ORIGINAL ARTICLE

Structural Modeling and Validation of Growth/Differentiation Factor 15 [NP_004855] Associated with Pregnancy Complication-Hyperemesis Gravidarum

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Abstract:

Background: Hyperemesis Gravidarum (HG) is a common pregnancy complication that occurs in 0.3–2% of pregnancies. Growth/Differentiation Factor (GDF) 15 serum levels are abnormally high in patients associated with HG. *In silico* analysis provides information about structure and function of GDF15. *Aim and Objectives:* The aim of this study was to enlist biochemical and functional properties of GDF15 protein and determine its three-dimensional structure, as GDF15 is known to be associated with risk of HG. *Material and Methods:* The PDB file of GDF15 [NP_004855] was created by RaptorX structure prediction server. The UCLA-DOE server was used to visual analysis of crystal structure of protein. The validation for structure models was performed by using PROCHECK. Model quality estimates were based on the QMEAN and ProSA. *Results:* The model showed good stereo-chemical property in terms of G-factor value -0.64, that indicates geometry of model corresponds to probability conformation with 95% residue in the favored region of Ramachandran plot, showing high accuracy of model prediction. The Z-score of -4.04 predicted by ProSA represents the good quality of the model. The energy plot shows the local model quality by plotting knowledge-based energies as a function of amino acid sequence position. *Conclusion:* The generated model could be supportive to understand the structure and functional characteristics of *Homo sapiens* growth/differentiation factor 15 [NP_004855]. As abnormal high serum levels of GDF15 were observe in patients associated with HG. Therefore, the structure model of GDF15 [NP_004855] is useful to understand its role in development of HG. *In Silico* docking study could be explain the molecular association of GDF15 [NP_004855] with HG and new drug designing, for that structure model is very useful.

Keywords: Hyperemesis gravidarum, GDF15, PDB, ProSA, Ramachandran plot

Introduction:

Nausea and Vomiting of Pregnancy (NVP) affect 50–90% of pregnant women [1-3] and as many as 18% of pregnant women take medication to treat this condition [4]. Hyperemesis Gravidarum (HG) is the most severe form and occurs in 0.3–2% of pregnancies [5]. Its clinical presentation includes severe intractable vomiting, often associated with dehydration, weight loss (>5% pre-pregnancy weight), ketonuria, nutritional deficiencies, and electrolyte disturbances [6]. HG remains the second leading cause of hospitalization during pregnancy [7].

The associated locus on chr19p13.11 contained genes GDF15. Further study supporting previously unknown biological connection between GDF15 and HG. GDF15 encodes a TGF-β superfamily member that is expressed at its highest levels in the trophoblast cells of the placenta [8]. The protein is found in maternal serum and increases significantly in the first two

trimesters [8]. GDF-15 is a member of TGF-β superfamily and often induced response associated with cellular stress [9]. GDF15 serum levels are abnormally high in patients associated with HG. The GDF15 levels may be used for diagnosis and develop treatments strategy for HG [10]. HG likely to have higher levels of Pregnancy-Associated Plasma Protein A (PAPP-A) and excessively high level of human Chorionic Gonadotropin (hCG) [11]. The HG associated with variation in genes encoding placental proteins (GDF15 and IGFBP7) and hormone receptors (GFRAL and PGR) [12].

GDF15 is believed to suppress production of pro-inflammatory cytokines in order to facilitate placentation and maintain pregnancy [13]. GDF15 also shown to be a regulator of physiological body weight and appetite via activation of neurons in the hypothalamus and area postrema (vomiting center) of the brainstem [14-15]. It is also notable that abnormal overproduction of GDF15 in cancer was recently found to be the key driver of cancer anorexia and cachexia which, like HG, exhibits symptoms of chronic nausea and weight loss [16]. Bioinformatics helps in management of complex biological data, sequence analysis and algorithmic designing [17-18]. However, by using the *in-silico* techniques protein sequences could be analyzed [19-20]. Therefore, the present study enlists the physiochemical and functional properties of GDF15 and provide information about its three-dimensional structure by using *in silico* tools and techniques.

