Cloning and Characterization of ADAMTS11, an Aggrecanase from the ADAMTS Family*

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Aggrecan is responsible for the mechanical properties of cartilage. One of the earliest changes observed in arthritis is the depletion of cartilage aggrecan due to increased proteolytic cleavage within the interglobular domain. Two major sites of cleavage have been identified in this region at Asn341-Phe342 and Glu373-Ala374. While several matrix metalloproteinases have been shown to cleave at Asn341-Phe342, an as yet unidentified protein termed “aggrecanase” is responsible for cleavage at Glu373-Ala374 and is hypothesized to play a pivotal role in cartilage damage. We have identified and cloned a novel disintegrin metalloproteinase with thrombospondin motifs that possesses aggrecanase activity, ADAMTS11 (aggrecanase-2), which has extensive homology to ADAMTS4 (aggrecanase-1) and the inflammation-associated gene ADAMTS1. ADAMTS11 possesses a number of conserved domains that have been shown to play a role in integrin binding, cell-cell interactions, and extracellular matrix binding. We have expressed recombinant human ADAMTS11 in insect cells and shown that it cleaves aggrecan at the Glu373-Ala374 site, with the cleavage pattern and inhibitor profile being indistinguishable from that observed with native aggrecanase. A comparison of the structure and expression patterns of ADAMTS11, ADAMTS4, and ADAMTS1 is also described. Our findings will facilitate the study of the mechanisms of cartilage degradation and provide targets to search for effective inhibitors of cartilage depletion in arthritic disease.

Aggrecan is the major proteoglycan of cartilage and is responsible for its compressibility and stiffness. Aggrecan contains two N-terminal globular domains, G1 and G2, separated by a proteolytically sensitive interglobular domain, followed by a glycosaminoglycan attachment region and a C-terminal globular domain (G3). The G1 domain of aggrecan interacts with hyaluronic acid and link protein to form large aggregates containing multiple aggrecan monomers that are trapped within the cartilage matrix. Cleavage of aggrecan has been shown to occur at Asn341-Phe342 and Glu373-Ala374 within the interglobular domain, with the cleaved aggrecan being free to exit the matrix since it lacks the G1 domain, which is responsible for formation of the high molecular weight complexes. Results from several studies suggest that cleavage at the Glu373-Ala374 site is responsible for the increased aggrecan degradation observed in inflammatory joint disease. Products resulting from cleavage at the Glu373-Ala374 site have been shown to accumulate in cartilage explants and chondrocyte cultures treated with interleukin-1 and retinoic acid (1–5) and in the synovial fluid of patients with osteoarthritis and inflammatory joint disease (6, 7). While several characterized matrix metalloproteases (MMP-1, -2, -3, -7, -8, -9 and -13)1 have been shown to cleave at the Asn341-Phe342 site (8–14), they are not responsible for the observed cleavage at Glu373-Ala374. A novel proteolytic activity, termed “aggrecanase,” has been hypothesized to be responsible for cleavage at the Glu373-Ala374 site, with the enzyme probably playing a pivotal role in the cartilage damage associated with osteoarthritis and inflammatory joint disease. Despite intensive work, the identity of aggrecanase has remained unknown for over 8 years.

The disintegrin and metalloproteinase (ADAM) family of proteases contains more than 20 members having extensive homology to the snake venom metalloproteases (15–17). The ADAMs have been shown to have similar domain arrangements, consisting of pre-, pro-, proteinase, disintegrin-like, cysteine-rich, epidermal growth factor-like, transmembrane, and cytoplasmic domains. A novel ADAM family member containing multiple carboxy thrombospondin motifs, ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motifs), has recently been described in mice (18). ADAMTS1 is structurally conserved with other members of the ADAM family; however, unlike the majority of ADAM family members, it lacks a transmembrane domain and contains unique thrombospondin motifs that are responsible for extracellular matrix binding (18, 19). Early ADAM family members were shown to play roles in sperm-egg fusion and myotube fusion (20, 21). Recently, the enzyme responsible for processing precursor tu-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF142099.‡‡ To whom correspondence should be addressed: Applied Biotechnology, The DuPont Pharmaceuticals Company, Experimental Station E336/237B, P.O. Box 336, Wilmington, DE 19880-0336; Tel.: 302-695-3859; Fax: 302-695-9420; E-mail: Timothy.C.Burn@dupontpharma.com.

