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Comparative Analysis And Characterization Of Various Syndromes Associated Human Collagen Proteins Using Computational Tools And Online Servers

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ABSTRACT

Collagen is the most abundant protein in the human body. Collagen forms a major part of connective tissue, which can be described as the supportive tissue of the organs of the body. Defect in collagen causes various syndromes. Here, we report the analysis and characterization studies on various syndromes [Alport syndrome (AS), Ehlers–Danlos syndrome (EDS), Goodpasture's syndrome (GPS) and Stickler syndrome (SS)] associated 13 human collagen proteins retrieved from Swiss-Prot database to provide more detailed description about these proteins. The results of primary structure analysis suggest that the AS, EDS and SS associated collagen proteins are rich in glycine (23% to 28%) and proline (15% to 23%) residues. The GPS associated collagen protein is rich in Glu (9%) and Ser (9%) residues. The computed pI values of collagens P02452, P02461, P20908, P05997 (EDS associated proteins), the collagen Q9Y5P4 (GPS associated protein), the collagens P12107, P13942 (SS associated collagens) reveals that these are acidic (pI<7) in character. The computed pI of all alport syndrome associated human collagen proteins (P29400, P53420, Q01955 and Q14031), the protein P08123 (EDS associated collagen) and the protein P02458 (SS associated collagen) infers that these are basic (pI>7) in character. The number of basic and acidic amino acids in each collagen proteins correlates well with the corresponding pI computed. The low aliphatic index (37-59) of most of the syndromes associated proteins infers that the collagen proteins may become unstable at high temperature. The biocomputed half-life of AS, GPS and SS associated collagen is 30hrs and greater than 20hrs for EDS associated collagens. Expasy's ProtParam classifies the GPS associated collagen as unstable on the basis of instability index (II>40) and all the other collagen proteins as stable (II<40). Secondary structure analysis shows that all the collagens are found to be predominant coil structure content. The SSCP server classifies the GPS associated collagen (Q9Y5P4) as mixed secondary structure class and all the other collagens as irregular secondary structure class proteins. The irregular structure of collagen proteins is due to the rich content of more flexible glycine and hydrophobic proline amino acids. The server SOSUI classifies all the four AS associated collagen proteins and the protein P02458 (SS associated collagen protein) as membrane protein and all the other proteins as soluble proteins. The identified transmembrane regions were visualized and analyzed using helical wheel plot generated by EMBOSS pepwheel tool and it is found that the helical wheel consists of more hydrophobic residues. This is the first report on characterization and comparative studies of AS, EDS, GPS and SS associated human collagen proteins.

KEYWORDS

Alport syndrome; Collagen proteins; Ehlers-Danlos syndrome; Goodpasture's syndrome; In Silico analysis; Stickler syndrome; Proteomics tools.

