Chapter

ADAMTS Proteases: Potential Biomarkers and Novel Therapeutic Targets for Cartilage Health

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Abstract

The equine locomotor system's health plays a key role on athletic performance. Bone and joint diseases are the major causes of lameness. Poor performance and diseases lead to great economic loss to equestrian sports and horse breeders. Therefore, prevention, early diagnosis, and therapy of joint diseases are important. A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTS) proteinase family plays an important role in many physiological processes such as tissue reorganization, coagulation, and angiogenesis. Aggrecan proteinases ADAMTS-4 and ADAMTS-5 are physiologically responsible for the restructuring with enzymatic cleavage of the cartilage, specific biomarkers in the synovium or body fluids for early diagnosis, and potential specific therapeutic targets in order to their role on degenerative joint diseases physiopathology in humans and various animals.

Keywords: ADAMTS, aggrecan, equine, lameness, metalloproteinase, proteoglycan

1. Introduction

A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTS) protease family plays an important role in many physiological and physiopathological processes. The ADAMTS family is an important potential biomarker for the evaluation of early diagnosis due to its roles in the physiopathological mechanisms of many diseases such as cancer, arthritis, and atherosclerosis. ADAMTS-4 and ADAMTS-5 have been reported to play an important role in the pathogenesis of osteoarthritis in humans and various animals, following their first molecular purification and cloning.

Articular cartilage is structurally composed of partially chondrocyte cells and a large number of extracellular matrix components. Many macromolecules have been identified in cartilage tissue, including collagen fibrils, aggregate proteoglycans, and glycoproteins. Although joint damage is caused by oxidative metabolism-induced free radicals and hypoxic conditions, the main reason is the increase in proteolytic enzymes. Matrix metalloproteinases (MMPs), pro- and anti-inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), and retinoic acid are the main biomarkers recommended for the diagnosis of joint damage [1–8].

In this chapter, we focus on ADAMTS proteases and their role on cartilage health for joints' stability, their possible usage as damage biomarkers for early diagnosis, and novel therapeutic properties.

2. ADAMTS proteases

Metzincins are a superfamily of zinc-dependent metalloproteases, metalloproteinases, or metalloendopeptidases, which are responsible for many physiological functions that also include cartilage turnover due to their regulatory activities on the extracellular matrix (ECM). The metzincins constitute by the zinc-binding catalytic motif consensus sequence HEXXHXXGXX (H/D) exactly; the binding of a zinc molecule is modulated by the three histidines (or an aspartic acid in the third position), the acid-base catalysis is facilitated by the glutamic acid residue in general, and the steric flexibility is acquired by the small glycine residue in the catalytic motif [9].

The first referenced equine gene mapping project was initiated in October 1995 by the "First International Equine Gene Mapping Workshop," in order to search answers of the main questions "speed gene" and "evidence of heritable trait", and the first construction of a low density, male linkage map in 1999 was reported [10, 11]. Thereafter, the first domestic horse gene map (EquCab2.0), a thoroughbred mare Twilight's gene sequence, was released in 2007 and published in November 2009 [12–16]. The latest version of high-quality equine gene map EquCab3.0 is available and enables to detailed data about genes and encoding proteins [16]. The Vertebrate Gene Nomenclature Committee (VGNC) [17] has standardized names to genes in vertebrate species including horse [18, 19]. According to these accessible latest data versions, the equine ADAMTS and ADAMTS-like family members with chromosomal locations are listed in **Table 1**.

