ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

An in silico based characterization and analysis of A Disintegrin MetalloProteinase with Thrombospodin Motifs (ADAMTS) in Homo Sapiens with respect to Angiognesis and/or Cancer

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Manuscript Info

Manuscript History:

Received: 15 September 2015
Final Accepted: 22 October 2015
Published Online: November 2015

Key words:

ADAMTS, phylogenetic analysis , disorder , cancer, angiogenesis, domain identification.

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Abstract

A Disintegrin MetalloProteinase with Thrombospodin Motifs (ADAMTS) belongs to the family of peptidase. 19 members of ADAMTS family are identified in humans. They play a role in enzyme activities.Out of the 19 ADAMTS present in humans 10 are found to be involved in Angiogenesis and/or cancer.ADAMTS8 is antiangiogenic. The following study deals with analysis of ADAMTS identified in humans. The ADAMTS having disease causing potential; were studied in detail. The identification of their domains and disordered regions was done using various bio-computational tools.

INTRODUCTION

ADAMTS which are also known as A Disintegrin MetalloProteinase with Thrombospodin Motifs. These ADAMTS belongs to the specified metalloproteinase superfamily that has 24 homosapiens genomes and 32 musmusculus genomes[1]. Both ADAM and ADAMTS are protein encoded gene families which have many functional and structural similarities between them[1]. There are 19 members present in the family of ADAMTS in humans[2]. All the ADAMTS family members are having enzyme activities and there is also a subfamily of ADAMTS which are known as ADAMTSL proteins that doesn't have any enzyme activity[3,13].ADAMTS also plays a very important role in various diseases like Arthritis, Angiogenesis and Cell migration[2]. There are 10 ADAMTS out of 19 ADAMTS that plays a major role in the disease such as Angiogenesis and/or cancer[4,14]. The 10 ADAMTS that takes part in cancer are ADAMTS1, ADAMTS2, ADAMTS4, ADAMTS5, ADAMTS8, ADAMTS9, ADAMTS12, ADAMTS13, ADAMTS15, ADAMTS18[4]. In this ADAMTS13 is the only member that has both Pro & Anti-Angiogenic properties in it [5].

2. Materials and Methods:

2.1 Protein sequence retrieval:

The protein sequences of all 19 ADAMTs were retrieved from UniProtKB[6], a high quality manually annotated and non-redundant protein sequence database. The retrieved sequences were used for further analysis.

2.2 Phylogenetic analysis:

The multiple sequence alignment of all 19 variants of ADAMTS family was done using CLUSTAL multiple sequence by MUSCLE 3.8 (MUltiple Sequence Comparison by Log- Expectation) [12], an online tool for aligning multiple sequences. The phylogenetic analysis was done using Phylip Drawtree of SDSC workbench[7] and a cladogram was generated showing relatedness between the sequences of ADAMTS family.

2.3 Physiochemical Analysis:

The various physical and chemical properties such as Number of amino acids, Molecular weight, Theoretical PI, Instability index, Aliphatic index, Aliphatic index and grand average of hydropathicity (GRAVY) was analyzed using Protparam[8] , an expassy's tool for analysing protein properties.

2.4 Secondary Structure Prediction:
The secondary structure prediction analysis was done using SOPMA[9]for all the 20 ADMT's in order to predict the composition of Alpha Helix, Beta Sheets, Turns and Coils from the ADMT sequences and the compositions are represented in a graphical method to identify the protein that is having highest residual property.
2.5 ADMTS in Angiogenesis and/or cancer:
ADAMTS have an eminent role in angiogenesis and/or cancer. ADAMTS13 is found to exhibit both pro- and anti-angiogenic activities. Along with it ADAMTS—ADAMTS1, ADAMTS2, ADAMTS4, ADAMTS5, ADAMTS8, ADAMTS9, ADAMTS12, ADAMTS13, ADAMTS15, and ADAMTS18 are also emerging as an important entity in angiogenesis and/or cancer. The mechanisms of action of above 10 ADAMTS are not shared by all family members of ADAMTS. This shows that not all members of ADAMTS family regulate cancer by influencing angiogenesis[4].
2.6 Domain identification:
The domain identification of disordered ADAMTS was done using SMART(Simple Modular Architecture Research Tool)[10]. It uses basic HMM approach for identification of protein domains. PFAm domains were identified using normal version of SMART for protein sequences.
2.7 Disorder analysis:
The disorder region analysis of the ADAMTS involved angiogenesis and/or cancer was done using DisEmbl 1.5 [11], a tool for determination of intrinsically disordered proteins. It is a widely used tool for disordered or unstructured region prediction.

3. Results and Discussion:

3.1 Phylogenetic analysis:
Phylogenetic analysis was done using PHYLIP DRAWTREE of SDSC workbench. The cladogram (Fig.1) was obtained by CLUSTALW alignment of all variants of ADAMTS family. The cladogram shows relatedness between the various ADAMTS family members. The phylogenetic relatedness was studied form the generated un-rooted tree of PHYLIP.

