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In Silico Characterization of Genetic Alterations Associated with Mal De Meleda

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Abstract

Mal de Meleda (MDM) is an autosomal recessive skin disorder majorly caused by mutations in the ARS gene encoding SLURP-1 protein secreted by Keratinocytes. A number of genetic alterations have already been reported in SLURP1 associated with MDM, theoretically proposed to affect the integrity of the downstream product. There are a no reports available to the best of our knowledge, which characterize the effect of respective mutations at the protein structural level, which is the focus of this study. The protein sequence of SLURP1 was obtained from the UniProt database and the disease associated alterations were retrieved and mined from the literature resources. Domain analysis showed that the protein belongs to the Ly6/uPAR superfamily, has antitumor activity and is also a marker of late skin differentiation. Complete tertiary structure of SLURP1 was predicted as shown in figure 4, for further structural analysis as was not previously determined and was submitted to the PMDB after systematic evaluation and validation (PMDB ID: PM0077826). Structural abnormalities in the protein due to mutations were explored through comparative structural analysis along with interspecies conservation of the respective residue through phylogenetic analysis. It is expected that this systematic structural analysis will enhance our understanding about the disease mechanism and will also help to develop better diagnosis and designing treatment strategies in the near future against MDM and other relevant disorders.

Keywords: Mal de Meleda; SLURP-1 protein; phylogenetic analysis; protein structure.

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1. Introduction

Mal de Meleda (MDM) also known as keratosis palmoplantaris transgrediens of Siemens is an autosomal recessive skin disorder characterized by diffuse palmoplantar keratoderma (PPK) and transgressive keratosis [2]. Mutations in the ARS gene located on chromosome 8q24.3 encoding SLURP-1, belongs to the family of Ly-6/uPAR, have reported to be associated with MDM [10]. The secreted and receptor proteins of Ly-6/uPAR family have their roles in a variety of processes including cell activation, signal transduction and cell adhesion [12,13]. The continuous cycle of keratinocyte generation and death is a self-sustained process partially controlled by locally produced acetylcholine (ACh) [9,15]. Keratinocyte secrete SLURP-1 protein, whose expression is positively regulated by extracellular $Ca2+$ in the presence of EGF and negatively regulated by retinoic acid and interferon- γ [15].SLURP1 is a marker of late differentiation, expressed in the granular layer of skin and is also found in some fluids like sweat, tears, saliva and urine [4]. It is also reported as a ligand involved in weak intracellular adhesions [1]. Several mutations have been reported in SLURP1 in MDM which are theoretically supposed to affect the protein integrity. It is to be noted that, the effect of SLURP1 mutations in MDM had never been investigated at the protein structural level, we therefore investigated it in an *in silico* annotation to further our understanding about the disease mechanism, which will help in future diagnosis and designing treatment strategies.

2. Materials and methods

The amino acid sequence of SLURP1 protein was retrieved from UniProt database (http://www.uniprot.org/) that is a central hub for the protein knowledge and provides information for sequence as well as the functional information of the proteins [17], with accession No. P55000. A thorough literature survey was performed to explore the reported genetic alteration in SLURP1 associated with Mal de Meleda and a total of four mutations were identified i.e. W15R [7,14], M32X, G86R [7] and R96X [9,7,14]. These mutations were incorporated manually into the seed sequence to get the mutants for further analysis. The effect of these mutations were explored at different levels in the protein structure to explore how these mutations affect the function and cause Mal de Meleda. Tertiary structure of SLURP1 was not previously determined so at the first step the tertiary structure of seed SLURP1 was predicted through Homology modeling by using MODELLER 9.9. which implements comparative modeling through fulfillment of spatial restraints [8], and can also execute some supplementary tasks like de novo loop modeling, multiple protein sequence alignment, model optimization and protein structure comparisons etc.

Energy minimization was conceded through GROMOS96 until all inconsistencies in the geometry were fixed and all the short interactions were assured. All the models were evaluated through multiple servers including WHATCHECK, PROCHECK [11], ERRAT [6] and VERIFY3D [5] for stereochemical and energetic properties. Protein structures-sequence, structure alignments and other manipulations were performed through SWISS-PDB VIEWER and ESPript 3.o [20]. Finally QMEAN Server [3] was used for the final model quality estimation, ProSA [22] for protein stereology and ANOLEA [16] for protein energy. The Ramachandran plot for all the models were generated through Rampage (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php), showing majority of the residues in each model in the favored regions.

