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ADAMTSL2 gene variant in patients with features of autosomal dominant connective tissue disorders

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Abstract

Ehlers-Danlos syndrome (EDS) consists of a heterogeneous group of genetically inherited connective tissue disorders. A family with three affected members over two generations with features of Dermatosparaxic EDS (dEDS) autosomal dominant transmission was reported by Desai et al. and having a heterozygous nonsynonymous missense variant of *ADAMTSL2* (c.1261G > A; p. Gly421Ser). Variation in this gene is also reported to cause autosomal recessive geleophysic dysplasia. We report five unrelated patients with the Gly421Ser variant identified from a large series of patients presenting with features of connective tissue disorders, each with a positive family history consistent with autosomal dominant transmission. Clinical features of a connective tissue disorder included generalized joint hypermobility and pain with fragility of internal and external tissues including of skin, dura, and arteries. Overall, our analyses including bioinformatics, protein modeling, and gene-protein interactions with the cases described would add evidence for the Gly421Ser variant in *ADAMTSL2* as causative for variable expressivity of autosomal dominant connective tissue disorders.

Keywords

1 | INTRODUCTION

Ehlers-Danlos syndrome (EDS) is a genetically heterogeneous group of connective tissue disorders characterized by joint hypermobility, skin extensibility, and tissue

fragility and subdivided EDS into six different categories based on clinical presentation and underlying genetic findings (Beighton et al., 1997). With continued improvement of genetic testing using next-generation sequencing (NGS) and elucidation of more causative genes, it became apparent that six categories were not sufficient. The International EDS Consortium proposed an expanded classification of EDS in 2017, bringing the total to 13 sub-types: Classic, Classic-like, Cardiac-valvular, Vascular, Hypermobile, Athrochalasia, Dermatosparaxis, Kyphoscoliotic, Brittle cornea syndrome, Spondylodysplastic, Musculocontractural, Myopathic, and Periodontal (Malfait et al., 2017). Overall, EDS is considered an uncommon disease with overall prevalence ranging from one in 2,500 to one in 5,000 as shown in existing literature (Joseph et al., 2018). Certain subtypes of EDS, however, are exceedingly rare. For example, EDS type VII C, Dermatosparaxis type (OMIM: 255410), has been identified in fewer than 20 patients (Desai et al., 2016; Van Damme et al., 2016). This form of EDS is delineated by extreme skin and tissue fragility as well as characteristic craniofacial features as defined by the 2017 classification.

Molecular diagnosis for Dermatosparaxic EDS (dEDS) is thought to require biallelic variants within the *ADAMTS2* gene (Malfait et al., 2017). However, Desai et al. (2016) reported a family with three affected members over two generations having a connective tissue disorder consistent with dEDS and a heterozygous nonsynonymous variant (c.1261G > A; p.Gly421Ser) in a related gene (ADAMTS-like protein 2 or *ADAMTSL2*) (OMIM:612277). No mutations were found in any other known EDS-associated genes using whole genome sequencing in their report. Prior to this report, biallelic *ADAMTSL2* gene variants were associated with geleophysic dysplasia (OMIM: 231050 [www.ncbi.nlm.nih.gov/OMIM]) where the phenotype includes joint limitation, severe short stature, facial dysmorphism, respiratory distress and cardiac valvular thickening. This gene encodes a glycoprotein that binds the cell surface and extracellular matrix interacting with transforming growth factor beta binding protein 1.

The proband reported by Desai et al. (2016) was a male child at 9 years of age having joint laxity and ankle crepitus producing a delay in walking. He had delay in speech, chewing difficulty, muscle weakness, and hypotonia. An abnormal gait and poor coordination noted at 5 years of age with weight and height at the 99th percentile. He was normocephalic with a slightly triangular shaped face with mid-face flattening, stretchable skin, and blue sclera. He had recurrent joint pain and hypermobility with patella dislocations that improved with age from three to 9 years and now decreased ankle movement. He had a heart murmur, urinary incontinence, hydroureter, and profound hydronephrosis requiring surgical intervention. He had exotropia, ADHD, autistic features and dysphagia with chronic constipation noted at 7 years. His father had multiple joint dislocations and fractures with minimal trauma and shoulder surgery following multiple dislocations along with wounds that did not heal normally. Several other family members including paternal aunts, uncles, and grandmother were suspected to have EDS due to skin elasticity and poor healing, multiple dislocations and fractures with minimal trauma and blue sclera. One family member had scoliosis. With these collection of findings, whole genome sequencing was performed on the mother, father and affected male proband nine-year-old son while other family members were studied using Sanger sequencing. Three of the affected family members over two generations were found

to have the *ADAMTSL2* gene variant. Prior to this report, it was believed that dEDS was caused by autosomal recessive mutations in *ADAMTS2*.

In an ongoing investigation of 100 consecutive patients presenting to the University of Kansas Medical Center (KUMC) Genetics Clinic with features of connective tissue disorder, next generation sequencing (NGS) of connective tissue gene panels were ordered. We found five unrelated patients (5%) all carrying the heterozygous nonsynonymous variant in the *ADAMTSL2* gene previously identified in three family members with potential dEDS (Desai et al., 2016). As other variants in this same gene are also reported causative for geleophysic dysplasia, we expect that the p.Gly421Ser variant is causative for expression of connective tissue disorders in the five reported KUMC patients.

