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ARTICLE

A novel nonsense mutation in *ADAMTS17* caused autosomal recessive inheritance Weill–Marchesani syndrome from a Chinese family

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Abstract

Weill–Marchesani syndrome (WMS) is a rare connective tissue disorder characterized by short stature, brachydactyly, joint stiffness, eye anomalies, including microspherophakia, ectopia of the lenses, severe myopia, glaucoma and occasionally heart defects. Given these complex clinical manifestations and genetic heterogeneity, WMS patients presented misdiagnosed as high myopia or angle closure glaucoma. Here, we report *ADAMTS17* mutations, a member of the extracellular matrix protease family, from a Chinese family. Patients have features that fall within the WMS spectrum. The exome (protein-coding regions of the genome) makes up ~1 % of the genome, it contains about 85% of known disease-related variants. Whole exome sequencing (WES) has been performed to identify the disease-associated genes, including one patient, his healthy sister, and his asymptomatic wife. Genome-wide homozygosity map was used to identify the disease caused locus. SNVs and INDELs were further predicted with MutationTaster, LRT, SIFT and SiPhy and compared to dbSNP150 and 1000 Genomes project. Filtered mutation was confirmed with Sanger sequencing in whole family members. The Genome-wide homozygosity map based on WES identified a total of 20 locus which were possible pathogenic. Further, a novel nonsense mutation c.1051A >T result in *p*.(lys351Ter) in *ADAMTS17* had been identified in a candidate loci. The Sanger sequencing data has verified two consanguineous WMS patients in the family pedigree and revealed autosomal recessive (AR) inheritance pattern. The nonsense mutation in *ADAMTS17* was analyzed in silico to explore its effects on protein function. We predicted the mutation produced non-function protein sequence. A novel nonsense mutation c.1051 A > T in *ADAMTS17* had been identified caused autosomal recessive WMS in the Chinese family.

Introduction

Weill–Marchesani syndrome (WMS) is a rare connective tissue disorder characterized by short stature, brachydactyly, joint stiffness, eye anomalies, including microspherophakia, ectopia of the lenses, severe myopia, glaucoma and occasionally heart defects. Mutations in the *ADAMTS10* (located in 19p13.2, MIM 277600) [1] and *FBN1*(located in 15q21.1, fibrillin-1, MIM 608328) [2] have been reported as primary causes of WMS. The ADAMTS10 protein is important for normal growth before and after birth, and it is involved in the development of the eyes, heart, and skeleton in human. ADAMTS10 inactivation in mice cause the same symptom as human [3]. The FNB1 protein has been identified as a structural macromolecule that polymerizes into microfibrils and helps to provide connective tissue strength and flexibility. Further, *ADAMTS17* (located in 15q26.3, MIM 613195) [4] and *LTBP2* (located in 14q24.3, MIM 614819) [5] has been

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identified as a gene that causes WMS. ADAMTS17 protein is a member of the secreted metalloproteinase family that is believed to bind to the extracellular matrix (ECM). However, its exact function remains unknown. The abundant LTBP2 protein, associated with fibrillin-containing microfibrils, promotes tissue development in aorta and elastic ligaments [6]. FBN1 may have a structural role in elastic-fiber architectural organization for LTBP2 by bonds between the C-terminal region of the LTBP2 protein and N-terminal [7], WMS had been identified in different inheritance modes. Autosomal dominant inheritance (AD) has been identified with the *FBN1* mutation [2] and autosomal recessive (AR) has been identified with *ADAMTS10*, *ADAMTS17* [8], and *LTBP2* [5] mutations among WMS patients. Further, the clinical features of WMS resulting from *FBN1*, *ADAMTS10*, *ADAMTS17*, and *LTBP2* mutations are similar, clinical homogeneity and genetic heterogeneity in WMS had been identified [9], which suggests a connection between these genes. We suspect that these genes are all involved in biogenesis of fibrils, and that the mutations may have a severe consequence for biogenesis of fibrils, resulting in disease.

Given the clinical value of next-generation sequencing as a diagnostic tool [10], we here describe the genetics and inheritance of Weill–Marchesani syndrome family from China using this approach.

