ADAM-TS5, ADAM-TS6, and ADAM-TS7, Novel Members of a New Family of Zinc Metalloproteases

GENERAL FEATURES AND GENOMIC DISTRIBUTION OF THE ADAM-TS FAMILY*  [8]

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We report the primary structure of three novel, putative zinc metalloproteases designated ADAM-TS5, ADAM-TS6, and ADAM-TS7. All have a similar domain organization, comprising a preproregion, a reprolysin-type catalytic domain, a disintegrin-like domain, a thrombospondin type-1 (TS) module, a cysteine-rich domain, a spacer domain without cysteine residues, and a COOH-terminal TS module. These genes are differentially regulated during mouse embryogenesis and in adult tissues, with Adamts5 highly expressed in the peri-implantation period in embryo and trophoblast. These proteins are similar to four other cognate gene products, defining a distinct family of human reprolisin-like metalloproteases, the ADAM-TS family. The other members of the family are ADAM-TS1, an inflammation-induced gene, the procollagen I/II amino-propeptide processing enzyme (PCINP, ADAM-TS2), and proteins predicted by the KIAA0366 and KIAA0688 genes (ADAM-TS3 and ADAM-TS4). Individual ADAM-TS members differ in the number of COOH-terminal TS modules, and some have unique COOH-terminal domains. The ADAM-TS genes are dispersed in human and mouse genomes.

Proteolysis of extracellular matrix (ECM) plays a critical role in establishing tissue architecture during development and in tissue degradation in diseases such as cancer, arthritis, Alzheimer's disease, and a variety of inflammatory conditions (1–3). The proteolytic enzymes responsible include members of diverse protease families and they may work in concert or in cascades to degrade or process molecules. Two groups of zinc metalloproteases in particular, ADAMs (a disintegrin and metalloprotease) (2–4) and MMPs (matrix metalloproteinases) (1), appear broadly relevant to extracellular proteolysis. These families include a large number of enzymes (over 20 gene products each) with demonstrated cleavage activity for matrix molecules as well as nonmatrix, bioactive molecules such as tumor necrosis factor-α (reviewed in Ref. 3). In other instances of extracellular proteolysis, such as cleavage of aggrecan at the Glu673,Ala674 peptide bond (5) or the shedding of 1-selectin from leukocytes (6), the responsible proteases have not yet been reported. Such activities may eventually be attributed to cognate proteases but they may well be due to one or more hitherto unknown enzymes. For these reasons, it is important to define the full repertoire of enzymes possessed by cells, their regulation, and their substrate preferences.

ADAMs, also referred to as MDC (metalloprotease-disintegrins with cysteine-rich domains, 2) have catalytic domains with zinc-binding signatures and disintegrin domains that are very similar to the snake venom metalloproteinases (reviewed in Ref. 7); together, the ADAMs and snake venom metalloproteinases are referred to as reprolysins (7). Most ADAM members are quite similar in domain organization (2, 4, 7), bearing from amino to carboxyl termini, a signal peptide, a proregion, a zinc-metalloprotease catalytic domain with the typical reprolysin signature HEXXHXXGGXXHXH (X is typically: a hydrophobic residue (superscript 1), glycine or a hydrophobic residue (superscript 2), asparagine (superscript 3)), a disintegrin domain, a cysteine-rich domain, an epidermal growth factor-like domain, and in many cases a membrane-spanning region and a cytoplasmic domain with signaling potential. A recently described murine gene encoded a secreted protein that differed substantially from the prototypic ADAM structure and was designated ADAM-TS12 (8). ADAM-TS12 lacks the epidermal growth factor-like repeat, does not have a canonical disintegrin sequence, and possesses three modules with similar thrombospondin type-1 (TS) repeats (8). This unique structure, the existence of three other similar gene products (ADAM-TS2–4) in the public domain, and our discovery of three novel, related gene products led us to recognize and to describe here the essential features of the ADAM-TS family.

The existence of procollagen I/II amino-propeptide processing enzyme  

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§ The on-line version of this article (available at http://www.jbc.org) contains the alignments of the ADAM-TS1–7 sequences.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF140673 (Adamts5), AF141293 (ADAMTS5), AF140674 (ADAMTS6), and AF140675 (ADAMTS7).