2 | SUBJECTS AND METHODS

2.1 | Genetic testing

Buccal cells were collected for DNA isolation from patients presenting for genetic services and sent to Fulgent Diagnostics (Temple City, CA), a CLIA approved commercial laboratory for testing using a custom connective tissue NGS panel expanded over the past 3 years ranging from 56–81 different genes. For variants of unknown clinical significance, the gene variants were analyzed using information about allele frequency in the Broad Institute gnomAD database (>120,000 individuals) and considered important if <0.01%; analysis of amino acid conservation amongst primates, mammals and nonmammal vertebrates (if found in >90%); physiological differences in nonsynonymous missense amino acid substitution based on Grantham's distance (>100); and in silico computational predictions. Information about each patient was obtained and made available during the clinical visit for further analysis and discussion with the patient and family.

2.2 | Protein structure prediction

The three-dimensional protein structure prediction was conducted using the I-TASSER server (Zhang, 2008; Roy et al., 2010) and the *ADAMTSL2* amino acid sequence spanning Asp 23 to Ser 951 (NCBI Reference Sequence: NP_001138792.1). Notably, the signal peptide residues from Met 1 to Gly 22 were omitted from the sequence that was uploaded to I-TASSER. The top model had a confidence score (C-score) of −1.14 and TM-score of 0.57 +/− 0.15. Analysis of the resulting structure and figure preparation were conducted using CCP4mg (McNicholas et al., 2011).

2.3 | Protein–protein interactions

To further assess the likelihood that dysfunctional variation in the *ADAMTSL2* gene influences expression of connective tissue defects clinically, we evaluated experimentally-derived evidence for direct downstream relationships between the encoded ADAMTSL2 protein and proteins encoded by genes known to influence other connective tissue related disorders using Ingenuity Pathway Analysis software (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis).

2.4 | Clinical reports

Five unrelated patients presenting for genetic evaluation and testing were found with the same heterozygous nonsynonymous variant in the *ADAMTSL2* gene as reported by Desai et al. (2016) will be described below and genomic data summarized in Table 1.

2.4.1 | Clinical report one—A 55-year-old female presented to the clinic with a significant history of vertebral arterial dissection and tissue fragility without trauma on two separate occasions, both occurring within the last 10 years, requiring stent placement. She sustained a cerebral vascular accident or stroke and currently is recovering. The patient also underwent a renal artery ultrasound in 2010, showing "pearly" renal arteries. In 2017, the patient developed Horner's syndrome with symptoms primarily localized to the left side, suggesting carotid artery dissection as recognized in other studies in the literature (Kasravi et al., 2010; Borgman, 2012) and confirmed by a CT angiogram of the head and neck identifying a left interior carotid focal pseudoaneurysm (2–3 mm in size), a sequalae from a prior dissection. A history of intermittent narrow complex tachycardia was noted which occurred every few months but resolves after moving to a supine position for 20–25 min. She also reports varicose veins of the lower extremities. Previous DNA testing for the vascular form of EDS was ordered and found to be normal. Her current Beighton hypermobility score was four out of nine, exhibiting positive signs for hyperextensibility of the fifth digits and thumb to wrist, bilaterally consistent with a connective tissue disorder at her current age. She has no history of kyphoscoliosis. Hyperflexibility of the lower extremities were noted at a younger age and her daughter has excessive hypermobility with a Beighton score greater than five. Her maternal aunt and grandmother also died due to an aortic aneurysm. Ectopic heartbeats requiring ablation were present in one nephew and a niece.

Given the patient's clinical picture, a connective tissue NGS panel was ordered from a CLIA approved commercial laboratory (Fulgent Diagnostics, Temple City, CA) and heterozygous missense variants were found in both *COL12A1* and *ADAMTSL2* genes. The *COL12A1* gene encodes a polypeptide that is homologous to type IX short-chain collagen peptides (Gordon et al., 1987) with homozygous mutations seen at a splice site associated with Ullrich Congenital Muscular Dystrophy 2. Autosomal dominant mutations are reported in less severe Bethlem Myopathy 2 (Zou et al., 2014). Both diseases are associated with progressive decreased mobility. Our patient's *COL12A1* variant, c.5221G > A (p. Glu1741Lys) has not been reported previously as pathogenic and observed at a frequency of 0.09% across controls in the entire BroadExAC database, above the cut-off for a rare variant and a low amino acid Grantham's distance, indicating a only a moderate change.

The *ADAMTSL2* gene encodes a secreted glycoprotein that interacts with transforming growth factor-beta (TGFB) binding protein-1, a protein that aids in the storage of latent TGF beta within the extracellular matrix (Le Goff et al., 2008). Homozygous (biallelic) mutations in this gene have been associated with geleophysic dysplasia-1, a progressive condition which resembles a lysosomal storage disorder characterized by short stature, progressive joint limitations, distinct facial features, progressive cardiac valvular disease, and thickened skin (Marzin & Cormier-Daire, 1993). Fulgent Diagnostics laboratory uses

NGS testing which includes 100% of the coding sequence for the NM_01469.3 transcript of *ADAMTSL2* gene with sequence to a minimum depth of 20x. The presence of mutations in the deep intronic or regulatory regions of this gene cannot be ruled out.

