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Full Length Research Article

Identification of the Domain Regions and the Mutated Sites of Neuropeptide S Receptor 1 (NPSR1) and its 3D Structure Prediction Using Molecular Mechanics

Janani N[1] and L. Shakila[2]

[1-2] P. G. and Research Department of Zoology, Ethiraj College for Women, Chennai - 600 008, Tamil Nadu, India

Abstract

The neuropeptide S receptor is a member of the G-protein coupled receptor superfamily of integral membrane proteins which binds neuropeptide S. It was formerly an orphan receptor, GPR154, until the discovery of neuropeptide S as the endogenous ligand. In this In-silico work, we found out the total number of mutated amino acids of the NPSR1 protein present in the domain's regions. The normal and mutated structure of the NPSR1 protein sequence was modelled using automated molecular modelling techniques. The modelled structure was validated and the 3D structure was visualized using advanced molecular visualization tool in order to view the molecular structural details with its mutated amino acids domains region in 3D form. The overall results clearly deliver the involvement of the mutated amino acids in the domains region of the modelled NPSR1 protein. Finally, we conclude that the identified domain regions of the amino acids act as potential drug binding sites which will be beneficial further in the field of drug designing for NPSR1 related diseases.

***Key words*:** Domains, GPR154, *Insilico*, Neuropeptide, NPSR1, Drug designing

Positional cloning was used to identify Neuropeptide S receptor 1 (NPSR1, also known as GPRA, GPR154) as an asthma susceptibility gene [1]. Significant single nucleotide polymorphism (SNP) and haplotype links to asthma were found in three different groups. The association between NPSR1 and asthma and allergies has been replicated in seven distinct populations to date. [2-8]. NPSR1 has also been linked to inflammatory illnesses of the skin and gut [9-10], neurally associated features such as sleep and circadian phenotypes [11] and anxiety [12].

NPSR1 is a 7-transmembrane G-protein coupled receptor (GPCR) that is related to neuropeptide Y (NPY), neurotensin, and tachykinin receptors phylogenetically [13]. Neuropeptide S (NPS), a natural NPSR1 ligand, has been shown to promote downstream signalling via intracellular coupling to Gaq and Gas [14-15]. There are several NPSR1 splice variants, but only two, NPSR1-A and NPSR1-B, are efficiently transported to the plasma membrane [16]. The C-terminus of a GPCR protein is essential for several stages of its lifecycle, and changes might alter, for example, delivery to the cell membrane, anchoring, and downstream signalling [17]. Conformational changes reveal phosphorylation sites on the C-terminus when the receptor is activated. These locations are phosphorylated by G protein coupled receptor kinases (GRKs) and can bind arrestins, which regulate GPCR activity. When an arrestin binds a receptor, it is either internalized and recycled, diverted to G-protein independent signalling pathways such the mitogen-activated protein kinase (MAPK), or destroyed [18].

Asthma-related genes include matrix metallopeptidase 10 (MMP10) and tissue inhibitor of metallopeptidase inhibitor 3 (TIMP3). MMP10 and TIMP3 have been discovered to colocalize with NPSR1-A in the bronchial epithelium. MMP10 expression is enhanced in NPSR1-An overexpressing cell treated with NPS. This finding implies that the NPS–NPSR1 signalling pathway causes TIMP3 and MMP10 overexpression and may be involved in the pathophysiology of asthma. Subsequently, this study aims to illustratethe involvement of the mutated amino acids in the domain region of the modelled NPSR1 protein; which can be potential drug binding regions, using the bioinformatics tools.

MATERIALS AND METHODS

Sequence retrieval

The target protein sequence NPSR1 (UniProt ID: Q6W5P4) was selected based on the UniProt clinical references and the sequence was retrieved from UniProt database (https://www.uniprot.org/) in FASTA format and the respective mutated positions/sequence conversion were changed manually

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Correspondence to: Janani N, P. G. and Research Department of Zoology, Ethiraj College for Women, Chennai - 600 008, Tamil Nadu, India, Tel: +91 9443771465; E-mail: jananikpd@gmail.com

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using EMBOSS seqret tool (https://www.ebi.ac.uk/Tools/sfc/emboss_seqret/).