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¹ The abbreviations used are: ECM, extracellular matrix; ADAM-TS, a disintegrin-like and metalloprotease domain with thrombospondin type I motifs; MMP, matrix metalloproteinase; PCINP, procollagen I/II amino-propeptide processing enzyme; EDS-VIIC, Ehlers-Danlos syndrome type VIIC; RACE, rapid amplification of cDNA ends; PCR, polymerase chain reaction; kbp, kilobase pairs; bp, base pairs; cm, centimeter; RFLV, restriction fragment length variants; EST, expressed sequence tag; I.M.A.G.E., integrated mapping of genomes and their expression; ORF, open reading frame.

² Nomenclature: gene nomenclature has been assigned in agreement with the Human Gene Nomenclature Committee. ADAMTS5, ADAMTS6, and ADAMTS7 etc. are human genes; Adamts5, Adamts6, and Adamts7, etc. are the mouse orthologs. The protein products of the respective genes are designated as ADAM-TS5, ADAM-TS6, and ADAM-TS7.

This paper is available on line at http://www.jbc.org
ing enzyme (PCINP) has been known for decades (9), and the recent cDNA cloning of bovine PCINP (10) demonstrated similarities of this enzyme with ADAM-TS1. The gene is now designated ADAMTS2 in keeping with nomenclature recommended by the Human Gene Nomenclature Committee. Lack of ADAM-TS2 is known to cause dermatosparaxis in cattle (11) or Ehlers-Danlos syndrome type VIIC (EDS-VIIC) in humans (12, 13). EDS-VIIC is a recessively inherited disorder, characterized clinically by severe skin fragility and biochemically by the presence in skin of procollagen incompletely processed at the amino terminus. The precise mutations in ADAMTS2 have not yet been reported in the literature. ADAM-TS3 and ADAM-TS4 designate the proteins predicted by mRNAs transcribed from the KIAA0366 (14) and KIAA0688 (15) genes, respectively. These genes have been deposited in GenBank™ by the Kanzusa DNA Institute and are designated ADAMTS3 and ADAMTS4, respectively.

We now describe here: 1) the discovery of three novel genes, ADAM-TS5–7 belonging to this family; 2) the relationship of their predicted protein products to essential structural features of the ADAM-TS family; 3) analysis of expression of our novel genes in various tissues; and 4) the distribution of the ADAM-TS genes in human and mouse genomes.

**EXPERIMENTAL PROCEDURES**

cDNA Cloning and Sequence Analysis—Using the BLAST (basic local alignment search tool) programs (16), we scanned the data base of expressed sequence tags (dbEST, Ref. 17) using the protein sequence of a Caenorhabditis elegans encoded ADAM-TS as well as ADAM-TS 1–4 and identified similarities in several human and mouse ESTs. These ESTs were derived from clones held by the I.M.A.G.E. consortium (integrated mapping of genomes and their expression): EST AA288689 (clone 569515) and EST W72552 (clone 345484) for mouse and human ADAM-TS5, respectively, EST AA400393 (clone 742630) for ADAM-TS6, and EST N48932 (clone 272089) for ADAM-TS7. Translation of these ESTs predicted peptides with similarity, but not identity to invertebrate ADAM-TS members or of snake venom disintegrins but with no identity in GenBank™ or other protein and nucleotide data bases. The I.M.A.G.E. clones were purchased (Research (integrated mapping of genomes and their expression): EST AA288689 (clone 569515) and EST W72552 (clone 345484) for mouse and human ADAM-TS5, respectively, EST AA400393 (clone 742630) for ADAM-TS6, and EST N48932 (clone 272089) for ADAM-TS7. Translation of these ESTs predicted peptides with similarity, but not identity to invertebrate ADAM-TS members or of snake venom disintegrins but with no identity in GenBank™ or other protein and nucleotide data bases. The I.M.A.G.E. clones were purchased (Research Genetics, Huntsville, AL) and their inserts sequenced in their entirety. Using oligonucleotide primers based on the sequences at the ends of the EST inserts and human fetal brain or 7-day-old mouse embryo cDNA (Marathon cDNA, CLONTECH) as template, we performed RACE (rapid amplification of cDNA ends, Ref. 18) by PCR at 5 °C, 30 s, 68 °C, and 72 °C; then 5 cycles of 95 °C for 0.5 min, 70 °C for 5 min, and 20 cycles of 95 °C for 0.5 min; and finally, 68 °C for 5 min. The PCR products were analyzed by Southern blotting, initially using [a-32P]dCTP-labeled inserts of the I.M.A.G.E. clones. For subsequent rounds of RACE, the inserts of clones obtained in the previous rounds were used as probes. For higher resolution of the ADAMTS3 and ADAMTS5 loci, we performed linkage analysis using DNA from the Stanford Human Genome Center G3 human radiation hybrid panel (Research Genetics, Huntsville, AL) for gene-specific PCR. Using oligonucleotide primer sets specific for each gene (for ADAMTS3, forward primer 5′-TCATC-CAGTGAAGAATGTG-3′; reverse primer 5′-CGACATTGCTCATC-3′; for ADAMTS5, forward primer 5′-ACTGCTGTACTAG-3′; and reverse primer 5′-ACATTTATTAAGGTC-3′) and stringent PCR conditions (for ADAMTS3: 95 °C for 30 s, 55 °C for 30 s, 68 °C for 30 s for 30 cycles; for ADAMTS5 95 °C for 30 s, 58 °C for 30 s, 68 °C for 30 s for 30 cycles), we obtained specific amplification in DNA from the Chinese hamster control DNA, which were not seen in DNA from the Chinese hamster somatic cell recipient. The retention or nonretention of the bands specific to the human gene was determined in the 83 hybrid DNA’s that comprised the panel. These results were compared with previously defined markers to obtain the linkage data together with the lod score for linkage (Table I).

