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In-Silico Molecular Characterization of Human TSHβ Subunit Protein

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Abstract: ***Hypothyroidism is a common disorder of endocrine system in which the thyroid gland does not produce enough thyroid hormone. The most common cause of hypothyroidism is inflammation of the thyroid gland, which damages the gland's cells. It can affect growth, cellular processes and many other body functions. As more damage accumulates, the risk of hypothyroidism increases. The molecular processes, mutations activating the dominant cellular genes and structure-function relationships of TSH permitted better understanding of the role of specific protein and carbohydrate domains in the synthesis, bioactivity, and clearance of the hormone. TSHβ subunit is a protein that in humans is encoded by the TSHB gene. Mutations located within the coding region of the TSHβ subunit gene responsible for hypothyroidism. The normal TSHB gene performs an essential function in normal production of thyroid hormone and the mutation of a TSHB gene is a key step leading to low hormone production. The present investigation was carried out to understand the molecular features of TSHβ protein through In silico characterization by retrieving the protein sequence information from major protein database, analysis of physicochemical properties, prediction of secondary structural elements of normal and mutated TSHβ proteins, tertiary structure prediction and structure visualization of normal and mutated TSHβ. This preliminary analysis forms the base for the detailed understanding of molecular mechanism of TSHβ subunit and further functional analysis of it can pave a new dimension for the treatment of hypothyroidism via structure based drug designing.***

Keywords: Thyroid, TSHβ subunit, gene mutation, hypothyroidism, *In-silico* etc

1. Introduction

Hypothyroidism is a common disorder of endocrine system in which the thyroid gland does not produce enough thyroid hormone. The most common cause of hypothyroidism is inflammation of the thyroid gland, which damages the gland's cells. It can affect growth, cellular processes and many other body functions [1]. Hypothyroidism is the commonest clinical disorder of thyroid function [3]. Hypothyroidism can caused by defect in the anatomy of the thyroid gland, abnormal development of the hypothylamus, disorders of the metabolism of the thyroid hormone, iodine deficiency, thyroid surgery, radiation treatment etc.[2] Among the various varieties of thyroid disorder the hypothyroidism is probably the most important, as it is requires an early diagnosis which is usually followed by appropriate therapy that can prevent the onset of brain damage. In a clinic based study from Mumbai out of 800 patients with thyroid diseases 79% had hypothyroidism [12]

TSHβ subunit is a protein that in humans is encoded by the TSHB gene. Cytogenetic location of the TSHβ is present at the short arm of chromosome no 1 at position 13. molecular location on chromosome 115,029,824 to 115,034,309bp [4]. TSHβ subunit consist of a 5' untranslated exon and two exons that encode a 138 aa chain that is cleaved into 118 aa mature protein [11]. The normal TSHB gene performs an essential function in normal production of thyroid hormone, and the mutation of a TSHB gene is an essential step leading to low hormone production [5]. TSHB gene mutation prevent the Production of functional thyroid stimulating hormone or its release from the pituitary gland as a result, thyroid hormone production is not stimulated. Leading to low hormone levels that are characteristics of hypothyroidism [15]. Five mutations located within the coding region of the TSHβ subunit gene responcible for hypothyroidism. But most frequent mutation in TSHβ subunit gene leading to a cysteine 105 to valine conversion (C105V) and to a frameshift with a premature stop codon at position 114 [6]. the risk of Hypothyroidism increases. In order to make an impact on Hypothyroidism, we must understand the molecular processes, mutations activating the dominant cellular genes and structure-function relationships of TSH permitted better understanding of the role of specific protein and carbohydrate domains in the synthesis, bioactivity, and clearance of this hormone [7].

With this detailed understanding of hypothyroidism and literature survey, the present study was carried out to understand the molecular features and role of TSHβ in hypothyroidism using bioinformatics approach like Primary protein sequence comparative analysis of normal and mutated by sequence retrieval from protein databases, Secondary structure prediction and understanding the basic secondary structural elements involved in normal and mutated TSHβ, Comparative studies of tertiary structures of normal and mutated TSHβ by modelling their 3D structures, Analysis of 3D structures of normal and mutated TSHβ with respect to their mutational sites and modifications.