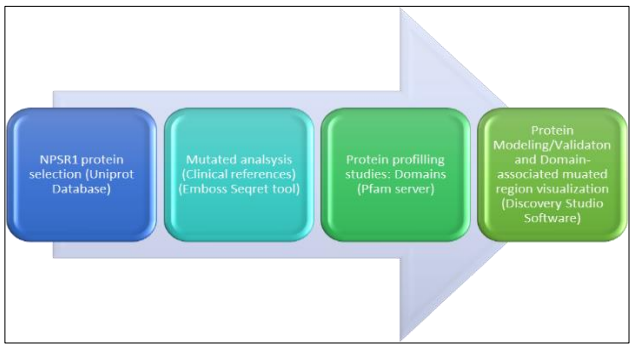

Fig 1 The workflow for bioinformatics analysis of NPSR1 and its 3D structure prediction

Protein sequence function analysis

The NPSR1 protein sequence was analysed using Pfam server (https://pfam.xfam.org/) in order to view the Motif and Domain present in NPSR1.

3D modelling and validation

The selected NPSR1 sequence was modelled using an automated protein homology modelling server called Swiss Model server (https://swissmodel.expasy.org/). The modelled 3D protein structure was evaluated using ProCheck server (https://saves.mbi.ucla.edu/).

3D structure visualization

The modelled protein structure of NPSR1 was viewed using advanced molecular visualization software, Discovery Studio software (Fig 1).

RESULTS AND DISCUSSION

The target protein responsible for Asthma, NPSR1 (UniProt ID: Q6W5P4, OMIM ID: 608595) (Neuropeptide S receptor 1) is present in the 7th chromosome and the length of its gene sequence is 1116 nt and the length of its protein sequence is 204 aa. In the primary step of the investigation, we performed motif and domain analysis. (Fig 2) shows the FASTA sequence of the amino acids in NPSR1 and the mutated amino acid sequence with respective positions are shown in (Fig 4). (Fig 2) shows the sequence format of the Normal amino acid content of NPSR1 with amino acid positions (N108I, R122Q, S1443G, C198F, T212I, S241R, I315T, and Q344R) highlighted in yellow. (Fig 3) shows the 3D view of the normal protein structure of NPSR1 shown in secondary structure colour model with amino acid labels (N108I, R122Q, S1443G, C198F, T212I, S241R, I315T, and Q344R), visualized using Discovery Studio Software.

```
      10         20         30         40         50
MPANFTEGSF DSSGTGQTLD SSPVACTETV TFTEVVEGKE WGSFYYSFKT
      60         70         80         90        100
EQLITLWVLF VFTIVGNSVV LFSTWRRKKK SRMTFFVTQL AITDSFTGLV
     110        120        130        140        150
NILTDINWRF TGDFTAPDLV CRVVRYLQVV LLYASTYVLV SLSIDRYHAI
     160        170        180        190        200
VYPMKFLQGE KQARVLIVIA WSLSFLFSIP TLIIFGKRTL SNGEVQCWAL
     210        220        230        240        250
WPDDSYWTPY MTIVAFLVYF IPLTIISIMY GIVIRTIWIK SKTYETVISN
     260        270        280        290        300
CSDGKLCSSY NRGLISKAKI KAIKYSIIII LAFICCWSPY FLFDILDNFN
     310        320        330        340        350
LLPDTQERFY ASVIIQNLPA LNSAINPLIY CVFSSSISFP CREQRSQDSR
     360        370
MTFRERTERH EMQILSKPEF I
```

Fig 2 Protein sequence of NPSR1– (EMBOSS Seqret tool)