Our patient's heterozygous nonsynonymous missense *ADAMTSL2* variant, c.1261G > A (p. Gly421Ser) has not been previously reported in controls across the BroadExAC database and the Grantham distance score of 56 represents an amino acid substitution of moderate change. Analysis of amino acid conservation indicates that the wild-type amino acid Gly421 is found in greater than 90% of primates (12/12), mammals (49/54) and nonmammal vertebrates (21/26) supporting conservation. Computational prediction tools supported a deleterious change with four predictive algorithms (i.e., FATHMMMKL, MUTATIONASSESSOR, MUTATIONTASTER, SIFT-Alamut). This variant is classified as a "Disease Mutation" in the Human Gene Mutation Database (HGMD) and identical to that reported by Desai et al. (2016) in a family with features of an autosomal dominant connective tissue disorder, specifically dEDS. Of the two gene variants identified and the patient's clinical presentation with tissue fragility, arterial dissections, varicose veins and hypermobility, the *ADAMTSL2* gene would be the most likely causative defect.

2.4.2 | Clinical report two—A 67-year-old female presented to the clinic with chronic pain including most joints, abdomen, and cervical spine with a diagnosis of cervical spondylosis, spinal stenosis, presence of dural cysts with spinal fluid leaks (wears a neck brace), umbilical hernia, mitral valve prolapse, blue sclera and poor skin healing most consistent with a connective tissue disorder. The patient had spinal cord compression with surgical fusion of C5 through C7 vertebrae. An MRI of the lumbar spine showed bulging discs at L1-L2 and L4-L5, mild spinal stenosis and lateral recess narrowing. She also has mild urinary incontinence. She reported a history of intracranial hypotension related to a presumed spinal leak, tissue fragility and spinal diverticula. She also had surgery for clipping of T5 and T6 nerve root cysts. Other relevant history included fatigue, progressive headaches, postural orthostatic hypotension with severe pressure in her right ear and tinnitus. Both of her pregnancies resulted in premature delivery at 28 weeks gestation due to early spontaneous rupture of membranes. Due to her complex history and generalized joint pain with movement, a Beighton hyper-mobility score assessment was attempted but not successful.

The patient's family history was significant for osteoarthritis amongst her four sisters and one brother having multiple orthopedic procedures. One of her sisters has joint hypermobility with a Beighton score greater than five. She has two nieces with joint hypermobility, but Beighton scores were not available. Her mother died at 85 years of age from an arrhythmia with a history of hypertension. The patient reported that two maternal uncles died from possible thoracic dissections and several relatives died due to myocardial infarctions.

Given the patient's clinical presentation and family history, a connective tissue NGS panel was ordered from Fulgent Diagnostics for NGS testing and a heterozygous missense gene variant was identified in *ADAMTSL2*, c.1261G > A(p. Gly421Ser), as reported by Desai et

al. (2016) and could account for several of her connective tissue related findings with joint involvement, spinal problems and tissue fragility.

2.4.3 | Clinical report three—A 48-year-old female presented to the clinic with chronic major and minor joint pain, dizziness, fatigue and unable to work. She also experiences spontaneous knee and hip crepitations, joint hyper-mobility, varicose veins with skin and tissue fragility. In 2012, she was diagnosed with chronic refractory migraines. She had a history of seven miscarriages with four livebirths and diagnosed with Factor V Leiden deficiency thought contributing to her miscarriages. She had undergone multiple Cesarean section deliveries. On exam, the patient exhibited mild skin marbling, easy bruising, varicose veins on lower extremities, loose and stretchable skin on her neck with multiple widened abdominal striae. She exhibited a Beighton score of seven out of nine with positive signs for thumb-wrist flexion, elbow hyperextension, and knee hyperextension, all bilateral as well as palms on the floor. She has not had an echocardiogram to examine for cardiac defects or x-rays to rule out kyphoscoliosis.

The patient has three children: a 25-year-old daughter with migraines, with history of fatigue or low energy, joint pain, and gastroparesis; a 27-year-old son with migraines and sinus issues, and a deceased son who passed at 7 months due to Pompe disease, an autosomal recessive disorder caused by a deficiency in the lysosomal enzyme acid-α-glucosidase (Kishnani et al., 2006). Her fourth child has Down syndrome and lactose intolerance. She has two nephews and one niece with syncopal episodes and a 72-year-old mother with essential tremors and migraine headaches.

Given the patient's joint hypermobility, skin fragility and high Beighton score, a connective tissue NGS panel was ordered from Fulgent Diagnostics and heterozygous variants were found in both *PTCH1* and *ADAMTSL2* genes. *PTCH1* gene codes for a transmembrane protein that serves as a receptor for hedgehog proteins, a family of embryonic organizing proteins that act to sequester (Chen & Struhl, 1996). Autosomal dominant mutations in *PTCH1* have been associated with holoprosencephaly-7 and basal cell nevus syndrome, both syndromes associated with diverse developmental abnormalities (Johnson et al., 1996; Ming et al., 2002), not seen in the proband or family members. This specific variant c.3487G > A (p. Gly1163Ser) was previously reported in an individual with bicuspid aortic valve (Bonachea et al., 2014) but classified as unknown clinical significance. The variant seen in the *ADAMTSL2* gene was c.1261G > A (p. Gly421Ser), as reported by Desai et al. (2016). Her clinical features are similar to those reported by to Desai et al. (2016) with the same *ADAMTSL2* gene variant.