**RESULTS**

Cloning of Novel ADAM-TS cDNAs—Using the BLAST algorithm to scan dbEST for novel ESTs that were homologous to cognate ADAM-TS genes, we identified a number of nonoverlapping sequences deposited in dbEST. Full-length sequencing of the inserts of these clones

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3 S. S. Apte, unpublished data.
confirmed that they were not identical to any other gene sequences deposited in GenBankTM. Using nested oligonucleotide primers based on the 5\(^\text{th}\) and 3\(^\text{th}\) ends of the sequences of the I.M.A.G.E. clones, we did successive rounds of RACE to extend the sequences as shown in Fig. 1 (a–c). RACE primers were generated 50–200 bp from the ends of the sequences so that the contiguity of RACE clones with I.M.A.G.E. clones could be clearly established. The predicted domain organization relative to the cDNA clones (thin line) and the extent of overlap between clones is shown. The original I.M.A.G.E. clones used are underlined. Intronic regions of incompletely spliced transcripts are shown by the angled dotted lines. DNA scale marker (in base pairs) and amino acid scale marker are at upper right. Location of the probe used for in situ hybridization (ISH) is shown in a, d, domain organization of ADAM-TS1–4 based on the primary structure predicted from cDNA sequences. Sequences of ADAM-TS1 (mouse, GenBankTM accession number D67076) and ADAM-TS2 (bovine, GenBankTM accession number X96389; human, GenBankTM accession number AJ003125) have been published, and those of ADAM-TS3 (human KIAA0366 gene, GenBankTM accession number AB002364) and ADAM-TS4 (human KIAA0688 gene, GenBankTM accession number AB014588) have been deposited in the data bases. The cDNA sequence of ADAM-TS3 does not extend to the start codon.
to the cloned cDNA and the relationship of the products of these novel genes to the four cognate ADAM-TS members are shown in Fig. 1.

**Cloning of Adamts5 and ADAMTS5**—A single round of 5’ and 3’ RACE sufficed for cloning of the entire coding sequence of Adamts5 and part of the catalytic zinc binding site through to the stop codon of ADAMTS5. The ADAMTS5 clone 345484-52 contained an incompletely processed mRNA (Fig. 1a) revealing a splice junction splitting the zinc binding sequence in the ADAM-TS5 cDNA. Although the complete primary structure of ADAM-TS5 could be deduced from the mouse cDNA the corresponding human cDNA sequence has not yet been completely cloned. At its 5’ end, clone TH5 contains a methionine codon (ATG) within a consensus Kozak sequence for initiation of translation (25), but we have been unable to extend the 5’ sequence further to see if this is the first methionine in the predicted open reading frame (ORF). The predicted amino acid sequences of Adamts5 and ADAMTS5 are shown in alignment in Fig. 2a.