2.1 ADAMTS family

Towards the end of 1990s, Kuno et al. [20] described a new family of metalloproteinase, which consists of sequence similarity with snake venom disintegrin that was upregulated in colon adenocarcinoma cell line as a specific gene for cachexigenic tumor and was named a disintegrin-like and metalloproteinase with thrombospondin type-1 motif (ADAMTS). The equine ADAMTS and ADAMTS-like proteins are a superfamily comprised of 19 and 7 members, respectively (**Table 1**). The ADAMTSs are secreted proteinases and multidomain enzymes constituted of zinc-binding active site motif similar to adamalysin (ADAMs) and ensued by a metalloproteinase domain with that of reprolysins (snake venom metalloproteinases) and disintegrin-like domain (**Figures 1** and **3**). ADAMTS-like (ADAMTSL) family and papilin are newly identified and secreted ECM-related proteins which are relatives to ADAMTS proteases. Additionally, they lack catalytic activity due to the absence of prometalloprotease and the disintegrin-like domain which are found in the ADAMTSs [21–24]. ADAMTSs differ from ADAMs with the lack of a transmembrane domain and the inclusion of well-conserved thrombospondin 1-like repeats, a cysteine-rich domain, and the CUB (complement subcomponents C1s/C1r, Uegf, BMP1) domain, thus being soluble extracellular proteases [25–31].

The main physiological functions of equine ADAMTSLs and papilin are extensively unknown; thus they have some troubles and need to further detailed investigations. However, recent studies on genome-wide association analysis and transgenic animals have indicated that ADAMTS, ADAMTSL, and papilin gene mutations cause lethal embryonic defects and autosomal recessive Mendelian disorders such as human Ehlers-Danlos syndrome [32], bovine dermatosparaxis

Gene/protein name	Chromosomal Location	VGNC_ ID	ENSEMBL	UniProt
ADAMTS1	26	15061	ENSECAG00000016339	F6YLN3
ADAMTS2	14	55397	ENSECAG00000016328	F6X633
ADAMTS3	3	—	ENSECAG00000019061	F6ZC90/F7A3A4
ADAMTS4	5	15070	ENSECAG00000024172	F6YRD3/ A0A3Q2H7G6
ADAMTS5	26	59233	ENSECAG00000006500	F7ACI3/ A0A5F5PZN1
ADAMTS6	21	15071	ENSECAG00000029347	F6X9L7
ADAMTS7	1	15072	ENSECAG00000007527	F6W0M1
ADAMTS8	7	15073	ENSECAG00000014164	F6ZXN7
ADAMTS9	16	15074	ENSECAG00000019880	F6VTC7
ADAMTS10	7	55772	ENSECAG00000016210	F6QIB9
ADAMTS12	21	15062	ENSECAG00000016121	F6TW13
ADAMTS13	25	—	(NCBI Gene ID: 100069281)	—
ADAMTS14	1	15063	ENSECAG00000014713	F7D1G5
ADAMTS15	7	15064	ENSECAG00000015715	F6V0J9
ADAMTS16	21	15065	ENSECAG00000000787	F6W504
ADAMTS17	1	15066	ENSECAG00000000579	F7DNJ9
ADAMTS18	3	15067	ENSECAG00000019006	F7A7V7
ADAMTS19	14	15068	ENSECAG00000023694	F6YNK0
ADAMTS20	6	15069	ENSECAG00000020835	F6PZV0
ADAMTSL1 (Punctin-1)	23	15075	ENSECAG00000015972	F6W1K7
ADAMTSL2	25	15076	ENSECAG00000011887	F6TEW7
ADAMTSL3 (Punctin-2/ SH3GL3)	1	22941	ENSECAG00000012008	F6T6C4
ADAMTSL4	5	15077	ENSECAG00000019154	F7A7L3
ADAMTSL5	7	50328	ENSECAG00000009642	F6X928
ADAMTSL6 (THSD4)	1	51434	ENSECAG00000022944	F6UWV1
PAPLN (Papilin)	24	21150	ENSECAT00000008176	F6VT48

Table 1.
The equine ADAMTS and ADAMTS-like family members with chromosomal locations and accession numbers.

[33, 34], human Weill-Marchesani syndrome [35], canine ectopia lentis [36], human Geleophysic dysplasia [37], canine Musladin-Lueke syndrome [38], and thrombotic thrombocytopenic purpura [39]. In consideration of this knowledge, ADAMTSs, ADAMTSLs, and papilin could be responsible as most commonly screened genetic disorders among horses as early embryonic death and abnormalities, junctional epidermolysis bullosa [40], hereditary equine regional dermal asthenia [41], thrombocytopenia and, von Willebrand disease [42].