Material and Methods:

Sequence Retrieval, Alignment and Homology Modeling:

The sequence of *Homo sapiens* growth/ differentiation factor 15 [NP_004855] protein was retrieved from NCBI. The RaptorX structure prediction server was used to generate PDB file of GDF15 [NP_004855] from FASTA sequence. Model construction and regularization of model were done by optimization protocol of RaptorX. Simulated annealing protocol used for energy minimization of model. In order to build a model of protein domain, multiple sequence alignment performed between full length GDF15 [NP_004855] sequence and another protein sequences of database. To build the model of GDF15 [NP_004855] protein with more homology, structure of GDF15 [NP_004855] protein model in RaptorX server was selected as template.

Model Reputation:

The UCLA-DOE server provides quality analysis of protein crystal structure and it requires structure in PDB format. Verify 3D expects this crystal structure to be submitted in PDB format [21]. PROCHECK server used for validation of structure model [22-23], and its results suggesting reliability of model [24]. The model was selected based on various factors such as overall G-factor, number of residues in core that fall in generously allowed and disallowed regions in Ramachandran plot. The model was further analyzed by WHATIF [25, 26] and QMEAN (https://swissmodel.expasy.org/qmean / version 3.1.0) [27-28] and ProSA [29]. The protein stability was analyzed by using ProSA and QMEAN Z-score.

Results and Discussion:

Building of Protein Model:

The alignment between target and template was performed by using homology modeling [30]. The sequence alignment of GDF15 [NP_004855]

revealed sequence homology with catenin binding domain (ID = 99%), which selected as template for the model building. To build the model, PSI-BLAST was done with the maximum E-value allowed for template being 0.005. The ribbon model of GDF15 [NP_004855] was generated by using RaptorX structure prediction server (Fig. 1).

Model Reputation:

The overall G-factor -0.64 indicates good stereo chemical property of model and represents that model geometry resembles to conformation with 90.5% residues in core section of Ramachandran plot [31]. Resulted percentage of residues in allowed and outer section was 2.7% and 2.3% respectively (Figs. 2a, b). The above results indicate reliability of protein model [31].

The compatibility and score profile of (3D) amino acid atomic model illustrated by verify 3D graph [32]. The high score of 0.58 indicates good quality of model (Fig. 3). Profile score beyond zero of verify 3D indicates acceptable model output [33].

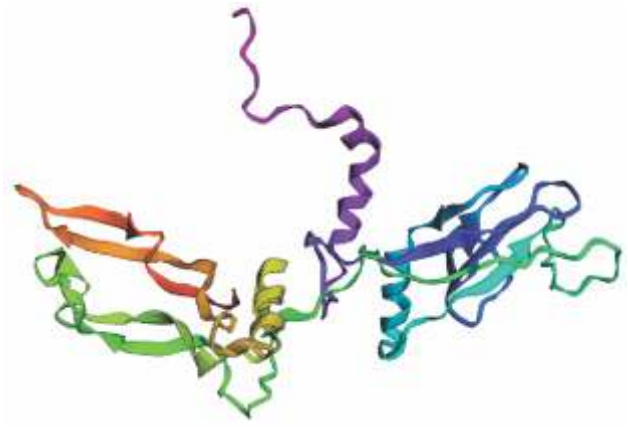

Fig. 1: GDF15 [NP_004855] Protein Ribbon Model Generated using RaptorX Structure Prediction Server

Model Validation:

ProSA was used to figure out potential errors in 3D model of GDF15 [NP_004855]. The archived ProSA Z-score score -4.04 indicates two aspects: overall model quality and energy deviation of GDF15 [NP_004855] protein. The values of Z-score thus predicted indicates less erroneous structures [34]. Reliability of projected model based on scoring function of QMEAN that stated as 'Z-score' [35].

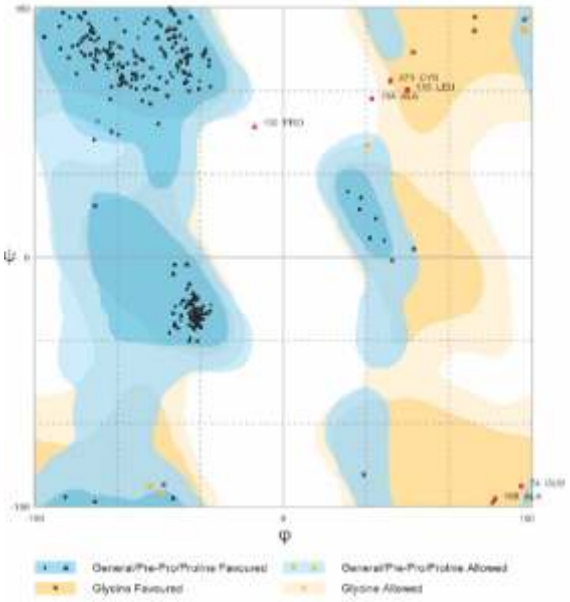

Fig. 2a: Ramachandran Plot of 3D Model of GDF15 [NP_004855]: Total number of residues were 249 with 95% in most favoured regions [A, B, L], 2.7% in allowed regions [a, b, l, p], 2.3% in outlier regions.

