Comparative analysis of human matrix metalloproteinases: Emerging therapeutic targets in diseases

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Abstract:
The identification of specific target proteins for any diseased condition involves extensive characterization of the potentially involved proteins. Members of a protein family demonstrating comparable features may show certain unusual features when implicated in a pathological condition. Advancements in the field of computational biology and the use of various bioinformatics tools for analysis can aid researchers to comprehend their system of work in primary stages of research. This initial screening can help to reduce time and cost of testing and experimentation in laboratory. Human matrix metalloproteinase (MMP) family of endopeptidases is one such family of 23 members responsible for the remodeling of extracellular matrix (ECM) by degradation of the ECM proteins. Though their role has been implicated in various pathological conditions such as arthritis, atherosclerosis, cancer, liver fibrosis, cardio-vascular and neurodegenerative disorders, little is known about the specific involvement of members of the large MMP family in diseases. A comparative *in silico* characterization of the MMP protein family has been carried out to analyze their physico-chemical, secondary structural and functional properties. Based on the observed patterns of occurrence of atypical features, we hypothesize that cysteine rich and highly thermostable MMPs might be key players in diseased conditions. Thus, a plausible grouping of disease responsive MMPs that might be considered as promising clinical targets may be done. This study can be used as a fundamental approach to characterize, analyze and screen large protein families for the identification of signature patterns.

Background:

The past two decades have seen an exponential rise in the accumulation of genomic and proteomic data stored in the form of countless numbers of nucleotide and protein sequences in the data banks. Massive efforts by thousands of research scientists are being done to annotate the structures and functions of proteins in biological organisms. Conversely, the understanding of the function of newly discovered proteins that may potentially play a designated role in normal or diseased conditions is greatly aided by this amassing collection of data [1]. The systematic annotation of these protein sequences with the help of bioinformatics tools is one of the major thrust areas of application biology today. The progress in the field of bioinformatics is marked by the development of numerous tools through which the classification and identification of certain significant proteins has been made systematic and easier, thus saving the time and cost of experimentation by repeated trial and error in the laboratory. Such a prior analysis may also provide a direction to wet laboratory studies and thus help to integrate the fields of *in silico* and experimental work together. Protein families consist of proteins that have evolved during the course of time from a common ancestor and exhibit a threshold level of relationship [2]. MMPs are a family of zinc containing endopeptidases, which is a subset of the metzincin superfamily of metalloproteinases. These regulatory proteases are the extracellular matrix (ECM) remodelers characterized by their substrate specificity to degrade ECM proteins. Based on this, they have been classified as collagenases, gelatinases, stromelysins, matrilysins, membrane type MMPs (MT-MMPs) and other unclassified MMPs [3]. Structurally, MMPs consist of four domains: an amino terminal hydrophobic pro- domain, a Zn^{2+} containing catalytic domain, a flexible hinge region and a carboxy terminal hemopexin-like domain responsible for their substrate specific nature [4]. Activity of MMPs is regulated by Tissue Inhibitors of Matrix Metalloproteinases (TIMPs). Under normal conditions, this control is responsible for maintenance of the ECM. An imbalance in the regulation of activity may thus, disrupt the integrity of ECM [5]. MMPs have been implicated as clinical targets in numerous physiological and pathological conditions, such as arthritis, atherosclerosis, cancer, eye diseases, skin diseases, cardio-vascular and neurodegenerative disorders [6].

Out of the 26 MMPs reported till date, 23 have been identified in humans [7]. Our study reports an *in silico* comparative characterization and analysis of human MMPs using various bio-computational tools, pertaining to their physico-chemical, secondary structural and functional features. Any atypical but significant feature may have various connotations with respect to the role of MMPs in pathological conditions. The aim here is to identify potential disease responsive MMPs that might possibly be implicated for their role in diseases. Moreover, such an in depth knowledge of all human MMPs would greatly aid researchers to identify the MMPs of interest relevant to their respective working systems. This would further set a precedent for similar comparative characterization studies for other large protein families, using the numerous resources from the field of computational biology.

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Methodology:

Protein sequence retrieval:
UniProtKB/Swiss-Prot, a high quality manually annotated and non-redundant protein sequence database, was used to retrieve the complete sequences of the 23 human MMPs [8]. These sequences were used for further analysis using various online bio-computational tools.

Physico-chemical analysis:
The computation of various physical and chemical parameters, such as amino acid composition, molecular weight, isoelectric point (pI), total number of negative and positive charged residues, extinction coefficient, instability index, aliphatic index and Grand Average of Hydropathy (GRAVY), was done using ExPASy's ProtParam tool (http://us.expasy.org/ tools/protparam.html). ExPASy's ProtScale tool was used to analyze the number of codons, bulkiness, polarity, refractivity, recognition factors, hydrophobicity, transmembrane tendency, percent buried residues, percent accessible residues, average area buried, average flexibility and relative mutability (http://us.expasy.org/tools/protscale.html) [9].