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1 The abbreviations used are: MMP, matrix metalloprotease; EST, expressed sequence tag; PCR, polymerase chain reaction; kb, kilobase pairs; TSP, thrombospondin type I; HPLC, high pressure liquid chromatography.
A100 mM NaCl, pH 7.5). Proteins were eluted from the column with buffer B containing 10% (w/v) ammonium sulfate without Brij-35. Fractions containing aggrecanase activity were pooled and loaded onto a Macro S column. The column was washed with buffer A containing 1.0 mM NaCl. The eluted material was passed through a gelatin-agarose column to remove contaminating MMPs (Table I). Prior to the gelatin-agarose column, the eluate from the Macro S column was adjusted to a final concentration of 0.1–1.0 M NaCl gradient in buffer B, and fractions with high enzymatic activity employing the BC-3 neoepitope antibody, were eluted with buffer B containing 1.0 mM NaCl. The eluted material was passed through a gelatin-agarose column to remove contaminating MMPs. The open reading frame encoding ADAMTS11 was PCR-amplified with appropriate consensus Kozak sequences for expression in the Drosophila system and subcloned into the pRHMA3 Drosophila expression vector (2). Recombinant protein was expressed as described (2, 3).

MATERIALS AND METHODS

Purification of Bovine ADAMTS11—6.5 liters of bovine cartilage conditioned media containing the aggrecanases were supplemented with 1 μM leupeptin, 1 μM pepstatin, 1 mM phenylmethylsulfonyl fluoride, and 0.05% Brij-35, passed through a 1.2-μm filter, and loaded onto a Macro S column. The column was washed with buffer A (50 mM HEPES, pH 7.5, 10 mM CaCl2, 0.1 mM NaCl, 0.05% (v/v) Brij-35), and aggrecanases were eluted with buffer A containing 1.0 mM NaCl. The eluted material was passed through a gelatin-agarose column to remove contaminating MMPs. The open reading frame encoding ADAMTS11 was PCR-amplified with appropriate consensus Kozak sequences for expression in the Drosophila system and subcloned into the pRHMA3 Drosophila expression vector (2). Recombinant protein was expressed as described (2, 3).

Northern Analysis—A commercially available Northern blot (CLONTECH, Palo Alto, CA) was hybridized with ADAMTS4 and ADAMTS11 probes derived from the 3′-untranslated regions. Blots were labeled by random priming as described (34). Blots were hybridized and washed as recommended by the manufacturer.