**Cloning of ADAMTS6**—The I.M.A.G.E. clone 742630 contained an ORF flanked by consensus splice sequences, indicating the presence of introns (Fig. 1b). Two successive rounds of RACE at the 5’ end and a single round of RACE at the 3’ end provided the complete coding sequence of ADAMTS6. The putative ATG codon is within a Kozak consensus sequence and encodes the first methionine within the ORF.

**Cloning of ADAMTS7**—The I.M.A.G.E. clone 272098 encoded a putative preproregion and was extended in the 3’ direction by two successive rounds of RACE (Fig. 1c). Attempts to extend the sequence at its 5’ end have not been successful. However, we identified a typical signal peptide sequence downstream of the first methionine in the translated ORF; this methionine codon lay within a satisfactory Kozak consensus for translation initiation, and we believe it likely that it encodes the start codon.

**Comparison of the Predicted Structure of ADAM-TS5, ADAM-TS6, and ADAM-TS7**

Fig. 2 (a–c) displays the deduced primary sequence of each protein. An alignment of these sequences (including ADAM-TS1–4) may be viewed as supplemental data on-line.

The three novel conceptually gene products described here share a common domain organization. From amino to carboxyl termini, we describe them as follows.

A Preproregion—A typical signal sequence of variable length is followed by a putative proregion of variable length but demonstrating short stretches of sequence similarity (see alignment on-line). Three cysteine residues are predicted within each novel prodomain. The COOH-terminal most of these lies within a sequence context similar to the cysteine “switch” of the MMPs (26) (Fig. 2, a–c). All three novel genes predict consensus cleavage signals for furin, three in the case of ADAM-TS5 and one each in the case of ADAM-TS6 and ADAM-TS7. The most carboxyl-terminal furin cleavage site in ADAM-TS5 likely predicts the processing site for generation of the mature protease. The amino terminus of the mature proteins is predicted to start at the residue immediately following the cleavage site (Fig. 2, a–c).

A Catalytic Domain—The catalytic domains are very similar to each other and contain eight cysteine residues and a typical replysins-type zinc binding signature (Fig. 2, a–c, and Fig. 3a). Five cysteine residues are upstream of the zinc binding sequence, while three residues are downstream, an arrangement that is shared with other ADAM-TS members. Like all MMPs and replysins, the zinc binding signature is followed in all ADAM-TS proteins by a methionine residue within a conserved sequence context (Fig. 3a). We designate this as being the methionine of the “Met-turn” a structural landmark present in all the MMPs and ADAMs; the Met-turn is a tight turn arranged as a right handed screw in the adamalysin and MMP polypeptides COOH-terminal to the third zinc-binding histidine (32). The methionine of the Met-turn is at a similar, but not constant, interval from the ADAM-TS zinc-binding signature, and in all the ADAM-TS members, a constant cysteine residue is present in that interval.

A Disintegrin-like Domain—The catalytic domain is followed by a domain of 60–90 residues with 35–45% similarity to snake venom disintegrins, but without the canonical cysteine arrangement seen in the latter. We term this the disintegrin-like domain; while of comparable length in ADAM-TS5 and ADAMTS7, it is considerably shorter in ADAM-TS6. The disintegrin-like domain contains eight cysteine residues (except for ADAM-TS6 which has six).

A TS Module—The first TS repeat is very similar in all three novel proteases and very similar to the first TS repeat of other ADAM-TSs (Fig. 3b). It contains the same number of residues (52) in all three novel gene products (Fig. 3b).

The Cysteine-rich Domain—This TS domain is followed by a conserved cysteine-rich sequence termed the cysteine-rich domain (to distinguish it from the cysteine-free spacer domain). It contains 10 conserved cysteines in each case and demonstrates high sequence homology with the cysteine-rich domain of other ADAM-TS proteins.

The Spacer Domain—This domain is of variable length, in all ADAM-TSs, and lacks the sequence landmarks so characteristic of all the other domains. It shows the least homology of all the domains. ADAMTS7 has the longest of all the spacer domains (221 amino acids), while ADAM-TS6 has the shortest of all spacer domains (127 amino acids). The spacer domain of ADAM-TS5 is intermediate in length (158 amino acids).

A COOH-terminal TS Module—The sequence of the second TS module is more variant between the members of the ADAM-TS family than the first TS module, despite the conservation of the number and spacing of cysteine residues. The second TS module of ADAM-TS7 is followed by a short sequence containing two cysteine residues.