Secondary structural analysis:
SOPMA tool (Self-Optimized Prediction Method with Alignment) of NPS@ (Network Protein Sequence Analysis) server was used to characterize the secondary structural features of the proteins such as, alpha helix, 3_{10} helix, Pi helix, beta bridge, extended strand, beta turn, bend region, random coil, ambiguous states and other states [10].

Functional analysis:
The analysis of the MMP motifs was done with the help of Motif Scan tool (http://myhits.isb-sib.ch/cgi-bin/motif_scan) [11]. The SOSUI server prediction yielded the transmembrane regions of the human MMPs, which were further classified as membrane bound and soluble proteins [12].

Results and Discussion:
MMPs are secreted in latent form as pro-MMPs and these zymogens are required to be cleaved for activation. They are found to exhibit pro and active forms, characterized by a difference in molecular weights **(Table 1 see Supplementary material)**. The exceptional behavior of MMP-12, with two active forms (45 kDa and 22 kDa), is because of an internal autolytic processing mechanism causing its carboxy terminal domain to be cleaved from its catalytic domain, thus yielding three products [13].
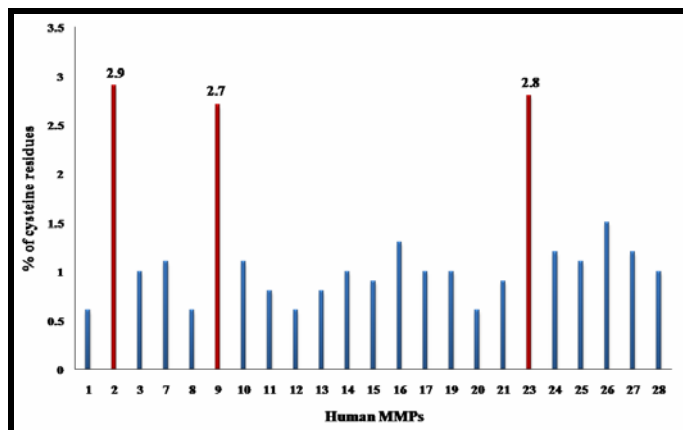

Figure 1: Percentage of cysteine residues in human MMPs computed by ExPASy's ProtParam tool. The amino acid composition of the 23 human MMPs was analyzed. Cysteine showed an abnormal trend as the percentage of cysteine residues in MMP-2, 9 and 23 was found to be exceptionally high as compared to other MMPs.

Analysis of amino acid composition indicates that while the percentage of cysteine residues in majority of MMPs lies in the range of 0.6-1.3%, MMP-2, 9 and 23 show a significant rise with values 2.9, 2.7 and 2.8 percent, respectively **(Figure 1) (Table 2 See supplementary material)**. High percentage of cysteine residues in MMP-2 and 9 might be correlated with presence of cysteine switch motif and role of these MMPs in pathological conditions. These gelatinases have been previously implicated in carcinomas and cardio-vascular

disorders. High cysteine content of the unclassified MMP-23 might be attributed to the presence of cysteine array in its structure. Highly significant presence of cysteine suggests its role as a critical residue for MMP activity and thus these MMPs may be investigated for possible role in diseased conditions. Further analysis of the amino acid composition can help to locate amino acid presence at an unusual level and be correlated with specific pathological conditions [14].
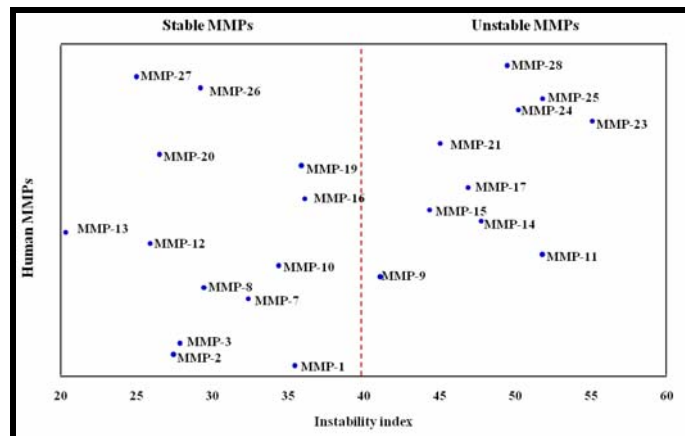