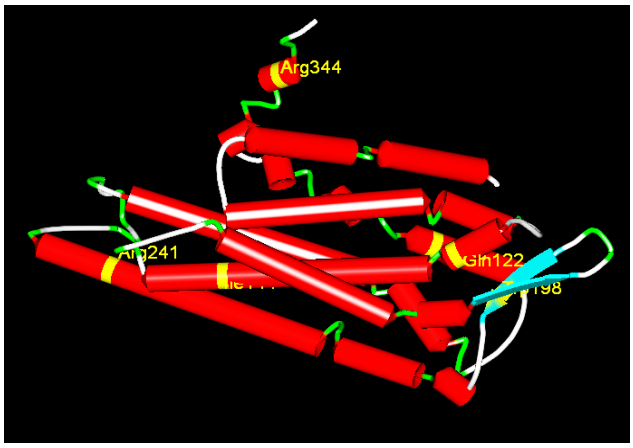

Fig 3 Protein modelling: 3D structure of NPSR1

```
      10         20         30         40         50
MPANFTEGSF DSSGTGQTLD SSPVACTETV TFTEVVEGKE WGSFYYSFKT
      60         70         80         90        100
EQLITLWVLF VFTIVGNSVV LFSTWRRKKK SRMTFFVTQL AITDSFTGLV
     110        120        130        140        150
NILTDIIWRF TGDFTAPDLV CQVVRYLQVV LLYASTYVLV SLGIDRYHAI
     160        170        180        190        200
VYPMKFLQGE KQARVLIVIA WSLSFLFSIP TLIIFGKRTL SNGEVQFWAL
     210        220        230        240        250
WPDDSYWTPY MIIVAFLVYF IPLTIISIMY GIVIRTIWIK RKTYETVISN
     260        270        280        290        300
CSDGKLCSSY NRGLISKAKI KAIKYSIIII LAFICCWSPY FLFDILDNFN
     310        320        330        340        350
LLPDTQERFY ASVITQNLPA LNSAINPLIY CVFSSSISFP CRERRSQDSR
     360        370
MTFRERTERH EMQILSKPEF I
```

Fig 4 Mutated sequence of NPSR1 - (EMBOSS Seqret tool)

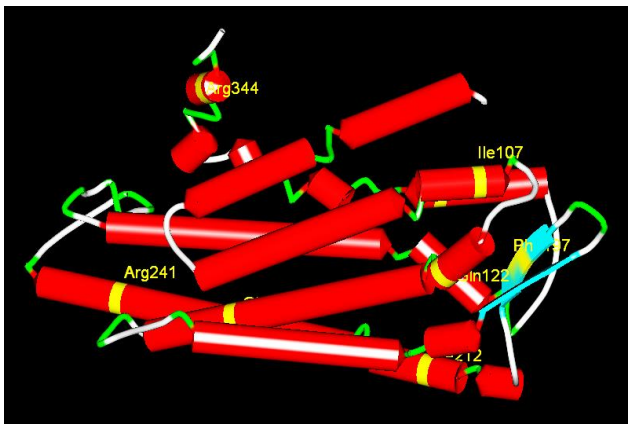

Fig 5 Protein modelling: Mutated 3D structure of NPSR1

(Fig 4) shows the sequence format of the mutated amino acid content of NPSR1 with amino acid positions (N108I, R122Q, S1443G, C198F, T212I, S241R, I315T, and Q344R) highlighted in yellow.

(Fig 5) shows the 3D view of the mutated protein structure of NPSR1 shown in secondary structure colour model with amino acid labels (N108I, R122Q, S1443G, C198F, T212I, S241R, I315T, and Q344R), visualized using Discovery Studio Software.