Overall, the predicted mature forms of these proteases show 20–40% similarity to each other and to ADAM-TS1–4, although this may be considerably higher or lower for individual domains as described above. The dendrogram in Fig. 3c indicates specific relationships between individual ADAM-TS members. The predicted molecular weights (Mr) of the full-length gene products are 108,633 (mADAM-TS5), 97,115 (hADAM-TS6), and 116,607 (hADAM-TS7). The actual Mr will almost certainly be different due to processing of these proenzymes to the mature forms and post-translational modifications. All the primary sequences predict the possibility of N-linked glycosylation at a number of potential sites. ADAM-TS5 and ADAM-TS7 contain three and two potential N-glycosylation sites, respectively, in the mature protease, of which two are in similar positions; one of these is in a constant position just upstream of the start of the spacer domain and the other lies within the spacer domain (Fig. 2, a and c). ADAM-TS5 possesses an additional site near the start of the disintegrin domain (Fig. 2a). In ADAM-TS5, the N-linked glycosylation sites are conserved in both mouse and human sequences. ADAM-TS6 has four potential N-linked glycosylation sites within the prodomain and two others in the mature protease. These are at different positions relative to the sites in ADAM-TS5 and ADAM-TS7 (Fig. 2, a–c).
FIG. 2. Predicted amino acid sequences of ADAM-TS5 (a) (alignment shows mouse sequence above, partial human sequence below), ADAM-TS6 (b), and ADAM-TS7 (c). The active site sequences and proposed Met-turn are enclosed in boxes. Potential furin cleavage site(s) are indicated by arrows. Thrombospondin type-1 modules are underlined. Other cysteine residues are indicated by asterisks. The preproregion extends until the furin cleavage site, and the catalytic domain extends from the furin cleavage site to the disintegrin-like sequence (Dis). The start of the spacer domain is indicated; the region between the NH₂-terminal TS domain and the spacer domain is the cysteine-rich domain. The single letter amino acid code is used.
smaller band of 2.0 kbp was detected in gestational day 17 embryo mRNA (Fig. 4a).

In Situ Hybridization—Specific hybridization of the antisense Adamts5 probe to sections of 8.5-day-old mouse embryos was obtained, whereas no hybridization or low level background staining was noted with the control sense probe (Fig. 5a). Staining was uniform throughout the 8.5-day-old embryos (Fig. 5b); we could not distinguish between the staining of various developmental components at this early stage. In addition, there was labeling of mRNA in trophoblastic cells lining the uterine cavity (Fig. 5, a and b) as well as in the developing placenta (Fig. 5, c and d). The decidual reaction (primarily cells in uterine glands) within the uterus also showed up-regulation of Adamts5 mRNA relative to the negative controls (Fig. 5, a and b). In sections from 10.5-day-old embryos, labeling was widespread but less intense compared with the 8.5-day-old embryo. Labeled cells were seen in mesenchyme and somites as well as in the neural tube and developing hindgut (Fig. 5d).

Chromosomal Mapping of Genes of the ADAM-TS Family

Human monochromosomal assignment was obtained using cloned cDNA probes for ADAMTS1 and ADAMTS5–7, and in addition, linkage mapping of ADAMTS3 and ADAMTS5 was obtained using PCR (Table I). For ADAMTS6 and ADAMTS7, we have yet to assign loci by linkage mapping in human or mouse genomes; the mapping of these genes is presently only available at the resolution of a single human chromosome (Table I).

Linkage analysis of the mouse chromosomal loci for Adamts1, Adamts2, Adamts4, and Adamts5 was made possible by the existence of I.M.A.G.E. clones representing these genes. For Adamts1, informative EcoRV RFLVs were detected (C3H/HeJ-gld, 12.0 kbp; Mus spretus, 5.0 kbp); for Adamts2, informative TaqI RFLVs were detected (C3H/HeJ-gld, 6.4 kbp; Mus spretus, 7.0 kbp); for Adamts4, informative BglII RFLVs were detected (C3H/HeJ-gld, 5.8 kbp; Mus spretus, 7.0 kbp), and for Adamts5, informative BglI RFLVs were detected (C3H/HeJ-gld, 24.0 kbp, Mus spretus, 7.0 kbp).