Figure 2: Distribution plot of Stable and Unstable MMPs as computed by ExPASy's ProtParam tool. The instability index classified MMP - 1, 2, 3, 7, 8, 10, 12, 13, 16, 19, 20, 26 and 27 as stable (Instability index <40) and MMP - 9, 11, 14, 15, 17, 21, 23, 24, 25 and 28 as unstable (Instability index >40) metalloproteinases.
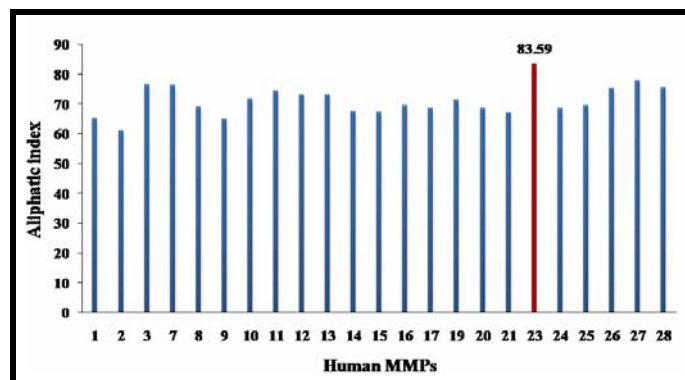
Figure 3: Computation of aliphatic index by ExPASy's ProtParam tool. The aliphatic index indicates the thermostability of proteins. MMP-23 was found to be the most thermostable MMP with a high aliphatic index of 83.59.

Other physico-chemical parameters also signify the behavior of MMPs in different conditions **(Table 3(a) and 3(b) see Supplementary material)**. pI values for majority of MMPs (MMP-7, 12, 14, 15, 16, 19, 20, 21, 23, 24, 25, 27 and 28) lie in the alkaline range (pH>7) while for the others, (MMP-1, 2, 3, 8, 9, 10, 11, 13, 17 and 26) it falls in the acidic range (pH<7). In addition to this, the instability index classifies MMP - 1, 2, 3, 7, 8, 10, 12, 13, 16, 19, 20, 26 and 27 as stable (Instability index <40) and remaining as unstable metalloproteinases (Instability index <40) **(Figure 2)**. Furthermore, aliphatic index, signifying the relative volume of protein occupied by aliphatic side chains helps to study thermo stable properties of an enzyme. It is found to span within a range of 61.09 to 83.59 **(Figure 3)**. Stability of human MMPs in a small range suggests their unstable nature over wide temperature range, though MMP-23 is observed as the most thermostable MMP. Moreover, high extinction coefficients are observed for MMP- 2, 15, 16, 21, 24 and 28, which is correlated with a high concentration of lysine, tryptophan and tyrosine residues in the sequence and may be useful in protein-protein and protein-ligand interaction studies in solution. Hydrophobicity values range from -0.6720 of MMP-19 (most hydrophilic) to 0.4615 of MMP-14 (most hydrophobic).

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 6(1): 23-30 (2011)   24   © 2011 Biomedical Informatics

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Secondary structural analysis indicates a pre-dominance of random coils, followed by α-helices, extended strands and β-turns in 20 MMPs while the extended strands exceed α-helices in MMP-9, 11 and 19 **(Figure 4) (Table 4 see Supplementary material)**. This is useful to predict three dimensional structures of proteins and can help in approximation of some aspects of protein function and their classification into families **[15]**.
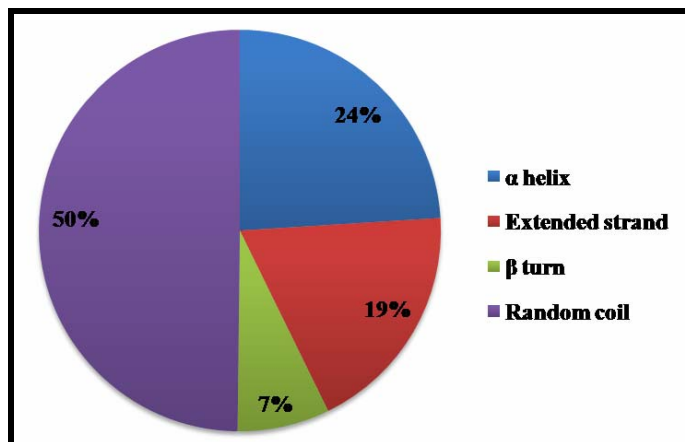
Figure 4: Analysis of secondary structural features through SOPMA. The computation of 23 human MMPs showed a pre-dominance of random coils, followed by α-helices, extended strands and β-turns in 20 MMPs, while extended strands exceeded α-helices in MMP-9, 11 and 19. The figure shows an average plot of the data of all 23 human MMPs.