2.4.4 | Clinical report four—An 18-year-old male presented to the clinic with chronic joint and jaw pain with hypermobility with subluxations of the shoulders, arms and jaw, chronic headaches, sleep issues, anxiety, and attention-deficit disorder. His mother experienced preterm labor beginning at 3 months gestation for the duration of the pregnancy. The patient was born at 37 weeks gestation after labor induction with no significant congenital anomalies. He experiences Raynaud's phenomenon in his hands and reports syncopal episodes when standing quickly. He has myopia corrected with glasses and ongoing pain in his lower back. No x-rays have been performed. He has a history of

spontaneous bruising, thin skin with fragility, and calloused feet. His hands are sensitive to trauma causing bleeding and wear gloves due to skin and tissue fragility. On exam, he also has soft stretchable skin with striae on upper arms, knees, legs, and back. There is increased space between the first and second toes, bilaterally. His right shoulder is lower than left upon standing but without recognize kyphoscoliosis. The patient's Beighton score was eight out of nine, exhibiting all positive signs for hyper-mobility except for palms on floor.

The patient has four brothers, a 22-year-old with chronic migraines and temporal mandibular joint (TMJ) pain, a 24-year-old with speech and learning issues, a 30-year-old with fibromyalgia, and a 32-year-old with congenital brain malformation, schizophrenia, and progressive hearing loss. He has a 3-year-old niece with speech and language issues. His mother is a 53-year-old female with preterm labor for each of her pregnancies and clinically diagnosed with EDS with joint hyperflexibility, flat feet, pelvic floor dysfunction, and scoliosis. Her Beighton score is greater than five by history. The patient's father is a 57-year-old with early-onset osteoporosis, bladder hernia, heart arrhythmia, and TMJ. He also has a 60-year-old paternal aunt with heart arrhythmia and mitral valve prolapse. His maternal grandmother died at age 80 years of unknown causes but had a history of hernias, slow healing, and polymyositis.

Given the patient's joint hypermobility, thin fragile skin with excessive stretch marks, a high Beighton score, and positive family history of similar findings consistent with a connective tissue disorder, a connective tissue NGS panel was ordered from Fulgent Diagnostics. Two heterozygous missense variants of unknown significance were found in both *COL3A1* and *ADAMTSL2*. The *COL3A1* gene encodes type III collagen, a protein expressed from early embryo stage through adulthood and is a major component of the extracellular matrix found in a variety of organs including dermis (Liu et al., 1997). Autosomal dominant mutations in this gene are associated with EDS Type IV (vascular) and rarely, type III (hypermobility) (Narcisi et al., 1994; Liu et al., 1997; Schwarze et al., 1997). Vascular EDS is characterized by joint and dermal manifestations, as well as spontaneous bowel and large artery rupture. Hyper-mobile EDS is characterized by joint hypermobility, skin hyperextensibility and tissue fragility with extra-musculoskeletal manifestations (Malfait et al., 2017). The *COL3A1* gene variant, c.2233G > A (p. Glu745Lys) was not previously reported in controls across the BroadExAC database. In silico, predictive analysis reported a deleterious change in eight computational algorithms. The amino acid position was highly conserved amongst mammals and primates. This patient also had a nonsynonymous *ADAMTSL2* gene variant at c.1261G > A (p. Gly421Ser), as reported by Desai et al. (2016). He has features consistent with the *ADAMTSL2* gene variant specifically excessive skin fragility, joint hypermobility and pain as similarly seen in his mother.

2.4.5 | Clinical report five—A 34-year-old female presented with joint hypermobility and subluxation, mild pectus excavatum but without evidence of kyphoscoliosis, chronic fatigue and migraines, mild mitral valve prolapse by echocardiogram, jaw pain, mobility issues, orthostatic and neurocardiogenic intolerance, pelvic floor dysfunction indicating loose connective tissue, and numbness with tingling of the extremities. The patient was noted to have a small umbilical cord at birth following a 42 weeks pregnancy. She developed tachycardia and ectopic atrial fibrillation that was ablated. She was later diagnosed with

bilateral subependymal gray matter heterotopia and episodes of cognitive impairment with loss of executive functioning. During the evaluation, she had undergone a lumbar puncture which was negative and a normal MRI of the cervical spine. On exam her Beighton score was six out of nine indicating a possible connective tissue disorder.

Her mother was 61 years old with mitral valve prolapse and chronic pain in her feet and lower back. Due to her mother being adopted, the patient had limited maternal family history information. Her father is a 57-year-old with mild cognitive impairment, chronic joint pain, multiple abdominal hernias, high homocysteine levels and a history of syncope with unknown origin. The patient's 34-year-old sister has Hashimoto's thyroid disease, supraventricular tachycardia, Wolf-Parkinson-White syndrome and migraines. Her 30-year-old brother has mild Chiari malformation and mitral valve prolapse but a Beighton score was unavailable.