INTRODUCTION

Collagen is the most plentiful protein in the body and about one third of all our protein is made up of collagen. Collagen forms a major part of connective tissue, which can be described as the supportive tissue of the organs of the body. Collagen acts like glue that allows muscles to stretch and contract. Defect in collagen causes various syndromes such as Alport Syndrome (AS), Ehlers-Danlos syndrome (EDS), Goodpasture's syndrome (GPS) and Stickler syndrome (SS) etc. Alport syndrome (AS)[1-2] is a group of hereditary disorders characterized by progressive deterioration of parts of the kidney known as basement membranes. This deterioration may lead to chronic kidney (renal) disease. Some types of alport syndrome may also cause deafness and ocular changes. Alport Syndrome is caused by mutations in type IV collagen genes[3-4], which is a major constituent of basement membranes[5]. Mutations in one of four collagen genes COL4A3, COL4A4, COL4A5 and COL4A6 cause alport syndrome. These collagen genes are involved in the production of type IV collagen proteins. Mutations in any of these genes disrupt the production, processing, or assembly of type IV collagen proteins. One recent estimate, based on data from Finland, was that the birth prevalence of AS is 1 in 53000[6]. Goodpasture syndrome (GP)[7] is a rare autoimmune disorder in childhood characterized by inflammation of the filtering structures of the kidneys and excessive bleeding into the lungs. Normally, autoimmune syndromes occur when the body's natural defenses against invading organisms begin to attack the body's own tissue, often for unknown reasons. Goodpasture syndrome occurs due to the damage in collagen IV by an immune attack. This attack disrupts the function of attached epithelia and leads to organ impairment. In 1958, Stanton and Tange[8] described nine patients with a pulmonary-renal disorder that they called Goodpasture syndrome after an earlier report in 1919 by Ernest Goodpasture[9]. Goodpasture's syndrome occurs primarily in young men in their late 20s and in men and women over 60 years of age[10]. Ehlers-Danlos syndrome [EDS][11-13], is a rare, heterogeneous group of genetic connective tissue disorder characterized by joint hypermobility (loose joints), easy bruising (contusion), skin extensibility (hyperelasticity or laxity of the skin), tissue fragility (weakness of tissues) and a bleeding diathesis[14]. Structural and genetic mutation studies of the skin in the EDS reveal that the syndrome is a disorders of the collagen fibril[15] due to he mutations in the type I, III and V collagen encoding genes[16-23]. Dr. Van Meekeren originally described EDS in 1682. The syndrome derives its name from reports by Edward Ehlers (a Danish dermatologist) in 1901 and by Henri-Alexandre Danlos (a French physician) with expertise in chemistry of skin disorders, in 1908. According to the Ehlers-Danlos National Foundation, one in 5,000 to one in 10,000 people are affected by some form of EDS. Mutations in one of five collagen genes COL1A1, COL1A2, COL3A1, COL5A1 and COL5A2 cause Ehlers-Danlos syndrome. These collagen genes are involved in the production of type I, III and type V collagen proteins. Mutations in any of these genes disrupt the production, processing, or assembly of type I, III or type V collagen proteins. There are different types of EDS and these were classified into six major types according to signs and symptoms. Stickler syndrome[24] (hereditary progressive arthro-ophthalmopathy) refers to a group of disorders of the connective tissue[25] (collagen) due to a genetic malfunction in the tissue that connects body's organ systems such as bones, heart, eyes, and ears. This disorder is associated with problems of vision, hearing, bone and joint, facial and cleft palate, and heart. Dr Gunnar B Stickler identified it in a family with ocular, orofacial, auditory, and musculoskeletal abnormalities[26]. Mutations in one of three collagen genes COL2A1, COL11A1 and COL11A2 cause stickler syndrome. These collagen genes are involved in the production of type II and type XI collagen proteins. Collagen type II is a homotrimer of three COL2A1 gene products and type XI is a heterotrimer containing one each of the COL2A1, COL11A1 and COL11A2 gene products[27]. Mutations in any of these genes disrupt the production, processing, or assembly of type II or type XI collagen proteins. There are four types of stickler syndrome, three types are reasonably well differentiated and the fourth remains not well understood. Type I stickler syndrome

FULL PAPER

(STL1)[28] is responsible for about 75% of reported cases and presents with a full array of symptoms (i.e., in eye, ear, jaw, cleft and joints). STL1 is also termed as membranous vitreous type. Stickler syndrome type II (STL2)[29] also presents with a full array of symptoms and it is termed as beaded vitreous type. Stickler syndrome type III (STL3)[30] caused by a mutation in the gene, present with a 'Sickler-like' syndrome [also known as Oto-spondylo-megaepiphyseal dysplasia[31] (OSMED)] that affects the joints and ears without involving the eyes[32] STL3 is also termed as nonocular type. Type I, II and type III stickler syndromes (STL1, STL2 and STL3) are caused by the mutations in the COL2A1[33-37], COL11A1[38-40] and COL11A2[41,42] genes respectively. Mutations in other genes[43] may also cause the stickler syndrome because not all individuals with the condition have mutations in one of the three identified genes. Due to these mutations, wrong shaped amino acid is put into the collagen protein, or is missing, and produces defective collagens. Defective collagen (wrong shape) protein molecules or reduced amounts of collagen affect the development of bones and other connective tissues, leading to the characteristic features of stickler syndrome. Stickler syndrome is usually passed from parent to child. The symptoms and severity of stickler syndrome is variable from patient to patient, even within a family. Medical professionals believe that one in 10,000 persons may be affected by stickler syndrome. Many researchers have reported clinical[44], disease mechanism[45] genetics studies[46] on AS, mutation studies[47] and clinical case reports[48] on EDS, molecular studies[49], clinical studies[50, 51] on GPS. The relation between the Goodpasture syndrome and type IV collagen[52, 53] were also reported. The clinical[54, 55] and genetics studies[56] on SS associated collagen proteins were also reported by various researchers. However, the complete sequence analysis, physicochemical characterization and comparative analysis of Alport syndrome (AS), Ehlers-Danlos syndrome (EDS), Goodpasture's syndrome (GPS) and Stickler syndrome (SS) associated human collagen proteins have not been done so far. In this paper we report the analysis and characterization studies on AS, EDS, GPS and SS collagen proteins retrieved from Swiss-Prot database to provide more detailed description about these proteins.

