BRIEF REPORT
Biallelic variants in ADAMTS15 cause a novel form of distal arthrogryposis


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ABSTRACT
Purpose: We aimed to identify the underlying genetic cause for a novel form of distal arthrogryposis.
Methods: Rare variant family-based genomics, exome sequencing, and disease-specific panel sequencing were used to detect ADAMTS15 variants in affected individuals. Adamts15 expression was analyzed at the single-cell level during murine embryogenesis. Expression patterns were characterized using in situ hybridization and RNAscope.
Results: We identified homozygous rare variant alleles of ADAMTS15 in 5 affected individuals from 4 unrelated consanguineous families presenting with congenital flexion contractures of the interphalangeal joints and hypoplastic or absent palmar creases. Radiographic investigations showed physiological interphalangeal joint morphology. Additional features included knee, Achilles tendon, and toe contractures, spinal stiffness, scoliosis, and orthodontic abnormalities. Analysis of mouse whole-embryo single-cell sequencing data revealed a tightly regulated Adamts15 expression in the limb mesenchyme between embryonic stages E11.5 and E15.0. A perimuscular and peritendinous expression was evident in in situ hybridization in the developing mouse limb. In accordance, RNAscope analysis detected a significant coexpression with Osr1, but not with markers for skeletal muscle or joint formation.
Conclusion: In aggregate, our findings provide evidence that rare biallelic recessive trait variants in ADAMTS15 cause a novel autosomal recessive connective tissue disorder, resulting in a distal arthrogryposis syndrome.

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Introduction

The distal arthrogryposes (DAs) are characterized by contractures involving 2 or more body parts, primarily affecting the wrists, hands, ankles, and feet.¹ The contractures can vary in severity, but usually are not progressive and do not affect previously unaffected joints.² To date, >10 DA types and 15 associated genes have been identified.¹,³ Most DA types are caused by heterozygous pathogenic variants in genes encoding sarcomeric components of skeletal muscle fibers.⁴ Besides these myopathic forms, arthrogryposis can be categorized etiologically into neuropathic or connective tissue associated types.⁵

The ADAMTS/L superfamily comprises 19 metalloproteases and 7 structurally related glycoproteins (ie, ADAMTS-like proteins) that play prominent roles in connective tissue homeostasis.⁶ The ADAMTS proteases share a similar structure containing a conserved N-terminal protease domain and a modular organized C-terminal ancillary domain, which mediates binding to extracellular matrix (ECM) components.⁷ Although most of these enzymes participate in cleaving various substrates, such as procollagen, versican, and aggrecan, some proteases appear to function more as ADAMTS-like proteins and are implicated in microfibril assembly and ECM regulating pathways such as TGFβ and BMP signaling.⁵,⁷ To date, 8 members of the superfamily have been linked to different autosomal recessive (AR) disease traits.⁸ Using exome sequencing (ES) and disease-specific panel analysis, we detected biallelic variants in ADAMTS15 (HGNC:16305, OMIM 607509), in 5 affected individuals from 4 independent consanguineous families with congenital distal contractures. ADAMTS15 is located on chromosome 11q24.3. The canonical transcript (ENST00000299164.3; NM_139055.3) consists of 8 exons and encodes a 950 residue protein that presumably acts as an extracellularly activated protease (versicanase) that hydrolyses versican.⁹ Detailed knowledge about the function and tissue-specific expression is lacking.

Our data implicate biallelic ADAMTS15 pathogenic variants as causative for a Mendelian disease trait involving distal contractures. Given our RNA data, we hypothesize that ADAMTS15 plays a critical role in perimuscular connective tissue and tendon development, which is disturbed in distal arthrogryposis caused by ADAMTS15 loss-of-function (LoF).

Materials and Methods

Parental consent was obtained for all clinical and molecular studies in this report and for the publication of clinical photographs. Next-generation sequencing was performed at Charité-Universitätsmedizin Berlin (individual 1: trio ES and individual 5: trio ES), Baylor College of Medicine (individual 3: single ES), and University Medical Center Göttingen (individual 4: panel sequencing). Given the virtual absence of ADAMTS15 expression in all typical cell lines and accessible human tissues (for an overview, consult https://www.proteinatlas.org/ENSG00000166106-ADAMTS15), we investigated the cell types expressing the gene during embryogenesis at the single-cell level using the Mouse Organogenesis Cell Atlas.¹⁰ Furthermore, we performed droplet digital polymerase chain reaction (ddPCR), in situ hybridization, and RNAscope for further functional characterization of Adaptms15 expression. Further details can be found in Supplemental Material and Methods.