Given the patient's elevated Beighton score, clinical presentation and family history, a connective tissue NGS panel was ordered from Fulgent Diagnostics. Three heterozygous missense variants were found in the *FLNA*, *MTHFR*, and *ADAMTSL2* genes. *FLNA* encodes for filamin A, which is a widely expressed actin-binding protein that regulates reorganization of actin cytoskeleton (Vadlamudi et al., 2002). This X-linked dominant condition [OMIM: 300017 (www.ncbi.nlm.nih.gov/OMIM)] has been associated with terminal osseous dysplasia, periventricular heterotopia, Melnick-Needles syndrome, and otopalatodigital syndrome types I and II with or without features of EDS, while X-linked recessive mutations have been associated with FG syndrome 2, cardiac valvular dysplasia, congenital short bowel syndrome, and frontometaphyseal dysplasia. The patient's heterozygous variant, c.410 T > A (p. Ile137Asn) was not previously reported in controls across the BroadExAC database and computational data indicate highly evolutionary conservation. This variant was classified as likely pathogenic.

The *MTHFR* gene encodes methylenetetrahydrofolate reductase, which catalyzes a co-substrate reaction for homocysteine remethylation to methionine (Goyette et al., 1998). Biallelic mutations in this gene (OMIM: 607093 [www.ncbi.nlm.nih.gov/OMIM]) have been associated with homocystinuria, early infantile epilepsy and blood clots. The patient's heterozygous variant, c.665C > T (p. Ala222Val) is common in the general population indicating a carrier status of this classical autosomal recessive gene. This patient also had the *ADAMTSL2* heterozygous variant c.1261G > A (p. Gly421Ser), as reported by Desai et al. (2016). Several features such as gray matter heterotopia, jaw pain, and cognitive impairment could be consistent with the FLNA gene variant while other features including hypermobility, chronic fatique, pectus excavatum, and orthostatic hypotension could be consistent with the *ADAMTSL2* gene variant and family history as reported by Desai et al. (2016).

3 | DISCUSSION

ADAMTSL2 (ADAMTS-like protein 2) is a member of the ADAMTS superfamily of genes. ADAMTS proteins are zinc metal-loendopeptidases with a majority of their substrates being extracellular matrix (ECM) components leading to strength and flexibility whereas

ADAMTS-like proteins lack the metalloprotease and propeptide and disintegrain-like domains but serve as regulators of ECM assembly and/or ADAMTS activity (Mead & Apte, 2018). The product of the ADAMTS2 gene is a procollagen I N-proteinase which cleaves the N-propeptide of type I and II procollagen (Colige et al., 1999). Overall, the structure of the ADAMTSL2 protein includes seven thrombospondin type 1 repeats, ADAMTS spacer modules, areas of glycosylation, and a PLAC module (Apte, 2009) (Figure 1). The high degree of homology between ADAMTS and ADAMTSL proteins suggests a functional relationship, though ADAMTSL2 lacks the metalloprotease domain which may impact its function and specificity for disease causation.

The seven-thrombospondin type 1 (TSP1) repeats within ADAMTSL2 flank a cysteine/glycan rich domain which harbors residue Gly421. Protein structure prediction approaches were utilized in an effort to obtain a putative three-dimensional model that could potentially provide structure/function insight. The cysteine/glycan rich domain is predicted to be mainly composed of α-helices and is buttressed by TSP1 repeats in the N-terminus (Figure 2). Interestingly, Gly421 is predicted to be in proximity to Asn 367 (~6 Å) which is a glycosylation site. Although the mutation from a Gly to a Ser residue is a subtle change, point mutations such as this can often affect protein folding or alter functions (Chhum et al., 2016; Sun et al., 2016). As such, one could envision that the Gly421Ser mutation could alter hydrogen bond interactions, affect local secondary structure conformations and/or perturb glycan interactions thereby leading to disease causation.

ADAMTSL2 has been found to interact with latent transforming growth factor beta-binding protein 1 (LTBP1) involved in storage and availability of transforming growth factor beta (TGFB), critical for controlling growth, cell proliferation, differentiation, motility and apoptosis [Genetics Home Reference (ghr.nlm.nih.gov/gene/ADAMTSL2)]. Similarly, one may postulate that structural differences may affect the mode of inheritance depending on the protein structure and folding, domains and function impacting the location of the protein-coding gene variants within the gene. Though somewhat limited, this relationship has been shown in mice where ADAMTSL-2 and -4 join with ADAMTS-10 and -17 to modulate fibrillin-1 function (Sengle et al., 2012).