MATERIALS AND METHODS

Sequence retrieval

We retrieved the various syndromes [Alport syndrome (AS), Ehlers-Danlos syndrome (EDS), Goodpasture's syndrome (GPS) and Stickler syndrome (SS)] associated human collagen protein sequences from the manually curated public protein database Swiss-Prot. Swiss-Prot is scanned for the key words 'Alport syndrome, Ehlers-Danlos syndrome, Goodpasture's syndrome and Stickler syndrome'. The search for AS protein sequence yielded 9 protein sequences. In this 4 sequences were human collagen proteins. The search for EDS protein sequence yielded 9 protein sequences. In this 5 sequences were human collagen proteins. The search for GPS protein sequence yielded 7 protein sequences. In this 1 sequence is human collagen protein. The search for SS protein sequence yielded 3 human collagen proteins. We downloaded the 4 AS associated human collagen proteins, 5 EDS associated human collagen proteins, 1 GPS associated human collagen protein and 3 SS associated human collagen proteins in FASTA format and organized a nonredundant data set (TABLE 1). The aim was to analyze, compare and characterize the various syndromes associated human collagen proteins using bioinformatics tools and online servers.

Computational tools and servers

1. Primary structure analysis

The amino acid composition (Supplementary data-1) of various syndromes associated human collagen proteins were computed using the tool CLC free Workbench. 1 (http://www.clcbio.com/index.php?id=28).

2. Hydrophobicity analysis

Percentages of hydrophobic and hydrophilic residues were calculated from the primary structure analysis results and tabulated in TABLE 2.

3. Physico-chemical characterization

The physico-chemical parameters such as theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction

FULL PAPER

TABLE 1: Human collagen protein sequences associated with various syndromes retrieved from swiss-prot database

Accession Number	Sequence Description	Disease
P29400	Collagen alpha 5(IV) chain precursor	Defects cause X-linked alport syndrome
P53420	Collagen alpha 4(IV) chain precursor	Defects cause autosomal recessive alport syndrome and familial benign hematuria
Q01955	Collagen alpha 3(IV) chain precursor	Defects cause autosomal recessive alport syndrome
Q14031	Collagen alpha 6(IV) chain precursor	Defects cause alport syndrome
P02452	Collagen alpha 1(I) chain [Precursor]	Defects cause EDS type I, VII which includes VII-A1, osteogenesis imperfecta type I,II, III and IV and dermatofibrosarcoma protuberans
P08123	Collagen alpha 2(I) chain [Precursor]	Defects cause EDS type VII-A2, osteogenesis imperfecta type I, II, III and IV
P02461	Collagen alpha 1(III) chain [Precursor]	Defects cause EDS type III and IV and Gottron type acrogeria
P20908	Collagen alpha 1(V) chain [Precursor]	Defects cause EDS type I and II
P05997	Collagen alpha 2(V) chain [Precursor]	Defects cause EDS type I and II
Q9Y5P4	Collagen type IV alpha 3 binding protein	Defects cause GP Syndrome
P02458	Collagen alpha 1(II) chain [Precursor]	Defects cause Stickler syndrome type I, Wagner syndrome type II and Kniest syndrome
P12107	Collagen alpha 1(XI) chain [Precursor]	Defects cause Stickler syndrome type II and Marshall syndrome
P13942	Collagen alpha 2(XI) chain [Precursor]	Defects cause Stickler syndrome type III and Weissenbacher-Zweymueller syndrome

TABLE 2: Hydrophilic and hydrophobic residues content

Accession Number	Percentage of hydrophobic residues	Percentage of hydrophilic residues	Net hydrophobic residues content
P29400	50.0	50.0	-
P53420	50.9	49.1	-
Q01955	50.6	49.4	-
Q14031	53.3	46.7	High
P02452	49.6	50.3	-
P08123	52.5	47.4	High
P02461	49.9	50.0	-
P20908	46.0	53.9	Low
P05997	48.1	51.8	Low
Q9Y5P4	42.1	57.8	Low
P02458	50	50	-
P12107	47	53	Low
P13942	48	52	-

coefficient[57], half-life[58-61], instability index[62], aliphatic index[63] and grand average hydrophathy[64] (GRAVY) were computed using the Expasy's ProtParam (http://us.expasy.org/tools/otparam.html) prediction server and tabulated in TABLE 3. The computed theoretical isoelectric point (pI) and the total number of acidic and basic amino acids were compared and shown in TABLE 4.