Results

Clinical data

All 5 affected participants displayed similar distal congenital contractures of the fingers and toes (Figure 1C). The fingers were bent in the proximal interphalangeal joints, whereas the distal parts were tapered and had hypoplastic or absent flexion creases. The musculature of the hands was partially atrophic, and all had a mild appearance of fetal finger pads and clinodactyly of the fifth finger. Further clinical findings included contractures of knee, Achilles tendon, and ankle (4/5), spine involvement (kyphoscoliosis and/or spinal stiffness) (4/5), and orthodontic features (small mouth, dental crowding, missing teeth, or arched palate) (4/5). No involvement of the central nervous system or other organs was noted, and radiographs of the hands and feet excluded a primary involvement of the bones or joints (Figure 1C). Detailed clinical descriptions are available in Supplemental Clinical Information and in Supplemental Table 1. Additional pheno
tic aspects and a comparison with overlapping entities are given in Supplemental Figure 1 and in Supplemental Table 2.

Molecular findings

Using ES, rare variant family-based genomics, and disease-specific panel analyses, we identified 4 rare homozygous variants in ADAMTS15 (NM_139055.3). The variant c.123C>G, p.(Tyr41*) is predicted to result in a premature termination codon (PTC) in the first exon. Analysis of complementary DNA synthesized from RNA extracted from patient-derived fibroblasts was performed by droplet digital PCR (ddPCR) and Sanger sequencing of an RT-PCR amplicon. This revealed that the variant c.1903-2A>G leads to a complete skipping of exon 7 (r.1903_2078del), which is predicted to result in a frameshift and premature stop of translation p.(Val635Alafs*30) (Supplemental Figure 2). The missense variants c.2281G>A, p.(Gly761Ser) and c.2715C>G, p.(Cys905Trp) affect highly conserved amino acids within the ADAMTS spacer 1 and thrombospondin type 1 domain, respectively (Supplemental Figure 3). All variants except c.2281G>A are not found in Genome Aggregation Database. The location of the identified variants...
Figure 1  Pedigrees, structure of ADAMTS15 protein and location of the identified variants, and clinical features of individuals with biallelic variants in ADAMTS15. A. Pedigrees of the 4 consanguineous families included in this study. Affected and unaffected individuals are indicated by filled and open squares (males) and circles (females), respectively. B. Identified variants and schematic overview of their location within the ADAMTS15 protein. Red arrows point to the locations of the 4 variants identified in this study. C. Clinical pictures and
and bioinformatic in silico predictions are summarized in Figure 1B and Supplemental Table 3. Segregation analysis is provided in Supplemental Figure 4.

Calculations for individuals 1, 2, and 5 revealed large absence of heterozygosity blocks surrounding ADAMTS15, likely resulting from homozygosity on recently configured haplotypes due to parental consanguinity. Inbreeding coefficients calculated from ES data confirmed known family histories of consanguinity (Supplemental Table 4).

**Adams15 expression during embryogenesis**

Using the Mouse Organogenesis Cell Atlas database, we observed that Adams15 expression is mainly restricted to the mesenchyme (Supplemental Figure 5A). Therefore, we performed further characterization of the mesenchyme trajectory, which showed that most of the Adams15-positive cells mapped to the connective tissue, skeletal muscle, and chondrocyte trajectories (Figure 2A).

In the developing limb, which is pathogenetically most relevant, Adams15 expression plateaued between E13.0 and E15.0 and disappeared almost completely by E15.5 (Figure 2B). In addition, in the developing limb, most positive cells were mesenchymal, only few were of muscle or vascular origin (Supplemental Figure 5B). To gain insight into the cell types potentially affected by loss of Adams15, we analyzed its coexpression with marker genes within the limb mesenchyme. Almost no coexpression was found for muscle, or tendon markers Scx and Osr2, which is associated with an overlapping disorder, with Adams15 having the highest coexpression (Supplemental Figure 5C). Moreover, significant overlaps were detected with Osr1 and Osr2, transcription factors expressed in muscle connective tissue, as well as the tendon markers Scx and Tnmd (Figure 2C, Supplemental Figure 5B).