2. Materials and Methods

2.1 Retrieval of protein sequence information of TSHβ

For the study of TSHβ molecule, its amino acid sequence was retrieved from the major protein sequence databases like UniprotKB. The UniProt Knowledgebase (UniProtKB) is the central hub for the collection of functional information on proteins, with accurate, consistent and rich annotation. The sequence obtained was stored in Fasta format with its accession number.

2.2 Analysis of Physicochemical properties

The analysis of physicochemical properties of TSHβ was done by using Protparam tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered protein sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) according to the method of john M. Walker, 2005.

2.3 Secondary structure prediction of TSHβ

The secondary structure prediction of TSHβ was carried out by using online secondary structure prediction tool SOPMA. self-optimized prediction method (SOPMA) has been described to improve the success rate in the prediction of the secondary structure of proteins,which gives the information of Alpha helix, beta sheets, extended strands and random coils according methods of Geourjon and Deleage, 1995.

2.4 Prediction of Tertiary structure of TSHβ

The Tertiary structure of TSHβ was obtained by using SWISS Model tool at ExPASy by selecting the template with maximum homology and with optimised parameters. SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the ExPASy web server, or from the program DeepView (Swiss Pdb-Viewer). The obtained structure was stored in pdb format for visualization.

2.5 Visualization of tertiary structure of TSHβ

The predicted Tertiary structure of TSHβ was visualized by using structure visualization tool Rasmol. RasMol is a computer program written for molecular graphics visualization intended and used primarily for the depiction and exploration of biological macromolecule structures. Visualization was done using different models and formats to understand structural features of TSHβ

Results and Discussion

3.1 Retrieval of protein sequence information of TSHβ.

The amino acid sequence was retrieved from protein sequence databases like UniprotKB and NCBI protein database. The sequence obtained was stored in Fasta format with accession_number P01222. The length of the sequence was found to be 138 aa as shown in figure 1(a)(b).

>sp|P01222|TSHB_HUMAN Thyrotropin subunit beta OS=Homo sapiens GN=TSHB PE=1 SV=2MTALFLMSMLFGLTCGQAMSFCIPTEYTMHIER RECAYCLTINTTICAGYCMTRDINGKLFLPKYALSQD VCTYRDFIYRTVEIPGCPLHVAPYFSYPVALSCKCGKC NTDYSDCIHEAIKTNYCTKPQKSYLVGFSV

Figure 1(a): Showing Protein sequence of normal TSHβ.

>sp|P01222|TSHB_HUMAN Thyrotropin subunit beta OS=Homo sapiens GN=TSHB PE=1 SV=2MTALFLMSMLFGLTCGQAMSFCIPTEYTMHIER RECAYCLTINTTICAGYCMTRDINGKLFLPKYALSQD VCTYRDFIYRTVEIPGCPLHVAPYFSYPVALSCKVGKC NTDYSDCIHEAIKTNYCTKPQKSYLVGFSV

Figure 1(b): Showing Protein sequence of mutated TSHβ.

3.2 Analysis of Physicochemical Properties

The analysis of physicochemical properties of TSHβ was done by using protein prediction tool .ProtParam tool computed that the TSHβ was basic in nature based on theoretical pi and unstable on the basis of instability index. The positive score indicates a higher hydrophobicity; according to the GRAVY index TSHβ were hydrophilic. Computed parameters for normal and mutated TSHβ were shown in below Table1 and Table2.

Table 1: Physicochemical properties of normal TSHβ

Properties	Values
No. of amino acid	138
Molecular weight	15639.3
Theoretical Pi	7.92
Total no. of negative charged residue (Asp+Glu)	10
Total no .of positively charged residue(Arg +Lys)	12
Atomic composition	
1)Carbon(C)	696
2)Hydrogen(H)	1071
3)Nitrogen(N)	173
4)Oxygen(O)	198
5)Sulphur(S)	19
Total no .of atoms	2157
Extinction coefficient No.	17140
Half life	30hrs
Instability index	48.97
Aliphatic index	74.93
Grand average of a hydropathicity	0.164

