# WARP is a new member of the von Willebrand factor A-domain superfamily of extracellular matrix proteins

Jamie Fitzgerald*, Su Tay Ting, John F. Bateman

*Cell and Matrix Biology Research Unit, Murdoch Childrens Research Institute and Department of Paediatrics, University of Melbourne, Royal Children's Hospital, Parkville, Vic. 3052, Australia*

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**Abstract We report a new member of the von Willebrand factor A-domain protein superfamily, WARP (for von Willebrand factor A-domain-related protein). The full-length mouse WARP cDNA is 2.3 kb in size and predicts a protein of 415 amino acids which contains a signal sequence, a VA-like domain, two fibronectin type III-like repeats, and a short proline- and arginine-rich segment. WARP mRNA was expressed predominantly in chondrocytes and in vitro expression experiments in transfected 293 cells indicated that WARP is a secreted glycoprotein that forms disulphide-bonded oligomers. We conclude that WARP is a new member of the von Willebrand factor A-domain (VA-domain) superfamily of extracellular matrix proteins which may play a role in cartilage structure and function.** 

*Key words:* Von Willebrand factor A-domain; Fibronectin type III repeat; Extracellular matrix

## 1. Introduction

The extracellular matrix (ECM) is a complex mixture of collagens, non-collagenous glycoproteins, and proteoglycans that interact to provide a structural scaffold as well as specific cues for the maintenance, growth and differentiation of cells and tissues. The protein cores of a large number of ECM molecules are composed of different combinations of a finite collection of modules [1].

One module present in a number of proteins is the type A-domain, first described in von Willebrand factor (reviewed in [2]). Members of the expanding von Willebrand factor type A-domain (VA-domain) protein superfamily participate in a variety of functions including hemostasis, cell adhesion and interactions between matrix molecules. ECM components that contain one or more VA-domains include collagens types VI [3,4], VII [5], XII [6], XIV [7], XX [8] and XXI [9], matrilins-1, -2, -3, -4 (reviewed in [10]), cochlin [11], polydom [12] and nine transmembrane α-integrin chains (α1, α2, α10, α11, αL, αM, αX, αD and αE) (reviewed in [13]) where they are also known as 'I'-domains.

The crystal structures for several VA-domains have been solved [13–17] and show that the VA module is an independently folding protein unit that attains a classic α/β 'Rossman' fold consisting of a parallel β-sheet surrounded by amphipathic α-helices. The majority of VA-domains contain a metal ion-dependent adhesion site (MIDAS) at the C-terminal end of the β-sheet, however not all VA-domains contain this motif [15]. Although the role of VA-domains in ECM proteins has not been precisely determined, they appear to play important roles in protein–protein interactions [18–20] and in multimeric protein assembly [21,22] and may be crucial in providing linkages between ECM structural networks [23,24].

We have identified a new member of the VA-domain protein superfamily by interrogation of the mouse expressed sequence tag (EST) database. WARP, von Willebrand factor A-domain-related protein, is a 415 amino acid modular protein containing a single VA-domain, two fibronectin type III (F3) repeats and a short proline/arginine-rich C-terminal segment. Our data indicate that WARP is a novel, secreted ECM glycoprotein expressed by chondrocytes.

## 2. Materials and methods

### 2.1. Identification of WARP cDNAs

The mouse EST database was conceptually translated into six reading frames and interrogated with the 200 amino acid sequence of the α3(VI) N8 VA-domain of human collagen VI [3] using the tblastn programme (v2.0) at the National Center for Biotechnology Information (NCBI). Several overlapping cDNA clones with significant similarity to α3(VI) N8 at the protein level were identified and we obtained three of these clones, ui42d08, ue22e08 and ml15f02 (Genome Systems). DNA sequencing (Amplicycle sequencing kit, Perkin Elmer Biosystems) revealed that clones ue22e08 (1026 bp) and mt15f02 (551 bp) lie entirely within the ui42d08 (2308 bp) sequence and exactly matched the larger clone spanning nucleotides 1282–2308[1] and 1833–2227, confirming that the three cDNAs represent a single gene. The collections of mouse and human WARP ESTs have been assigned to the Unigene (http://www.ncbi.nlm.nih.gov/UniGene/) entries Mm. 26515 and Hs. 110443 respectively.

### 2.2. WARP plasmid constructs and expression in transfected cells

The ui42d08 cDNA in pME18 (GenBank accession number AI115125) (Fig. 1A) was subcloned into the pBluescript SK$^-$ vector (Stratagene) as a *Xho*I fragment and translated in vitro using the TNT coupled transcription and translation system (Promega) [25] to confirm the open reading frame. To enable immunoprecipitation of

*Corresponding author. Fax: (61)-3-9345 7997.
*E-mail address:* fitzgerj@cryptic.rch.unimelb.edu.au (J. Fitzgerald).