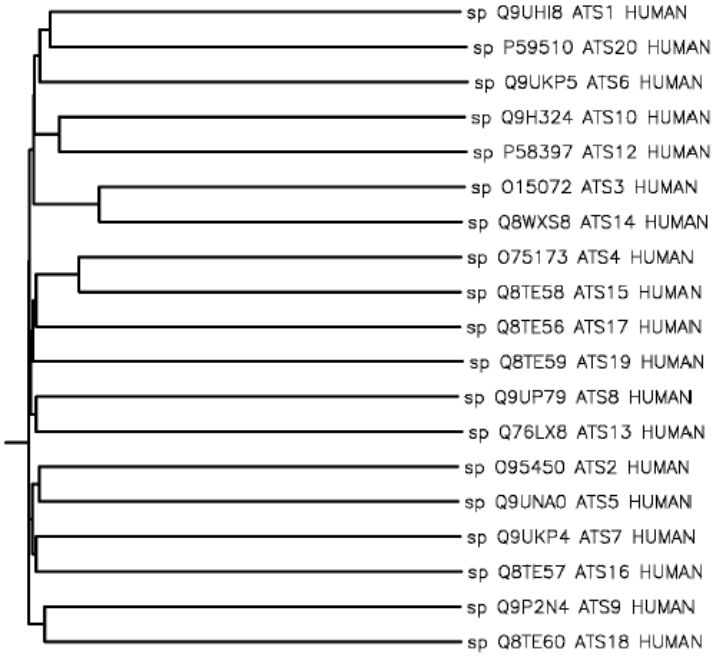


Figure 1: Phylogenetic analysis of 19 ADAMTS

Physiochemical analysis:
The identification of various properties of retrieved ADAMTS sequences was done using Protparam. Detail properties of each member of ADAMTS family are present in Table-1.The aliphatic index of each ADAMTS was analyzed by generating a graph of index against ADAMTS members (fig 2). Identification of aliphatic index can be done directly by analysing the generated graphical representation.

Table-1: ProtParam Analysis/Physiochemical Analysis:

Accession Number	No.of Amino Acid	Molecular Weight	Theoretical PI	Instability Index	Aliphatic Index	Hydrpathic (GRAVY)	Formula
Q9UHI8	967	10537.6	6.40	42.88 Unstable	64.36	-0.480	$C_{4576}H_{7141}N_{1311}O_{1428}S_{62}$
O95450	1211	134754.8	6.76	50.13 Unstable	70.05	-0.498	$C_{5862}H_{9166}N_{1734}O_{1760}S_{83}$
O15072	1205	135602.8	6.76	51.44 Unstable	65.36	-0.618	$C_{5884}H_{9150}N_{1722}O_{1808}S_{83}$
O75173	837	90196.8	8.18	50.14 Unstable	75.78	-0.247	$C_{3969}H_{6202}N_{1148}O_{1164}S_{49}$
Q9UNA0	930	101717.7	9.13	43.16 Unstable	70.40	-0.412	$C_{4455}H_{7006}N_{1312}O_{1319}S_{52}$
Q9UKP5	1117	125272.9	6.24	47.48 Unstable	64.58	-0.521	$C_{5463}H_{8380}N_{1554}O_{1676}S_{82}$
Q9UKP4	1686	184094.8	5.81	59.93 Unstable	64.45	-0.522	$C_{8033}H_{12372}N_{2352}O_{2439}S_{99}$
Q9UP79	889	96460.2	5.78	48.84 Unstable	74.32	-0.369	$C_{4222}H_{6601}N_{1209}O_{1283}S_{51}$
Q9P2N4	1935	216491.1	8.13	46.03 Unstable	57.07	-0.659	$C_{9293}H_{14452}N_{2780}O_{2900}S_{155}$
Q9H324	1103	120874.2	8.34	58.85 Unstable	68.64	-0.387	$C_{5254}H_{8211}N_{1565}O_{1567}S_{78}$
P58397	1594	177675.5	8.25	50.69 Unstable	63.28	-0.575	$C_{7733}H_{12078}N_{2236}O_{2347}S_{117}$
Q76LX8	1427	153604.4	6.96	56.66 Unstable	71.14	-0.269	$C_{6645}H_{10446}N_{1978}O_{2010}S_{106}$
Q8WXS8	1223	133888.1	6.81	49.19 Unstable	66.49	-0.482	$C_{5840}H_{9055}N_{1715}O_{1760}S_{76}$
Q8TE58	950	103286.7	8.94	42.26 Unstable	72.79	-0.342	$C_{4516}H_{7119}N_{1327}O_{1339}S_{58}$
Q8TE57	1224	136203.4	8.99	55.14 Unstable	61.94	-0.501	$C_{5946}H_{9244}N_{1754}O_{1733}S_{99}$
Q8TE56	1095	121126.9	8.47	46.87 Unstable	69.73	-0.473	$C_{5249}H_{8271}N_{1575}O_{1566}S_{82}$
Q8TE60	1221	135167.1	8.91	52.01 Unstable	63.91	-0.449	$C_{5927}H_{9199}N_{1703}O_{1744}S_{92}$
Q8TE59	1207	134047.7	7.80	50.43 Unstable	66.84	-0.503	$C_{5819}H_{9141}N_{1697}O_{1754}S_{97}$
P59510	1910	214721.2	6.98	46.10 Unstable	62.90	-0.530	$C_{9288}H_{14353}N_{2681}O_{2866}S_{165}$