The possible domains and characteristic motifs and patterns contained in SLURP1 were investigated by using Pfam v27.0 [18] and InterPRO2 [21]. All the models including seed and mutants were aligned along with secondary structures through ESPript 3.o [20]. Physiochemical analysis including molecular weight, theoretical pI, amino acid composition, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) of SLURP1 and its mutants were performed by using ProtParam tool (http://web.expasy.org/protparam/) as shown in Table 1.

Table 1: Representation of physiochemical properties seed protein vs mutated proteins

Proteins	No. of amino acids	Mol. Wt.	Theoretical PI	Instability index	Aliphatic Index	GRAVY
Seed	103	11185.9	5.21	53.29 (unstable)	72.04	0.202
M32X	31	3417.1	7.71	31.80 (stable)	85.16	0.429
R96X	96	10366.2	5.66	54.33 (unstable)	69.17	0.276
W15R	103	11155.9	5.71	57.55 (unstable)	72.04	0.167
G86R	103	11285.0	5.71	54.11 (unstable)	72.04	0.162

Phylogenetic tree was obtained by using ML (maximum-likelihood) method; the reliability was evaluated by the bootstrap method with 1000 replications. The conservation of domain present on SLURP1 across different species was also evaluated through phylogenetic analysis as shown in Figure 2.

3. Results

The amino acid sequence of SLURP1 (Secreted Ly-6/uPAR-related protein 1), was obtained from the UniProt database and Mal de Meleda associated genetic alteration were manually retrieved from the reported literature. Domain analysis results showed that SLURP1belongs to the Ly6/uPAR superfamily (InterPro number (IPR027103). SLURP1 has antitumor activity. It was found to be a marker of late differentiation of the skin. SLURP1 play role in maintaining the structural integrity and physiological of the keratinocyte layers [4].

The optimized model and final averaged passed all the WHATCHECK suite (tests implemented in the stereochemistry-evaluating) and in the VERIFY3D program, which uses contact potentials to evaluate whether the modeled amino acid residues occur in the environment typical for globular proteins with solvent-exposed surface and hydrophobic core. Plain wrong model is even might be accepted with regarding to its stereochemistry but the energy calculated would show the polypeptide chain as misfolded.

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B2014102593SZG6KPTC    -MASRWAVQLLLVAAWSMGCGEALKCYTCKEPMTSASCRTITRCKPEDTACMTTLVTVEA 59
TR|H2QWS9|H2QWS9_PANTR -MASRWAVQLLLVAAWSMGCGEALKCYTCKEPMTSASCRTITRCKPEDTACMTTLVTVEA 59
SP|P55000|SLUR1_HUMAN  -MASRWAVQLLLVAAWSMGCGEALKCYTCKEPMTSASCRTITRCKPEDTACMTTLVTVEA 59
TR|G3QIW3|G3QIW3_GORGO -MASRWAVQLLLVAAWSMGCGEALKCYTCKEPMTSASCRTITRCKPEDTACMTTLVTVEA 59
TR|G1QKS9|G1QKS9_NOMLE -MASRWAVQLLLLAAWSMGCGEALKCYTCQQPMTSASCRTITHCKPEDTACMTTLVTVEA 59
TR|F7HHQ4|F7HHQ4_MACMU -MASRWAVQLLLVAAWSMGCGEALKCYTCEQPMVSAACKTITYCKPEDTACMTTLVTVEA 59
TR|G7PD24|G7PD24_MACFA -MASRWAVQLLLMAAWSMGCGEALKCYTCEQPMVSAACKTITYCKPEDTACMTTLVTVEA 59
TR|I3MBE1|I3MBE1_SPETR -MASSWATRLLLLAAWSMGCGEAFRCYSCERPTPIYACKNITYCKLEDTACKTTLEKVEN 59
TR|D4A7L8|D4A7L8_RAT   -MTLRWAVWLLLLAAWSMGYGEAFRCYTCEQPTAINSCKNIVHCKLEDTACKTVLETVEA 59
SP|Q9Z0K7|SLUR1_MOUSE  -MTLRWAMWLLLLAAWSMGYGEAFRCYTCEQPTAINSCKNIAQCKMEDTACKTVLETVEA 59
TR|E2RBP1|E2RBP1_CANFA ------ALLLLLLAAASLRTGEAFRCFTCELPTPVSLCKNISVCKPEDTACKTTLLQIES 54
TR|D2HHG9|D2HHG9_AILME ----RAVLPLLLVAASSLRPGEAFKCFTCEQPTAVPLCKHITQCKPEATACKTTLVQMES 56
TR|M3VU51|M3VU51_FELCA -LKLREAQLLLLLAASCLHAGEAFRCFTCEQPTAIPLCKNITRCKPEATACMTTLVLMEA 59
TR|E1BP47|E1BP47_BOVIN MAVPRALLALLLAAASAVHLGEALSCITCEQPTALPLCKNITYCKPNEIACKTTLVTVEA 60
TR|W5NUK2|W5NUK2_SHEEP MAVPRALLALLLAAASAVSLGEALSCITCEQPTALPLCKNITYCKPDEIACKTTLMTMEA 60
                                *** ** .:  ***: * :*: *     *: *  ** :  ** *.*  :*