The association between the ADAMTS superfamily and connective tissue disorders (Mead & Apte, 2018) as well as evidence of functional relationship between ADAMTS and *ADAMTSL2* may provide further evidence that a mutation within *ADAMTSL2* can confer a connective tissue disorder phenotype. For example, *ADAMTSL2* has been experimentally observed to directly interact with four other proteins encoded by genes that are causal for other connective tissue disorders (Figure 3). Specifically, *ADAMTSL2* binds to the extracellular matrix protein FIBULIN-1(Wang et al., 2011) and mutations in the FBLN1 gene are associated with synpolydactyly 2 associated with metacarpal and metatarsal synostoses (OMIM: 135820). Direct relationships have also been observed between *ADAMTSL2* and three different lysyl oxidase binding proteins that cross-link extracellular matrix proteins playing a role in connective tissue stability, including LOX, LOXL2 and LOXL3 (Aviram et al., 2019). Lysyl oxidase is a copper dependent enzyme that plays a critical in the synthesis of connective tissue matrices. Notably, a homozygous mutation (p.M292R) in the mouse Lox protein is observed to increase the number of aneurysms

of the ascending aorta in newborn mice (Lee et al., 2016). Moreover, upregulation of LOXL2 mRNA in human muscle is associated with Duchenne muscular dystrophy (Dadgar et al., 2014). Finally, a rare, autosomal recessive form of the [OJV4] collagenopathy known as Stickler syndrome, a classical connective tissue disorder, was observed to relate to homozygous mutations in LOXL3 (Chan et al., 2019). Furthermore, in situ hybridization of *ADAMTSL2* mRNA has demonstrated expression in cardiomyocytes, epidermis, dermis, the tracheal wall, developing skeletal muscle, and pulmonary arteries (Le Goff et al., 2008). Widespread expression of *ADAMTSL2* lends credence to the idea that patients' clinical presentations would be compatible with a dysfunction in the *ADAMTSL2* gene product, altering its regulatory activities as a recessive gene to a structural-related protein of a typical dominant gene impacted by glycosylation sites within the protein near the amino acid substitution at position 421 of the 951 amino acids comprising the *ADAMTSL2* protein seen in our patients. This area of heavy glycosylation is located between the ADAM-spacer domain and TSP1 domains (Figure 1). Is it possible that mutations in this region may alter the glycosylation pattern subsequently changing the three-dimensional structure of the protein and function? To test this hypothesis, more research is needed.

The five reported clinical cases with features of a connective tissue disorder have varying degrees of similarity relative to the proband and affected family members described by Desai et al. (2016) with dEDS including chronic joint pain, hypermobility and orthopedic procedures, amblyopia, gross motor delay, speech issues and ADHD, poor healing, stretchable skin and striae, facial flattening, heart murmurs or issues, ureter problems and hydronephrosis, asthma, and chronic constipation. Four of our five cases met the clinical diagnostic criteria for generalized joint hypermobility, as patients with a Beighton score of   six for prepubertal children and adolescents,   five from puberty to adulthood until 50 years, and   four for adults who are >50 years (Malfait et al., 2017). Clinical presentations and family histories for Clinical Reports One, Two, Three and Five could be consistent with an autosomal dominant transmission with variable expressivity of the *ADAMTSL2* gene variant c.1261G > A (p. Gly421Ser). Clinical Report Four had a likely pathogenic autosomal dominant variant for *COL3A1* which may account for several vascular-related clinical findings in this patient but other features including excessive skin fragility, hyper-mobility and joint pain also present in his mother are similarly reported in patients by Desai et al. (2016) with the same *ADAMTSL2* gene variant. Other first and second degree relatives of our patients also reported features consistent with a connective tissue disorder by history such as hypermobility and joint pain, scoliosis, thoracic dissections, flat feet, Chiari malformation, hernias and slow healing but none had DNA testing available to date. Hence, our clinical reports, *ADAMTSL2* protein description and function and *ADAMTSL2* protein structure prediction studies as well as the presentation of three affected family members reported by Desai et al. (2016) generates a growing body of evidence that heterozygosity for this variant, previously recognized as only autosomal recessive, may cause a connective tissue phenotype.

We would concur that the nonsynonymous missense *ADAMTSL2* gene variant ostensibly impacts the functional role of this gene in relationship with extracellular matrices, cross-linking and biogenesis including transforming growth factor beta (TGFB) and connective tissue disorders generating a phenotype consistent with EDS (e.g., stretchable fragile skin,

joint laxity, scoliosis and vascular abnormalities). However, no functional assays were performed in our clinical report and beyond the scope of our study. This is recognized as a limitation and will require more research.