*Abbreviations:* WARP, von Willebrand factor A-domain-related protein; VA-domain, von Willebrand factor A-domain; F3, fibronectin type III repeat; ECM, extracellular matrix; EST, expressed sequence tag; RT-PCR, reverse transcription-polymerase chain reaction; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis

[1] Nucleotides are numbered from the start of the WARP cDNA clone deposited in GenBank (AYO30094). Amino acid sequence numbers are from the translational start site.

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WARP protein from transfected cells, a His-tagged full-length expression construct was also produced. Six histidine residues were incorporated at the N-terminus immediately following amino acid 21, between the signal peptide and the start of the VA-domain, by strand overlap extension polymerase chain reaction (PCR) [25] and subcloned into the pBluescript SK$^-$ vector. To allow episomal expression in mammalian cells, WARP-His was subcloned from pBluescript SK$^-$ into pCEP4 (Invitrogen) as a *Xho*I fragment. WARP-His in pCEP4 was transfected into 293-EBNA cells (Invitrogen) grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine serum using FuGene transfection reagent (Roche) according to the manufacturer's instructions and grown for 14 days in the presence of 250 μg/ml hygromycin B (Roche) to select for transfected cells.

### 2.3. Cell culture

Human embryonic kidney 293-EBNA cells, mouse MC3T3 osteoblast [26], Mov13 fibroblast [27], 3T3 fibroblast, C2C12 myoblast [28], and C57 primary fibroblast cell cultures were maintained in culture in DMEM containing 10% bovine serum. Primary chondrocytes were isolated as previously described [29]. Briefly, rib cages were dissected from newborn mice and incubated in DMEM containing 5% bovine serum and 2 mg/ml collagenase (Worthington Biochemical Corp.) for 30 min at 37°C. Loose connective tissue and bone was removed and the rib cartilage incubated in fresh collagenase solution for 16 h. Chondrocytes released from cartilage were centrifuged and grown as a pellet culture for 16 h in DMEM containing 10% foetal calf serum prior to RNA isolation.

### 2.4. mRNA expression analysis

Total RNA was isolated from mouse cell lines and primary rib chondrocytes using the mini RNeasy™ RNA isolation kit (Qiagen) according to the manufacturers instructions and from mouse tissues using the guanidinium thiocyanate and phenol/chloroform method of Chomzynski [30]. To ensure that no genomic DNA was carried through the isolation procedure all RNA samples were digested with DNA-free™ DNase treatment and removal kit (Ambion). Reverse transcription (RT)-PCR was performed using the GeneAmpR RNA PCR kit (Perkin Elmer). 2 μg of total RNA was added to each RT reaction in a total volume of 40 μl and 10 μl of cDNA was used in the subsequent PCR in a 50 μl reaction volume. The optimal $Mg^{2+}$ concentration was found to be 0.35 mM for the WARP amplification and 1 mM for the internal control, hypoxanthine guanine phosphoribosyltransferase (HPRT), a housekeeping gene involved in purine metabolism. In the PCR step, NR1 ($^{1666}$5′-CTCAAAGCCATGCGTAGTCC-3′$^{1685}$), and NF4 ($^{953}$5′-AGAACGCATCGTCATCTCGC-3′$^{972}$) primers were used to amplify a 693 bp region of WARP. mHPRT1 ($^{231}$5′-CCTGCTGGATTACATTAAAG-3′$^{251}$) and mHPRT2 ($^{581}$5′-TCAAGGGCATATCCAACAAC-3′$^{601}$) primers were used to amplify a 350 bp fragment of the mouse HPRT gene (GenBank accession number NM_013556). The cycle number for each gene was selected so that amplification was in the linear range (not shown), allowing the level of PCR products to be compared between samples. Simultaneous amplification of HPRT derived from the same cDNA reaction allowed correction for small variations in amount of template.

For Northern blot analysis, 60 μg of total RNA was poly(A)-selected using oligo dT Dynabeads (Dynal), fractionated on a 1% (w/v) agarose formaldehyde gel and transferred to Hybond N$^+$ nylon membrane (Amersham). A [$^{32}$P]dCTP-labelled WARP probe was hybridised to the blot in Ultrahyb hybridisation solution (Ambion) at 42°C overnight. The blot was washed to a stringency of 0.1×SSC/0.1% sodium dodecyl sulphate (SDS) (w/v) at 65°C, exposed to a phosphor-screen and scanned with a Storm phosphor-imager (Molecular Dynamics).