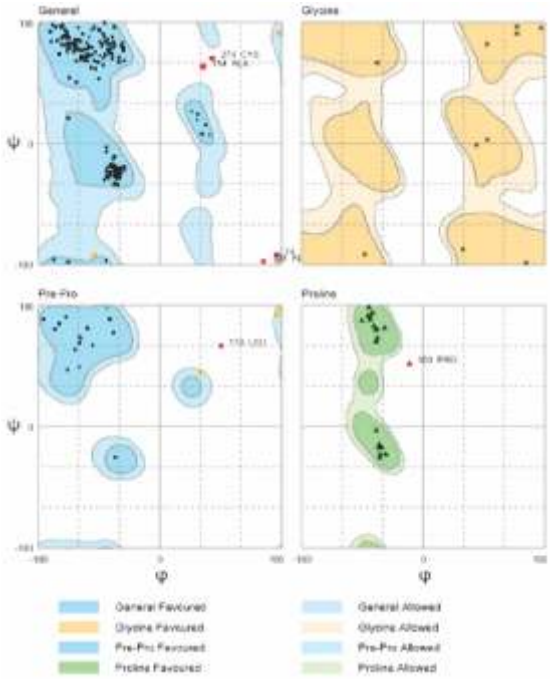

Fig. 2b: Non-proline Residues and Non-Glycine Residue Regions

The Local Distance Difference Test (lDDT) was performed [http://swissmodel.expasy.org/lddt] for assessing local correctness of models, including stereochemical plausibility. The QMEAN Z-score -4.16, which was very close to 0 and its illustrations acceptable value. Assessed validity of model predictable among 0 and 1, that could be concluded from the density plot locus set for QMEAN score [36-37] (Fig. 5b). Figure 5a illustrations QMEAN scores for biological unit reference set that used as a tool for oligomeric protein assessment.

A comparison between normalized QMEAN score (-4.16) and protein size in non-redundant set of PDB structures in the plot revealed different set of Z-values for different parameters such as C-beta interactions (-4.60), interactions between all atoms (-4.62), solvation (-2.73) and torsion (-2.37) (Fig. 5b) [36-37]. The Z-score measures the total energy deviation of the GDF15 [NP_004855] protein structure with respect to an energy distribution derived from random conformations [35].

Fig. 3: Verified 3D Graph of GDF15 [NP_004855] GDF15 [NP_004855]: 61.28% of the residues have averaged 3D-1D score >= 0.2. Fewer than 80% of the amino acids have scored >= 0.2 in the 3D/1D profile.

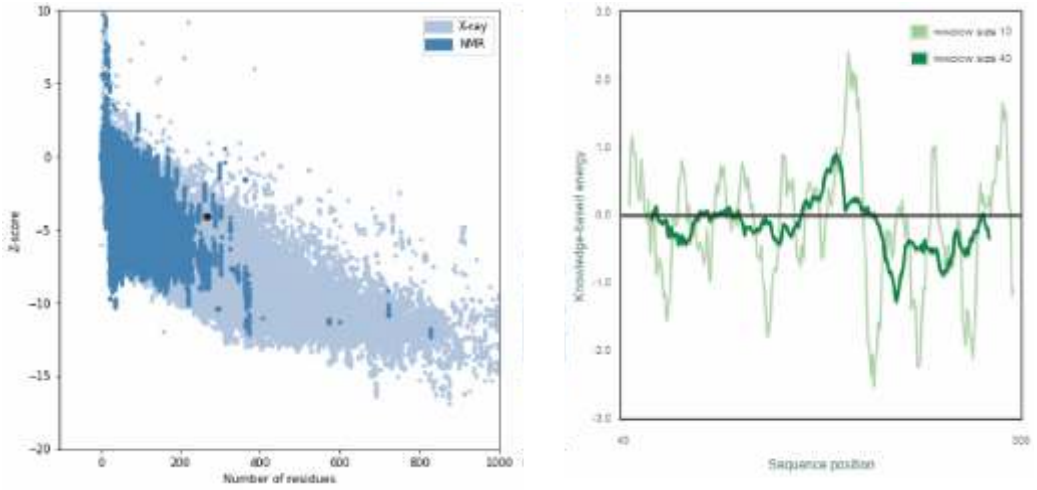

Fig. 4: ProSA Web Service Analysis of GDF15 [NP_004855] Overall Model Quality (A) and Local Model Quality (B)