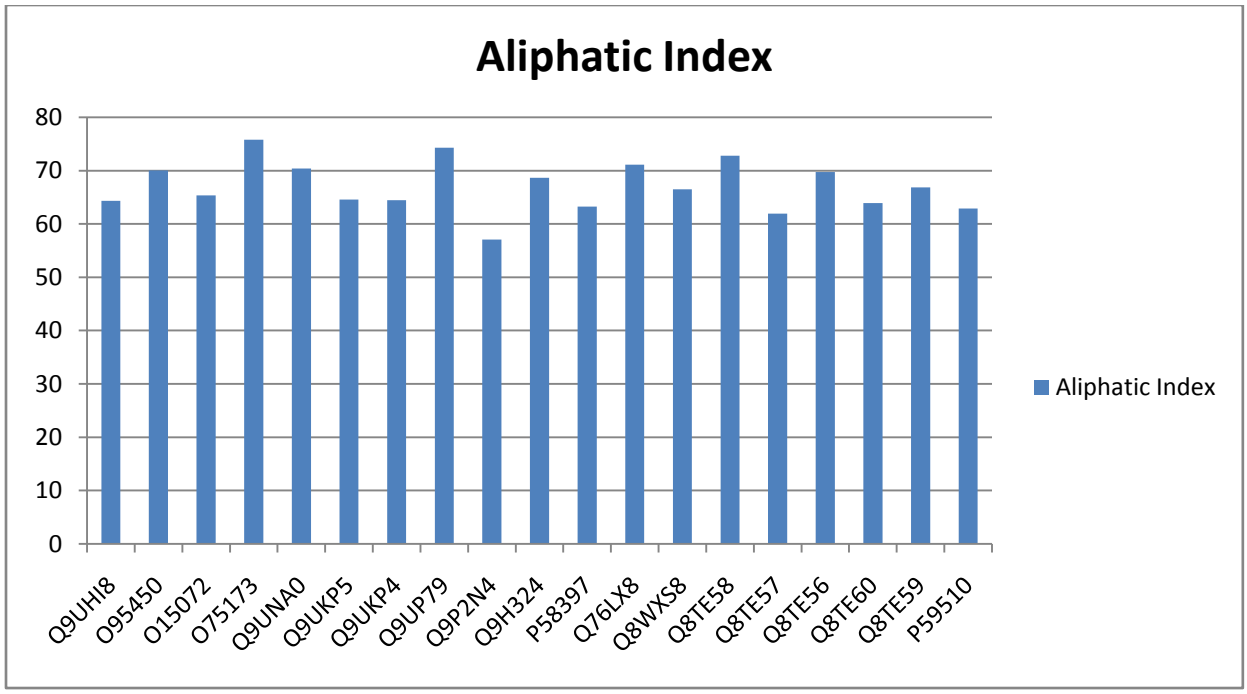


Figure 2: Analysis of aliphatic index by ExPASy's ProtParam tool.

Secondary structure prediction:

The identification of secondary structure of all ADAMTS was done using SOPMA which determines alpha helix, beta turns and coils. The alpha helix, beta turns and coils were represented graphically to identify the highest residual property containing secondary structural elements (fig- 3,4).

