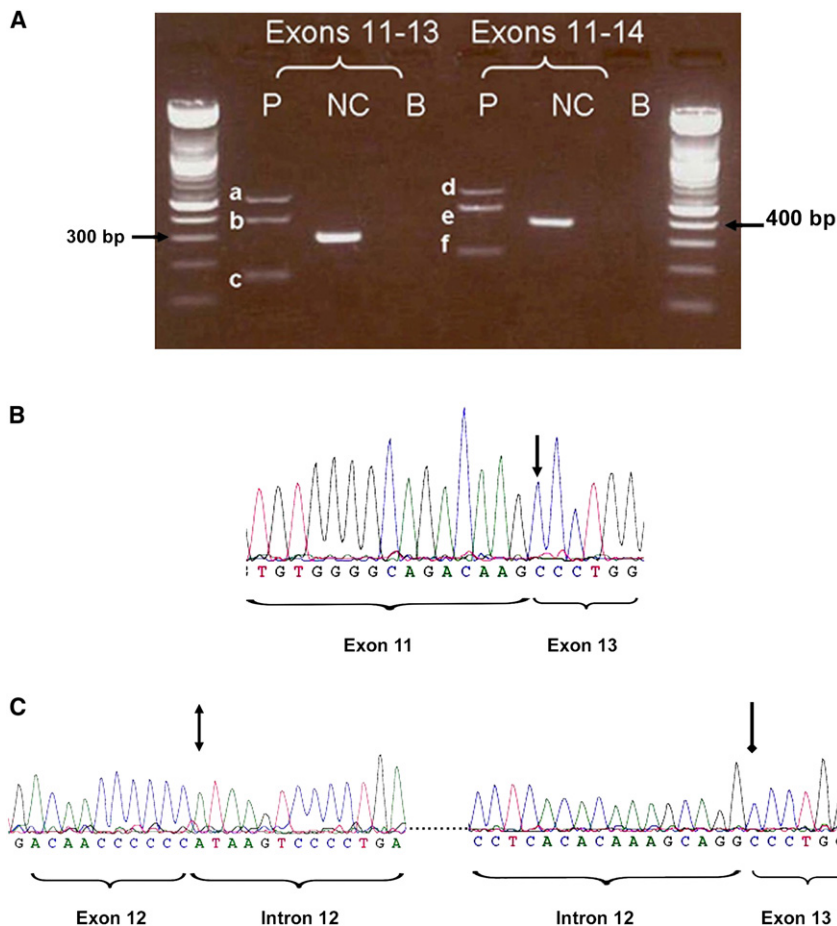


Figure 3. Splicing Patterns of the c.1721+1 G > A Mutation in *ADAMTS17* in Patient WMS-D1

(A) PCR products of cDNA from the patient (P) and a normal control (NC). Two sets of primers were used for the PCR. The patient cDNA gave three PCR products of different sizes with both sets of primers, whereas the NC gave only the wild-type expected product size.

(B) The sequence chromatogram of band "c" from PCR using the forward primer confirms exon 12 skipping. An arrow points to the end of exon 11 and the beginning of exon 13.

(C) Sequence chromatogram of PCR band "a." A double-headed arrow points to the end of exon 12 and the beginning of intron 12, and a single-headed arrow points to the end of the "spliced-in" part of intron 12 and the beginning of exon 13.

cellular matrix (ECM). Immunohistochemistry for which a mouse monoclonal *ADAMTS17* antibody was used on different normal and cancer human tissues showed strong staining in the spleen and testis and moderate staining in the liver, kidney, placenta, and lymph node, whereas the expression was weak in the heart, lung, colon, ovary and uterine wall smooth muscle. The pancreas and thyroid gland lacked expression of *ADAMTS17* (Table S4). Importantly, eye sections from E14.5 mouse embryos showed some diffuse expression of *ADAMTS17* within the area of a future ciliary body (Figure S1).