Real Time PCR—Real time PCR was performed essentially as described (35). Primers and probes were designed from human ADAMTS4 (primers, GACACTGTTGGTGCCAGATG and TCACTGTTAGCAGGTAGTTTGA; probe, CAAGATGGCCGATTCCACCGT), ADAMTS11 (primers, GCTGCCACACCTCAAGAA and TGGTGACCTCCAGCGTGT; probe, CAAGATGGCCGATTCCACCGT), and partial ADAMTS11 sequences (primers, GGCGCAATCTGCGGTGC and GCACATCACCCTCCAGCGTGG; probe, TCCCGAAACCCGACCTGCG) using Primer Express (Perkin-Elmer). Data obtained with commercially available mammalian 18 S ribosomal RNA primers and probes were used to normalize between tissues (Perkin-Elmer). Primers were labeled at the 5′-end with the reporter dye 6-FAM and on the 3′-end with the quencher dye TAMRA (Perkin-Elmer). Probes were labeled by random hexamers and Moloney murine leukemia virus reverse transcriptase (CLONTECH, Palo Alto, CA). Each PCR utilized the cDNA from 50 ng of starting RNA. All PCRs were performed in triplicate, with copy numbers being calculated by comparing the threshold values for each reaction with a standard curve produced using linearized cDNA for the respective gene. The concentration of the linearized DNAs used in the standard curves were measured using the Molecular Express, Inc. (Eugene, OR) PicoGreen assay as recommended by the manufacturer. All expression levels are relative, since the initial efficiency of the reverse transcription reaction was not accounted for in the copy number calculations. Expression levels between tissues were normalized with 18 S ribosomal RNA. RNAs from normal and diseased tissues were obtained from a commercial vendor (Biochain Institute Inc., San Leandro, CA) with quality being assessed in ethidium bromide-stained agarose gels prior to real time PCR experiments. The arthritic tissues used in these studies included fibrous tissue and joint capsule from the femur of a 33-year-old patient with arthritis.

RESULTS

Purification of a Bovine Aggrecanase—Interleukin 1-stimulated bovine nasal cartilage conditioned medium was chosen as a source for aggrecanase. Purification was followed using an enzymatic activity employing the BC-3 neoepitope antibody,
TABLE I
Protein purification of bovine ADAMTS11

| Fraction     | Volume (ml) | Total protein (mg) | Total activity (ng) | Specific activity (ng/ml) | Fold purification |
|--------------|-------------|--------------------|---------------------|--------------------------|------------------|
| Media        | 6500        | 656                | 9425                | 14                       | 1                |
| S            | 750         | 99                 | 28,350              | 26                       | 20               |
| Gelatin      | 140         | 86                 | 32,795              | 351                      | 26               |
| Phenyl-Sepharose | 88       | 14                 | 17,800              | 1257                     | 87               |
| CM           | 4           | 2.2                | 15,840              | 7200                     | 496              |
| Superdex 2   | 12          | 0.15               | 7744                | 51,626                   | 3560             |
| HPLC         | 4           | 0.0022             | 3200                | 1,454,545                | 100,313          |

Fraction Volume Total protein Total activity Specific activity Fold purification

Table I summarizes the purification of 6.5 l of conditioned media from interleukin-1-stimulated bovine nasal cartilage cultures. Activity was assessed using the BC-3 antibody as described under “Materials and Methods.” An increase in total activity was seen consistently following purification on the S-column and is likely to be due to the removal of an inhibitory activity present in the starting material.

An Aggrecanase from the ADAMTS Family (ADAMTS11)

Our initial cloning efforts relied on human heart cDNA, since murine ADAMTS1 was shown to be expressed in heart (18), and human osteoarthritic cartilage RNA was unavailable. We were able to amplify a 163-base pair product from human heart cDNA utilizing PCR primers designed from the murine sequence. Primers designed from the human PCR product and a partial 3′-rapid amplification of cDNA ends clone were then used to screen 2.5 × 10^6 clones from a human liver cDNA library, which had been prescreened by PCR and shown to contain ADAMTS11 sequences. Two 5.5-kb cDNA clones were obtained, with DNA sequence analysis revealing a 2793-base pair open reading frame preceded by two in-frame stop codons. The deduced protein sequence is 980 amino acids in size and has four potential glycosylation sites (Fig. 1). Sequences from the deduced human protein are 95% (39/41) identical to the N-terminal peptide sequence of bovine ADAMTS11 (Fig. 1A).