Methods

Cases and clinical assessment

Detailed interviews were conducted with family members to gather information on pedigree structure, onset of disease, and initial symptoms. Clinical data were obtained and supported clinical WMS diagnosis of patients. Visual acuity was assessed by using the standard Snellen chart. Intraocular pressure was measured by using Goldmann applanation tonometry, the gold standard measuring intraocular pressure, and the finger tension estimation technique. The family pedigree was generated using R.3.5.1. The red color denotes available samples, arrow designates the proband, and solid means patients in pedigree diagram of family. Peripheral blood samples, including probands, 2 consanguineous patients and 5 family members, were collected for DNA extraction. Genomic DNA was extracted from 200 µl of whole blood with the TaKaRa Genomic DNA Kit (TaKaRa Biotechnology, Dalian, China) according to the manufacturer's instructions.

WES and bioinformatics analysis

Whole exome sequencing was performed to identify the disease-associated variants. Enrichment of the whole-exome region libraries was performed using Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, Palo Alto, CA, USA). DNA libraries were sequenced using paired-end 150 bp reads on a NovaSeq system (Illumina, San Diego, CA, USA). Raw sequencing files were subjected to quality control using FASTQC. The reads data were mapped to the hg19 human genome (UCSC human genome hg19 build) by using BWA. Single-nucleotide variants (SNVs) and insertion–deletion variants (INDELs) were identified by SAMTOOLS and annotated by ANNOVAR [11]. Genome-wide homozygosity mapping was performed using HomozygosityMapper (http://www.homozygositymapper.org/). All genetic variants were compared to dbSNP150 and the 1000 Genomes project. The pathogenicity of variants was predicted with MutationTaster, LRT, SIFT, and SiPhy. The protein region function was obtained from UniProt database.

PCR and sanger sequencing

Sanger sequencing was used to confirm the segregation of identified variants in the family. The exon 7 of *ADAMTS17* gene was amplified using PCR primers of WMS-L (TGT TATCATTCGCTGTGGC) and WMS-R (CCCTTAGGG-TAAAGCTCAAT). All PCRs were performed in a reaction mixture (50 µl) containing 1 µl of dissolved DNA, 0.5 µl of each primer (10 µM concentration), 25 µl TaKaRa Ex mix (TaKaRa Biotechnology, Dalian, China) and ddH_2O. PCR cycling conditions consisted of 94 °C for 30 s, 55 °C for 35 s, 72 °C for 35 s for 35 cycles followed by a final extension at 72 °C for 7 min. The 600 bp fragments contain exon7 of *ADAMTS17* was amplified. The PCR products were analyzed on a 1.5% agarose gel and sequenced by ABI 3730xl DNA Sequencer.

Results

Clinical manifestation

The family pedigree is depicted in Fig. 1. All patients in the family were short of stature (Fig. 2a). When recruited, the 12 years old female proband (IIIe), had experienced decreased vision for one year. Routine clinical examination found she has a normal axial length (23.24 mm for the left eye and 23.66 mm for right eye, Fig. 2b). However, corneal refractive power (45.00D for right eye and 45.75D for the left eye) differed with typical near-sighted people. In addition, shallow anterior chamber was found (Fig. 2c) and indicated the disease originates in the lens. Lens removal surgery leads to symptom remission. The golden ring [12] of the lens could be seen when the pupil was dilated (Fig. 2d). The proband had brachydactyly (Fig. 2e). Based

on the above all clinical manifestation, the proband was diagnosed with WMS. Upon inquiry, her father (IIb) and sister (IIIb) also had impaired vision, short stature, brachydactyly (Fig. 2f, g) and shallow anterior chamber. Secondary glaucoma and lens dislocation were diagnosed additionally in her father (IIb) and her sister (IIIb). Her sister (IIIb) had impaired vision and bursting pain in the eyes for 5 years without obvious inducement, optic atrophy was identified due to continuous high intraocular pressure. Her father (IIb) had an impaired vision since youth and was blind in the left eye, the eyesight of right eye was going

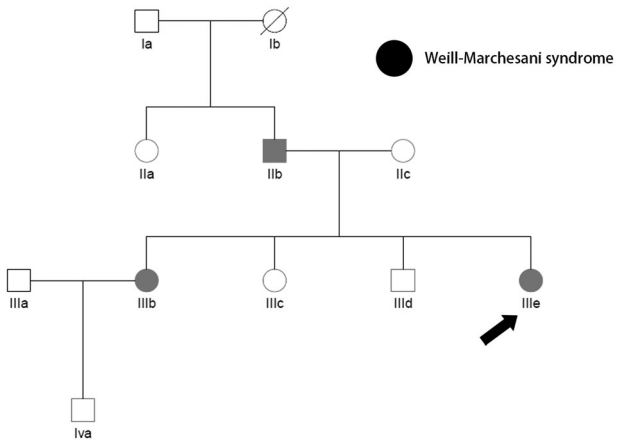

Fig. 1 Pedigree diagram of family. IIIe was proband, her father (IIb) and her sister (IIIb) were patients in this family. Red means samples were available

during a short interval of time. Clinical information has been shown in Table 1. We found that symptoms will aggravate gradually over time. No heart disease was evident in any family member.