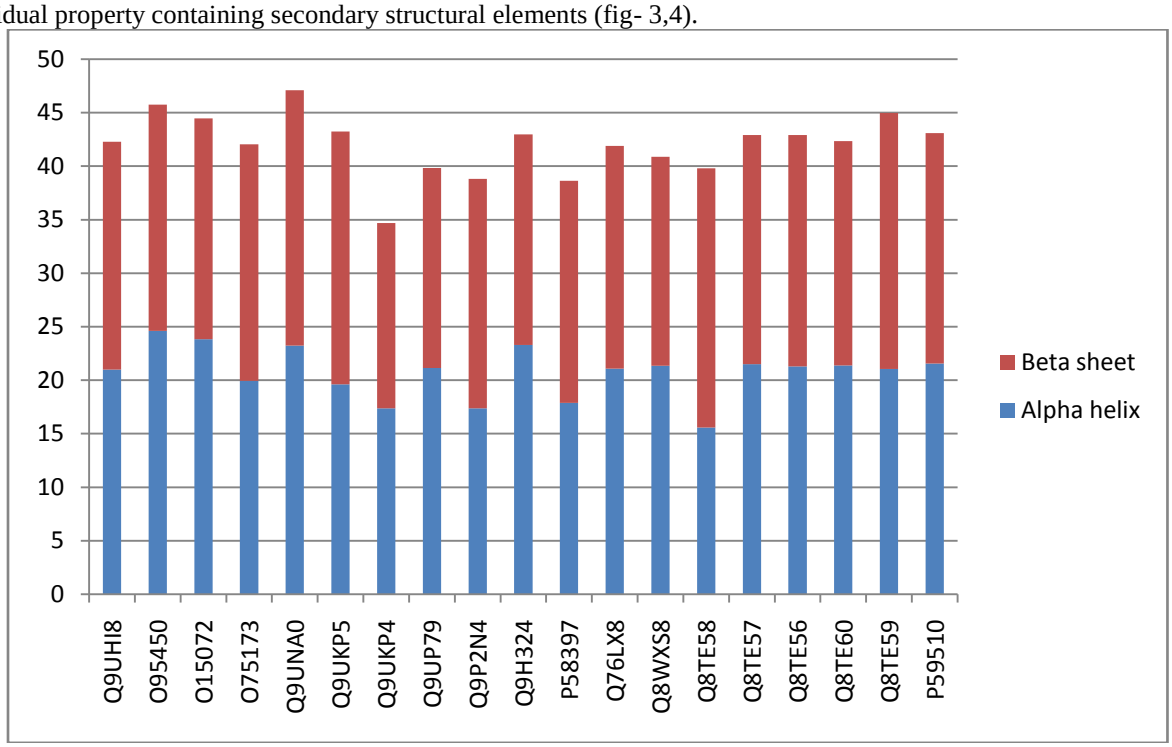


Fig 3: Alpha Helix and Beta Sheets

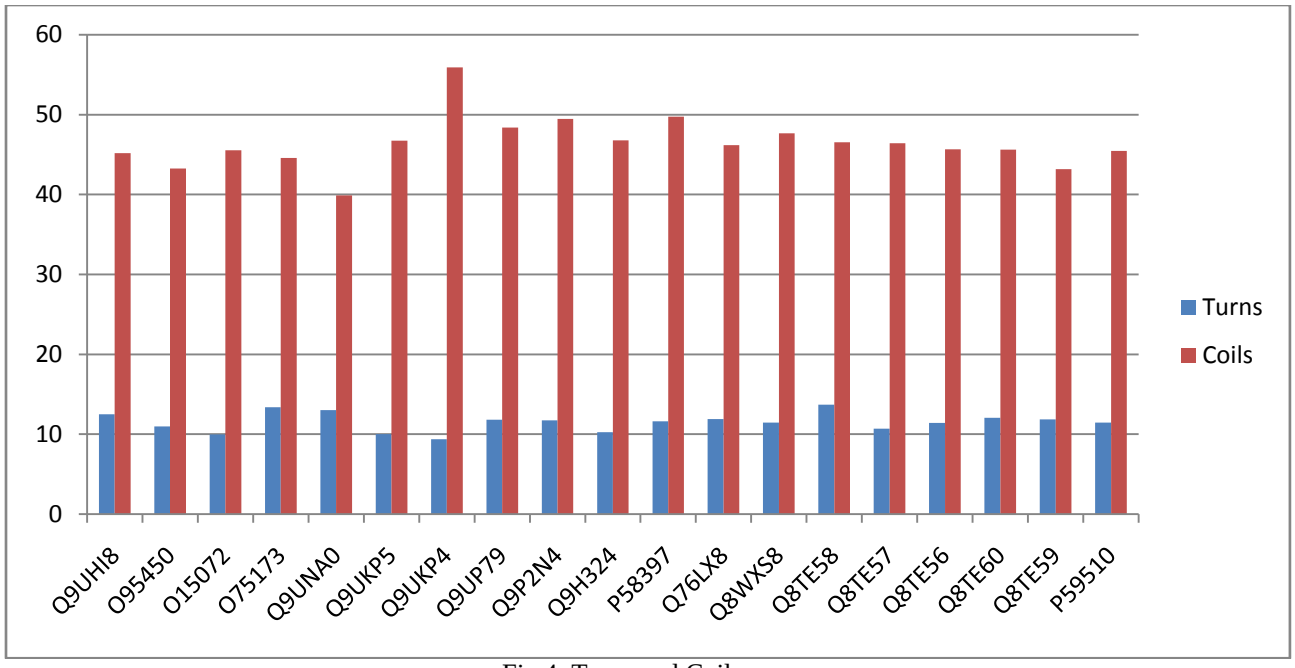


Fig 4: Turns and Coils:

Domain identification:

The domains of ADAMTS involved in angiogenesis and/or cancer were identified using SMART. The TSP domain was comman to all the ADAMTS involved in angiogenesis and/or cancer(fig-5) .TSP stands for Thrombospondin type 1 repeats which bind and activate TGF-beta. This protein plays a role in latelet aggregation, angiogenesis, and tumorigenesis.TSP1 bind to the CD47 receptors. Blocking the binfing activity of TSP1 can result in making normal tissue

immune to cancer radiation therapy and thus it will assists tumour death. Detail information about each ADAMTS was obtained from SMART results(Table-2). The identifiers, alternativing splicing units and the source gene for each ADAMT was also obtained.

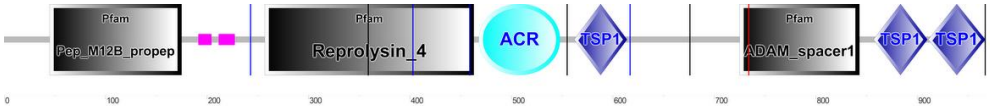
Table 2 : Details of each ADAMTS obtained from SMART results.

ADMTS	Length	Identifiers	Source gene	Alternative splicing
ADMTS1	967 aa	ATS1_HUMAN, Q9UHI8, ENSP00000284984, D3DSD5, Q9NSJ8, Q9P2K0, Q9UH83, Q9UP80	ENSG00000154734	ATS1_HUMAN, ENSP00000429557, H7C206_HUMAN, ENSP00000431065
ADMTS2	1211 aa	ATS2_HUMAN, O95450, ENSP00000251582	ENSG00000087116	ATS2_HUMAN, O95450-2
ADMTS4	837 aa	ATS4_HUMAN, O75173, ENSP00000356975, Q5VTW2, Q6P4Q8, Q6UWA8, Q9UN83	ENSG00000158859	ATS4_HUMAN, Q5VTW1_HUMAN
ADMTS5	930 aa	ATS5_HUMAN, Q9UNA0, ENSP00000284987, Q52LV4, Q9UKP2	ENSG00000154736	-

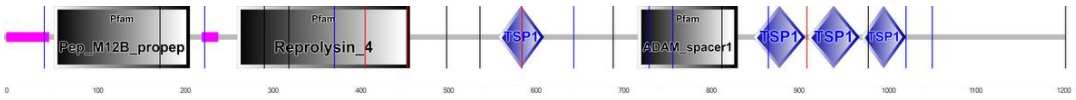
ADMTS8	889 aa	ATS8_HUMAN, Q9UP79, ENSP00000257359, Q9NZS0	ENSG00000134917	-
ADMTS9	1935 aa	ATS9_HUMAN, Q9P2N4, ENSP00000418735, A1L4L0, B7ZVX9, B9ZVN0, Q9NR29, B4E0E4_HUMAN, B4E0E4	ENSG00000163638	Q9P2N4-4, ATS9_HUMAN, H0Y859_HUMAN, C9JWI2_HUMAN
ADMTS12	1594 aa	ATS12_HUMAN, P58397, ENSP00000422554, A2RRN9, A5D6V6, Q6UWL3	ENSG00000151388	ATS12_HUMAN, P58397-3, D6REX0_HUMAN
ADMTS13	1427 aa	ATS13_HUMAN, Q76LX8, ENSP00000456186, ENSP00000360997, Q6UY16, Q710F6, Q711T8, Q96L37, Q9H0G3, B3KWF7_HUMAN, B3KWF7	ENSG00000260099	ATS13_HUMAN, UPI000000107E, Q76LX8-2, Q76LX8-3, E7EV88_HUMAN, ENSP00000454450
ADMTS15	950 aa	ATS15_HUMAN, Q8TE58, ENSP00000299164, Q32MI6	ENSG00000166106	-
ADMTS18	1221 aa	ATS18_HUMAN, Q8TE60, ENSP00000282849, Q6P4R5, Q6ZWJ9	ENSG00000140873	H3BMG1_HUMAN, ATS18_HUMAN, B4DEX3_HUMAN, H3BTZ3_HUMAN