A whole mount in situ hybridization confirmed that Adams15 is expressed at perimuscular and peritendinous areas in the developing limbs (Figure 2D). Additional RNAscope analysis corroborated the partial colocalization of Adams15 and Osr1 (Figure 2E). At E14.5, strong Adams15 signals were observed around tendons and at tendon attachment sites (Figure 2F). These data indicate that the pathomechanism in this AR trait could involve muscle and tendon development.

**Discussion**

Our study identified 4 different homozygous variants in ADAMTS15 in 5 individuals from 4 unrelated families that shared similar rare disease traits. The phenotype is characterized by congenital contractures, primarily affecting the small joints of the fingers and toes. Additional features included contractures of the knee and Achilles tendon, spinal stiffness, scoliosis, and orthodontic abnormalities. Radiographic investigations excluded bony abnormalities of the affected joints. The sequence changes included an early nonsense variant: c.123C>G, p.(Tyr41*) and c.2715C>A, p.(Cys905Trp).

Interestingly, other ADAMTS superfamily members and direct interaction partners have been described in association with congenital contractures. Clinical features of ADAMTS10- and ADAMTS17-associated Weill–Marchesani syndrome (WMS) types 1 (OMIM 277600) and 4 (OMIM 613195) include short stature, microspherophakia, brachydactyly, thickened skin, and stiffness of small and large joints.11,12 Besides strabismus, our affected participants did not show any additional ophthalmological features. Biallelic pathogenic variants of ADAMTS12 are associated with geleophysid dysplasia type 1 (GPHYS1D; OMIM 231050), a progressive musculoskeletal disorder characterized by severe short stature, brachydactyly, progressive joint contractures, cardiac valvular involvement, and thinned skin.13 In contrast to WMS and GPHYS1D, the individuals reported herein do not consistently show skeletal anomalies, skin stiffening, or pulmonary involvement. Compared with FBN2-associated congenital contractual arachnodactyly syndrome (OMIM 121050), also previously known as DA type 9, our participants did not display arachnodactyly, ear deformities, or dolichostenomelia.14 A detailed list of the clinical features in our participants compared with the typical clinical findings of different types of WMS, FBN2-associated congenital contractual arachnodactyly syndrome, and GPHYS1D is available in Supplemental Table 2.

The variants identified in this study presumably result in an ADAMTS15 LoF. Remarkably, no homozygous individuals for likely damaging predicted LoF variants are present in the Genome Aggregation Database (last access date March 3, 2022). The variant c.123C>G, p.(Tyr41*) in family A causes a PTC in the first exon and the intronic variant c.1903-2A>G leads to skipping of exon 7, resulting in a frameshift and PTC: p.(Val635Alafs*30). In both cases, the PTGs presumably result in nonsense-mediated decay or a truncated protein, effectively deleting many functional domains. The missense variant p.(Gly761Ser) in family C affects a highly conserved amino acid residue within the ADAMTS spacer region, whereas the missense variant in family D p.(Cys905Trp) localizes to the second thrombospondin type 1 repeat. Notably, a variant at a homologous position in ADAMTS13 p.(Cys1024Gly) is listed as
pathogenic in ClinVar (ID: 5803). Analysis of ES-derived absence of heterozygosity data revealed, that the ultrarare variants are located within long-sized runs of homozygosity, further supporting the Clan Genomics hypothesis.15 The AR inheritance and presence of 2 likely LoF alleles as the underlying disease mechanism are compatible with enzymatic disease and the thus far reported spectrum of pathogenic variants in the ADAMTS family members.8

In general, our understanding of the biological mechanisms of ECM regulation by ADAMTS proteoglycanases is only emerging and essentially depend on their substrates and other ECM-binding partners.16 ADAMTS15 is 1 of 7 members (ADAMTS1, 4, 5, 8, 9, 15, and 20) that belong to an evolutionary distinct subset of proteoglycanases that are implicated in versican turnover.17,18 Because the other members of the ADAMTS/L superfamily that are already associated with joint contractures play a pivotal role in fibrillin microfibril assembly and ECM-associated signaling pathways (TGF-β, BMP), a functional interaction between these members and ADAMTS15 seems plausible and parsimoniously explains the aggregate data.