### 2.5. WARP biosynthetic labelling and analysis

293-EBNA cells transfected with WARP were grown to confluence in a 60-mm dish and labelled for 16 h with 300 μCi of L-[$^{35}$S]methionine (1398 Ci/mmol, NEN Research Products) in DMEM without L-methionine and L-cysteine (Life Technologies). The medium was removed and centrifuged to pellet any cells and NP-40 added to the supernatant to 1% (v/v) together with a cocktail of protease inhibitors (1 mM 4-(2 aminoethyl)-benzenesulfonyl-fluoride (AEBSF); 1 mM phenylmethylsulfonyl fluoride (PMSF); 20 mM *N*-ethylmaleimide (NEM)). The cell layer was dispersed in 1 ml of lysis buffer (150 mM NaCl; 50 mM Tris–HCl, pH 7.5; 5 mM EDTA; 20 mM NEM; 1 mM AEBSF; 1 mM PMSF; 1% (v/v) NP-40) on ice for 30 min then centrifuged briefly to remove insoluble material. Following a pre-clear step with 100 μl protein G-Sepharose (20% slurry in PBS), anti-His antibody (Roche) (1 in 100 dilution) was added to each fraction together with 100 μl fresh protein G-Sepharose and mixed gently at 4°C for 16 h. The antibody–Sepharose complex was washed twice with 50% lysis buffer/50% NET (150 mM NaCl; 50 mM Tris–HCl, pH 7.4; 1 mM EDTA; 0.1% NP-40) for 30 min each then twice with NET. Immunoprecipitated material was separated from the Sepharose beads by heating at 65°C for 15 min in SDS–polyacrylamide gel electrophoresis (PAGE) sample buffer containing 20 mM dithiothreitol (DTT), fractionated on a 10% (w/v) SDS–polyacrylamide gel and subjected to fluorography.

### 2.6. N-glycosidase treatment

WARP-His protein was deglycosylated by N-glycosidase F (Roche) treatment according to the manufacturers guidelines. Immunoprecipitated WARP-His was denatured by boiling in 1% SDS for 2 min then diluted 1 in 10 with sodium phosphate buffer (20 mM sodium phosphate, pH 7.2; 10 mM sodium azide; 50 mM EDTA; 0.5% (v/v) NP-40) and boiled again for 2 min. Following addition of 0.4 units of N-glycosidase F the sample was incubated for 20 h at 37°C then heat denatured in SDS–PAGE sample buffer containing 20 mM DTT and analysed by SDS–PAGE.

### 2.7. SDS-PAGE

Samples were resolved on 10% (w/v) polyacrylamide separating gels with a 3.5% (w/v) acrylamide stacking gel in the absence of urea as described previously [25,31].

## 3. Results and discussion

### 3.1. Analysis of WARP sequences

To identify novel ECM proteins that contain VA-like domains, the mouse EST database at the NCBI was searched with the N-terminal N8 VA-domain of the α3 chain of human collagen VI [3]. We identified several overlapping EST clones that when fully sequenced clearly represent a novel gene that contains a predicted VA-like protein module. The longest EST clone, ui42d08, appeared to be full-length with a start methionine codon at nucleotides 30–32 and an in-frame TGA stop codon at 1275–1277, indicating an open reading frame of 1248 bp with 29 bp of 5′-untranslated region and 1063 bp of 3′-untranslated region (refer to the WARP GenBank entry for cDNA sequence, AAK38350). The 3′-end of the clone includes a poly(A) tail and a predicted polyadenylation site at nucleotides 2279–2285. The full-length WARP cDNA was transcribed and translated in vitro and SDS–PAGE analysis demonstrated a single protein product indicating that no stop codons were present within the open reading frame (data not shown). Since the full-length WARP nucleotide and protein sequences have not been previously reported and the VA-domain is related to, but distinctly different from, those described in existing family members (Fig. 2A), we conclude that this gene is a new member of the VA superfamily. We named this gene WARP, for von Willebrand factor A-domain-related protein.

The human homologue of WARP was identified by searching the genome data with the mouse WARP protein sequence. A match with a predicted protein sequence (hypothetical protein FLJ22215) with very high homology to the mouse WARP was found. The human WARP gene, which maps to chromosome 1p36.3 (contig NT_025635), is composed of four exons each of which encodes a separate protein domain. The first exon (73 bp in size) encodes the signal peptide, exon 2 (558 bp) the VA-domain, exon 3 (279 bp) the first F3 repeat and