Haplotypic analyses indicated that Adamts1 and Adamts5 co-segregated with Grik1 on mouse chromosome 16 in all 38 and 114 meiotic events examined, respectively. Adamts2 co-segregated with the Ii3 locus on mouse chromosome 11 in 113/114 meiotic events examined, while Adamts4 co-segregated with the Fcgr3, Fcgr2, Mpz, Apoa2, and Fcε1g loci on mouse chromosome 1 in all 114 meiotic events examined. The best gene order ± the S.D. indicated the loci as outlined in Table I.

Based on linkage mapping of mouse or human genes, we were able to strongly suggest chromosomal position for the corresponding ortholog using the mouse-human homology maps. Overall, our results (Table I) demonstrate that the genes of this novel family are dispersed in the human and mouse genomes, with the exception of the Adamts1 and Adamts5 genes which are both linked to Grik1 on mouse chromosome 16. ADAMTS2 and ADAMTS6 both lie on human chromosome 5, but we do not yet know if these genes are linked.

DISCUSSION

dBEST is a unique resource for identification of novel mRNAs using the BLAST programs. This, combined with the availability of the I.M.A.G.E. clones, provides a means of cloning full-length coding sequences using the inserts of I.M.A.G.E. clones as probes for RACE or library screening. The ESTs that we initially identified were small, did not encode similar peptides, and were not identical to each other, suggesting that they might represent different genes or nonoverlapping sequences from the same gene. Analysis of the monochromosomal map-
signal peptides, the cloned nucleotide sequences do not extend far enough upstream to confirm that these are the first methionines in the ORFs we have defined. Despite many attempts to extend the sequences further using various mRNA templates, PCR conditions, and polymerases, we have not been successful.

**ADAM-TS Genes Are Highly Regulated**—Previous work of Kuno et al. (8) and Colige et al. (10), respectively, showed that ADAM-TS1 and ADAM-TS2 mRNA were either not detectable by Northern analysis in normal tissues or were expressed at very low levels. Colige et al. (10) used RT-PCR to detect mRNA, while Kuno et al. (8) showed a dramatic up-regulation in capecinogenic carcinoma cells and in mice stimulated with lipopolysaccharide. Our own studies demonstrate that ADAMTS5 and ADAMTS6 are expressed at low levels, primarily in the placenta. ADAMTS5 showed prominent expression in the 7-day-old embryo but lower expression thereafter, suggesting that this enzyme may play a role in proteolytic processing mostly during the peri-implantation period. The ADAM-TS genes (ADAMTS7 excepted) may be very highly regulated and inducible under certain circumstances only, such as has been demonstrated for ADAM-TS1 (8) and ADAM-TS5 (this study).

Since all members of this family are likely to be processed by furin and are therefore likely to be constitutively activated during secretion, perhaps regulation at the transcriptional level may be required to control their activity. In this context, nothing is known of the functionality of the putative cystein-switch in the ADAM-TS family. It is also not known whether inhibitors of the MMPs and ADAMs, such as TIMP-3 (3, 27), will act as effective natural regulators of these enzymes or whether other, more specific inhibitors exist.

**Genomic Distribution of ADAM-TS Genes**—We determined the ADAM-TS gene loci to facilitate exploration of their possible role in genetic diseases. Our studies demonstrate that unlike many of the MMPs (28) the ADAM-TS genes (with the exception of ADAMTS1 and ADAMTS5) are not clustered within the genome. Linkage mapping in the human or mouse genomes is a powerful tool for genetic studies, and as well, it permits assignment of a locus in the other species using human-mouse linkage homology maps. We have assigned linkage in human or mouse genomes for five out of the seven genes now...
known in this family. The Online Mendelian Inheritance in Man (OMIM) data base shows that a number of human diseases for which the causative mutations have not been identified are mapped to the vicinity of the ADAM-TS loci. Dentinogenesis imperfecta and amelogenesis imperfecta map to 4q21 in the vicinity of ADAMTS3. Usher syndrome-2A (autosomal recessive) maps to 21q21-q22, a familial platelet disorder with associated myeloid malignancy to 21q22-q22, in the vicinity of ADAMTS1 and ADAMTS5. Dominant nonsyndromic sensorineural deafness seven maps to 1q21-q23, limb girdle muscular dystrophy-1B to 1q11-q21, and familial partial lipodystrophy to 1q21-q24, the region of ADAMTS4. Mapping of ADAMTS2 to 5q23-q24 indicates the locus for the EDS-VIIC mutations.