B2014102593SZG6KPTC    EYPFNQSPVVTRSCSSSCVATDPDSIGAAHLIFCCFRDLCNSEL---------------- 103
TR|H2QWS9|H2QWS9_PANTR EYPFNQSPVVTRSCSSSCVATDPDSIGAAHLIFCCFRDLCNSEL---------------- 103
SP|P55000|SLUR1_HUMAN  EYPFNQSPVVTRSCSSSCVATDPDSIGAAHLIFCCFRDLCNSEL---------------- 103
TR|G3QIW3|G3QIW3_GORGO EFPFNQSPVVTRSCSSSCVATDPDSIGAAHLIFCCFRDLCNSEL---------------- 103
TR|G1QKS9|G1QKS9_NOMLE EYPFNQSPVVTRSCSRSCMATDPDSIGAAHLIFCCFRDLCNSEL---------------- 103
TR|F7HHQ4|F7HHQ4_MACMU EFPFNQSPVVTRSCSRSCMATDPDSIGAAHLIYCCFRDLCNSEL---------------- 103
TR|G7PD24|G7PD24_MACFA EFPFNQSPVVTRSCSRSCMATDPDSIGAAHLIYCCFRDLCNSEL---------------- 103
TR|I3MBE1|I3MBE1_SPETR EYPFDQSPMVTRSCSDSCLATDPDSIGAAHPVFCCFRDLCNSVEAAQLGAGT-------- 111
TR|D4A7L8|D4A7L8_RAT   EFPFNHSPMVTRSCSSSCLATDPDGIGVAHPVFCCFRDLCNSGFPGLVTGL--------- 110
SP|Q9Z0K7|SLUR1_MOUSE  AFPFNHSPMVTRSCSSSCLATDPDGIGVAHPVFCCFRDLCNSGFPGFVAGL--------- 110
TR|E2RBP1|E2RBP1_CANFA EYPFHQSPMVTRSCSSSCIATDPDSIGAAHLVFCCFHDLCNS------------------ 96
TR|D2HHG9|D2HHG9_AILME EYPFNQSPMVTRSCASSCIATDPDSMGVAHPVYCCFHDLCNS------------------ 98
TR|M3VU51|M3VU51_FELCA EYPFNQMPLVTRSCSNSCFATDPDSIGSTRAVYCCFRDLCNSVQATRLGV---------- 109
TR|E1BP47|E1BP47_BOVIN EFPFNESPVVTSTCASSCEATDPDSIGAAHPIFCCFHDLCNSVGIARLSAGALAPLGAVV 120
TR|W5NUK2|W5NUK2_SHEEP EFPFNESPVVTSTCASSCEATDPDSIGAAHPIFCCFHDLCNSVGIARLSAGALAPLGAVV 120
                        :**.. *:** :*: ** *****.:* :: ::***:*****
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Fig:1: Multiple sequence alignment of SLURP1 amino acid sequence with other homologues.