Advanced genomic technology with NGS allows for identification of gene variants in patients for genetic testing. Given the associated clinical findings presented in this report and that of Desai et al. (2016), further support recognized autosomal recessive inheritance patterns may also present with a dominant mode of transmission of specific variants impacting protein domains or other unstructured regions (e.g., areas of heavy glycosylation) and different clinical findings. For example, increased use and experience with NGS technology have identified patients with heterozygous variants of recognized autosomal recessive genes with signs and symptoms of a recessive disease (e.g., ZNF469 causing brittle cornea syndrome [OMIM #229200] with keratoconus and corneal thinning in a heterozygous state [Lechner et al., 2014]). The authors would encourage other clinicians to report their clinical and molecular genetic testing experiences with patients having features of connective tissue disorders such as EDS to gain a better understanding of the pathogenesis of these disorders, genes involved and description with inheritance patterns leading to better care and treatment and more accurate genetic counseling for affected families.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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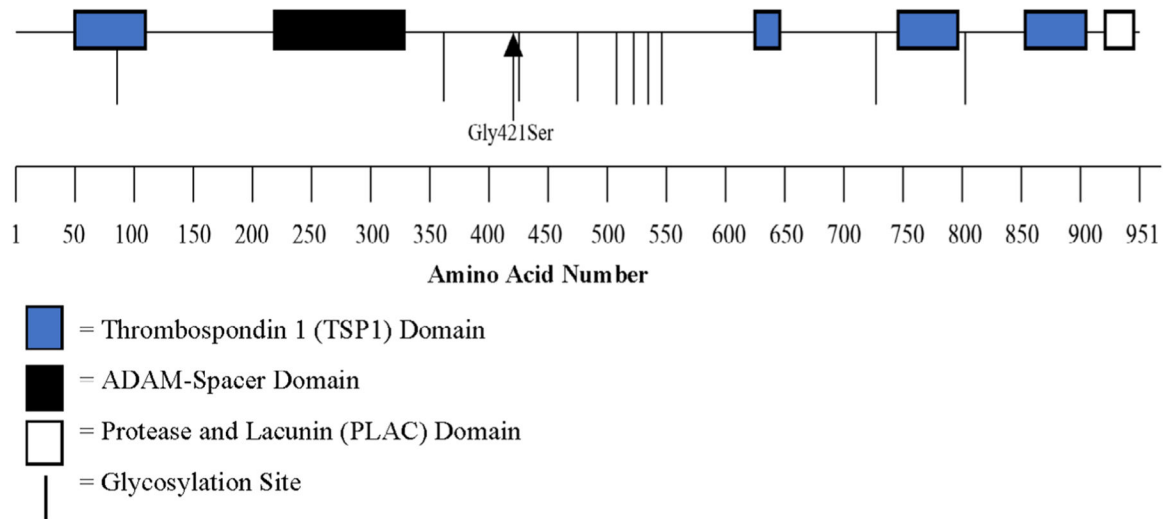
FIGURE 1.
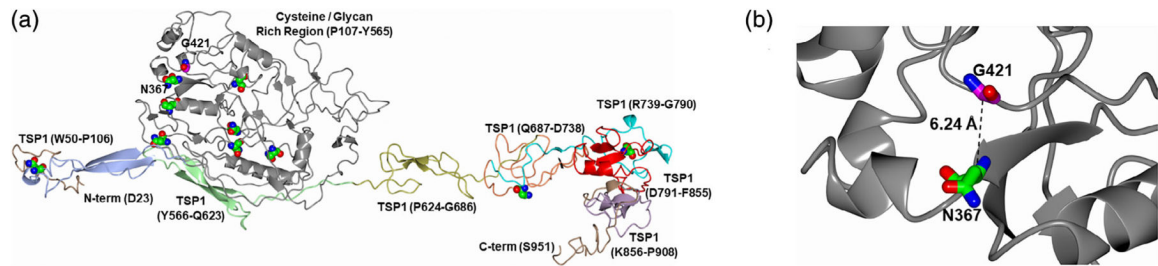ADAMTSL2 protein structure and amino acid location

FIGURE 2.
ADAMTSL2 model predicted from I-TASSER. (a) The TSP1 and Cysteine/Glycan rich domains rendered as ribbons. The N-glycosylated asparagine (N367) and Gly 421 (G421) residues are drawn as spheres. (b) Zoomed-in view of the N367 and G421 residues in the cysteine/glycan rich region showing their proximity in the structural model
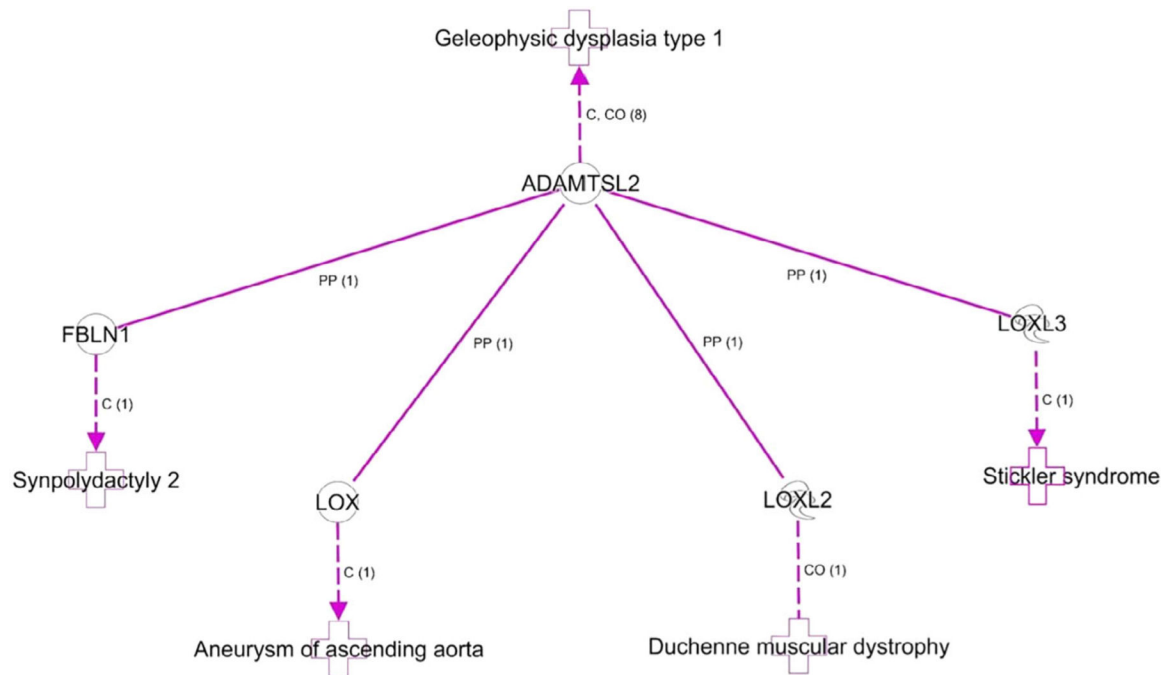
FIGURE 3.