4. Secondary structure prediction

The tools SOPM, SOPMA[65] and the secondary structural content prediction (SSCP method-I) server[66] were used for the secondary structure prediction, secondary structure class identification and for the computation of percentages of α-helical, β-strand and coiled regions (TABLE 5).

5. Transmembrane regions analysis

The SOSUI[67] server performed the identification of transmembrane regions (TABLE 6). The predicted transmembrane helices were visualized and

FULL PAPER

TABLE 3: Parameters computed using expasy's protparam tool

Accession number	M.Wt	pI	- R	+ R	EC	II	AI	GRAVY
P29400	161043.8	7.71	115	117	46290	36.65	50.02	-0.618
P53420	164095.7	8.9	130	153	82570	32.64	45.95	-0.658
Q01955	161740.4	9.3	119	154	58620	29.7	47.74	-0.624
Q14031	163796.7	9.31	109	143	65920	32.78	59.11	-0.427
P02452	138980	5.66	141	129	51860	30.27	37.9	-0.789
P08123	129553	9.03	110	122	49410	22.57	46.9	-0.662
P02461	138652	6.19	129	122	60350	30.11	37.3	-0.798
P20908	183656	4.94	225	168	91750	33.04	45.3	-0.873
P05997	144817	6.34	142	135	50460	26.05	43	-0.819
Q9Y5P4	70831	5.29	99	74	106060	46.81	70.58	-0.641
P02458	134492.1	8.66	128	135	45660	23.98	38.95	-0.838
P12107	181120.9	5.08	221	175	98570	30.80	45.07	-0.855
P13942	171781.6	5.89	189	173	46510	34.11	49.97	-0.801

M.Wt.-Molecular weight,pI - Isoelectric point, -R - Number of negative residues, +R - Number of positive residues, EC- Extinction coefficient at 280nm, II - Instability Index, AI - Aliphatic Index, GRAVY - Grand Average Hydropathy

TABLE 4: Computed theoretical isoelectric point (pI) and number of acidic and basic amino acids

Accession number	pI	No. of basic amino acids	No. of acidic amino acids	Property
P29400	7.71	117	115	Basic
P53420	8.9	153	130	Basic
Q01955	9.3	154	119	Basic
Q14031	9.31	143	109	Basic
P02452	5.66	129	141	Acidic
P08123	9.03	122	110	Basic
P02461	6.19	122	129	Weakly Acidic
P20908	4.94	168	225	Acidic
P05997	6.34	135	142	Weakly Acidic
Q9Y5P4	5.29	74	99	Acidic
P02458	8.66	143	128	Basic
P12107	5.08	191	221	Acidic
P13942	5.89	193	189	Weakly Acidic

analyzed using helical wheel plots generated by the program Pepwheel[68] included in the EMBOSS 2.7 suite[69].

TABLE 5: Percentage of residues forming alpha, beta and coil structure

Accession number	Alpha	Beta	Coil	Class
P29400	1.5	8.7	89.9	Irregular
P53420	3	9.5	87.5	Irregular
Q01955	2.6	9.8	87.6	Irregular
Q14031	1.2	12.5	86.2	Irregular
P02452	0	0	100	Irregular
P08123	0	0	100	Irregular
P02461	0	0	100	Irregular
P20908	0	0	100	Irregular
P05997	0	0	100	Irregular
Q9Y5P4	28.2	21.9	49.9	Mixed
P02458	6.8	4.7	88.6	Irregular
P12107	8.6	12.7	78.7	Irregular
P13942	10.4	9.3	80.02	Irregular

RESULTS AND DISCUSSION

The following discussions are based on the results tabulated in TABLES 2 - 6.

Primary structure analysis

The results of primary structure analysis (Supplementary data-1) suggest that the AS, EDS and SS

TABLE 6: Transmembrane regions identified by SOSUI server

Accession number	Transmembrane region	Type	Length
P29400	Rgvslaaglfllalslwg	Primary	18
P53420	Slatgpwslililfsvqyvygsg	Primary	23
Q01955	Prpqvlllplllvllaaapaask	Primary	23
Q14031	Mlinklwlllvtlclteelaaa	Primary	22
P02458	Irlgapqslvlltllvaavlrcq	Primary	23

associated collagen proteins are rich in glycine (23% to 28%) and proline (15% to 23%) amino acids. In GPS associated collagen proteins the amino acids Glu (9%) and Ser (9%) are high in content. The high hydrophobicity of Q14031 (Collagen alpha 6(IV) chain precursor), P08123 (Collagen alpha 2(I) chain precursors) collagen protein is due to the presence of high non-polar residues (TABLE 2).