Table 2: Physicochemical properties of mutated TSHβ

Properties	Vlues
No. of amino acid	138
Molecular weight	15639.3
Theoretical Pi	7.95
Total no. of negative charged residue (Asp+Glu)	10
Total no .of positively charged residue(Arg +Lys)	12
Atomic composition	
1)Carbon(C)	698
2)Hydrogen(H)	1075
3)Nitrogen(N)	173
4)Oxygen(O)	198
5)Sulphur(S)	18
Total no .of atoms	2162
Extinction coefficient No.	17140
Half life	30hrs
Instability index	47.74
Aliphatic index	77.03
Grand average of a hydropathicity	0.177

3.3 Secondary structure prediction of TSHβ

Secondary structure prediction of TSHβ was carried out by using SOPMA and all the secondary structural elements like

alpha helix, beta sheets, random coils and extended strands for normal and muatated TSHβ were predicted as shown in table 2 (a)(b) and figure 2(a)(b)

Table 2(a): Showing secondary structure information of normal TSHβ

Structural components	*Residues*	*Percentage*
Alfa helix (Hh)	29	21.07%
3_{10} helix (Gg)	0	0.00%
Pi helix (Ii)	0	0.00%
Beta bridge (Bb)	0	0.00%
Extended strands(Ee)	39	28.26%
Beta turn (Tt)	18	13.04%
Bend region (Ss)	0	0.00%
Random coil (Cc)	52	37.68%
Ambiguous	0	0.00%

Table 2(b): Showing secondary structure information of mutated TSHβ

Structural components	*Residues*	*Percentage*
Alfa helix (Hh)	31	22.46%
3_{10} helix (Gg)	0	0.00%
Pi helix (Ii)	0	0.00%
Beta bridge (Bb)	0	0.00%
Extended strands(Ee)	39	28.26%
Beta turn (Tt)	16	11.59%
Bend region (Ss)	0	0.00%
Random coil (Cc)	52	37.68%
Ambiguous	0	0.00%

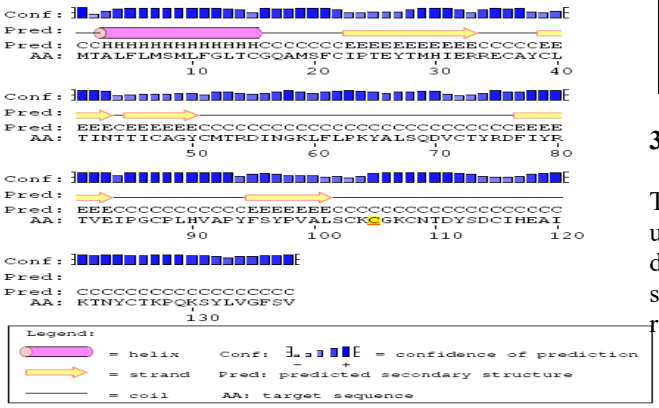

Figure 2(a): Secondary structure information of normal TSHβ

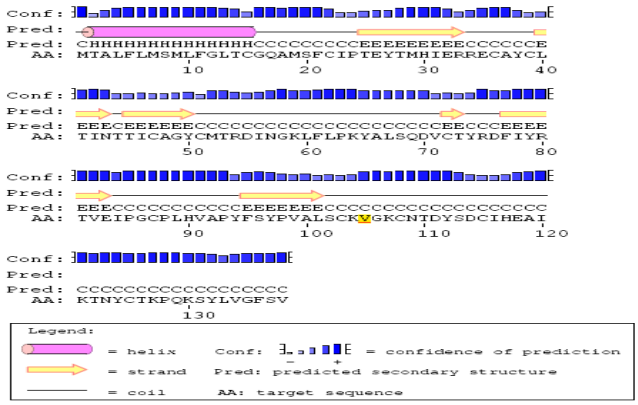

Figure 2(b): Showing secondary structure information of normal TSHβ

3.4 Prediction Tertiary structure of TSHβ

The Tertiary structure of TSHβ was obtained by using SWISS Model tool by selecting the template with maximum homology and with optimised parameters. The obtained structure was stored in pdb format for visualization. The details of template selected for structure prediction was as shown on table 3(a) (b).