The Motif Scan tool predicts the presence of a cysteine switch, a zinc protease and a hemopexin motif in human MMPs which have been the subject of discussion in various literatures **(Table 5 see Supplementary material)**. The cysteine switch regulates activity of MMPs via complex formation between cysteine residue of prodomain and zinc atom of catalytic domain **[16]**. The hemopexin domain is an essential part of MMPs performing multiple functions in activation and inhibition, homodimerization and multimerization, binding and cleavage of substrates, attachment to cell surface and degradation of MMPs **[17]**. The primary sequence motif HExxH is present in the catalytic domain of zinc-dependant MMPs. While the two conserved histidine residues coordinate the zinc atom, the glutamic acid residue is a member of the active site of enzyme **[18]**. The zinc binding region signature has been defined as (uncharged)-(uncharged)-H-E-(uncharged)-(uncharged)-H-(uncharged)-(hydro phobic) **[19]**. Furthermore, an extra type II domain of fibronectin is found in MMP-2 and 9 at three regions within the catalytic domain, playing a pivotal role in the collagen binding region of these enzymes. MMP-17, 19, 21, 23, 25, 26 and 28, classified as 'other MMPs', show the presence of zinc protease motif only. Also, transmembrane regions of length 20-23 base pairs are predicted in 14 MMPs using SOSUI server **(Table 6 see Supplementary material)**. MMP-15, 16, 24 and 25 are found to possess two transmembrane regions.

Conclusion:
Intensive characterization and comparative analysis of the MMP family of proteins with the help of numerous bio-computational tools yielded new insights and perspectives which can be used to identify and group MMPs that play a crucial role in a pathological condition. In this study, physico-chemical, secondary structural and functional analysis of the large human MMP family was carried out. The findings through this study may be used by researchers working on MMPs in context of any experimental system. The amino acid composition shows a considerably high percentage of cysteine residues in MMP-23, along with MMP-2 and 9. Also, MMP 23 is found to be the most thermo stable MMP. We, thus, hypothesize that MMP-23, along with MMP-2 and 9, might be a key player in pathological conditions. Further studies with the help of experimental research and testing need to be carried out to validate this proposal. In this manner, certain other groupings and clustering of disease responsive MMPs can be made by analysis of the various parameters of MMPs computed using bioinformatics tools. Additionally, this study may be taken as a prototype for similar in silico investigational studies with regard to other large proteins families, wherein such comparative analysis might aid in giving a direction and help to streamline the conduct of experimentation

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Supplementary material:

Table 1: Functional properties and molecular weights of human MMPs

MMP	Accession No.	Function	Mol. Wt (kDa) Pro form	Active form
MMP-1	P03956	Cleaves collagens- I, II, III,VII,X	55	45
MMP-2	P08253	Remodeling of vasculature, angiogenesis, tissue repair, tumor invasion, inflammation, atherosclerotic plaque rupture, degradation of ECM, myocardial cell death etc.	72	66
MMP-3	P08254	Degrades fibronectin, laminin, gelatins-I, III, IV,V; collagens-III, IV, X, IX & cartilage proteoglycans	57	45
MMP-7	P09237	Degrades casein, gelatin-I,III,IV,V & fibronectin	28	19
MMP-8	P22894	Degrade fibrillar collagens- I,II,III	75	58
MMP-9	P14780	Local proteolysis of ECM, leukocyte migration; cleaves fibronectin, collagen- IV,V	92	86
MMP-10	P09238	Degrade fibronectin, gelatin-I,III,IV,V, collagens-III, IV,V	57	44
MMP-11	P24347	Progression of epithelial malignancies	51	44
MMP-12	P39900	Tissue injury & remodeling, significant elastolytic activity	54	45, 22
MMP-13	P45452	Degrades collagen-I; may be involved in tumoral process	60	48
MMP-14	P50281	Specifically activate progelatinase A; trigger invasion by tumor cells on the tumor cell surface	66	56
MMP-15	P51511	Degrades ECM components; may activate progelatinase A	72	50
MMP-16	P51512	Degrades collagen-III & fibronectin; activates progelatinase A; ECM remodeling of blood vessels	64	52
MMP-17	Q9ULZ9	Degrades fibrin; may be involved in the activation of membrane-bound precursors of growth factors or inflammatory mediators	57	53
MMP-19	Q99542	Degrades aggrecan, collagen-IV, laminin, nidogen, nascin-C isoform, fibronectin & type I gelatin; May play role in neovascularization or angiogenesis	54	45
MMP-20	O60882	Degrades amelogenin, aggrecan & cartilage oligomeric matrix protein (COMP)	54	22
MMP-21	Q8N119	May function in tumor progression & embryogenesis; cleaves alpha-1-antitrypsin	62	49
MMP-23	O75900	Degrades ECM components	28	19
MMP-24	Q9Y5R2	Activates progelatinase A; may cleave proteoglycans, fibronectin	57	53
MMP-25	Q9NPA2	May activate progelatinase A	34	28
MMP-26	Q9NRE1	May hydrolyze collagen-IV, fibronectin, fibrinogen, beta-casein, type I gelatin & alpha-1 proteinase inhibitor; activates progelatinase B	28	19
MMP-27	Q9H306	Degrades ECM components	N.D.	N.D.
MMP-28	Q9H239	Degrades casein; could play role in tissue homeostasis & repair	56	45