The rational energies are seldom observed for misfolded structures. Thus, the scores reported for our model by WHATCHECK (Z-score –0.07) and VERIFY3D (average score 0.3, no regions scored lower than 0) suggest that the conformation of individual residues and both its 3D fold are reasonable. In comparative structural analysis of mutant and seed SLURP1, it was observed that, the wild-type residue is bigger than the mutant residue in the case of W15R as shown in Table 2. The wild-type residue was neutral while the mutant is positively charged. The wild-type residue is more hydrophobic than the mutant residue. The mutation is located within the signal peptide, which is important because it is recognized by other proteins and often cleaved of to generate the mature protein.

The new residue that is introduced in the signal peptide differs in its properties from the original one. It is possible that this mutation disturbs recognition of the signal peptide. It was also observed that the mutant residue was not among the residue types observed at this position in other, homologous sequences which might

indicate that the mutation is possibly damaging to the protein. This residue is part of an interpro domain named "Secreted Ly-6/uPAR-related protein 1" (IPR027103). There is a difference in charge between the wild-type and mutant amino acid. The mutation introduces a charge, this can cause repulsion of ligands or other residues with the same charge. The wild-type and mutant amino acids differ in size. The mutant residue is smaller, this might lead to loss of interactions. The hydrophobicity of the wild-type and mutant residue differs. Hydrophobic interactions, either in the core of the protein or on the surface, will be lost.