Fig 5 : Identified domains from SMART results

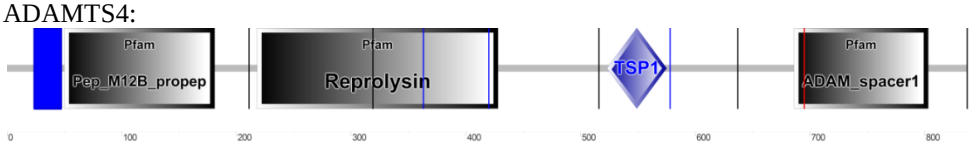
ADAMTS1:



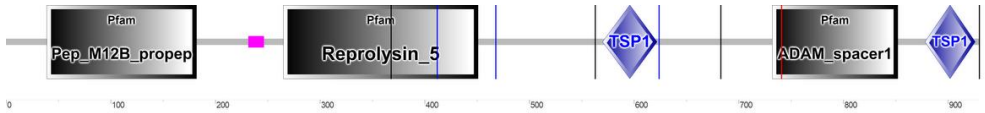
ADAMTS2:



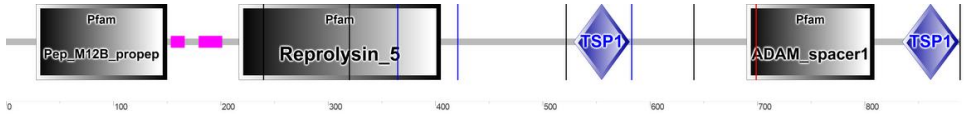
ADAMTS4:



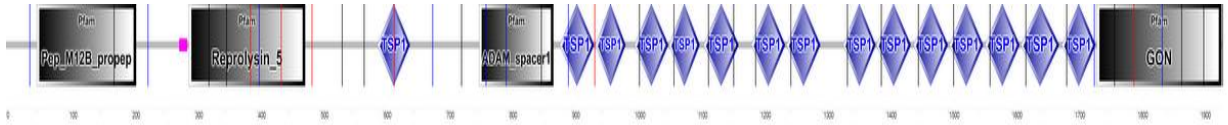
ADAMTS5:



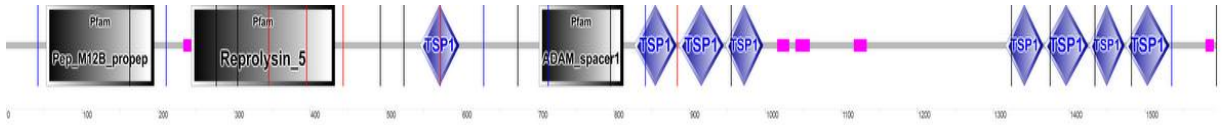
ADAMTS8:



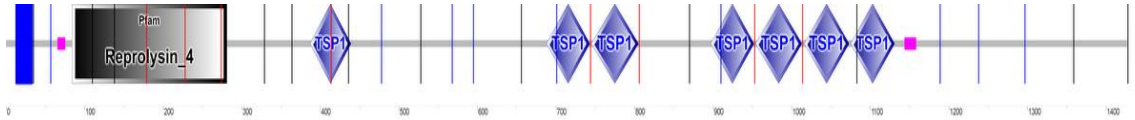
ADAMTS9:



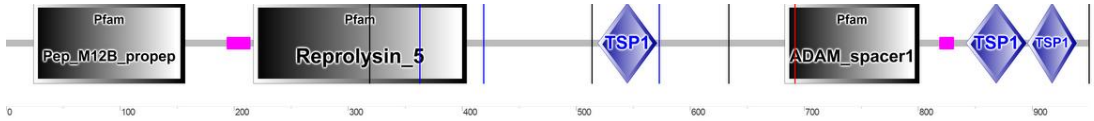
ADAMTS12:



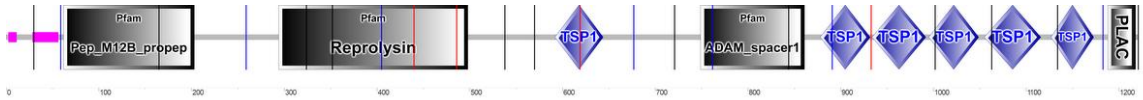
ADAMTS13:



ADAMTS15:



ADAMTS18:



DISEMBL(Disorder analysis):

The intrinsic disorderness of all the ADAMTS which takes part in angiogenesis/cancer are predicted by using the server DISEMBL 1.5 with default parameters. This server gives us the disorderness of the protein by three different criteria's such as Loops/coils, Hot-loops definition, Remark-465 definition. Remark-465 means the missing coordinates in the X-ray structure (table-3). The disorderness can also be seen in a graph where blue lines of the graph represents coils, Green line of the graph represents Remark-465 and Joinframe,Peakwidth and Threshold are the parameters used to interpret the graph (fig-6).

Table-3: Results of the disorderness of the ADAMTS that takes place in angiogenesis/cancer.