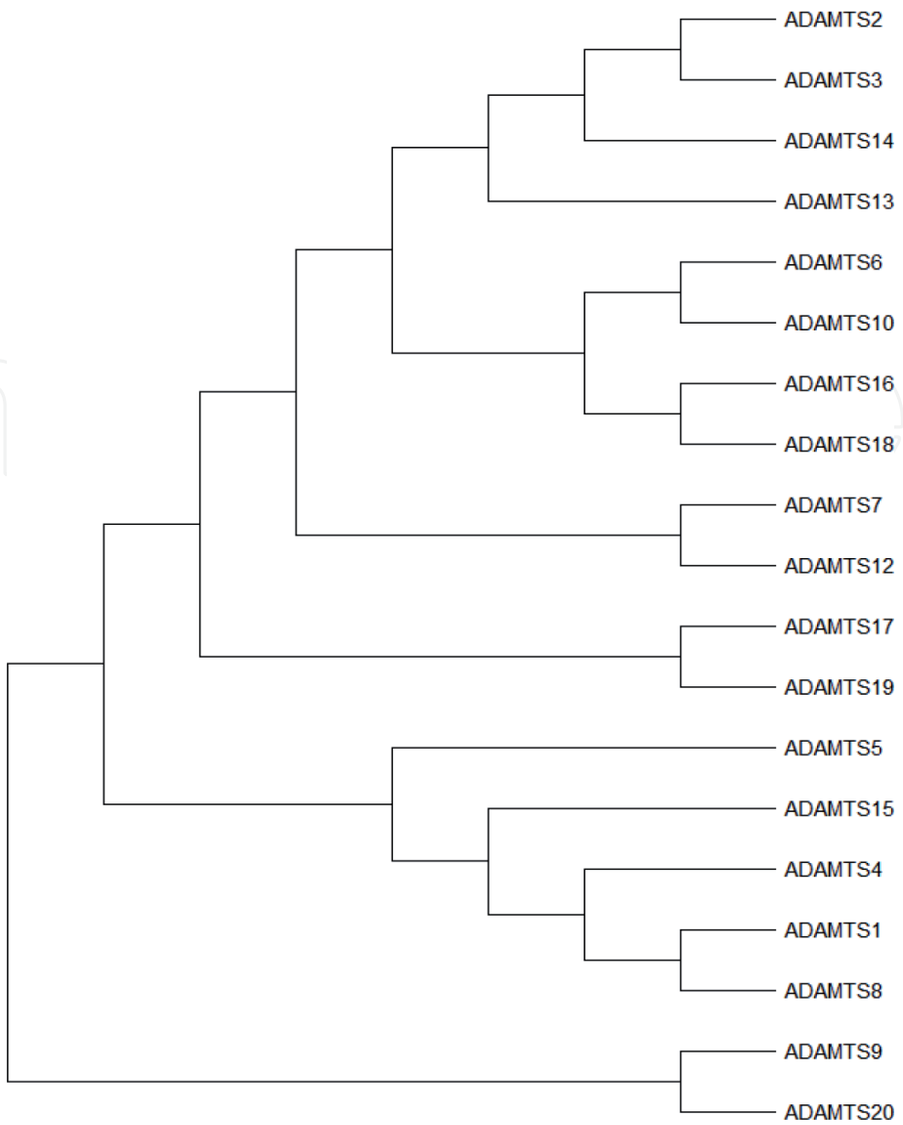

Figure 1.
Phylogenetic analysis of equine ADAMTS protein family. The evolutionary history was inferred using the maximum parsimony method. The most parsimonious tree with length = 9505 is shown. The consistency index is (0.751174), the retention index is (0.551189), and the composite index is 0.440603 (0.414039) for all sites and parsimony-informative sites. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches [43]. The MP tree was obtained using the subtree-pruning-regrafting (SPR) algorithm (p. 126 in Ref. [44]) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). Branch lengths were calculated using the average pathway method (see p. 132 in Ref. [2]) and are in the units of the number of changes over the whole sequence. They are shown next to the branches. This analysis involved 19 amino acid sequences. There were a total of 2768 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [45].