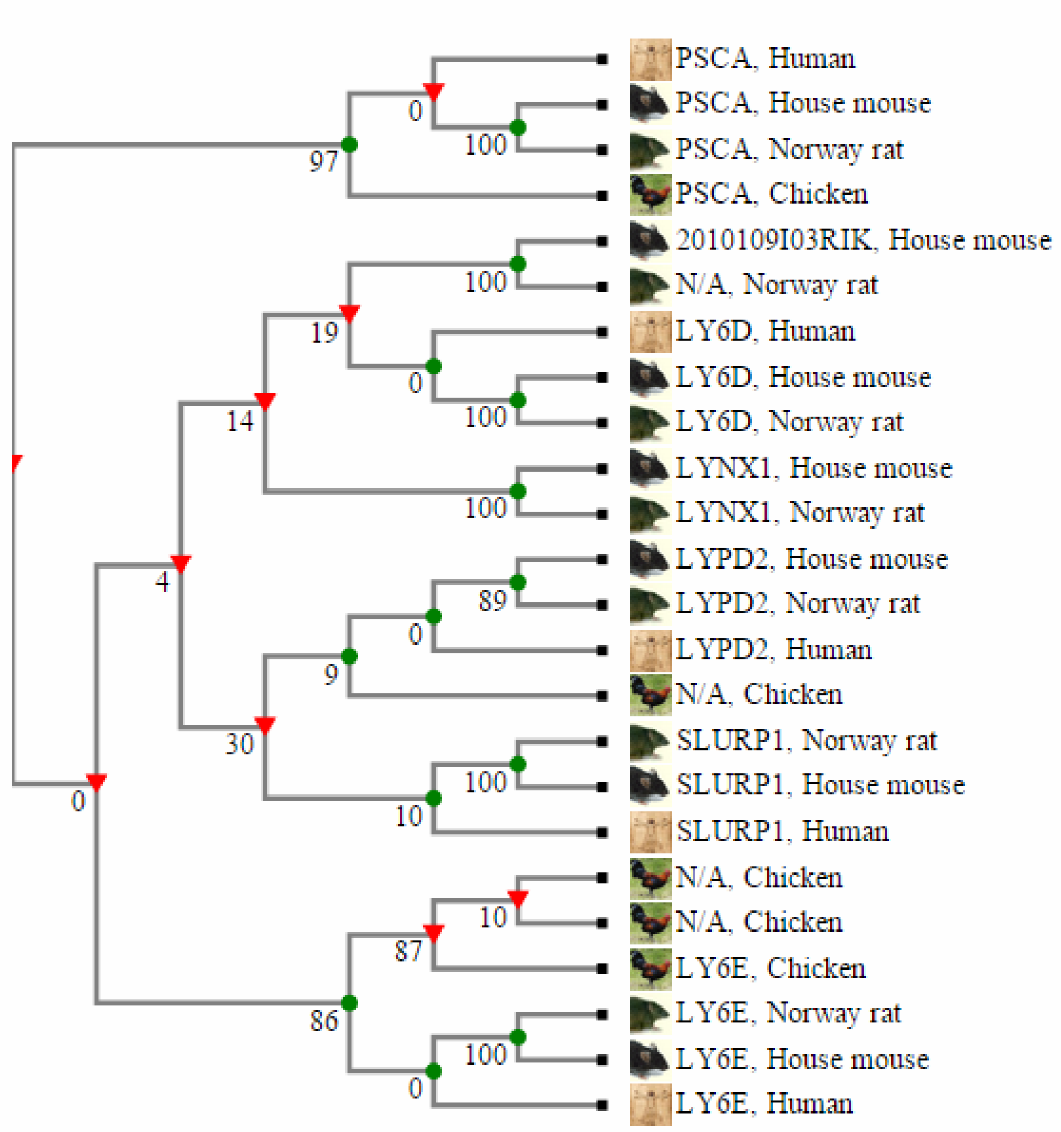

Fig-2: Phylogenetic relationship of SLURP1 amino acid sequence with other homologues across different organisms.

The wild-type residue is smaller than the mutant residue in case of G86R (Table 2). The mutant residue is positively charged and wild-type residue was neutral. Glycine is the wild-type residue has high flexibility of all residues which is helpful in protein's function. This function can be abolished with the mutation in this glycine, leading to sever genetic disease. The wild-type residue less susceptible to mutation but few other susceptible residue have also been observed.

Table 2: Structural view of amino acid change from wild to mutant in the structure of SLURP1.

No	Mutation	Wild amino acid	Mutant amino acid
1.	**W15R**	NH, H_2N, OH, O Tryptophan	H_2N, NH, NH, H_2N, OH, O Arginine
2.	**M32X**	S, H_2N, OH, O Methionine	Stop codon
3.	**G86R**	H_2N, OH, O Glycine	H_2N, NH, NH, H_2N, OH, O Arginine
4.	**R96X**	H_2N, NH, NH, H_2N, OH, O Arginine	Stop codon

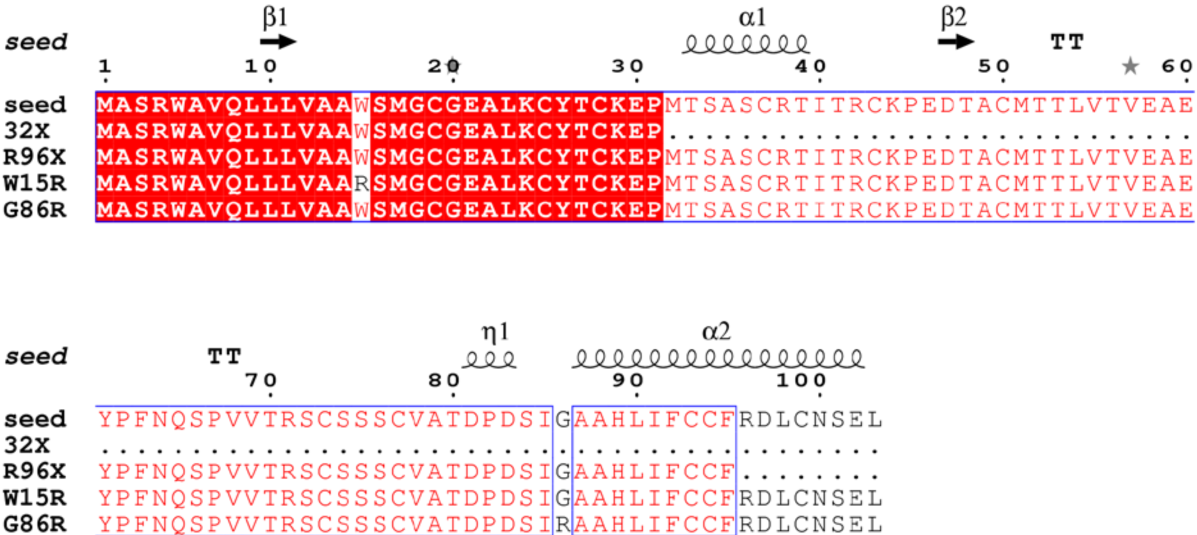

Fig-3: Sequences and second structure analysis of SLURP1 with its respective mutant sequences along with its secondary structure.