Analysis of cell lines frequently used for functional in vitro investigations indicated a surprising restriction of ADAMTS15 expression. This was corroborated by single-cell sequencing data of whole mouse embryos showing an expression in the mouse limb only between E11.5 and E15.0. During this developmental phase, mesenchymal condensations of the skeletal elements are converted into cartilage and endochondral ossification begins.19 In parallel, the musculotendinous apparatus develops and tendon-bone connections are formed. Although, Adamts15 expression is partially found in chondrocytes and muscle cells, the strongest coexpression in the limb is with the tendon marker scleraxis (Scx), the perimuscular connective tissue marker Osr1, and with versican (Vcan). The latter coexpression is in line with the suggested function of Adamts15 as a versicanase, although expression is not conclusive evidence for enzyme

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**Figure 2** Expression of Adams15 RNA during mouse embryonic development. A. Whole mouse embryo single-cell analysis of mesenchymal cells showing Adams15 expression. The strongest accumulation of positive cells was found in the c.t. trajectory. B. Single-cell analysis of Adams15-expressing cells among mesenchymal cells in the developing limbs. Highest expression was found between E13.0 and E15.0. C. Co-expression of Adams15 and marker genes Scx and Osr1 RNA in cells from developing limbs. Colored numbers correspond to cells displaying expression for the respective gene, black numbers indicate double positive cells. D. WISH for Adams15 in developing limbs at E12.5 and E13.5. Note perimuscular and tendinous expression pattern at E13.5. E. RNAscope colabeling of Adams15 (green) and Osr1 (white) in the distal limb at E13.5. Scale bar = 200 mm. F. ISH for Adams15 in E14.5 forelimb in comparison with pan-MyHC immunostaining (red) for muscles and DAPI (blue) for nuclei on the same section. Inserts show muscle-tendon connection and arrowheads indicate tendon attachment sites at digits. Scale bar = 400 mm. ch, chondrocytes; c.m., cardiac muscle; c.t., connective tissue; DAPI, 4′,6-diamidino-2-phenylindole; i.m., intermediate mesoderm; ISH, in situ hybridization; l.m., limb mesenchyme; ob, osteoblasts; s.m., skeletal muscle; t, tendon; WISH, whole mount in situ hybridization.
A direct role of Adamts15 in the fusion of myoblasts was suggested on the basis of expression analysis in C2C12 cells but has never been experimentally proven. The expression data presented in this article does not indicate significant expression in muscle cells. Co-expression with Osr1 and Scx suggests a role of Adamts15 in the formation of perimascular connective tissue and tendons. Because the depletion of Osr1 in the limb leads to abnormal skeletal muscle development through altered ECM production, the loss of ADAMTS15 might also have a similar, non–cell autonomous effect on muscle cells.

In conclusion, these studies describe a new rare syndrome with a distal arthrogryposis phenotype that is associated with biallelic pathogenic variants in ADAMTS15. Owing to the clinical features and the fact that ADAMTS15 belongs to the ADAMTS/L family, we would classify this disease trait as a novel connective tissue related DA type. We hypothesize that impaired ADAMTS15 function causes tissue-specific ECM dysregulation. Further experimental investigations are necessary to identify these tissue-specific molecular mechanisms.

Data Availability

Data supporting this paper are contained within the article and Supplemental Information. Any additional data not compromised by ethical issues will be available upon request.

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Author Information


Ethics Declaration

This study adheres to the principles in the Declaration of Helsinki. Permission for the study was obtained from the Ethics Committee of the University Medical Center Göttingen (proposal no. 10/4/21 Ü). Written informed consent was obtained from all participants including consent for publication of photographs. Consent forms are archived and available upon request.

Conflict of Interest

J.R.L. has stock ownership in 23andMe, is a paid consultant for Regeneron Genetics Center (RGC), and is a coinventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, genomic disorders, and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic and genomic testing conducted at Baylor Genetics; J.R.L. serves on the Scientific Advisory Board of Baylor Genetics. U.K. has been a consultant for Alexion Pharmaceuticals, Inc. All other authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2022.07.012) contains supplementary material, which is available to authorized users.

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References