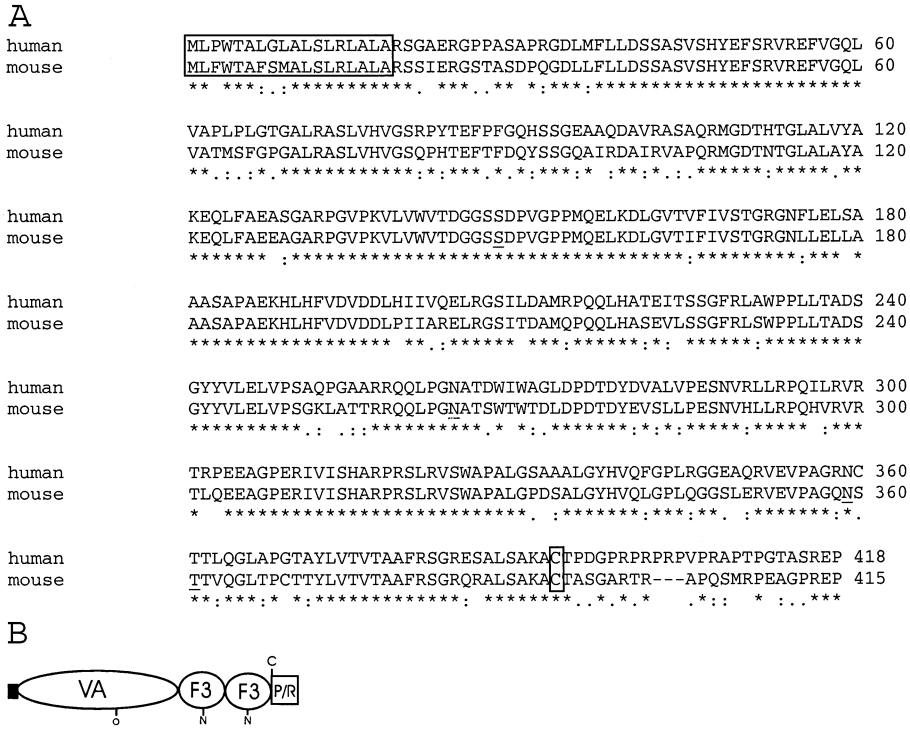


Fig. 1. Sequence and modular organisation of mouse and human WARP protein sequences. A: Deduced amino acid sequence of human and mouse WARP. The predicted N-terminal signal sequence is boxed and the position of potential N-linked ($Asn^{264}$ and $Asn^{359}$) and O-linked ($Ser^{148}$ and $Thr^{361}$) glycosylation sites conserved in both sequences are underlined. The conserved C-terminal cysteine residue ($Cys^{393}$) available for disulfide bond formation is boxed. Alignments were performed using CLUSTALW (http://www.ch.embnet.org/software/ClustalW.html) [41]. Sites where amino acids are identical in both sequences are marked with an asterisk, conserved substitutions are marked with a colon and semi-conserved substitutions with a full-stop. B: The modular structure of WARP is shown using standard symbols to represent conserved ECM protein modules [42]. VA, VA-domain; F3, fibronectin type III repeat; P/R, proline- and arginine-rich segment. Approximate positions of N- and O-linked glycosylation sites and Cys residue, conserved in both mouse and human sequences, are indicated.

exon 4 (347 bp) the second F3 repeat, the P/R-rich C-terminal segment and the 3′-untranslated region. These sequences are clearly homologues of each other because they share 79% amino acid identity and if conserved changes are considered, share 95% identity.

The mouse WARP open reading frame encodes a 415 amino acid protein with a predicted molecular weight of 45 kDa although the human sequence is slightly larger with a three amino acid (PRP) insertion in the C-terminal domain (Fig. 1A). Both homologues contain an 18 amino acid signal sequence with a cleavage site between $Ala^{18}$ and $Arg^{19}$ as indicated by signal sequence prediction programme SignalP (v2.0) (http://genome.cbs.dtu.dk/services/SignalP-2.0) [32] (not shown). The signal sequence is followed by a VA-domain of approximately 200 amino acids with a putative MIDAS motif [13] and three potential O-linked sites at $Ser^{148}$, $Thr^{362}$ and $Thr^{401}$, as predicted by NetOGlyc software (http://genome.cbs.dtu.dk/services/NetOGlyc) [33] although only the first two are conserved in the human sequence (Fig. 1A). Adjacent to the VA-domain are two F3 repeats of approximately 80 amino acids in length each containing a potential N-linked glycosylation site at $Asn^{264}$ and $Asn^{359}$ that fits the consensus sequence NxS/T. The C-terminus at the end of the second F3 repeat is 21 amino acids in length (24 in the human sequence) and is rich in proline and arginine residues but did not show homology to any other protein by extensive database searching. The domain structure of the WARP proteins is shown in Fig. 1B.

### 3.2. Similarity of WARP to other ECM proteins

The protein sequences of the two domains present in WARP (VA and F3) were used to search the non-redundant and conserved domain databases at NCBI. A high degree of amino acid similarity exists between the WARP VA-domain and those found in other ECM proteins with most similarity to VA-domains present in the FACIT collagens XII, XIV [34] and the recently described FACIT collagens XX [8] and XXI [9], the matrilins [10] and cochlin [11] (Fig. 2A). The amino acids within the MIDAS motif which are critical for ion binding, $Asp^{40}$, $Ser^{42}$, $Ser^{44}$, $Thr^{113}$ and $Asp^{144}$, are conserved in both mouse and human WARP although biochemical and crystallographic studies are required to directly demonstrate a functional MIDAS motif. In addition, the overall arrangement of α-helices and β-sheets that form the important secondary structural framework [17] shared between all VA-like domains is conserved in WARP. The two F3 repeats are less conserved than the VA-domain, although the overall framework of seven hydrophobic strands that form the β-sandwich typical of F3 repeats is conserved [35] (Fig. 2B). The first F3 repeat, F3-1, is most similar to those found in tenascins and collagen XIV and F3-2 is most similar to those in collagen VII and the FACIT collagens (data not shown).