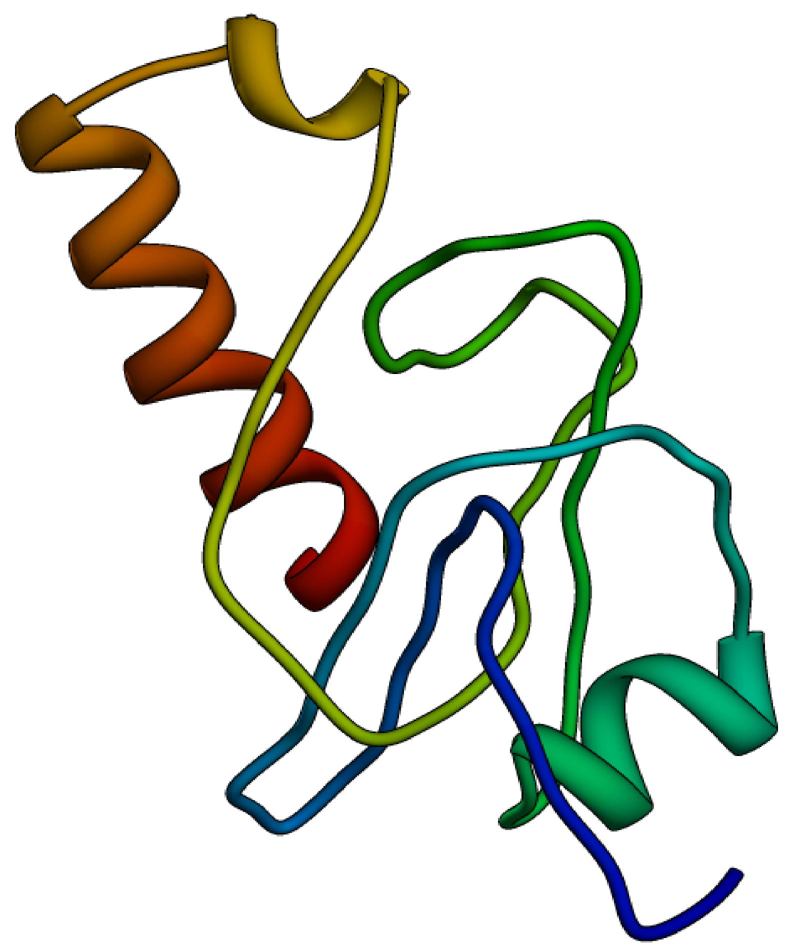

Fig-4: Tertiary Structure of Seed SLURP1, predicted through homology modeling.