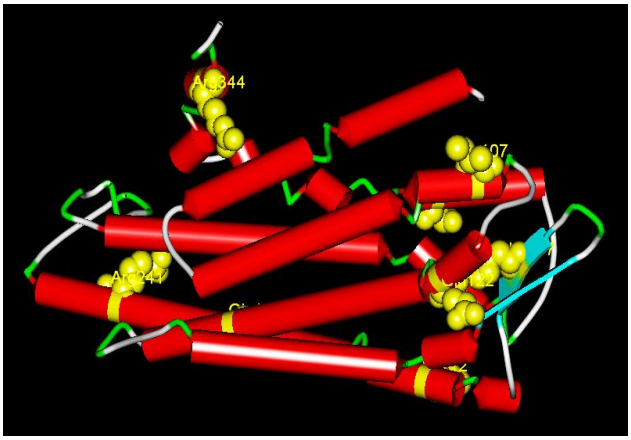

Fig 6 Protein modelling: Mutated 3D structure of NPSR1 (Space filling model)

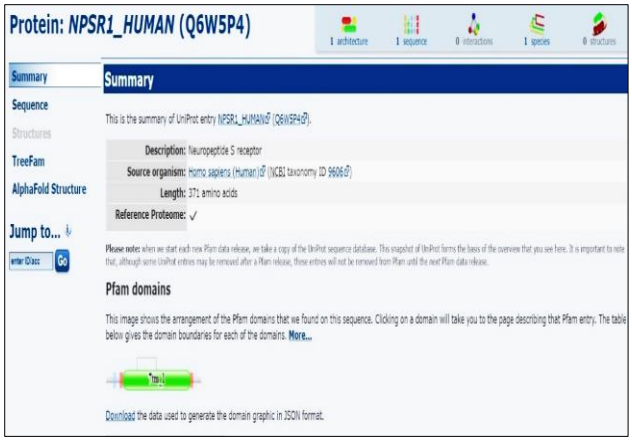

Fig 7 Protein domain prediction using Pfam database

(Fig 6) shows the 3D view of the mutated protein structure ofNPSR1 shown in secondary structure colour model with amino acid labels in space-filling model (N108I, R122Q, S1443G, C198F, T212I, S241R, I315T, and Q344R), visualized using Discovery Studio Software. (Fig 7) depicts the amino acid positions in the domain region in NPSR1 protein sequence.

Proteins are generally composed of one or more functional regions, commonly termed as domains. We utilised Pfam [19] server to view the domain regions present in the NPSR1 protein sequence. The results obtained from Pfam for NPSR1 show that the domain regions belong to superfamily members which include the rhodopsin-like GPCRs, the secretin-like GPCRs, the cAMP receptors, the fungal mating pheromone receptors, and the metabotropic glutamate receptor family according to the information gained from the specialised database for GPCRs [20].