### 3.3. WARP mRNA is expressed in mouse chondrocytes

The sites of WARP mRNA expression were determined by RT-PCR analysis using primers specific for WARP and a control, HPRT. cDNA template that was made from RNA

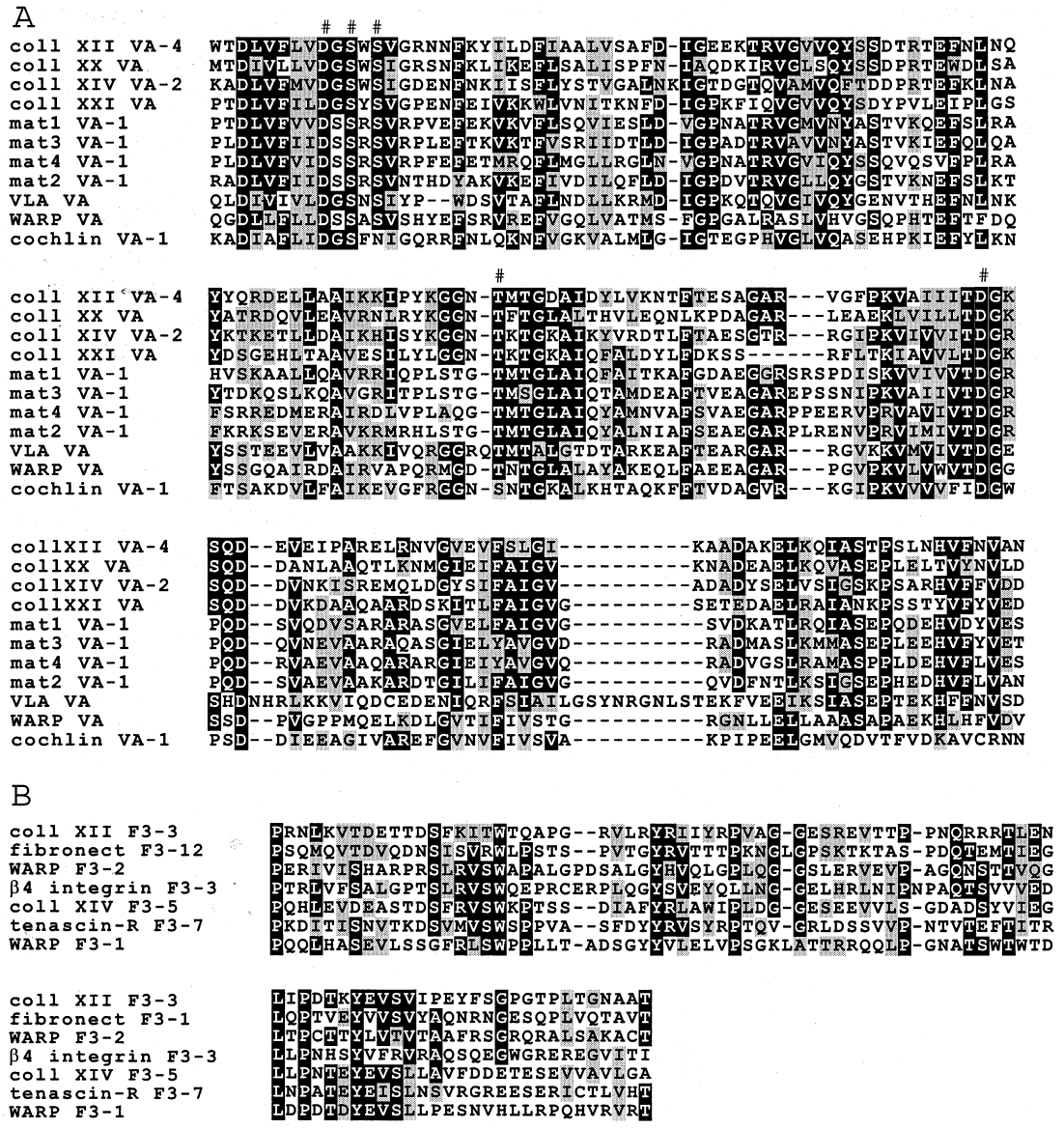


Fig. 2. Alignment of VA-domain and F3 repeats of WARP with homologous domains in other ECM proteins. Identical positions are shown within dark boxes and conserved substitutions in grey boxes. Alignments were performed using CLUSTALW (http://www.ch.embnet.org/software/ClustalW.html) [41] with some changes performed visually and shaded using BOXSHADE (http://www.ch.embnet.org/software/BOX_form.html). A: Alignment of VA-domains from several ECM proteins. Sequences are mouse matrilin-2 (GenBank accession number NP_058042, amino acids 55–221), matrilin-4 (NP_038620, 34–200), matrilin-3 (NP_034900, 76–242), matrilin-1 (NP_034899, 43–209), and WARP (AAK38350, 32–195), and chicken collagen XIV (S78476, 1040–1204), collagen XX (AAK58847, 239–302), and cochlin (O42163, 160–323), and human collagen XII (NP_004361, 138–301), collagen XXI (AAL02227, 35–196), and VLA-1 α-integrin (P56199, 142–316). Conserved amino acids important for the MIDAS motif are indicated (#). B: Alignment of F3 repeats from a sample of ECM proteins. Sequences are mouse WARP F3-domain 1 (AAK38350, 215–301) and 2 (308–394), human collagen XII (NP_004361, 726–810), β4-integrin chain (NP_000204, 1461–1548), fibronectin (P11276, 1635–1720) and tenascin R (2211329A, 867–951), and chicken collagen XIV (S78476, 627–711).

was isolated from various mouse tissues including heart, skeletal muscle, testis, brain and lung, and cell lines including Mov13 fibroblast, MC3T3 osteoblast, 3T3 fibroblast, C2C12 myoblast cell lines, and primary cells including mouse rib chondrocytes and skin fibroblasts (Fig. 3A). To control for variation between RT reactions, WARP and HPRT were amplified in separate reactions using the same template cDNA. Following 36 cycles of amplification, a WARP PCR product was present in chondrocyte RNA (upper panel, lane 6) but not in any other tissue or cell line. The presence of a band representing HPRT in all lanes (lower panel) indicates that for all samples the starting RNA was intact and the RT reactions were successful. To confirm WARP expression in chondrocytes, mRNA from chondrocytes isolated from newborn rib cartilage was subjected to Northern blot analysis (Fig. 3B). Following hybridisation to a WARP cDNA probe, a band representing WARP was detected in chondrocytes (lane 1) but not in Mov13 fibroblasts, MC3T3 osteoblasts and C2C12 myoblasts. WARP migrates as a 2.3 kb mRNA which is in agreement with the size of the full-length WARP cDNA which is 2308 bp in length. We conclude that WARP is expressed highest in chondrocytes and at a much lower level in

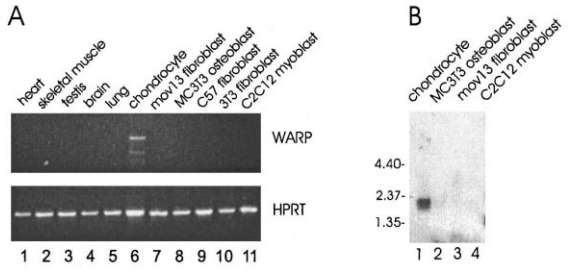