ADAMTS11 Is a Disintegrin Metalloproteinase—Human ADAMTS11 is a multidomain protein containing a signal sequence, pro-domain, metalloproteinase domain, disintegrin-like domain, and “spacer domain” located between a thrombospondin type I (TSP) motif and TSP submotif (Fig. 1B). The metalloproteinase domain contains a consensus sequence for a zinc-dependent metalloproteinase with homology to the snake venom metalloproteinases and other members of the ADAM family, with the conserved aspartate after the third cysteine (35% identical to ADAMTS11). The metalloproteinase domain of murine ADAMTS1 (18), a Caenorhabditis elegans metalloproteinase of unknown function (f25 h.3 protein, GenBank™ accession no. 1181986) predicted from genomic sequence (39), and ADAMTS4 (25). This latter sequence has recently been deposited in the data bases as clone KIAA0688 (ADAMTS4), an unidentified human gene from a set of size-fractionated human brain cDNA libraries (40, 41). Not surprisingly, the lowest levels of conservation are seen in the pro-domains of these proteins, while the remainder of ADAMTS4 and ADAMTS1 are 48 and 50% identical to ADAMTS11, respectively. Other sequences having homology, but at a significantly lower level than those mentioned above, included an unidentified human gene from a brain cDNA library, KIAA0366 (ADAMTS3) (41, 42) and procollagen I N-proteinase (ADAMTS2) (42, 43), both of which are less than 35% identical to ADAMTS11. In contrast, ADAMTS2 and ADAMTS3 are 65% identical and 73% similar to each other. Based on these homologies and sequence alignments, the ADAMTS family members can be clustered into two subfamilies, with ADAMTS1, ADAMTS4, ADAMTS11, and f25 h.8.3 being on one branch and ADAMTS2 and ADAMTS3 being on a divergent branch (Fig. 1C). Further analysis indicates that ADAMTS4 and ADAMTS1 are more closely related to each other than ADAMTS11, with the former pair likely to have resulted from a duplication after an initial duplication involving ADAMTS11 (Fig. 1C). This interpretation is consistent independent of whether the full-length proteins, the metalloproteinase domains, or disintegrin-like domains are used in the analysis.

Analysis of the deduced protein sequences revealed multiple consensus glycosylation sites in ADAMTS11, ADAMTS1, and f25 h.8.3, while ADAMTS4 lacks potential sites for glycosylation (Fig. 1B). At present, the relevance of these potential differences in glycosylation status is unknown.

The metalloproteinase domain of ADAMTS11 is preceded by a pro-domain having a potential cysteine switch at Cys209 as well as a cleavage site for furin (residues 257–261), a serine
**A.** Protein sequence of human ADAMTS11 and comparison with other closely related proteins. Amino acid sequence deduced from the ADAMTS11 cDNAs is shown aligned to activated forms of murine ADAMTS1 and human ADAMTS4. The pre- and pro-domains were excluded from the alignment, since they are significantly less conserved between the three family members. Residues matching the consensus sequence of the three proteins are shaded. Domains are labeled above the sequence and are delineated by arrows. Sequences corresponding to the N-terminal peptide sequence from bovine ADAMTS11 enzyme are denoted by the line at the start of the metalloprotease domain; residues that are different in the bovine sequence are shown above the line. The conserved zinc-binding motif and “Met turn” are boxed. A filled circle denotes the location of a potential Cys switch, while asterisks designate potential N-linked glycosylation sites.

**B.** The organization of the signal sequences (SS), pro-domains (Pro), metalloprotease domains (Protease), disintegrin-like domains (Disint), spacer regions, and thrombospondin motifs (TSP) and submotifs (TSP-sub) are shown for ADAMTS11, murine ADAMTS1, ADAMTS4, and the *C. elegans* f25 h8.3 protein. The filled circles show the relative positions of glycosylation sites. Only a portion of the 18 C-terminal TSP submotifs are shown for the *C. elegans* f25 h8.3 protein.