Physico-chemical analysis

Although Expasy's ProtParam computes the extinction coefficient (EC) for a range of (276, 278, 279, 280 and 282nm) wavelength, 280nm is favoured because proteins absorb strongly there while other substances commonly in protein solutions do not. Extinction coefficient of all collagens computed at 280nm is ranging from 45660 to 106060 M^{-1} cm^{-1}. EC of various syndromes associated human collagen proteins at 280nm is ranging from 46290 to 82570 M^{-1} cm^{-1} (for AS associated collagens), 49410 to 91750 M^{-1} cm^{-1} (for EDS associated collagens), 106060 M^{-1} cm^{-1} (for GPS associated collagen) and 45660 to 98570 M^{-1} cm^{-1} (for SS associated collagens) with respect to the concentration of aromatic amino acids. The high EC of collagen P53420 is due to the presence of Cys (1.9%), Trp (0.5%) and Tyr (1.4%) residues. The high EC of collagen P20908 is due the presence of Tyr (2.18%) residue and for collagen P12107, it is due to the presence of Trp (0.5%) and Tyr (2%). Comparatively the EC of GPS associated collagen protein is very high (106060 M^{-1} cm^{-1}). It is found that the high EC of GPS associated collagen protein is due to the presence of high concentration of Cys (2%), Trp (2%) and Tyr (3%). The computed protein concentration and extinction coefficients help in the quantitative study of protein-protein and protein-ligand interactions in solution. The biocomputed half-life of AS, GPS and SS associated collagen is 30hrs and greater than 20hrs for EDS associated collagens. Expasy's ProtParam classifies the GPS associated collagen as unstable on the basis of instability index (II>40) and all the other collagen proteins as stable (II<40). The low aliphatic index (37-59) of all the syndromes associated proteins infers that the collagen proteins may become unstable at high temperature. The aliphatic index (AI) that is defined as the relative volume of a protein occupied by aliphatic side chains (A, V, I and L) is regarded as a positive factor for the increase of thermal stability of globular proteins. Grand Average hydropathy (GRAVY) index of all collagen proteins is ranging from -0.4 to -0.8, the very low GRAVY index infers its higher hydro solubility, which could result in a better interaction with water (TABLE 3). GRAVY index of all the three stickler syndrome collagen proteins is around -0.8, this indicates that all the three proteins may interact equally with water. Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of the protein is zero. At pI proteins are stable and compact. The computed pI values (TABLE 4) of collagens P02452, P02461, P20908, P05997 (EDS associated proteins), the collagen Q9Y5P4 (GPS associated protein), the collagens P12107, P13942 (SS associated collagens) reveals that these are acidic (pI<7) in character. The computed pI of all alport syndrome associated human collagen proteins (P29400, P53420, Q01955 and Q14031), the protein P08123 (EDS associated collagen) and the protein P02458 (SS associated collagen) infers that these are basic (pI>7) in character. The number of basic and acidic amino acids in each collagen proteins correlates well with the corresponding pI computed (TABLE 4). The computed isolelectric point (pI) will be useful for developing buffer systems for purification of proteins by isoelectric focusing method.

Secondary structure analysis

The secondary structure predicted with the help of programs SOPM, SOPMA and SSCP show that all the collagens are found to be predominant coil structure content (>78%) except the GPS associ-