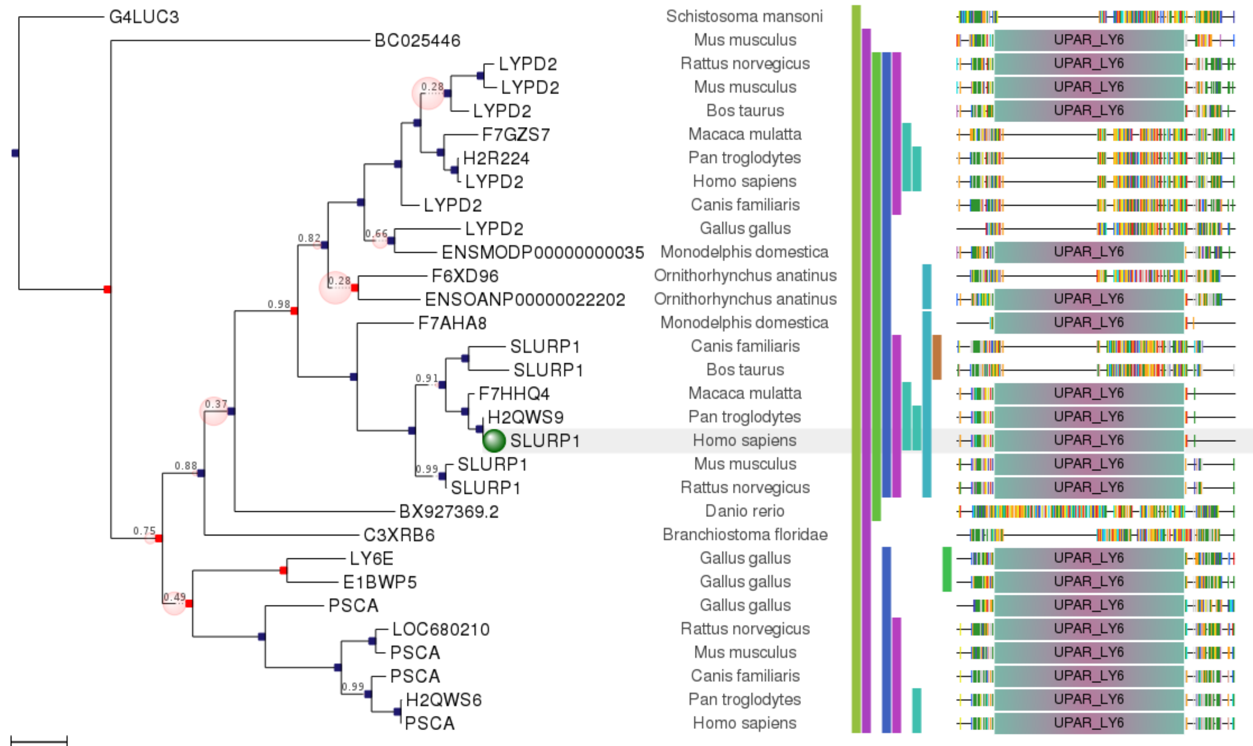

Fig-5: Evolutionary conservation of UPAR_LY6

This residue is part of an interpro domain named "CD59 antigen" (IPR001526) which is a part of an interpro domain named "Ly-6 antigen / uPA receptor -like" (IPR016054). Which belongs to an interpro domain named "Secreted Ly-6/uPAR-related protein 1" (IPR027103). The mutant and wild-type amino acids are different on the basis of its charge, with the insertion of mutation a charge is introduced; which results in the repulsion of ligands or other residues with the equal charge. The mutant and wild-type amino acids also have different sizes. A special backbone conformation to facilitate movement of the protein is done by glysine residue due to its flexible nature. Disturbance in this conformation or movement may occur with the mutation in glycine .

To estimate inter-species conservation of certain segments and to identify regions shared by other members of the Ly-6/uPAR family of proteins, A multiple sequence alignment was performed for SLURP-1 protein given in Figure 1.

The uPAR proteins family members were not the same, excluding the conserved cysteins that play a part in disulphide bonds. Alignment of the human SLURP-1 sequence to other members of the Ly-6/uPARprotein family (hPSCA, hCD59, hE48 and huPAR) showed that the amino acid corresponding to the position of the novel p.Cys94Ser alteration is conserved in the vast majority of proteins (data not shown). Alignment of the human SLURP-1 sequence to other species also showed conservation of the cysteine corresponding to position 94 in the human SLURP-1.

4. Discussion

SLURP-1 protein is involved in keratinocyte differentiation [15] and alterations of the protein can disturb normal skin development as has been found in patients with MDM [8, 10, 14, 16–22]. The SLURP-1 protein is encoded by the 3 exons of the SLURP1 gene (previously ARS) and contains a signal peptide as well as conserved cysteines important in forming disulphide bonds that are critical for the function of the protein. Human SLURP-1 is a member of the large Ly-6/uPAR protein superfamily; proteins that contain 8–10 highly conserved cysteines that form disulphide bonds.