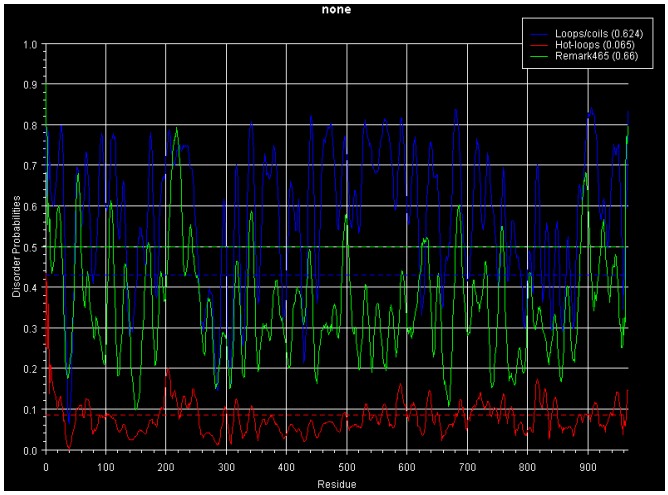
ADMTS	Disordered by Loops/coils definition	Disordered by Hot-loops definition	Disordered by Remark-465 definition
ADMTS1	1-33, 49-135, 154-182, 188-254, 293-301, 314-	1-29, 66-76, 189-221, 226-254, 314-321, 582-600,	1-8, 17-27, 51-59, 106-113, 205-228, 682-691,

	323, 334-351, 358-391, 402-425, 435-449, 455-621, 630-657, 664-699, 704-750, 755-790, 812-823, 829-837, 846-854, 865-872, 881-967	633-642, 686-695, 707-739, 756-764, 813-835, 842-850, 943-955	890-904
ADMTS2	1-8, 21-67, 81-110, 116-163, 210-234, 259-268, 274-282, 301-309, 323-338, 348-401, 413-433, 463-619, 633-652, 673-747, 770-784, 804-814, 837-852, 861-889, 896-1039, 1045-1068, 1077-1190	76-107, 130-147, 247-265, 662-670, 701-738, 774-785, 838-848, 862-875, 913-924, 945-969, 976-986, 1010-1039, 1046-1067, 1148-1159, 1169-1193	78-90, 249-260, 951-971, 1146-1157
ADMTS4	1-29, 46-78, 97-106, 112-145, 157-210, 252-262, 272-285, 295-311, 317-353, 370-410, 416-582, 591-621, 631-708, 716-723, 730-782, 787-817, 830-837	1-13, 197-207, 253-263, 541-579, 799-818, 826-837	1-10, 643-652, 806-814
ADMTS5	14-71, 81-88, 103-142, 164-186, 195-229, 240-254, 346-357, 367-402, 416-436, 445-457, 464-513, 529-643, 655-732, 737-766, 781-794, 804-825, 835-922	24-34, 52-70, 164-185, 243-257, 431-438, 445-453, 532-555, 565-575, 594-605, 679-705, 737-769, 777-787, 851-883, 899-930	23-66, 207-232, 244-255, 856-877
ADMTS8	1-11, 22-51, 66-111, 122-214, 254-262, 275-312, 320-352, 367-392, 397-410, 416-444, 453-469, 476-588, 596-622, 642-653, 661-744, 775-826, 837-889	1-11, 82-95, 157-185, 192-210, 257-264, 278-289, 549-565, 576-586, 667-701, 867-889	1-8, 28-39, 168-207, 513-522, 572-585, 859-868, 875-889
ADMTS9	18-25, 30-37, 52-97, 113-122, 127-142, 154-161, 173-236, 268-292, 327-336, 348-359, 368-383, 395-426, 437-478, 489-682, 693-811, 857-957, 964-1019, 1027-1074, 1083-1128, 1141-1205, 1210-1259, 1269-1404, 1410-1559, 1569-1650, 1655-1752, 1768-1825, 1846-1885, 1893-1935	26-35, 53-68, 199-248, 266-294, 559-570, 611-631, 659-671, 823-843, 905-932, 980-996, 1009-1019, 1027-1059, 1154-1204, 1380-1390, 1412-1421, 1439-1448, 1521-1552, 1569-1609, 1706-1751, 1896-1935	84-98, 192-201, 211-241, 274-283, 976-996, 1010-1017, 1094-1112, 1169-1181, 1229-1237, 1287-1303, 1483-1495, 1539-1550
ADMTS12	23-89, 131-151, 160-215, 228-244, 280-289, 324-338, 348-385, 394-436, 446-489, 502-561, 566-643, 653-668, 675-701, 710-728, 740-790, 802-	72-85, 201-247, 254-263, 328-336, 514-545, 565-585, 598-608, 709-724, 855-879, 971-1028, 1035-1054, 1104-1116, 1124-1140, 1158-1176, 1219-	868-880, 1035-1076, 1095-1116, 1123-1132, 1155-1172, 1278-1288, 1500-1511, 1576-1594