FULL PAPER

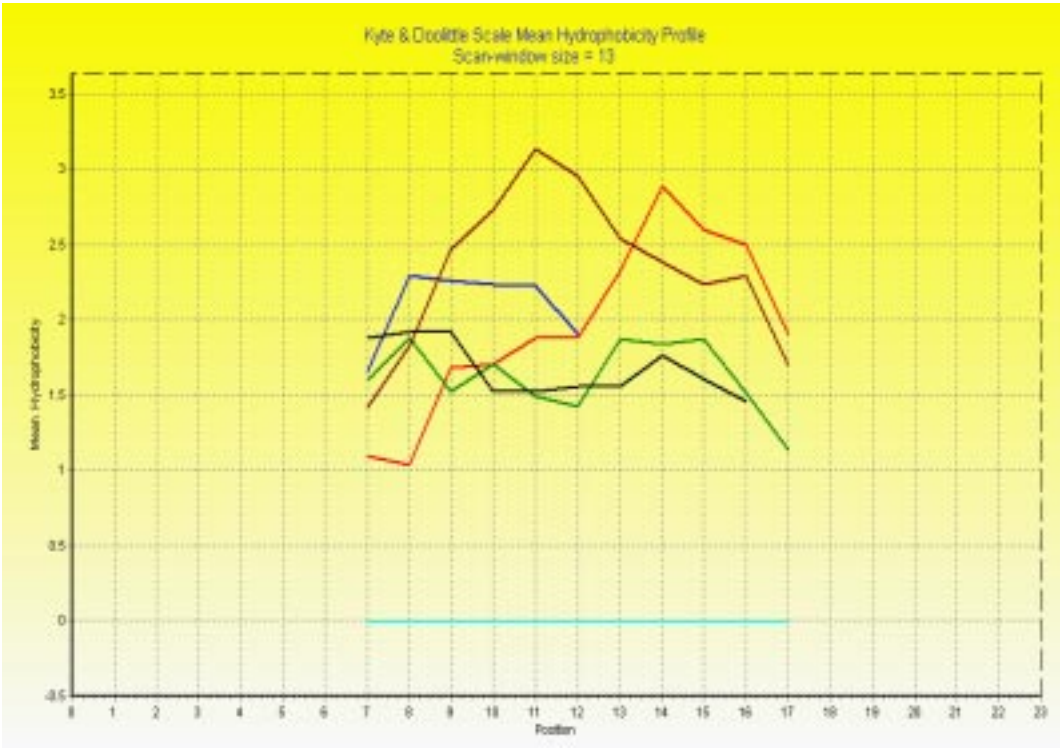

Figure 1: Kyte and Doolittle mean hydrophobicity profile computed for the transmembrane regions of collagens P02458 (SS associated protein), P29400, P53420, Q01955 and Q14031 (AS associated protein). _____ P02458, _____ P29400, _____ P53420, _____ Q01955 and _____ Q14031. All the peaks above the zero line indicates that residues in transmembrane regions are hydrophobic

ated collagen in which the coil content is 49.9% only. The secondary structure prediction server (SSCP) classifies the GPS associated collagen (Q9Y5P4) as mixed secondary structure class and all the other collagens as irregular secondary structure class proteins. The irregular structure of most of the collagen proteins is due to the rich content of more flexible glycine and hydrophobic proline amino acids. Proline has a special property of creating kinks in polypetide chains and disrupting ordered secondary structure. The percentage of residues forming α-helices, β-strands and coils computed by the SSCP server are shown in TABLE 5.

Transmembrane regions analysis

The server SOSUI classifies all the four AS associated collagen proteins (P29400, P53420, Q01955 and Q14031) and the protein P02458 (SS associated collagen protein) as membrane protein and all the other proteins as soluble proteins. The transmembrane region and their length are tabulated in TABLE 6. The transmembrane helix of all collagens found to have more hydrophobic residues (Supplementary data-2) and it is well documented by the Kyte and Doolittle mean hydrophobicity profile[70], (Figure 1) in which all the peaks are above the zero line.

CONCLUSION

Collagen proteins causing various syndromes such as Alport syndrome, Ehlers-Danlos syndrome, Goodpasture's syndrome and Stickler syndrome have been chosen from Swiss-Prot database mainly to analyze, compare and characterize their physicochemical properties, primary and secondary structures using bioinformatics tools and online servers. Primary structure analysis reveals that all the collagen proteins are rich in glycine and proline amino acids. The high hydrophobicity of Q14031 (Collagen alpha 6(IV) chain precursor) and P08123 (Collagen alpha 2(I) chain precursor) proteins are due to the presence of high non-polar residues. Physicochemi-

cal characterization studies give a good idea about the properties such as pI, EC, AI, GRAVY and Instability Index that are essential and vital in providing data about the proteins and their properties. Secondary structure analysis predicts that all the collagens are found to be predominant coil structure content. The server SSCP classifies the GPS collagen proteins as mixed secondary structure class and all the other collagen proteins as irregular class. The SOSUI server identified a membrane region in all the four AS associated collagen proteins (P29400, P53420, Q01955 and Q14031) and in the collagen P02458 (SS associated collagen protein) protein. The predicted transmembrane regions are rich in hydrophobic residues.

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FULL PAPER

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