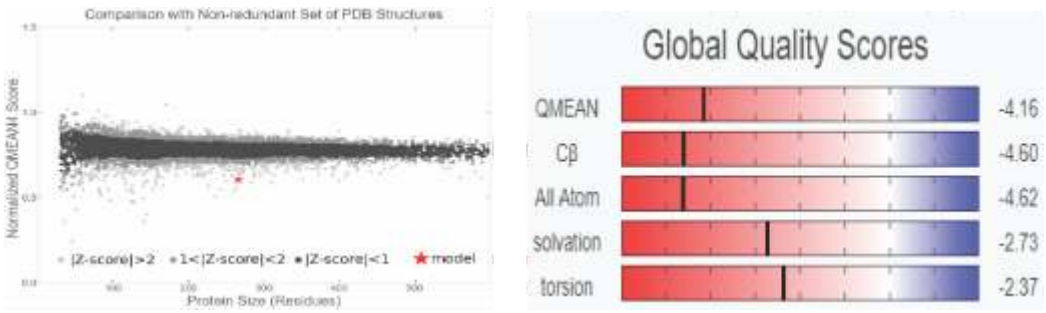

Fig. 5a: QMEAN Scores for Biological Unit Reference Set. [5b] Plot showing the QMEAN Value as well as Z-score

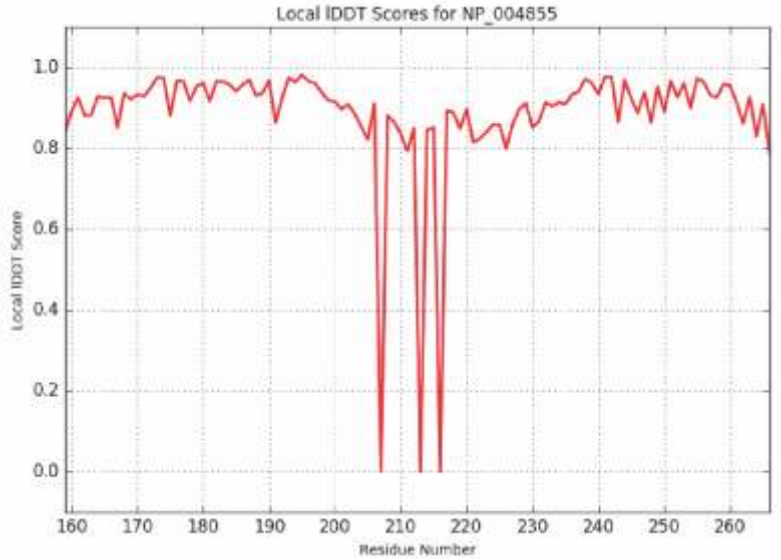

Fig. 6: Local IDDT Score of GDF15 [NP_004855]

The Z-score also tests variance of total structural energy with respect to the energy dispersal resulting from random conformation. Local Distance Difference Test (lDDT) score 0.8804 indicates a highly reliable structure (Fig. 6). The lDDT evaluates validation of stereochemical plausibility and local distance variances of atoms in model [38].

The GDF15 serum levels are observed abnormally high in patients associated with HG. Therefore, the structure model of GDF15 [NP_004855] is useful to understand its role in development of HG [39].

Conclusion:

The variants in GDF15 [NP_004855] associated with the risk of (HG). The functional characteristics of GDF15 [NP_004855] predicted by the generated mode. The structure, function and mechanism of proteins action could be studied through *in silico* modeling techniques. Methods ProSA, QMEAN, and PROCHECK build model reliability. The lDDT evaluates validation of stereochemical plausibility. Therefore, the structure model of GDF15 [NP_004855] is useful to understand its role in development of HG. *In-silico* docking study could explain the molecular association of GDF15 [NP_004855] with HG and new drug designing, for that structure model is very useful.

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