WES and homozygosity mapping analysis identified a novel nonsense mutation in *ADAMTS17*

To localize the disease gene accurately, sequencing was performed in one patient (IIb), his healthy sister (IIa) and his asymptomatic wife (IIc). The mean depths of the targeted region were 105.54 ×, 124.02 × and 103.78 × for IIb, IIa, IIc respectively. Targeted regions with depths greater than 10 × reads showed coverage more than 99.5%. Only SNVs or INDELs with quality >20 and DP > 10 were considered as a reliable mutation. More than 20,000 SNVs or INDELs in all exon regions were identified. An autosomal recessive inheritance pattern was evident in this family's pedigree. Therefore, a genome-wide homozygosity mapping analysis was performed based on SNV genotypes using HomozygosityMapper. A total of 20 homozygous loci with putative association to disease were identified, one of which contained a gene that has been reportedly associated with WMS (a 3.7 Mb locus of homozygosity on chromosome 15 (Fig. 3) containing *ADAMTS17* gene). A total 10 *ADAMTS17* gene variants were identified in patient (IIb), one a nonsense mutation (c.1051A>T) had been retained when exclude the genetics variants in dbSNP150 and the 1000 Genomes project. The rs4369638 allele, with a frequency exceeding 80 percent in the 1000 genomes project,

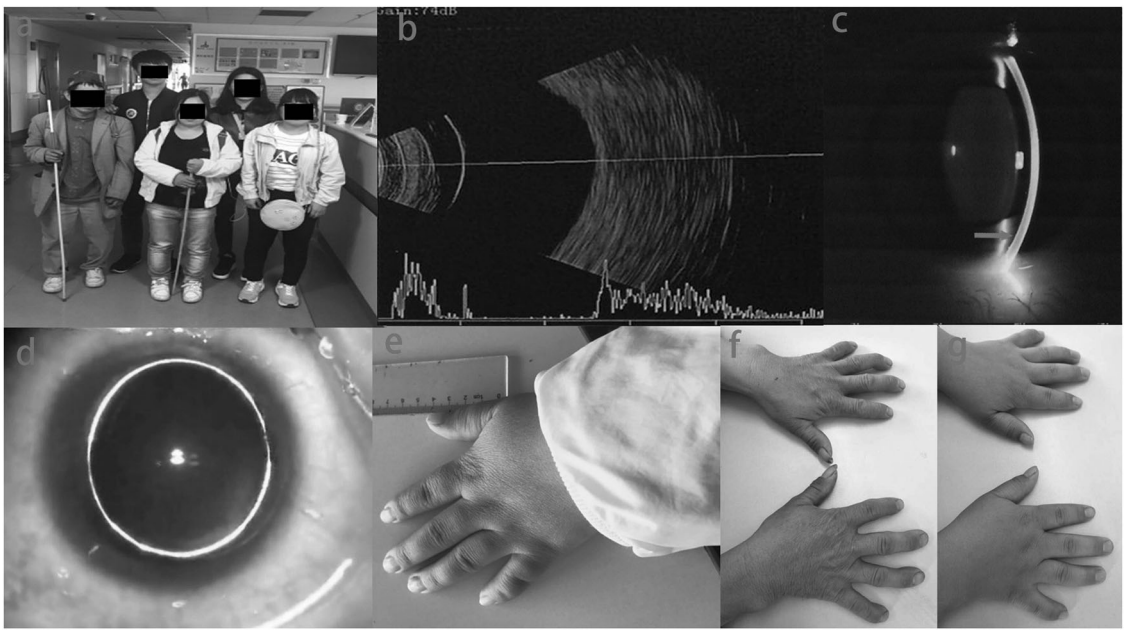

Fig. 2 Clinical manifestation. **a** Short stature: the people in the front row were WMS patients and those behind were asymptomatic carriers in the family. **b** Normal axial length in IIIe by using type-A ultrasound and type-B ultrasound, **c** shallow anterior in IIIe, **d** golden ring of lens in IIIe, **e** brachydactyly in IIIe, **f** brachydactyly in IIb, **g** brachydactyly in IIIb