Table 2: Amino acid composition of human MMPs (in %)

MMP	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
MMP-1	5.8	5.1	4.7	7.0	0.6	4.9	5.3	7.5	3.4	3.8	6.8	6.4	2.1	7.7	6.4	4.5	5.3	1.7	4.7	6.2
MMP-2	7.1	3.9	3.6	8.2	2.9	2.9	5.0	10.0	1.5	3.8	7.1	6.8	1.8	7.1	6.7	4.4	6.4	2.3	4.5	3.9
MMP-3	6.1	4.8	3.4	7.5	1.0	1.9	6.3	6.7	2.7	4.4	9.0	6.9	1.5	6.7	7.8	6.1	5.7	1.7	3.6	6.3
MMP-7	6.7	4.5	4.1	6.0	1.1	3.4	4.5	9.7	3.0	4.1	10.1	6.4	3.4	4.1	6.0	7.1	4.9	1.9	4.1	4.9
MMP-8	6.4	5.1	5.4	5.8	0.6	5.6	4.7	7.1	2.1	5.4	7.7	4.7	1.7	7.9	6.6	7.1	5.1	1.7	5.1	4.1
MMP-9	7.2	6.9	2.0	7.6	2.7	3.7	4.1	9.5	2.0	2.0	8.8	3.1	1.3	6.2	8.3	6.2	7.1	2.0	3.8	5.5
MMP-10	7.4	4.2	3.2	6.9	1.1	2.7	6.5	6.3	3.2	3.6	9.0	6.1	2.1	7.6	6.9	7.6	4.2	1.9	4.4	5.3
MMP-11	11.3	7.6	0.8	6.8	0.8	4.3	4.3	7.8	3.5	2.3	9.8	2.5	1.0	6.1	10.7	4.1	4.7	3.3	2.9	5.5
MMP-12	5.1	4.5	6.0	6.0	0.6	3.0	4.5	7.4	3.0	5.3	8.5	7.0	2.6	8.1	5.7	6.2	5.1	1.7	4.9	4.9
MMP-13	5.3	4.0	3.8	8.7	0.8	1.7	5.7	7.4	3.8	5.1	9.1	6.2	2.5	6.8	7.0	6.4	4.7	2.1	4.5	4.2
MMP-14	7.4	6.5	3.6	5.3	1.0	3.3	6.5	9.1	2.1	4.0	7.6	5.5	2.6	6.4	7.7	5.2	4.5	2.2	4.3	5.3
MMP-15	6.4	8.4	3.0	6.1	0.9	4.0	5.7	10.2	2.7	2.4	8.7	3.3	2.4	5.1	10.2	3.7	4.0	2.8	3.9	6.1

MMP-16	4.1	5.4	4.1	6.8	1.3	3.3	4.0	8.1	3.0	5.9	6.4	6.4	2.5	5.9	9.2	4.3	6.3	2.6	4.4	5.9
MMP-17	10.6	7.3	1.3	7.6	1.0	3.6	4.0	8.5	3.8	1.7	9.3	2.5	1.7	4.5	10.1	6.5	4.6	2.7	3.5	5.3
MMP-19	6.9	6.1	3.5	5.1	1.0	4.3	5.5	8.1	2.6	2.8	9.8	4.5	2.0	4.9	8.3	6.3	6.1	2.8	4.1	5.3
MMP-20	7.7	4.8	3.5	5.4	0.6	3.1	5.0	7.9	2.9	4.1	7.0	6.8	2.9	6.0	6.4	7.0	6.0	1.4	5.4	6.0
MMP-21	9.1	8.4	3.7	6.2	0.9	3.9	4.4	6.5	2.3	4.4	7.7	3.9	1.2	6.2	9.1	7.4	3.7	3.5	3.9	3.7
MMP-23	10.3	10.5	1.8	3.6	2.8	2.1	3.8	7.7	4.1	2.1	11.8	2.6	1.3	4.4	7.9	5.1	5.1	3.1	3.3	6.7
MMP-24	7.9	7.9	2.9	5.1	1.2	4.0	5.4	8.2	2.6	4.2	7.0	5.3	1.6	4.8	10.1	4.3	4.3	2.6	4.5	5.9
MMP-25	8.5	8.2	1.4	6.2	1.1	5.2	4.1	8.5	2.3	2.0	10.0	3.0	1.6	4.8	12.1	6.2	4.3	2.7	2.8	5.0
MMP-26	4.6	2.3	4.2	6.9	1.5	6.9	2.7	7.3	6.5	5.7	8.4	3.8	1.9	5.4	6.9	7.3	5.4	3.4	3.4	5.4
MMP-27	5.7	5.3	3.9	6.2	1.2	3.3	4.7	7.2	2.7	7.0	8.2	7.0	2.5	8.2	5.7	4.9	6.0	1.8	4.1	4.5
MMP-28	10.0	9.6	2.3	5.2	1.0	4.8	4.6	9.0	2.7	2.1	11.0	3.5	1.0	5.4	7.1	5.4	3.5	3.7	3.3	5.0