**C.** Dendrogram showing the relationship of sequences from the data bases that have the highest degree of homology to ADAMTS11.
endoprotease that has been implicated in the processing of a wide variety of proproteins (44, 45). It has been proposed that the catalytic activity of MMPs is masked by a conserved cysteine in the pro-domain, a “cysteine switch,” that binds to the active-site zinc to inhibit the enzyme (46, 47). Although there are no clear cysteine switch consensus sequences for the ADAMs family (48), Cys209 is conserved in other metalloproteinase-disintegrin family members (18), murine ADAMTS1, and ADAMTS4 and may function as the cysteine switch.

ADAMTS11 contains a number of conserved domains that have been shown to be involved in adhesion, including a disintegrin-like domain, a pair of TSP motifs, and a conserved spacer domain between the TSP motifs (16, 18, 19, 49–53). One of the noteworthy differences between the aggrecanase/ADAMTS1 family members is the number of TSP submotifs present in their respective C termini (Fig. 1B). In contrast to the tandem pair of TSP submotifs at the C terminus of ADAMTS1, ADAMTS11 has a single C-terminal TSP submotif, while ADAMTS4 lacks a TSP submotif (Fig. 1B). As mentioned above, data base searches also revealed extensive homology between ADAMTS11 and the C. elegans f25 h8.3 protein. The f25 h8.3 protein is of particular interest, since it contains up to 18 tandem copies of the C-terminal submotif (Fig. 1B).

Expression Analysis of the Aggrecanases and ADAMTS1—We initially compared the patterns of gene expression for human aggrecanases using Northern analysis (Fig. 2). A 4.3-kb band was seen in ADAMTS4 blots, with expression being present in heart, brain, placenta, lung, and skeletal muscle. For ADAMTS11, the highest expression was seen in placenta with much weaker signals also being observed in heart and brain (Fig. 2). A series of bands hybridized to the ADAMTS11 probe with a strong signal being seen at 12.4, 10.7, 8.6, and 6.6 kb, while a series of weaker bands were present between 5 and 6 kb. Our initial ADAMTS11 cDNAs were from a 5.6-kb transcript in liver; however, we have been able to amplify sequences from placenta using 3′-rapid amplification of cDNA ends that had longer 3′-untranslated regions (data not shown). Therefore, the longer ADAMTS11 transcripts detected by Northern analysis are likely to result from alternative cleavage/polyadenylation sites in the 3′-untranslated region.

We utilized real time PCR to more quantitatively measure the expression of the aggrecanases and ADAMTS1. Real time PCR also allowed us to perform experiments on tissues where we had limiting amounts of RNA. Real time PCR is an ex-

![Figure 2](image1.png)

**Fig. 2. Northern analysis of the aggrecanases.** Labeled cDNA sequences from the 3′-untranslated region of ADAMTS4 and ADAMTS11 were hybridized to a Northern blot. The positions of the RNA markers are shown at the left; the source of RNA is shown at the top of each lane.

![Figure 3](image2.png)

**Fig. 3. Expression levels of the aggrecanases and human ADAMTS1.** The expression levels of human ADAMTS11, ADAMTS4, and ADAMTS1 were determined by real time PCR in a variety of normal and arthritic tissues. The number of copies of each cDNA present in reverse transcribed total RNA were determined relative to a standard curve produced using cloned cDNAs. All values were normalized to 18 S ribosomal RNA and are displayed in arbitrary units.
and ADAMTS4 being expressed at approximately 4- and 10-fold higher levels, respectively. Since we did not have access to matched arthritic and nonarthritic samples, we could not compare the relative expression levels of these genes in normal and diseased tissues.