Table 1 Clinical information of patients

Patient ID	IIIe	IIIb	IIb
Age (year)	12	19	44
Disease course (year)	1	5	Since youth
High cup/disc ratio (left/right)	0.6/0.6	0.9/1.0	/
Intraocular pressure (left/right, mmHg)	27.0/28.0	37.3/48.0	41.0/33.0
Vision	Decrease vision	Impaired vision	Bind/impaired vision
Secondary glaucoma and lens dislocation	No happened	Happened	Happened

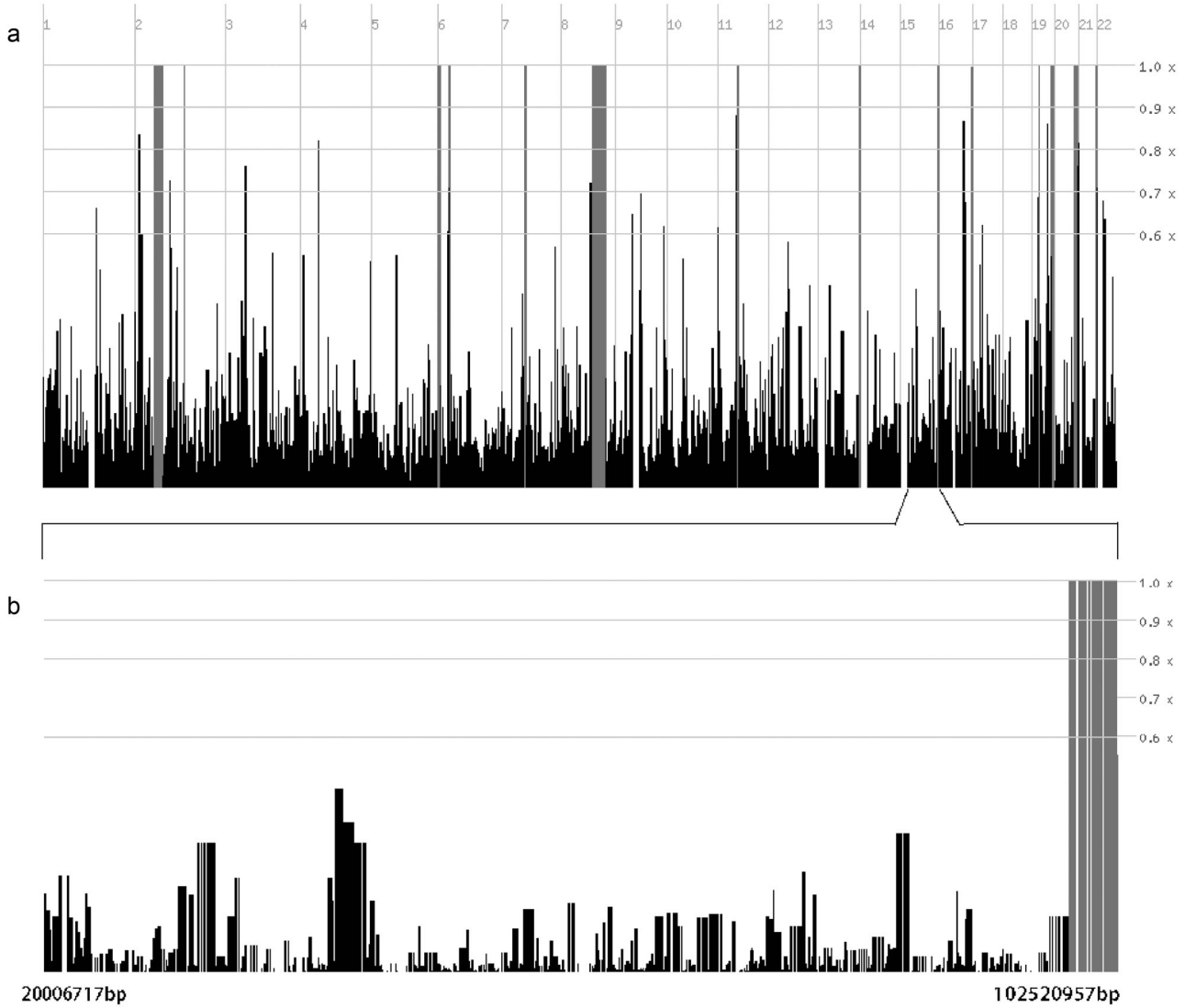

Fig. 3 Homozygosity mapping analysis. **a** Genome-wide homozygosity mapping. **b** Homozygosity mapping on chromosome 15

encodes an amino acid into which the rs4369638 mutation (TAA in lieu of TAG) introduces a stop codon. Mutation c.1051A>T results in termination of protein synthesis, truncating the protein from 1095 to 350 amino acids. This mutation therefore likely causes disease has a high possibility to cause disease (score equal to 1 with Disease_causing_automatic in MutationTaster and score equal to 0 with deleterious in LRT). Further, score equal to 5.47 in gerp++gt2 was identified, suggesting the mutation occurs in a conservative sequence will abrogate function.