3. Role of ADAMTS family on equine cartilage health

Musculoskeletal system health is crucial for equine locomotion. This system is responsible to deploy the mechanical energy to the joints for efficient movement and specific biomechanical functions. There are many types of joints presented, where a majority of the free movements are managed by diarthrodial or synovial joints in the body. Cartilage tissue, which is the most important part of the diarthrodial joints, is absorbed and the loading energy throughout locomotion is distributed. Alterations due to inaccurately loading or metabolic disruptions could lead to acute or chronic damage, namely as arthritis, osteoarthrosis, or osteoarthritis to the joint and its critical component cartilage tissue, and restrict the locomotor functions. It is important to understand the molecular mechanisms of healthy and damaged cartilage tissues by the novel candidate molecular biomarkers in order to

early detection, easily clinical application, and therapy. Hence, we will focus on the cartilage health and importance of the ADAMTS family in this section.

3.1 Healthy cartilage tissue

Cartilages are divided into three major types as histological and biochemical properties in the body as hyaline, elastic and fibrocartilage. The distinctive features among these cartilage types are water content/dry matter balance and fiber types. In the diarthrodial joints, hyaline cartilage is existed on the articular surfaces and is well resist to pressure stress during various locomotion by its unique structure [46, 47].

Avascularized, unnerved, alymphatic hyaline cartilage (Latin words "hyălĭnus" meaning "glassy; made of glass; transparent") tissue's matrix is fundamentally constituted by water (%63–70), collagens (the majority type II in normal hyaline cartilage), non-collagenous proteins, and proteoglycans (the majority of aggrecans), while the most compounds are glycosaminoglycans (GAG) [48, 49].

Proteoglycans are classified into four subgroups related to their function: intracellular, cell-surface, pericellular, and extracellular. In the cartilage tissue, hyaluronan- and lectin-binding proteoglycans (hyalectans; aggrecan, versican, neurocan, and brevican) and small leucine-rich proteoglycans exist. Hyalectans are compromised with a similar structure in their tridomain structure; the N-terminal domain binds to hyaluronan, a central domain with the core protein for attachment of GAG chains, and the C-terminal region that binds lectins [50, 51].

The major proteoglycans in the diarthrodial joints aggrecans are the crucial elements for the biomechanical function with well-balanced load distribution and transmissions in order to provide the viscoelastic properties, the tight junctions, and the bridges of the extracellular matrix (ECM) [50, 52–55]. Aggrecans have a large molecular mass that contains GAG side chains comprising of the mostly chondroitin sulfate and keratan sulfate. They have three globular domains (G1, G2, and G3) to maintain the stabilization of protein complexes and to ensure mechanical features of cartilage. These highly conserved globular domains among the vertebrates have specific cleavage sites for proteases such as ADAMTSs (**Figure 2**) [50, 55–57]. The GAG's chondroitin sulfate and keratan sulfate contents of aggrecan could directly affect the aggrecanase activity by ADAMTSs [58].