Discussion

This study reports the identification of *ADAMTS17* as another WMS gene, for which three mutations were identified in seven patients who had the typical WMS ocular manifestations in addition to short stature. The three mutations consist of a 1 bp insertion in exon 18, which is predicted to produce a truncated protein of 841 amino acids, a splice-site mutation that completely abolished the consensus donor sequence of intron 12 and produced three truncated transcripts, and a nonsense mutation in exon 4. The c.2458_2459insG mutation and the splice mutation c.1721+1 G > A are predicted to generate a truncated protein that retains the catalytic domain but

lacks the central and/or C-terminal TS repeats, which are thought to be essential for ECM binding.¹⁷ On the other hand, the nonsense mutation c.760 C > T in exon 4 would create a truncated protein that lacks the catalytic domain in addition to the central and C-terminal TS repeats. Interestingly, RNA analysis on lymphoblasts from patients suggests that all of these

mutations created stable mRNA transcripts. c.760 C > T and c.1721+1 G > A mutations affect all known *ADAMTS17* isoforms, whereas c.2458_2459insG in exon 18 only affects isoform "a." The nature of these mutations suggests that they are pathogenic, and we confirmed their absence in 600 ethnically matched normal control chromosomes.

Proteases have important roles in ocular physiology and pathophysiology. These proteases include caspases, calpains, matrix metalloproteases, ADAMs, ADAMTSs, those in the ubiquitin-proteasome pathway (UPP), tissue plasminogen activator, and secretase.¹⁸ ADAMTS is a family of 19 homologous secreted metalloproteinases that are believed to be anchored to the ECM via their C-terminal thrombospondin (TS) repeats.¹⁹ These proteins undergo N-terminal processing and removal of the prodomain during their transit through the membrane of the endoplasmic reticulum; they also undergo C-terminal processing that affects substrate specificity and ECM binding.²⁰ Previous characterization of the ADAMTS proteins showed their involvement in different cellular mechanisms^{17,19}, and some of these metalloproteinases have been implicated in a number of connective-tissue disorders and ectopia lentis.^{8,21,22} *ADAMTS17* is a member of the same protein family as *ADAMTS10*, which is mutated in AR WMS.^{8,17} However, only the structure of *ADAMTS17* was characterized, and its putative role and substrate remain to be elucidated.