Direct downstream relationships between ADAMTSL2 and proteins encoded by causal genes for other connective tissue disorders. Shown are protein–protein interactions (PP) between ADAMTSL2 and proteins encoded by genes where variation is causal (C) and correlated (CO) with connective tissue disorders

TABLE 1

Individual genetic data and description of five clinical report cases with ADAMTSL2 involvement

Clinical report	Age (year)	Gender	Gene	Beighton score	Variant (nonsynonymous)	Zygosity	Recognized gene inheritance	Classification	Allele frequency	In Silico prediction	Grantham distance	Evolutionary conservation
Case 1	55	Female	**Gene 1.** COL12A1 **Gene 2.** ADAMTSL2	4/9	**Gene 1.** C.5221G > A p.Glu74Lys **Gene 2.** C.1261G > A p.Gly421Ser	Heterozygous	**Gene 1.** AD & AR **Gene 2.** AR	**Gene 1.** VUS **Gene 2.** VUS	**Gene 1.** 0.09% (OMIM #120320) **Gene 2.** Variant previously reported by Desai et al. (2016)	**Gene 1.** 7/9 = deleterious **Gene 2.** 4/11 = deleterious	**Gene 1.** 56 **Gene 2.** 56	**Gene 1.** 62/62 mammals including 12 primates **Gene 2.** 49/54 mammals including 12/12 primates
Case 2	67	Female	ADAMTSL2	N/A	C.1261G > A p.Gly421Ser	Heterozygous	AR	VUS	0% (0/5012)	4/11 = deleterious	56	49/54 mammals including 12/12 primates
Case 3	48	Female	**Gene 1.** PTCH1 **Gene 2.** ADAMTSL2	7/9	**Gene 1.** C.3487G > A p.Gly1163Ser **Gene 2.** C.1261G > A p.Gly421Ser	Heterozygous	**Gene 1.** AD **Gene 2.** AR	**Gene 1.** VUS **Gene 2.** VUS	**Gene 1.** 0.06% (OMIM #601309) **Gene 2.** Variant previously reported by Desai et al. (2016)	**Gene 1.** 2/2 = deleterious **Gene 2.** 1/2 = deleterious	**Gene 1.** 56 **Gene 2.** 56	**Gene 1.** Conserved in all 100 vertebrates **Gene 2.** 49/54 mammals including 12/12 primates
Case 4	18	Male	**Gene 1.** COL3A1 **Gene 2.** ADAMTSL2	8/9	**Gene 1.** C.2233G > A p.Glu745Lys **Gene 2.** C.1261G > A p.Gly421Ser	Heterozygous	**Gene 1.** AD & AR **Gene 2.** AR	**Gene 1.** VUS **Gene 2.** VUS	**Gene 1.** Variant not previously reported **Gene 2.** Variant previously reported by Desai et al. (2016)	**Gene 1.** 8/10 = deleterious **Gene 2.** 4/11 = deleterious	**Gene 1.** 56 **Gene 2.** 56	**Gene 1.** 59/62 mammals including 12/12 primates **Gene 2.** 49/54 mammals including 12/12 primates
Case 5	34	Female	**Gene 1.** FLNA **Gene 2.** MTHFR **Gene 3.** ADAMTSL2	6/9	**Gene 1.** c.410 T > A p.Ile137Asn **Gene 2.** C.665C > T p.Ala222Val **Gene 3.** C.1261G > A p.Gly421Ser	Heterozygous	**Gene 1.** X-linked **Gene 2.** AR **Gene 3.** AR	**Gene 1.** Likely pathogenic **Gene 2.** VUS **Gene 3.** Risk allele	**Gene 1.** Variant not previously reported **Gene 2.** 30.79% (OMIM #607093) **Gene 3.** Variant previously	**Gene 1.** N/A **Gene 2.** 2/2 = deleterious **Gene 3.** 4/11 = deleterious	**Gene 1.** N/A **Gene 2.** 64 **Gene 3.** 56	**Gene 1.** N/A **Gene 2.** 96/96 vertebrates **Gene 3.** 49/54 mammals including 12/12 primates

Clinical report	Age (year)	Gender	Gene	Beighton score	Variant (nonsynonymous)	Zygosity	Recognized gene inheritance	Classification	Allele frequency reported by Desai et al. (2016)	In Silico prediction	Grantham distance	Evolutionary conservation