**ADAMTS11 Has Aggrecanase Activity**—In order to demonstrate that ADAMTS11 cDNA encoded a proteinase with aggrecanase activity, we expressed recombinant full-length ADAMTS11 in the Drosophila S2 system. Multiple immunoreactive bands were seen by Western analysis in conditioned medium from rADAMTS11 expressing Drosophila S2 cells and not in control cell lines (data not shown). Conditioned media from Drosophila cells expressing recombinant ADAMTS11 were able to cleave aggrecan at the Glu373-Ala374 bond as evidenced by detection of products with the BC-3 antibody, while media from uninduced cells and cells transfected with an empty expression vector lacked activity (Fig. 4A). The aggrecan cleavage patterns were indistinguishable for partially purified conditioned medium from bovine nasal cartilage cultures and Drosophila cells expressing recombinant ADAMTS11 (Fig. 4). No cleavage was seen at the Asn341-Phe342 site, which has been shown to be preferentially cleaved by matrix metalloproteinases (data not shown). Several BC-3-reactive products were seen in Western blots (Fig. 4), consistent with previous work that has identified additional cleavage sites for aggrecanases within the C-terminal region of aggrecan (54). The inhibition profile of three peptidic hydroxamates was compared between recombinant ADAMTS11 and the endogenous bovine aggrecanase, which had purified through the CM column step. The rank order of potency was consistent between the recombinant material and endogenous bovine material (Table II), with the IC50 values being slightly lower for the recombinant human material when compared with those observed with the bovine material. The difference in IC50 values is probably the result of species differences or differences in protein binding between the two samples.

**DISCUSSION**

We have described the cloning of a novel disintegrin metalloproteinase and demonstrate that it possesses aggrecanase activity. Our data indicate that the aggrecanase/ADAMTS1 family has multiple members, with the family being conserved from C. elegans through human. The two human aggrecanases and ADAMTS1 described in this report are expressed in a variety of tissues in addition to arthritic tissues; however, it is impossible to draw any broad conclusions about underlying themes of gene expression patterns based on the tissue examined. *In situ* hybridizations will be required to determine what cell types are responsible for the observed gene expression in each tissue. Based on the broad expression patterns of ADAMTS4 and ADAMTS11, it is likely that they have other roles in addition to cartilage remodeling. At present, it is unknown

![An Aggrecanase from the ADAMTS Family (ADAMTS11)](image)

**FIG. 4.** Comparison of recombinant ADAMTS11 (rADAMTS11) and native aggrecanase. A, conditioned media from induced Drosophila S2 cells transfected with empty expression vector (lane 1) and uninduced (lane 2) or induced (lane 3) cells transfected with the recombinant ADAMTS11 expression construct were incubated with aggrecan monomers. B, native aggrecan monomers were treated with conditioned medium from bovine nasal cartilage cultures that had been purified through to the CM step as described under “Materials and Methods.” EDTA was used to quench the reaction (lane 1) or in the absence of EDTA (lane 2). Glu373-Ala374 reactive material was detected by Western blot using the BC-3 antibody.

**TABLE II**

Comparison of inhibitor profiles for bovine aggrecanase and recombinant human ADAMTS11

| Inhibitor | Bovine CM material | IC50 (nM) | Recombinant human ADAMTS11 |
|-----------|--------------------|----------|---------------------------|
| SE206     | 137                | 40       |                           |
| BB-16     | 548                | 108      |                           |
| XS309     | >10,000            | 3744     |                           |
whether any of these genes are up-regulated in inflammatory joint disease due to the limited availability of human RNA from matched normal and arthritic tissues. Animal models will probably be required to address this latter issue. The fact that all three family members are expressed in the limited number of arthritic tissues examined in this report suggests that they are likely to play a role in inflammatory joint disease.

Sequence analysis of ADAMTS11 suggests that the enzyme is synthesized in an inactive pro-form that may be processed by furin during transit through the secretory pathway. This hypothesis is supported by data indicating that the N-terminal peptide sequence of the enzyme purified from bovine cartilage conditioned medium starts immediately C-terminal of a consensus furin cleavage site. Furthermore, inhibition of furin may be responsible for the previously described block in aggrecan cleavage seen in response to a serine-protease inhibitor (55).