Fig. 3. Expression of WARP mRNA in mouse tissues and cell lines. A: RT-PCR analysis of WARP mRNA expression. Total RNA was isolated from mouse tissues (lanes 1–6) and cell lines (lanes 7–11), treated with DNase to remove contaminating genomic DNA, and added to an oligo dT-primed RT reaction followed by PCR using primers specific for WARP (upper panel) and HPRT (lower panel). B: Northern blot analysis of WARP. Poly(A) mRNA isolated from primary mouse chondrocytes (lane 1), MC3T3 osteoblasts (lane 2), Mov13 fibroblasts (lane 3) and C2C12 myoblasts (lane 4) was fractionated on a 1% agarose gel and transferred to nylon membrane. The membrane was probed with [$\alpha$-$^{32}$P]dCTP-labelled WARP cDNA fragment and exposed to X-ray film. The migration position of RNA markers in kb is indicated on the left.

other tissues and cell lines although a more detailed immunochemical analysis is required to determine whether WARP is chondrocyte-specific.

### 3.4. Expression of WARP in transfected cells

To determine whether the predicted signal sequence is functional in directing WARP secretion from cells, and to determine if the putative N-glycosylation sites are utilised, a WARP cDNA expression construct with a poly-His-tag inserted between the signal peptide and VA-domain was transfected into 293-EBNA cells. The stably transfected cells were labelled overnight with [$^{35}$S]methionine and immunoprecipitated with anti-His antibodies. No material was immunoprecipitated from untransfected 293-EBNA cells (Fig. 4, lanes 1 and 2) indicating that no endogenous proteins are recognised by the anti-His antibody. In cells transfected with the His-WARP cDNA, His-tagged WARP protein migrated as an approximately 48 kDa band in both cell layer and media fractions (lanes 3 and 4). The majority of WARP is detected in the medium during these continuous labelling conditions, suggesting that WARP is efficiently secreted from cells and functions in the ECM environment. When the immunoprecipitated material was resolved under non-reducing conditions, a higher molecular weight form was present indicating that in these cells WARP forms higher-order structures via reducible disulfide bonds. The higher molecular weight species migrates at approximately 102 kDa suggesting that WARP assembles into a disulfide-bonded homo-dimer. Although both human and mouse WARP protein sequences contain two C-terminal Cys residues, only one is conserved in both species at Cys$^{393}$. Site-directed mutagenesis experiments will determine which Cys residue participates in intermolecular disulfide bond formation. When WARP was subjected to N-glycosidase digestion there was a mobility shift to approximately 45 kDa indicating that WARP has one or more N-linked oligosaccharide side chains (lane 6). The molecular weight of the deglycosylated protein is in good agreement with the predicted molecular weight of 45 kDa. There are two possible N-glycosylation sites at Asn$^{254}$ and Asn$^{359}$ located in similar positions in the centre of each of the two F3 repeats in a loop region between $\beta$-strands C and C′ (Fig. 2B). Although we might expect both sites to be equally available for glycosylation, our data do not provide information on whether one or both of these sites is glycosylated in vitro.