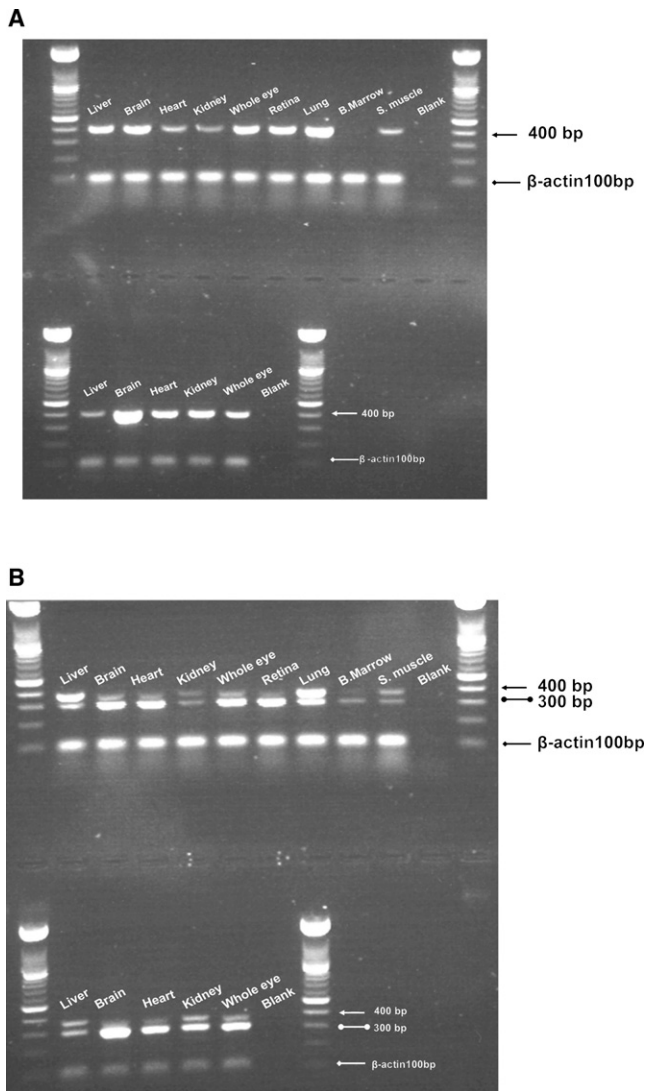


Figure 4. Expression of *ADAMTS17* in cDNA Libraries
 Expression of *ADAMTS17* in cDNA libraries from different human adult (top panel) and fetal (bottom panel) tissues.
 (A) An arrow points to a 400 bp *ADAMTS17* product specific for isoform "a."
 (B) A double-headed arrow indicates a 300 bp *ADAMTS17* product specific for isoform "b." A single-headed arrow points to a third transcript detected.

The previously reported *ADAMTS10* mutations identified in WMS patients disrupted the catalytic domain of the enzyme, and it was suggested that the catalytic domain of *ADAMTS10* could play a role in connective tissue remodeling and that this might explain the pathogenic mechanism of AR WMS.⁸ In contrast, two of our mutations in *ADAMTS17* resulted in transcripts that retained the catalytic domain but lacked the C-terminal TS repeats (Figure 5), suggesting that *ADAMTS17* might play more of a structural role in the assembly or integrity of ECM, similar to what was proposed for *ADAMTSL4*.²²

Our patients with truncating mutations in *ADAMTS17* had the typical eye anomalies described in WMS (these anomalies include spherophakia, ectopia lentis, high

myopia, and glaucoma secondary to shallow anterior-chamber angles) but lacked joint stiffness, brachydactyly, and cardiac valvular abnormalities. Expression analysis of all known normal transcripts of *ADAMTS17* on cDNA libraries from multiple fetal and adult tissues confirmed the previously reported high expression in the lungs¹⁹ and showed high expression in the whole eye and retina, which is consistent with the eye abnormalities observed in these patients. Moreover, *ADAMTS17* expression was observed in the ciliary body of E.14.5 mouse embryos, which might explain the role of this protein in zonule development. Because we did not examine the expression of *ADAMTS17* in developing limb buds, we cannot determine whether the lack of hand manifestations is due to a lack of expression or whether it results from redundant expression of other *ADAMTS* proteins.

We found two homozygous missense *ADAMTS10* mutations, c.1553 G > A and c.2098 G > T, in patients with autosomal-recessive inheritance and full WMS manifestations, including joint stiffness and brachydactyly. This confirms previous reports about the involvement of *ADAMTS10* in this condition and the genetic heterogeneity of the disorder already pointed out by Faivre et al.²³ Because we found *ADAMTS17* mutations only in patients with incomplete WMS manifestations, there is a possibility that different members of the *ADAMTS* family of proteins could be responsible for different subtypes of the syndrome. In addition, some *ADAMTS* proteins could have overlapping functions and interactions in such a way that a mutation in one of them can be complemented by the function of others or affect their activity in a limited fashion and thus cause a partial presentation of symptoms. However, *ADAMTS17* mutations seem to be capable of producing only the ophthalmic manifestations and short stature encountered in WMS.