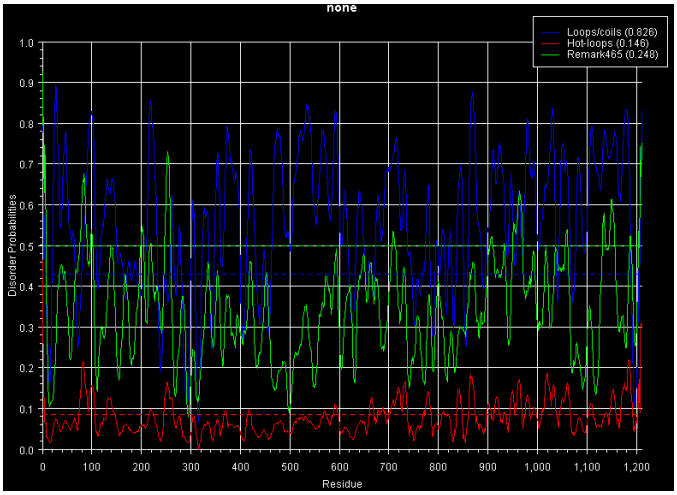
	820, 829-906, 915-1528, 1555-1577	1240, 1363-1375, 1570-1582, 1587-1594	
ADMTS13	1-16, 23-67, 90-99, 110-123, 134-144, 154-172, 182-215, 227-266, 278-319, 334-425, 449-479, 493-539, 545-566, 607-623, 634-654, 662-684, 690-708, 724-767, 774-911, 921-957, 980-1035, 1040-1049, 1058-1212, 1233-1241, 1273-1327, 1345-1356, 1363-1372, 1378-1387, 1409-1427	1-12, 161-169, 384-396, 549-567, 608-624, 632-656, 776-784, 934-944, 980-988, 1059-1067, 1199-1208, 1261-1282, 1411-1427	1-8, 57-73, 288-296, 389-403, 449-462, 722-732, 808-821, 875-885, 936-945, 1181-1192, 1417-1427
ADMTS15	12-52, 63-113, 122-216, 253-262, 273-285, 300-311, 319-374, 386-405, 415-444, 450-502, 507-579, 588-595, 603-724, 731-738, 756-780, 785-950	10-37, 185-220, 254-262, 278-288, 512-526, 543-556, 656-680, 763-819, 928-950	14-22, 144-154, 163-172, 198-210, 569-580, 804-817, 830-841, 852-874
ADMTS18	10-23, 47-62, 67-90, 109-119, 128-140, 145-157, 167-203, 214-242, 250-294, 306-313, 327-337, 349-361, 375-384, 393-427, 437-475, 487-533, 544-649, 656-686, 696-752, 757-778, 799-839, 850-879, 885-895, 902-1055, 1064-1221	75-86, 148-157, 249-295, 300-315, 912-924, 991-1001, 1021-1038, 1069-1112	82-93, 153-161, 232-253, 277-291, 946-956, 1023-1035

Fig 6: Disorder Prediction graphs of all the 10 ADAMTS that taks part in angiogenesis and/or cancer.

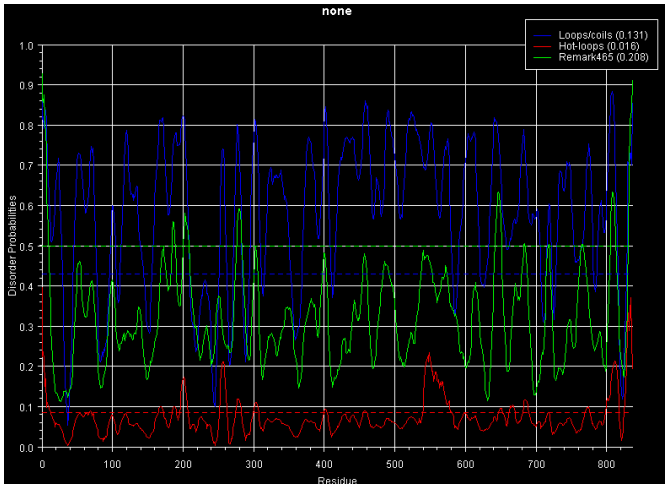
ADAMTS 1



ADAMTS2



ADAMTS 4



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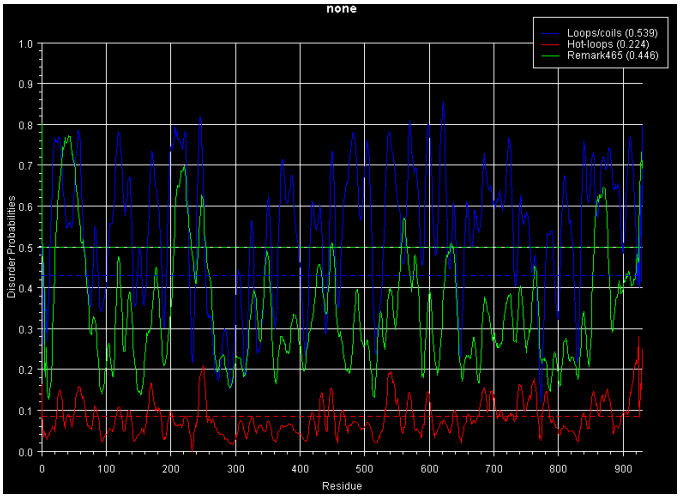
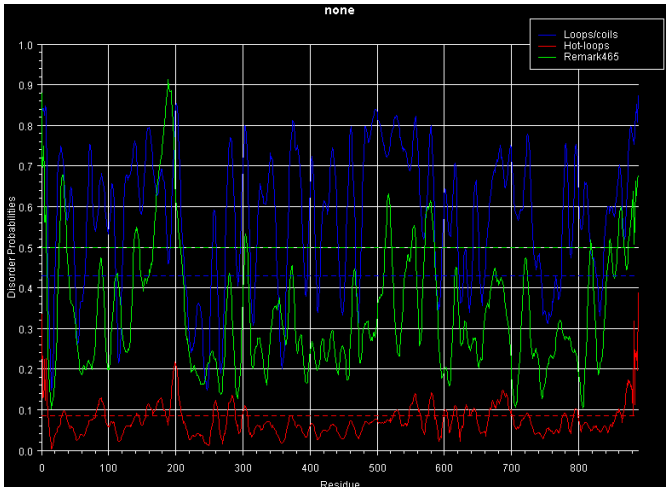