In this report we describe the identification and initial characterisation of WARP, a secreted von Willebrand factor A-domain-related protein, expressed by newborn mouse chondrocytes. The presence of a VA-domain identifies WARP as a new member of the VA-domain superfamily of ECM proteins whose other members include matrilins-1, -2, -3, and -4, collagens VI, VII, XII, XIV, XX, and XXI, nine $\alpha$-integrin chains, cochlin and polydom. The domain organisation of WARP is broadly similar to that of the matrilins (for review see [10]) and the FACIT collagens [8,9,34]. The four matrilin family members contain at least one VA-domain, a variable number of epidermal growth factor repeats and a short C-terminal domain. The C-terminus of matrilin-1 forms a coiled-coil structure composed of a heptad repeat of hydrophobic amino acids which directs the formation of matrilin multimers [36]. Multimers are then stabilised by interchain disulfide bonds provided by two Cys residues present within the C-terminus [37]. The C-terminal domain in WARP is not predicted to form a coiled-coil structure of the type found in matrilins because it does not contain a well-defined heptad repeat of hydrophobic residues. However the conserved Cys residue at Cys$^{393}$ at the end of the second F3 repeat, would be in a good position to stabilise WARP oligomerisation and it is tempting to speculate that the C-terminus of WARP is involved in the formation of WARP oligomers.

The modular arrangement of a VA-domain adjacent to F3 repeat domains in WARP is relatively rare in ECM proteins and found only in collagen VII, and the FACIT collagens XII, XIV and XX. Collagens XII and XIV are present in tissues rich in type I collagen and closely associate with the surface of fibrillar collagen [38] and have been proposed to mediate the assembly and growth of collagen fibrils [39,40]. It has been proposed that this regulatory function involves the

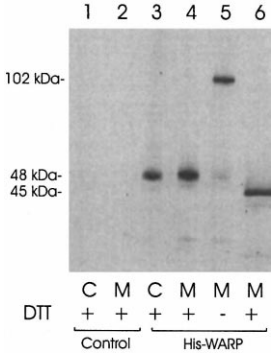


Fig. 4. In vitro expression of WARP. His-WARP cDNA in pCEP4 was transfected into 293-EBNA human embryonic kidney cells and His-WARP protein was immunoprecipitated from cell layer (lanes 1 and 3) and medium (lanes 2, 4–6) fractions of untransfected control 293-EBNA cells (control, lanes 1 and 2) or 293-EBNA cells transfected with His-WARP cDNA (His-WARP, lanes 3–6) using an anti-His antibody. Sample digested with N-glycosidase F following immunoprecipitation is shown in lane 6. All samples were reduced with 20 mM DTT prior to SDS–PAGE except for sample in lane 5. The migration position of protein molecular weight markers is indicated on the left.

lateral alignment of collagen triple helices with the FACIT collagen N-terminal domains that contain the VA and F3 repeats extending out from the fibril surface in a position to interact with other fibrils. Future experiments will explore the possibility that WARP VA- and F3-domains function in a similar manner to mediate an interaction between WARP and cartilage collagen II fibrils.