Table 3(a): Physico-chemical parameters of human MMPs

MMP	No. of A.A.	M.W (Da)	pI	"-" charged residues	"+" charged residues	Extinction coefficient	Instability index	Aliphatic index	GRAVY
MMP-1	469	54007	6.47	58	54	76905	35.46	65.27	-0.572
MMP-2	660	73882	5.26	87	71	128325	27.46	61.09	-0.446
MMP-3	477	53977	5.77	66	56	69580	27.9	76.65	-0.386
MMP-7	267	29677	7.73	28	29	44015	32.39	76.37	-0.369
MMP-8	467	53412	6.38	49	46	79885	29.47	69.16	-0.451
MMP-9	707	78458	5.69	83	71	118355	41.10	65.13	-0.394
MMP-10	476	54151	5.49	64	49	81040	34.39	71.74	-0.370
MMP-11	488	54618	6.38	54	49	109110	51.79	74.47	-0.327
MMP-12	470	54002	8.75	49	54	78395	25.91	73.23	-0.383
MMP-13	471	53820	5.32	68	48	86540	20.36	73.10	-0.435
MMP-14	582	65884	7.63	69	70	109125	47.75	67.73	-0.464
MMP-15	669	75807	7.03	79	78	143615	44.36	67.34	-0.567
MMP-16	607	69521	8.72	65	72	128730	36.13	69.51	-0.484
MMP-17	603	66653	6.08	70	59	119665	46.90	68.69	-0.454
MMP-19	508	57357	7.22	54	54	108540	35.90	71.44	-0.472
MMP-20	483	54360	8.84	50	56	77365	26.53	68.67	-0.401
MMP-21	569	65015	9.19	60	70	143030	45.06	67.14	-0.547
MMP-23	390	43935	9.94	29	51	85995	55.08	83.59	-0.183
MMP-24	645	73231	9.30	68	85	137210	50.21	68.53	-0.562
MMP-25	562	62554	8.76	58	63	106715	51.82	69.48	-0.499
MMP-26	261	29708	5.96	25	16	63160	29.25	75.44	-0.385
MMP-27	513	59026	8.83	56	63	81165	25.03	77.95	-0.278
MMP-28	520	58939	9.70	51	68	130080	49.47	75.50	-0.444

Table 3(b): Physico-chemical parameters of human MMPs

MMP	No. of codons	Bulkiness	Polarity	Refractivity	Recognition factors	Hydrophobicity	Transmembrane tendency	% buried residues	% accessible residues	Avg area buried	Avg flexibility	Relative Mutability
MMP-1	3.6110	15.1460	19.783	16.5695	87.0000	-0.1110	-0.2305	5.9945	5.5165	125.6165	0.4470	70.2775
MMP-2	3.5000	14.0970	19.827	16.1855	87.1115	-0.2110	-0.7410	5.7780	5.6665	128.3280	0.4340	70.8890
MMP-3	3.5555	15.3020	17.268	16.7545	89.3890	0.3280	-0.4410	6.2830	5.9835	127.0220	0.4365	74.1110
MMP-7	3.7220	14.1535	19.810	15.4755	88.1670	-0.0165	-0.5925	6.1055	6.0390	123.9110	0.4380	78.2225
MMP-8	3.3890	14.7670	17.577	17.3755	87.1110	-0.2220	-0.3885	5.4445	6.0500	129.4615	0.4395	74.7780
MMP-9	3.7220	15.4210	17.065	17.2705	88.7780	0.4000	-0.2795	7.0610	5.6335	123.6555	0.4425	73.2220
MMP-10	3.3335	15.5170	19.693	18.9495	88.4445	0.2115	-0.4760	6.1720	6.0280	133.1720	0.4185	76.0000
MMP-11	3.6665	15.9240	17.456	16.6195	87.8890	-0.1220	-0.2640	6.0335	5.3280	127.3445	0.4305	71.1110
MMP-12	3.5000	14.2855	16.941	16.5455	88.5000	-0.1615	-0.2895	5.5610	5.6220	128.8555	0.4475	76.2225
MMP-13	3.5555	14.4015	19.529	17.6950	88.6665	-0.1055	-0.5055	6.1725	5.6275	129.4775	0.4365	80.0555
MMP-14	3.6110	15.3545	17.051	16.1120	89.1110	0.4615	-0.2935	7.0445	5.5890	129.2110	0.4460	73.6110
MMP-15	3.7780	14.9395	17.583	16.5930	87.2220	0.0725	-0.3135	6.3275	5.3445	126.7835	0.4450	74.2780
MMP-16	3.3890	15.2295	17.297	16.7755	88.1670	0.0835	-0.5865	6.4170	5.4665	128.0390	0.4405	74.2775
MMP-17	3.8335	15.1760	17.463	15.7685	88.5000	0.0000	-0.3615	6.5720	5.4110	123.0110	0.4450	69.7225
MMP-19	3.5000	14.2875	22.683	15.5570	88.3335	-0.6720	-0.7225	6.1835	5.1780	123.2445	0.4410	70.5000
MMP-20	3.5000	14.3375	22.541	16.4980	89.7775	-0.0500	-0.5680	5.8335	5.6000	127.3500	0.4325	73.1110
MMP-21	3.8335	14.6015	19.892	18.0500	88.2775	-0.5500	-0.6645	5.9665	5.7555	129.3940	0.4415	73.0000
MMP-23	3.5555	15.3540	17.265	16.5920	88.2225	0.3665	-0.2880	6.9280	5.3500	125.1945	0.4280	74.6665
MMP-24	3.7225	14.9800	22.630	15.5350	88.0555	-0.1445	-0.5225	6.3275	5.4775	126.6665	0.4450	72.6665