To the best of our knowledge, this is the first report of homozygous mutations of *ADAMTS17* in patients with ectopia lentis and short stature. This highlights the importance of the *ADAMTS* protein family in connective-tissue biology, particularly on the crystalline lens zonules because the main impact in the eye seems to be through abnormalities of the elastic zonules. This in turn causes lens subluxation, spherophakia, and high myopia and causes the anterior chamber to become shallow, which results in glaucoma. *ADAMTS17* and *ADAMTS10* have structural similarities, but on the basis of the level of conservation of the protease active site^{19,24}, they belong to two different phylogenetic nodes, and so far we do not have an explanation for the fact that *ADAMTS17* seems to cause less systemic abnormalities. *ADAMTS10* seems to be more highly expressed than *ADAMTS17* in the heart, which could partially explain why patients with *ADAMTS17* mutations lacked congenital cardiac abnormalities. Because no echocardiographic studies were done, we cannot completely rule out subclinical cardiac abnormalities in these individuals. Among our cohort, we were not able to find any variants in the *ADAMTS17*, *ADAMTS10*, or *FBN1* genes

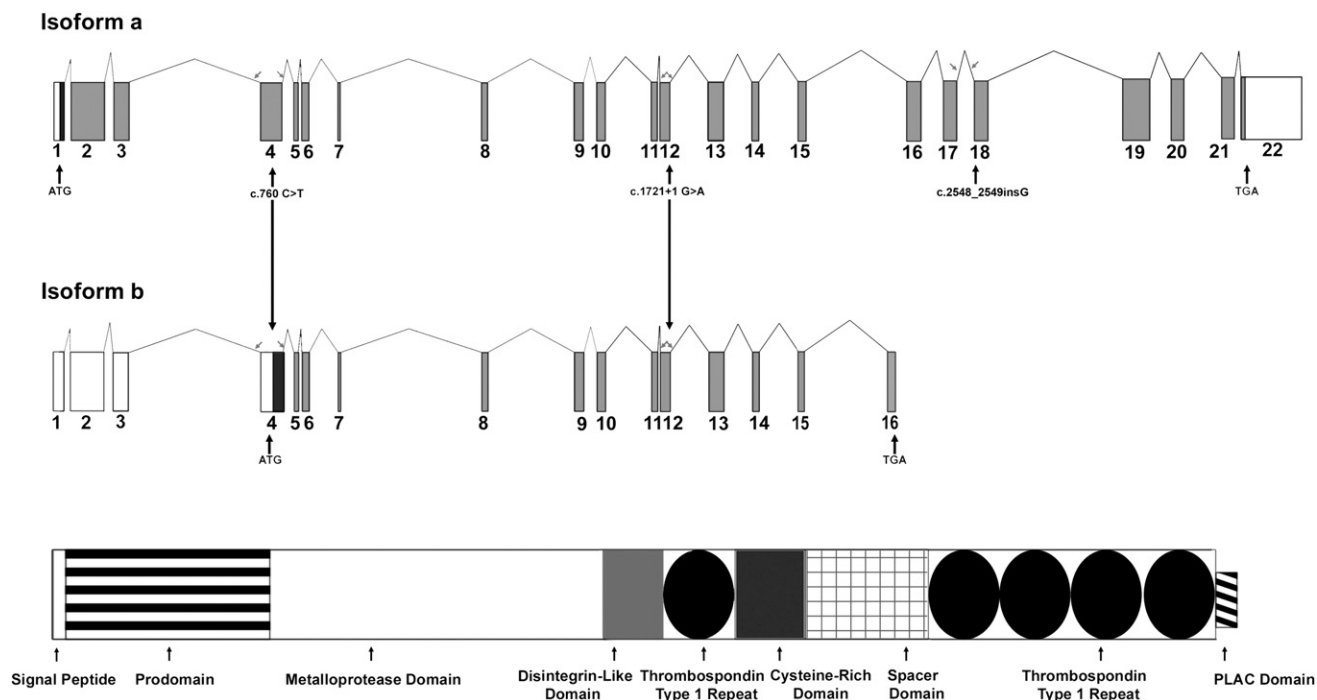


Figure 5. The Two Major Isoforms of the *ADAMTS17* Gene

Isoform “a” based on Ensembl transcript (ENST00000268070) and isoform “b” is based on Ensembl transcript (ENST00000378898). The protein domains are illustrated, and the position of each of the three mutations is specified. Arrows point to the position of primers flanking exons 4, 12, and 18.

that segregated with the disease in two patients. One of them (WMS-G1) had incomplete features of WMS without brachydactyly or joint stiffness. The second patient (WMS-II.1) seemed to have all the features, but the disease had a late onset, and lens subluxation and glaucoma appeared in middle age instead of childhood or adolescence. Although we cannot exclude deep intronic or regulatory-element mutations in these genes, our results suggest that additional loci might have caused WMS or WMS-like manifestations in these two patients.