ADAMTS11 exhibits the consensus motif, HExxxHxxGXXH, whose three conserved histidine residues are involved in binding of the catalytically essential zinc ion in members of the metzincin superfamily (38). This protein also has an aspartic acid following the third conserved histidine residue, as is found in the adamalysin family, and a conserved methionine downstream of the active site, which is found in members of the metzincin family as part of a “Met turn” (38, 56). While both ADAMTS4 and ADAMTS1 have a similar zinc-binding motif, with an aspartic acid residue following the third histidine and a downstream methionine, these two proteins have an asparagine residue between the second and third histidine instead of a glycine. In the metzincins whose crystal structures have been determined, the active site helix is terminated at this invariant glycine residue, which results in the main chain abruptly turning downward into the lower domain of the protein (38). The conserved glycine in each of the enzymes exhibits an identical conformation not accessible to non-Gly residues. This raises the possibility that ADAMTS11 may exhibit subtle structural differences in this region from ADAMTS4 and ADAMTS1. However, this has not been observed as any difference in cleavage of aggrecan by ADAMTS4 and ADAMTS1 or in inhibitor profiles against these two proteases. Further elucidation of the effect of this substitution awaits determination of the crystal structures of these proteases.

ADAMTS11 possesses several different domains that are likely to play a role in adhesion. Disintegrin proteins are found in snake venoms, where they act as inhibitors of platelet aggregation by binding to integrins through a conserved RGD motif (16, 49). Disintegrin-like domains, while lacking the RGD motif, have also been implicated in integrin binding (50, 51). The function of the disintegrin-like domain of ADAMTS11 is unclear; it may allow binding to integrins expressed on the articular chondrocytes (57). The TSP type I motif of thrombospondin has been implicated in binding to matrix macromolecules and cell adhesion (52, 53), with recent data indicating that the TSP motif and submotif of murine ADAMTS1 bind to heparin and the extracellular matrix (18, 19). The TSP motif and submotif of ADAMTS11 may be responsible for binding to the glycosaminoglycans of aggrecan. Consistent with this hypothesis is the observation that deglycosylation of aggrecan decreases the production of Glu373-Ala374 cleavage products by aggrecanases (58). Sequences within the spacer region between the TSP motifs of ADAMTS11 are conserved in all the family members and are likely to provide an additional adhesion motif, since recent studies demonstrate that sequences within the spacer region of murine ADAMTS1 can themselves bind tightly to the extracellular matrix (19). The variable number of TSP submotifs in the ADAMTS family members may lead to altered affinities for glycosaminoglycans, since the TSP submotifs of ADAMTS1 have been shown to play a role in extracellular matrix binding (19).

Comparison of the intron/exon structure of the murine AD- AMTS1 gene to the protein domains indicates that the two TSP submotifs are present in the final exon of the gene (59). Based on this observation, the evolution of the variable number of submotifs in ADAMTS4 and ADAMTS11 is likely to have involved more than the simple deletion of an internal TSP en-coding an individual TSP submotif. Determination of the exon/intron structure of the three family members is likely to shed light on evolutionary paths that have led to the present diversity in this gene family. A comparison of the promoters for the genes may also provide insight into the different regulatory elements controlling the expression of the family members.

In preliminary experiments, we have identified additional genes that have a high degree of homology to the members of the aggrecanase/ADAMTS1 subfamily. It is unclear if other family members besides ADAMTS4 and ADAMTS11 will possess aggrecanase activity. However, it is possible that ADAMTS1 has aggrecanase activity given its high degree of homology with ADAMTS4 and ADAMTS11, as well as its high level of expression in arthritic tissues. Additional work will focus on the role of the ADAMTS family of enzymes in inflammation and joint disease and the regulation of the respective genes. The availability of recombinant proteins for this family of genes will aid in the identification of specific inhibitors of the different enzymes that will be important tools for deciphering the biological role of the individual family members. In addition, the recombinant proteins will facilitate the development of potential therapeutics for inhibiting aggrecan degradation and the subsequent cartilage damage associated with inflammatory joint disease.

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