MMP-25	3.9445	15.1620	16.949	17.4410	88.7775	0.4335	-0.1095	6.9330	5.6720	127.9275	0.4375	70.3890
MMP-26	3.3335	15.4225	14.731	16.3125	89.0555	0.1445	-0.1935	6.0445	5.3555	126.4890	0.4360	74.3335
MMP-27	3.2780	15.6320	17.096	17.2455	87.1665	0.3000	-0.1730	6.0000	5.1500	130.9555	0.4340	74.4445
MMP-28	3.7220	14.2075	20.412	16.7435	88.5555	-0.5500	-0.6835	5.7720	5.1555	127.7220	0.4420	70.1110

Table 4: Secondary structural features of human MMPs (in %)

MMP	α helix	3_{10} helix	Pi helix	β bridge	Extended strand	β turn	Bend region	Random coil	Ambigous states	Other states
MMP-1	23.03	0	0	0	21.54	8.32	0	47.12	0	0
MMP-2	23.18	0	0	0	21.52	10.76	0	44.55	0	0
MMP-3	24.74	0	0	0	20.75	9.01	0	45.49	0	0
MMP-7	33.71	0	0	0	14.98	4.87	0	46.44	0	0
MMP-8	23.77	0	0	0	20.56	7.71	0	47.97	0	0
MMP-9	17.40	0	0	0	21.92	8.35	0	52.33	0	0
MMP-10	24.37	0	0	0	20.38	8.61	0	46.64	0	0
MMP-11	17.83	0	0	0	19.26	7.17	0	55.74	0	0
MMP-12	23.19	0	0	0	20.00	7.02	0	49.79	0	0
MMP-13	23.99	0	0	0	21.44	7.64	0	46.92	0	0
MMP-14	23.71	0	0	0	19.24	7.90	0	49.14	0	0
MMP-15	24.96	0	0	0	18.54	8.82	0	47.68	0	0
MMP-16	23.23	0	0	0	20.26	7.74	0	48.76	0	0
MMP-17	18.74	0	0	0	16.09	8.79	0	56.38	0	0
MMP-19	19.69	0	0	0	20.67	8.66	0	50.98	0	0
MMP-20	23.19	0	0	0	17.81	7.45	0	51.55	0	0
MMP-21	22.14	0	0	0	17.40	8.26	0	52.20	0	0
MMP-23	32.82	0	0	0	20.26	7.44	0	39.49	0	0
MMP-24	23.57	0	0	0	16.74	7.13	0	52.56	0	0
MMP-25	24.02	0	0	0	15.66	5.69	0	54.63	0	0
MMP-26	27.97	0	0	0	16.09	3.07	0	52.87	0	0
MMP-27	26.32	0	0	0	20.47	8.38	0	44.83	0	0
MMP-28	25.38	0	0	0	17.12	7.50	0	50.00	0	0