ADAMTS 8

ADAMTS9



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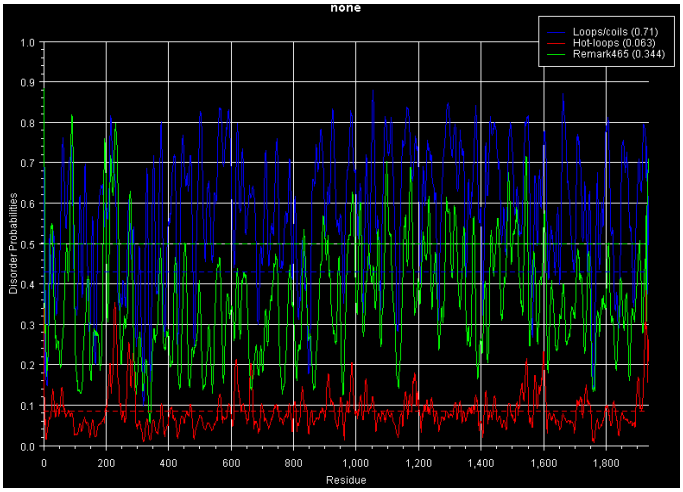

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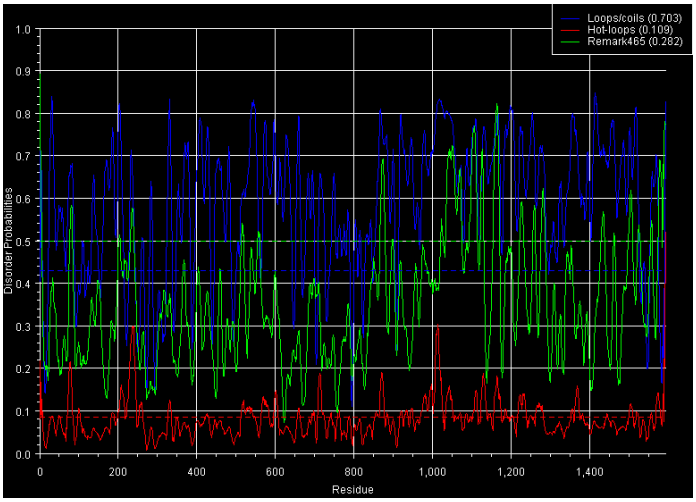

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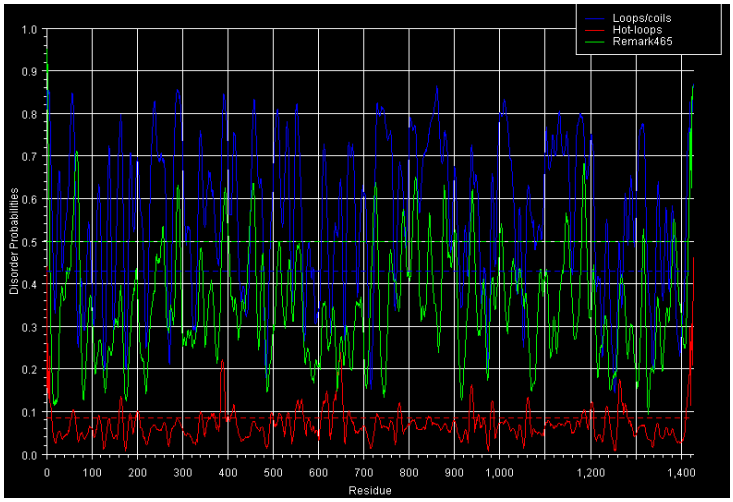

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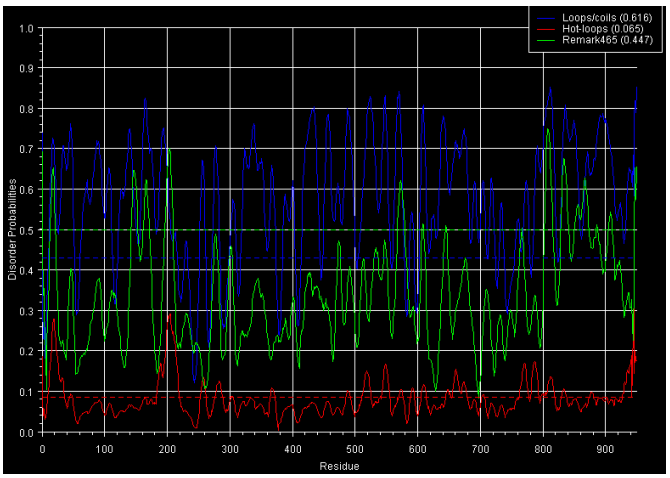

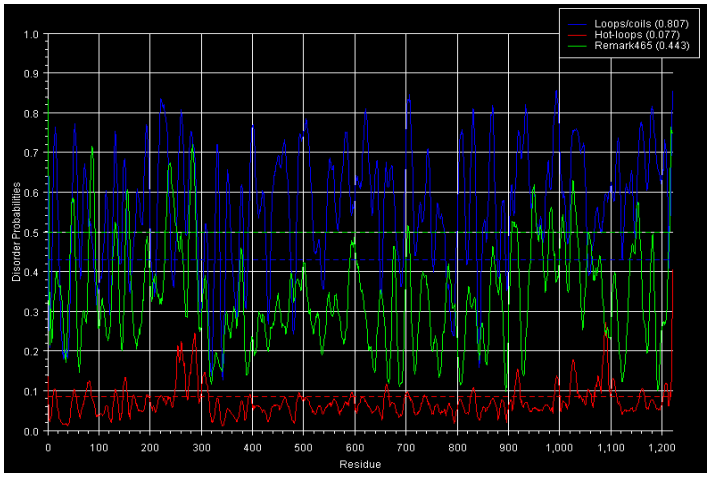

Conclusion:

The detail analysis of ADAMTS in Homo sapiens was done. The phylogenetic analysis depicted the evolutionary relatedness between all the 19 ADAMTS identified in humans. TSP1 a common domain was identified in all the proteins involved in disease characterization. Intrinsically disordered regions were identified for each member of ADAMTS involved in angiogenesis and/or cancer.

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