According to the UniProt database, the ADAMTS protein family has a highly homologous N-terminal protease domain and C-terminal ancillary domains. The mutation c.1051A>T in *ADAMTS17* causes protein synthesis termination *p*.(lys351Ter) in the catalytic module; destroying this module likely interrupts major protein function in this

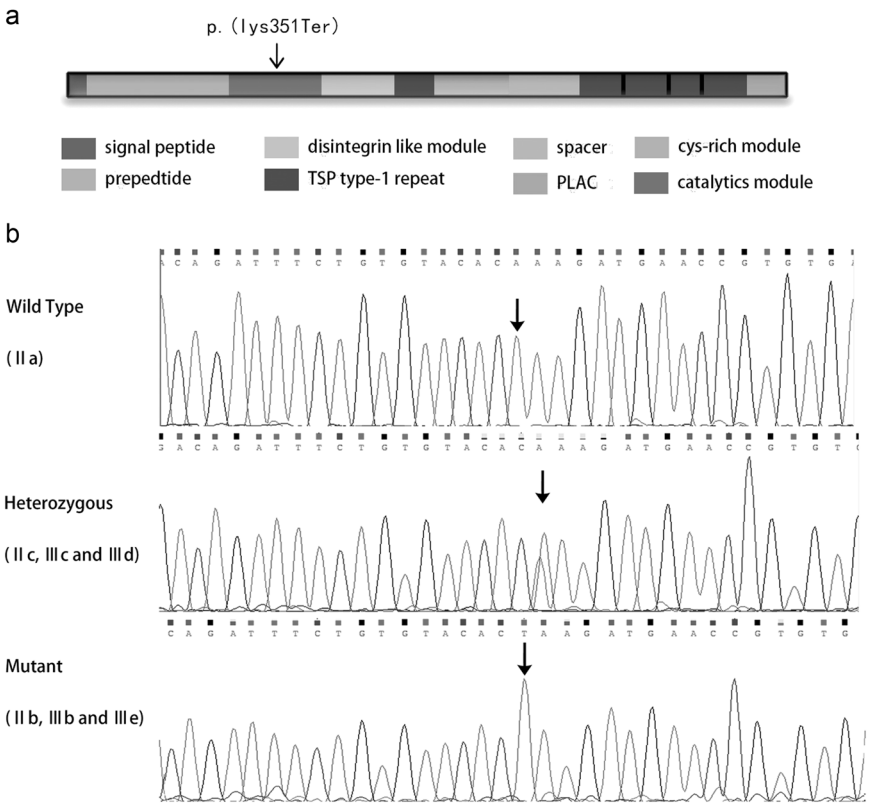

Fig. 4 Sequence analysis in *ADAMTS17*. **a** Structure of ADAMTS17 protein. **b** Sequencing chromatograms of representative individuals from family

family (Fig. 4a). Further, we found no other pathogenic variants in *ADAMTS10*, *LBTP2*, and *FBN1* which would have caused WMS or WMS-like symptoms in this family. Taken together, the homozygous variant c.1051A>T results in *p*.(lys351Ter) in the *ADAMTS17* gene likely caused this occurrence of WMS.

Sanger sequencing confirmation

In order to further confirm that c.1051A>T in *ADAMTS17* gene was disease-causing mutation and validate our conclusion, we assayed the mutation-carrying state in family members. Primers were designed for exon7 of the *ADAMTS17* gene to amplify and sequence a 600 bp DNA fragment. This confirmed c.1051A>T. IIa was the only one wild Type in our studied sample. IIb, IIIb, and IIIe were mutant with WMS. IIc, IIIc, IIId, and IV a were heterozygous without clinical symptoms as carriers of the *ADAMTS17* mutant gene (Fig. 4b). The results confirmed that the disease in this family derived from autosomal recessive inheritance of c.1051A>T in *ADAMTS17* as the basis for WMS disease in this family.