Table 5: Motifs of human MMPs

MMP	Motif found	Motif ID	Description	Start	End
MMP-1	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch	90	97
	HEMOPEXIN	PS00024	Hemopexin domain signature	317	332
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature	215	224
MMP-2	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch	100	107
	HEMOPEXIN	PS00024	Hemopexin domain signature	606	621
	FN2_1	PS00023	Fibronectin type-II collagen-binding domain signature	233	274
				291	332
				349	390
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature	400	409
MMP-3	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch	90	97
	HEMOPEXIN	PS00024	Hemopexin domain signature	329	344
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature	215	224
MMP-7	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch	85	92
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature	211	220
MMP-8	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch	89	96
	HEMOPEXIN	PS00024	Hemopexin domain signature	318	333
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature	214	223
MMP-9	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch	97	104
	HEMOPEXIN	PS00024	Hemopexin domain signature	556	571
	FN2_1	PS00023	Fibronectin type-II collagen-binding domain signature	230	271
				288	329
				347	388
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature	398	407
MMP-10	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch	89	96
	HEMOPEXIN	PS00024	Hemopexin domain signature	328	343
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature	214	223
MMP-11	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch	78	85
	HEMOPEXIN	PS00024	Hemopexin domain signature	332	347

	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		212	221
MMP-12	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch		90	97
	HEMOPEXIN	PS00024	Hemopexin domain signature		321	336
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		215	224
MMP-13	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch		94	101
	HEMOPEXIN	PS00024	Hemopexin domain signature		323	338
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		219	228
MMP-14	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch		91	98
	HEMOPEXIN	PS00024	Hemopexin domain signature.		357	372
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature.		236	245
MMP-15	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch		109	116
	HEMOPEXIN	PS00024	Hemopexin domain signature		408	423
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		256	265
MMP-16	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch		99	106
	HEMOPEXIN	PS00024	Hemopexin domain signature		381	396
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		243	252
MMP-17	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		245	254
MMP-19	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		209	218
MMP-20	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch		98	105
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		223	232
MMP-21	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		280	289
MMP-23	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		208	217
MMP-24	HEMOPEXIN	PS00024	Hemopexin domain signature		418	433
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		279	288
MMP-25	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		230	239
MMP-26	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		205	214
MMP-27	CYSTEINE_SWITCH	PS00546	Matrixins cysteine switch		89	96
	HEMOPEXIN	PS00024	Hemopexin domain signature		318	333
	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		213	222
MMP-28	ZINC_PROTEASE	PS00142	Neutral zinc metallopeptidases, zinc-binding region signature		237	246

Table 6: Transmembrane regions of human MMPs

MMP	Nature of protein	N terminal	Transmembrane region	C terminal	Length	Type
MMP-1	Soluble	-	-	-	-	-
MMP-2	Soluble	-	-	-	-	-
MMP-3	Transmembrane	2	KSLPILLLLCVAVCSAYPLDGAA	24	23	Primary
MMP-7	Transmembrane	1	MRLTVLCAVCLLPGSLALPLPQE	23	23	Primary
MMP-8	Soluble	-	-	-	-	-
MMP-9	Transmembrane	1	MSLWQPLVLVLLVLGCCFAA	20	20	Primary
MMP-10	Transmembrane	2	MHLAFLVLLCLPVCSAYPLSGAA	24	23	Primary
MMP-11	Soluble	-	-	-	-	-
MMP-12	Soluble	-	-	-	-	-
MMP-13	Soluble	-	-	-	-	-
MMP-14	Transmembrane	532	EGGGAVSAAAVVLPVLLLLLVLA	554	23	Primary
MMP-15	Transmembrane	28	RLLPLLLVLLGCLGLGVAAED	48	21	Secondary
		619	EVARTVNVVMVLVPLLLLLCVLG	641	23	Primary
MMP-16	Transmembrane	15	VHHSGVFFLQTLLWILCATVCGT	37	23	Secondary
		561	TVKAIAIVIPCILALCLLVLVYT	583	23	Primary
MMP-17	Soluble	-	-	-	-	-
MMP-19	Soluble	-	-	-	-	-
MMP-20	Soluble	-	-	-	-	-
MMP-21	Transmembrane	1	MLAASIFRPTLLLCWLAAPW	20	20	Primary
MMP-23	Transmembrane	31	LPALVLLARLGAPAVPAWSAAQG	53	23	Primary
MMP-24	Transmembrane	34	RLLLLLLLPALCCLPGAARAAAAA	56	23	Secondary

		598	GSVNAVAVVIPCILSLCILVLVY	620	23	Primary
MMP-25	Transmembrane	6	RLLALLLLLLAPPARAPKPSAQD	28	23	Primary
		541	GRWPAPIPLLLLPLLVGGVASR	562	22	Primary
MMP-26	Transmembrane	2	QLVILRVTIFLPWCFAVPVPPAA	24	23	Primary
MMP-27	Transmembrane	4	LLLLFLFFITFSSAFPLVRMT	24	21	Primary
MMP-28	Transmembrane	1	MVARVGLLLRALQLLLWGHLDAQ	23	23	Primary

*(-) – Does not exist.