Although, the hyaline cartilage consists of the chondrocytes which are the only cell type; this cell population has shown different morphological properties under the microscope and has been identified as dark, light, and adipocyte-like

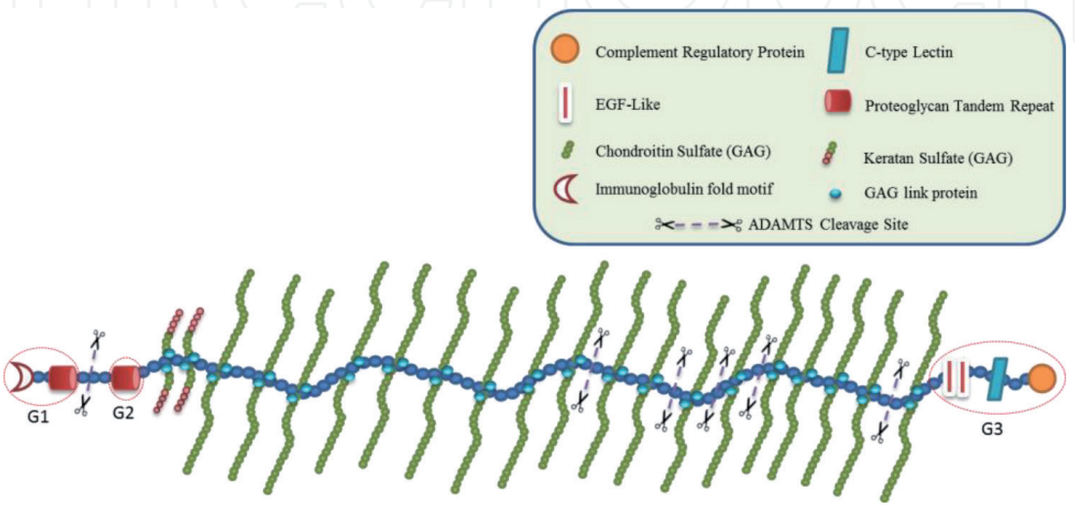

Figure 2.
Schematic view of aggrecan proteoglycan [50, 55, 56].

(adipochondrocytes) [59]. Chondrocytes manage the functional regulation of joint. These cell groups provide the synthesis and degradation of the ECM compnents, to support growth and regeneration, through the maintenance the gene expression and metabolism responded by mechanical stimuli. Extracellular matrix and proteoglycans are expressed by chondrocytes in articular joints [60–64].

The interleukins (ILs) and tumor necrosis factor α (TNF-α) cytokines are synthesized by chondrocytes, synovium, or inflammatory cells [7, 65]. Proteoglycan depletion has been stimulating physiopathologic processes, which is the main cause of degenerative joint diseases, e.g., osteoarthritis [66, 67]. Interleukins and TNF-α exert their effects on many diseases nonselectively from dental to cancer [68, 69], despite that a biomarker must be tissue-specific [70, 71]. Thus, in the last decade, the equine orthopedic researches have been deeply focused on proteoglycans, especially aggrecanases.

3.2 Aggrecanases on cartilage physiopathology

As it is well known, the proteases are responsible for proteolysis processes, which are catalytic enzymes to breakdown of the proteins into small polypeptides and amino acids by cleaved peptide bonds. The metalloproteinase superfamily members show their proteinase activity on osteoarthritis formation throughout the physiopathological processes. The matrix metalloproteinases (MMPs) and their endogenous inhibitors tissue inhibitors of metalloproteinases (TIMPs) are extensively studied; besides this, recent advances have indicated that the role of ADAMTS proteinase family is more considerable due to its abundant and specific aggrecanase activity by ADAMTS-4 and -5.

The aggrecan residues which cleaved at the glutamate 373-alanine 374 bond between the G1 and G2 interglobular domains were found at the synovial fluid analysis from various joint diseases (inflammatory or noninflammatory) in humans [72]. The first aggrecanase was purified and cloned by Tortorella et.al and named as aggrecanase-1 (currently termed as ADAMTS-4). They showed that ADAMTS-4 cleaves the aggrecan at the glutamic acid-373-alanine-374 bond [73]. After a while, Abbaszade et al. described aggrecanase-2 and named ADAMTS-11 (presently known as ADAMTS-5) [74]. ADAMTS-4 and ADAMTS-5 cleave the aggrecan at five common aggrecanase specific sites (Glu373-Ala374, Glu1480-Gly1481, Glu1667-Gly1668, Glu1771-Ala1772, and Glu1871-Leu1872,); nonetheless, ADAMTS-5 cleaves an additional site (Glu1480-Gly1481). Moreover, ADAMTS-5 is approximately twice slower than ADAMTS-4 [75, 76].