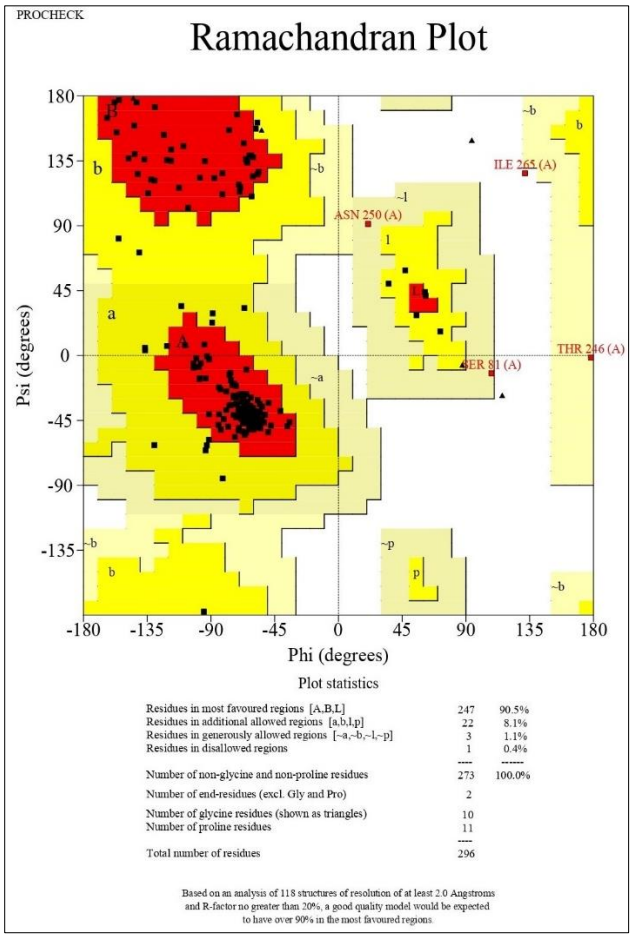

Fig 8 3D structure validation of NPSR1 using ProCheck server

NPSR1 belongs to the class -The rhodopsin-like GPCRs. The rhodopsin-like GPCRs themselves represent a widespread protein family that includes hormones, neurotransmitters, and light receptors, all of which transduce extracellular signals through interaction with guanine nucleotide-binding (G) proteins. Although their activating ligands vary widely in structure and character, the amino acid sequences of the receptors are very similar and are believed to adopt a common structural framework comprising 7 transmembrane (TM) helices [21-23].

We used SWISS-MODEL to convert the amino acid sequence of TSHR into 3D structure (Fig 3, 5). SWISS-MODEL [24- 27] was used to elaborately analyse the molecular and structural details of TSHR for the purpose of docking. SWISS-MODEL is a server for automated comparative modelling of three-dimensional (3D) protein structures. computed models by the SWISS-MODEL server homology modelling pipeline which relies on ProMod3, an in-house comparative modelling engine based on Open Structure. The modelled 3D protein was completely evaluated using ProCheck server [28] for assessment of Ramachandran plot.

(Fig 8) depicts the of Assessment of Ramachandran plot for the predicted mutated protein sequence of the modeled NPSR1.

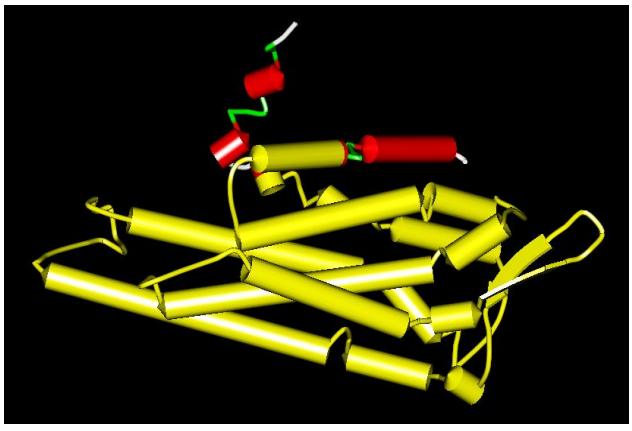

Fig 9 3D structure domain prediction using discover studio software

(Fig 9) depicts the 3D structure of NPSR1 protein with the domain regions shown in yellow colour viewed using Discovery studio software.

(Fig 8) shows the assessment of Ramachandran Plot which confirms that there is no error (93.9%) in the modelled protein (Fig 3, 5). The range of Pfam functional domains is (60-356 amino acids positions ranges). There are 7 mutated amino

acids (N107: R122Q: S143G: C197F:T212I:S241R: I315T:Q344R) which are directly involved in the functional part of the Domains of NPSR1 protein sequence. These identified domains (Fig 9) and the mutated amino acids form the basis for identification of novel drug candidates using structure-based drug designing.

CONCLUSION

In this study, we explored the 3D view of mutated amino acids and how they structurally influence within the limits of the functional part of (Domains) NPSR1protein.Our overall results clearly demonstrates that the identified mutated amino acids with its respective positions act as potential candidates for drug binding sites which can further used in drug designing for NPSR1 related diseases.

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