Domain Organization and Evolutionary Relationships in the ADAM-TS Family—The novel ADAM-TS proteases described here show all the hallmarks of the ADAM-TS family (Fig. 1). A recent review described the similarities and phylogenetic relationship between the first four members of the family (29). Our results (Fig. 3c) indicate that distinct and evolutionarily related subsets may exist within the ADAM-TS family. ADAMTS3 and ADAMTS4 are not only similar in organization and length, but are almost identical within the catalytic domain, particularly in the vicinity of the zinc-binding consensus sequence. This remarkable degree of identity, however, does not extend to other domains or to the unique COOH-terminal domains (Fig. 1d), which are totally unrelated to each other or to any other protein in the data base. The three new members we describe here are all very similar in domain organization, but it is also clear from the dendrogram that they do not form a distinct subset of proteases. In fact, each of the novel proteases we have described belongs to a distinct limb of the dendrogram (Fig. 3c). ADAMTS1 and ADAMTS4 have three and one TS domains, respectively, yet their zinc-binding signatures are very similar, and they are closely related phylogenetically in the ADAM-TS dendrogram, with some proximity to ADAMTS5 (Fig. 3c). In ADAMTS1 and ADAMTS4, but not in ADAMTS5, a conserved glycine residue (indicated by an asterisk in Fig. 3c) within the zinc binding signature, is altered to an asparagine.

The NH2-terminal most (first) TS domain shows striking conservation within the family (Fig. 3b). All cysteine residues, a pentapeptide, CSR/T/S/Y/C, a number of glycine residues, and tryptophans at TS domain positions 1 and 4 are conserved. The pentapeptide CSR/T/S/Y/C in ADAM-TS members is consistently different from the corresponding sequence in thrombospondin (CSVTC), which is known to bind to CD36 (reviewed in Ref. 30). A potential linear heparin binding motif (BBXB, B is a basic amino acid, X denotes any amino acid) found in thrombospondins (30) is present in the sequence of ADAM-TS1, ADAM-TS4, ADAM-TS6, and ADAM-TS7 (Fig. 3b). Cysteine residues and their spacing are highly conserved in all members within the pro, catalytic, disintegrin, and cysteine-rich domains (Fig. 2, a–c, and the alignment accessible on-line).

Assuming that the even numbers of cysteines in each of the domains of the mature ADAM-TS proteases participate in intrachain disulfide bond formation, one can begin to visualize the structure of ADAM-TS proteins. A catalytic domain folded like the metzincins (32) is likely to be followed by independently folded domains in a modular fashion, with the spacer domain providing the most variability in sequence, length, and flexibility. It will be interesting to investigate whether interdomain interactions are essential for structure and function.

Function of the ADAM-TS Family—ADAM-TS1 transiently expressed in COS cells is detected within the cell substratum, but not in culture medium, indicating that it is a component of ECM (31). It interacts with ECM components through the TS and spacer domains (31). Since heparin displaces ADAMTS1 from ECM, it is possible that heparin or heparan-sulfate proteoglycans mediate the ECM interaction (31). The substrates of ADAM-TS1 are presently unknown. ADAM-TS1 is thought to play a role in tumor cachexia and inflammation (8).

ADAM-TS2 cleaves native triple-helical, but not denatured procollagen I, and has an affinity for collagen XIV (33). A distinct NH2-terminal processing event, mediated by an enzyme other than ADAM-TS2, occurs in procollagen III (34). In view of the high degree of similarity of the catalytic domains of ADAM-TS2 and ADAM-TS3, but not of their COOH-terminal halves we speculate that these two members may be closely related functionally. Perhaps ADAM-TS3 may also represent a collagen processing enzyme, with a different specificity, such as for procollagen III. It is also possible that ADAM-TS3 may be a second procollagen I and/or procollagen II amino peptidase. The severity of the skin phenotype in EDS-VIIC relative to that in bone and cartilage may suggest the existence of additional procollagen III amino-processing enzymes. The functions of ADAMTS5, ADAMTS6, and ADAMTS7 are unknown at the present time.

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ADAM-TS Family 25563

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