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## References

[1] Engel, J., Efimov, V.P. and Maurer, P. (1994) Development Suppl. 35–42.
[2] Colombatti, A., Bonaldo, P. and Doliana, R. (1993) Matrix 13, 297–306.
[3] Chu, M.L. et al. (1990) EMBO J. 9, 385–393.
[4] Chu, M.-L., Pan, T., Conway, D., Kuo, H.-J., Glanville, R.W., Timpl, R., Mann, K. and Deutzmann, R. (1989) EMBO J. 8, 1939–1946.
[5] Parente, M.G. et al. (1991) Proc. Natl. Acad. Sci. USA 88, 6931–6935.
[6] Yamagata, M., Yamada, K.M., Yamada, S.S., Shinomura, T., Tanaka, H., Nishida, Y., Obara, M. and Kimata, K. (1991) J. Cell Biol. 115, 209–221.
[7] Trueb, J. and Trueb, B. (1992) Eur. J. Biochem. 207, 557.
[8] Koch, M., Foley, J.E., Hahn, R., Zhou, P., Burgeson, R.E., Gerecke, D.R. and Gordon, M.K. (2001) J. Biol. Chem. 276, 23120–23126.
[9] Fitzgerald, J. and Bateman, J.F. (2001) FEBS Lett. 505, 275–280.
[10] Deak, F., Wagener, R., Kiss, I. and Paulsson, M. (1999) Matrix Biol. 18, 55–64.
[11] Robertson, N.G. et al. (1997) Genomics 46, 345–354.
[12] Gilges, D., Vinit, M.A., Callebaut, I., Coulombel, L., Cacheux, V., Romeo, P. and Vigon, I. (2000) Biochem. J. 352, 49–59.
[13] Lee, J.-O., Rieu, P., Arnaout, M.A. and Liddington, R. (1995) Cell 80, 631–638.
[14] Emsley, J., Cruz, M., Handin, R. and Liddington, R. (1998) J. Biol. Chem. 273, 10396–10401.
[15] Bienkowska, J., Cruz, M., Atiemo, A., Handin, R. and Liddington, R. (1997) J. Biol. Chem. 272, 25162–25167.
[16] Qu, A. and Leahy, D.J. (1995) Proc. Natl. Acad. Sci. USA 92, 10277–10281.
[17] Emsley, J., King, S.L., Bergelson, J.M. and Liddington, R.C. (1997) J. Biol. Chem. 272, 28512–28517.
[18] Ruggeri, Z.M. (1997) J. Clin. Invest. 99, 559–564.
[19] Denis, C., Baruch, D., Kielty, C.M., Ajzenberg, N., Christophe, O. and Meyer, D. (1993) Arterioscler. Thromb. 13, 398–406.
[20] Tuckwell, D.S., Reid, K.B., Barnes, M.J. and Humphries, M.J. (1996) Eur. J. Biochem. 241, 732–739.
[21] Fitzgerald, J., Morgelin, M., Selan, C., Wiberg, C., Keene, D.R., Lamande, S.R. and Bateman, J.F. (2001) J. Biol. Chem. 276, 187–193.
[22] Chen, Q., Zhang, Y., Johnson, D.M. and Goetinck, P.F. (1999) Mol. Biol. Cell 10, 2149–2162.
[23] Kuo, H.-J., Maslen, C.L., Keene, D.R. and Glanville, R.W. (1997) J. Biol. Chem. 272, 26522–26529.
[24] Specks, U., Mayer, U., Nischt, R., Spissinger, T., Mann, K., Timpl, R., Engel, J. and Chu, M.-L. (1992) EMBO J. 11, 4281–4290.
[25] Chan, D., Weng, Y.M., Hocking, A.M., Golub, S., McQuillan, D.J. and Bateman, J.F. (1996) J. Biol. Chem. 271, 13566–13572.
[26] Sudo, H., Kodama, H., Amagai, Y., Yamamoto, S. and Kasai, S. (1983) J. Cell Biol. 96, 191–198.
[27] Schnieke, A., Harbers, K. and Jaenisch, R. (1983) Nature 304, 315–320.
[28] McMahon, D.K., Anderson, P.A., Nassar, R., Bunting, J.B., Saba, Z., Oakeley, A. and Malouf, N.N. (1994) Am. J. Physiol. 266, 1795–1802.
[29] Chan, D., Taylor, T.K. and Cole, W.G. (1993) J. Biol. Chem. 268, 15238–15245.
[30] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[31] Bateman, J.F., Mascara, T., Chan, D. and Cole, W.G. (1984) Biochem. J. 217, 103–115.
[32] Nielson, H., Engelbrecht, J., Brunak, S. and von Heijne, G. (1997) Protein Eng. 10, 1–6.
[33] Hansen, J.E., Lund, O., Engelbrecht, J., Bohr, H., Nielsen, J.O., Hansen, J.-E.S. and Brunak, S. (1995) Biochem. J. 308, 801–813.
[34] Ricard-Blum, S., Dublet, B. and van der Rest, M. (2000) in: Protein Profile, Oxford University Press, Oxford.
[35] Leahy, D.J., Aukhil, I. and Erickson, H.P. (1996) Cell 84, 155–164.
[36] Beck, K., Gambee, J.E., Bohan, C.A. and Bachinger, H.P. (1996) J. Mol. Biol. 256, 909–923.
[37] Haudenschild, D.R., Tondravi, M.M., Hofer, U., Chen, Q. and Goetinck, P.F. (1995) J. Biol. Chem. 270, 23150–23154.
[38] Schuppan, D. et al. (1990) J. Biol. Chem. 265, 8823–8832.
[39] Young, B.B., Gordon, M.K. and Birk, D. (2000) Dev. Dyn. 217, 439.
[40] Koch, M., Bohrmann, B., Matthison, M., Hagios, C., Trueb, B. and Chiquet, M. (1995) J. Cell Biol. 130, 1005–1014.
[41] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) Nucleic Acids Res. 22, 4673–4680.
[42] Bork, P., Downing, A.K., Kieffer, B. and Campbell, I.D. (1996) Q. Rev. Biophys. 29, 119–167.