Discussion

The *ADAMTS* gene superfamily includes 19 distinct secreted enzymes. ADAMTS proteases are zinc metalloendopeptidases with diverse roles in the formation, remodeling, and the destruction of the extracellular matrix. A catalytic domain at the N terminus and substrate-binding region at the C terminus has been identified in ADAMTS. ADAMTS participates in metabolic activities [13, 14]. Mutations in *ADAMTS13, ADAMTS2, ADAMTS10, ADAMTS17, ADAMTSL2*, and *ADAMTSL4* have been identified in distinct human genetic disorders, ranging from thrombotic thrombocytopenic purpura to acromelic dysplasia [15]. Here, we discovered a novel nonsense mutation c.1051A>T in *ADAMTS17* leads to WMS, inherited as an autosomal recessive trait. Those affected in this family had been verified brachydactyly, short stature, and visual impairment.

Previous studies have suggested that *ADAMTS17* is associated with height [16] and the copy number variant located in the locus near the *ADAMTS17* can also affect height [17]. Interestingly, short stature is an obvious feature of WMS. This suggests that *ADAMTS17* may play an important role in normal human growth and development. By examining the genetic mechanisms of the WMS in this Chinese family, we identified a novel *ADAMTS17* mutation in WES derived from a nonsense mutation c.1051A>T, which abrogated function of the protein with *p*.(lys351Ter). We inferred the ADAMTS17 mutant protein in the family had no catalytic function or substrate recognition, resulting in incomplete microfibril biogenesis and causing a series of connective tissue symptoms, but this must be established experimentally. Compared with other mutation types,

nonsense mutations can have larger effects by causing a complete loss of protein function.

FBN1 is the major microfibril component in adults. Mutations in *FBN1* typically cause Marfan syndrome. Mutations in the *ADAMTS10* and *ADAMTS*-like genes damage microfibrils, causing similar symptoms. The overlapping disease spectra suggested fibrillin microfibrils formed ab initio are not fully functional, rather, ADAMTS proteins play a crucial role in regulating microfibril formation. The interactions between ADAMTS10 and FBN1 had been reported in the extracellular matrix of cultured fibroblasts [18]. However, the exact function of ADAMTS17 is unknown. One thing which deserves attention is that ADAMTS10 and ADAMTS17 share a similar domain structure, characterized by a highly homologous N-terminal protease domain and a C-terminal ancillary domain. Therefore, we suggest the role of ADAMTS17 may involve the formation and maintenance of microfibrils. Further study of this is warranted.

Since WMS and WMS-like syndrome are severe illnesses, the genetic bases merit clarification. These include the *ADAMTS*, *FBN, LBP* superfamilies. How these mutations change metabolic pathways remains unclear. At present, no accepted treatments are known.

Differences among dog breeds provide a model to study the function of ADAMTS proteins and their contribution to pathology [19]. GWAS identified a Primary Open Angle Glaucoma (POAG associated 4.9 Mb inversion disrupting *ADAMTS17* in the Petit Basset Griffon Vendéen dog breed [20]. Different independent mutations in *ADAMTS* superfamily may be occur in different breeds [21]. The genetic heterogeneity in specific dog breed and difference in *ADAMTS* superfamily may be the result of molecular evolution.

In conclusion, we report for the first time a nonsense mutation (c.1051A>T) in *ADAMTS17* as a cause of WMS. Prior reports indicate other WMS-inducing mutations in this gene, including splice-site mutations (c.873+1 G>T [22] and c.1721+1G>A [4], indels (including c.2458_2459insG [4], c.652delG [23] and a 106.96 Kb deletion containing exon 1–3 regions [24], and a missense mutation c.760C>T [4] was identified in *ADAMTS17* gene with WMS or WMS-like syndrome. Our study further underscores the importance of *ADAMTS17* in normal growth and development and increases the total number of reported canine *ADAMTS17* mutations to seven.

Data availability

The datasets collected and/or analyzed during the current study are available from the corresponding author upon reasonable request. Please contact author for data requests.

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Author contributions YSH and HAY conceived designed, review and editing the study. XZ and YCZ conducted the experiments. HAY, XZ, JL, SZH, YBK, GJW and YLY collected the data, processed data analysis and interpretation of data. HAY wrote the original manuscript. YSH critically revising the article. All authors reviewed and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Editorial policies and ethical consideration The current study was approved by the Institutional Review Boards of Kunming Medical University. Written informed consent was obtained from all investigated individuals prior to the study. Before enrollment, the patients or their guardians gave informed consent.

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