Verloes et al. suggested the acronym GEMSS (glaucoma, ectopia lentis, microspherophakia, short stature, and stiffness of joints) for what they considered to be a dominant form of WMS.²⁵ We believe that rather than constraining the definition of the syndrome to a particular type of hereditary transmission, it might be more beneficial at this stage, when more mutations are being discovered, to reach better standardization and a consensus regarding what manifestations constitute WMS, as was done with Marfan syndrome.²⁶ It is worth highlighting that lenticular myopia, ectopia lentis, glaucoma, spherophakia, and short stature were present in patients with mutations in *ADAMTS10* and *ADAMTS17* as well as in the two patients with no identified mutations in the three genes.

Although not all patients developed glaucoma, most had shallow anterior chambers secondary to anterior lens subluxation and elevated intraocular pressures at some point, which would put them at risk of developing secondary angle-closure glaucoma. The average axial globe

length was shorter than usual. This confirms the lenticular component of the high myopia encountered in these patients and also suggests that some of these patients might be more predisposed to secondary angle-closure glaucoma due to the combination of a short globe and anterior crystalline lens subluxation’s crowding the anterior chamber angle and blocking the trabecular meshwork.

Six of the eight patients with WMS-like manifestations had peripheral irido-corneal adhesions, some of which were noted before any intraocular surgical procedure was undertaken. It is possible that they could have formed as a result of intermittent anterior lens movement and repeated contact between the iris and the cornea, but we cannot rule this out as another possible clinical ophthalmological feature in some patients with this condition. We did not observe these kinds of irido-corneal adhesions in any of the patients with classical WMS.

For phenotype classification, we prefer to describe the lens abnormality as spherophakia instead of microspherophakia because the smaller equatorial diameter was not clearly seen in all of our patients, whereas the globular shape of the lens was easier to identify by slit-lamp biomicroscopy and to document consistently by ultrasound measurements. Besides, it is possible that the lens is not actually anatomically small but that the globular shape and the decreased equatorial diameter are secondary phenomena due to abnormal laxity of the crystalline lens zonules. Although we did not observe broken or missing

zonules, we assume that there might have been significant zonular elongation, particularly in those individuals with significant lens displacement or clear visualization of the whole equator of the lens after dilation.

In summary, we have identified null mutations in *ADAMTS17* as a cause of an autosomal-recessive WMS-like syndrome. We have confirmed abnormalities in *ADAMTS10* in typical cases of WMS. Lenticular myopia, ectopia lentis, glaucoma (secondary to anterior lens subluxation and angle closure), spherophakia, and short stature were common denominators in patients with complete and incomplete manifestations of WMS. In our cohort of patients with these manifestations, *ADAMTS17* and *ADAMTS10* mutations were present in more than 80% of cases. Further work involving other ethnic groups and ectopia lentis patients might clarify the role that different mutations play in the pathogenesis of this condition.

Supplemental Data

Supplemental data include one figure and four tables and can be found with this article online at <http://www.cell.com/AJHG/>.

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Web Resources

The URLs for data presented herein are as follows:

GenBank, <http://www.ncbi.nlm.nih.gov/Genbank>

Ensembl project genome database, <http://www.ensembl.org/index.html>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/omim>

Single Nucleotide Polymorphism database, <http://www.ncbi.nlm.nih.gov/SNP/>

UCSC Genome Browser, <http://genome.ucsc.edu/>

Accession Numbers

Submitter SNP (ss) numbers for variants identified in this study are ss161151830, ss161151831, ss161151832, ss161151833.

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