ADAMTS-4 and -5 are distributed in equine hoof lamina [78] and joints [79] and are expressed more in cartilage tissue than other tissues [80]. In our study, we observed concour horses after 50 minutes of a regular exercise program. As a result the serum ADAMTS-5 levels significantly increased but ADAMTS-4 did not. We concluded that ADAMTS-4 and ADAMTS-5 are using different pathways to physiologic and physiopathologic response [81]. Additionally and interestingly, the owners, whose horses had higher individual ADAMTS-5 serum levels, called the local veterinarians to complain about an orthopedic problem two or three weeks after our observations (unpublished data).

ADAMTS-4 needs to interact with sulfated GAGs that are attached to aggrecan core protein in order to effectively aggrecan degradation [58, 82]. ADAMTS-4 lacks the thrombospondin repeat domain on C-terminal region (**Figure 3**). This unique configuration allows bind to the adhesive glycoprotein fibronectin [82, 83]. Fibronectin is a glycoprotein that is found in low levels under physiologic conditions at the articular surface of cartilage and increases on pathologic conditions by activating innate immune response with toll-like receptors that are responded to

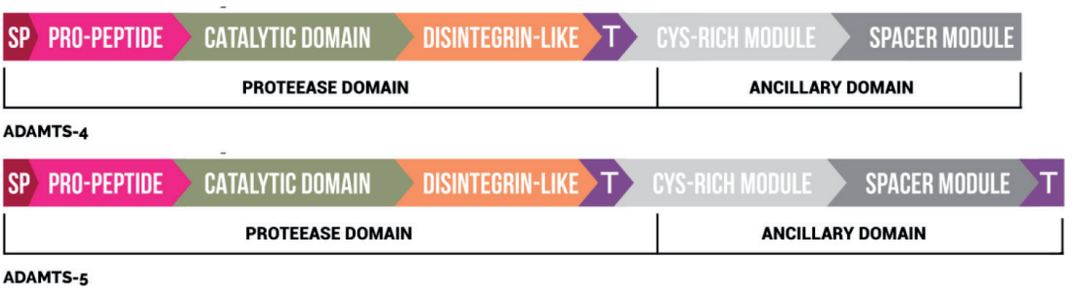

Figure 3.
Domain organization of ADAMTS aggrecanases. SP: signal peptide; T: thrombospondin type 1 motif; and CYS: cysteine [77].

regulate the innate immune system in case of pathogen-related inflammation [84, 85]. Hashimoto et al. reported that fibronectin is a novel inhibitor of ADAMTS4 activity in addition to its original endogenous inhibitor TIMP-3. Hence, fibronectin could be a potent preventive therapeutic against aggrecan degradation related to degenerative joint diseases [82]. While ADAMTS-4 is mediated by TNF-α, IL-1β, and nuclear factor-kappa B (NFκB) released from synovial macrophages, the regulation of ADAMTS-5 is not totally but predominantly independent of the these cytokine response [86].

4. Conclusion

The differences between synthesis pathways of ADAMTS-4 and ADAMTS-5 have to be taken into consideration on the TNF-α and IL-1 neutralization-targeted cytokine inhibitor therapies throughout degenerative joint diseases. In addition to classical therapy strategies, novel gene therapies are arising nowadays. An exciting work on this subject is a knockout murine model by the correction of ADAMTS-13 gene, which causes von Willebrand disease and leads to thrombotic thrombocytopenic purpura [87]. Transgenic animal studies with ADAMTS-4 and -5 double knockout mice [88, 89] revealed that aggrecan deletion protects from progressive osteoarthritis. These results have indicated that ADAMTS-4 and -5 may be potent therapeutic agents against laminitis and osteoarthritis, tendon, and ligament injuries for equine gene therapy.

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