Proteins play a vital role in almost all biological processes; either it is the process of transportation, storage, signaling regulation, etc. Any defect in the protein can cause the abnormality and lead a person towards the disease. SLURP1 is also one of the protein having 103 amino acids that cause the skin disorder if it goes wrong. To analyze what changes and at what level they affect the function in what way. For this purpose the protein structure was retrieved from Uniprot database having the treasure of the sequences of different proteins of different species. As w retrieved the sequence we applied it to find the domain using Pfam (http://pfam.sanger.ac.uk/). To find the domain is important as it allows us to know where will be the mutations in or out of the functional part of the protein. According to Pfam the domain of SLURP-1 is from 25 bps to 100 bps known as u-PAR/Ly-6 domain. Different mutations were found through the literature. These mutations include stop codon at amino acid position 32, Arginine to stop codon at position 96 (R96X), W15R, G86R.

These mutations were incorporated in seed sequence of protein manually. Then these mutated proteins and as well as the seed protein were applied for secondary structure of the protein. The secondary structure was observed keenly for all the proteins to notice the changes in helixes, coils and sheets before they develop into the functional protein that is the tertiary structure. HNN was used for the secondary structure predictions, the coils present in seed protein, early stop codon protein 32 position, R96X [9,7,14], W15R [7,14] G86R [7] are 55, 11,54, 54, 52 in number respectively similarly the helixes are 29,18,19 28, 32 and strands are 19, 2, 23,21,19 in number respectively. The tertiary structure was modeled for all the mutated and seed proteins to observe the changes. The homology modeling was failed as we didn't found any hit template that would be more than 30% in homology with the query sequence so threading was tried with the help of LOMETS (http://zhanglab.ccmb.med.umich.edu/LOMETS/). LOMETS is a Meta server based protein fold recognitions that selects 10 best models from 160 models depending upon confidence score. The confidence score is based on the z-score of the template, confidence score of the server, and the sequence identity. The model 1 is selected out of 10 models the model selected has the 77 alignment length and the best z-score. Then this model was subjected to Chiron (http://troll.med.unc.edu/chiron/login.php) [19] that is the program for protein model refinement. It removes the steric clashes and finally minimizes the energy. The steric clashes arises in result of the overlapping electron clouds of two atoms. It does not alter the structure very much but can make the structure firm. The next step was to validate the structure. It was proceeded with the help of What if and ProSA web as well as Ramachandran Plot statistics. According to the what if validation the clashes were reduced from 1119 to 17 and the repulsion energy from 112.178 to 13.3748 kcal/mol. The ProSA web gives the z-score of -0.07. the Ramachandran Plots were obtained using the Rampage. The models of the mutated proteins were also designed to observe the structure at tertiary level that what changes are affecting the protein. The LOMETS was

again used to model these structures. The first model of all the mutated proteins were selected as they have the higher confidence score and as well as the good Z-score with maximum alignment. Then all these models were subjected to Rampage. According to it 97% residues are in favoured region and only 3% is in allowed region while no residue was found in outlier region. In case of mutated proteins that are truncated proteins whose stop codon is at amino acid 32 and R96X, W15R and G86R have 96.6%, 94.7%, 92.1% and 91.1% amino acids in favoured region respectively while 3.4%, 2.1% amino acids of mutated protein having stop codon at 32 and R96X are in allowed region, W15R and G86R have 5.9% residues in allowed region. The outlier region contains 3.2%, 2.0% and 3.0 % residues of R96X, W15R and G86R respectively while no residue of mutated protein having stop codon at 32 position was found in outlier region. According to protParam the isoelectric point of all the proteins are slightly different. The truncated protein has the highest theoretical PI. It can affect the solubility of a molecule at a particular pH. The stability index was also measured the truncated protein at amino acid 32 has the stability index less than 40 that is 31.80 so its considered to be stable in the test tube while the seed protein, R96X, W15R, G86R has instability index greater than 40 that is 53.29, 54.33, 57.55, 54.11 respectively so they are considered to be unstable, the aliphatic index of protein that stops at 32 codon and R96X that are both truncated proteins are affected while no change was observed in all other proteins that is 72.04. Gravy was also affected by these mutations slightly. The gravy of the normal protein is 0.202 while the others are 0.429, 0.276, 0.167, 0.162 respectively. Similarly the molecular also affected. The molecular weight of all the mutated proteins reduced while the seed protein has the highest molecular weight (Table 2).

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