Table 3 (a): Showing Template details for normal TSHβ

Sr no	**Name**	**Title**	Identity	Oligostate
1.	4ay9.1.B	follitropin subunit beta	42.59%	hetero-oligomer
2.	1xwd.1.B	Follitropin beta chain	42.59%	hetero-oligomer
3.	1xwd.2.B	Follitropin beta chain	42.59%	hetero-oligomer
4.	1fl7.1.B	follicle stimulating protein beta chain	41.67%	homo-dimer
5.	1fl7.2.B	follicle stimulating protein beta chain	41.67%	homo-dimer

Table 3 (b): Showing Template details for Mutated TSHβ

Sr no	**Name**	**Title**	Identity	Oligostate
1.	1xwd.2.B	follitropin subunit beta	41.67%	hetero-oligomer
2.	4ay9.1.B	Follitropin beta chain	41.67%	hetero-oligomer
3.	1xwd.1.B	Follitropin beta chain	41.67%	hetero-oligomer
4.	1fl7.1.B	follicle stimulating protein beta chain	40.74%	homo-dimer
5.	1fl7.2.B	follicle stimulating protein beta chain	40.74%	homo-dimer

3.5 Visualization of tertiary structure of TSHβ

The predicted Tertiary structure of TSHβ was visualized by using structure visualization tool Rasmol. Visualization was done using different models and formats to understand structural features of TSHβ. The various models were represented as shown in figures 3 & 4.

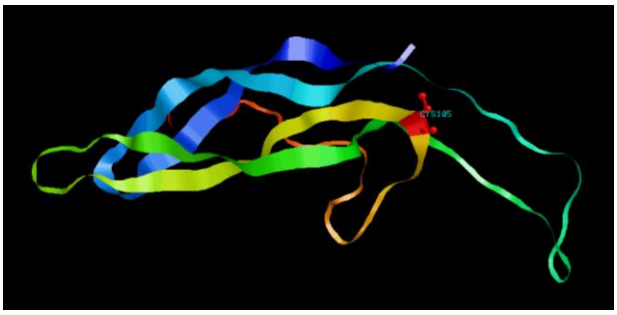

Figure 3: Showing 3D structure of normal TSHβ highlighting residue with position

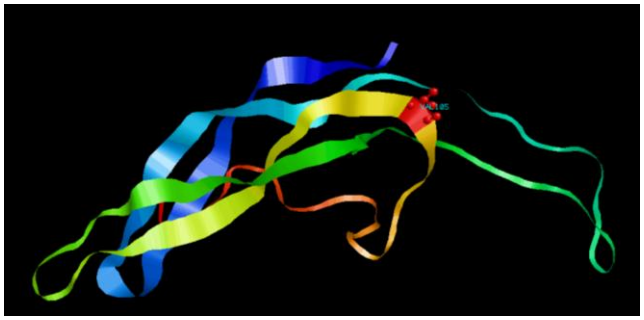

Figure 4: Showing 3D structure of normal TSHβ highlighting residue with position

References

[1] Dumont, JE., Lamy, F., Roger, P. & Maenhaut, C. "Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors," Physiological Reviews, Vol.72, No.3, pp. 667-697, ISSN 0031-9333,1992.

[2] *Garber, JR; Cobin, RH; Gharib, H; Hennessey, JV; Klein, I; Mechanick, JI; Pessah-Pollack, R; Singer, PA; et al.* "Clinical Practice Guidelines for Hypothyroidism in Adults," *(PDF) Thyroid 22 (12): 12001235,*doi:10,1089/thy,0205,PMID 22954017, 2012.

[3] Sunderesan, S. Progress achieved in universal salt iodization programme in India, In: Prakash R, Sunderesan S, Kapil U,editors, "Proceedings of symposium on elimination of IDD through universal access to iodized salt," New Delhi: Shivnash Computers and Publications, p. 28-42, 1998.

[4] Deladoëy J, Vuissoz JM, Domené HM, Malik N, Gruneiro-Papendieck L, et al. "Congenital secondary hypothyroidism due to a mutation C105Vfs114X thyrotropin-beta mutation," genetic study of five unrelated families from Switzerland and Argentina,Thyroid 13: 553-559, 2003.

[5] J Clin, "Pituitary stalk interruption syndrome and isolated pituitary hypoplasia may be caused by mutations in holoprosencephaly-related genes," Endocrinol Metab 98: E779-784, 2005.

[6] Dacou-Voutetakis C, Feltquate DM, Drakopoulou M, Kourides IA, Dracopoli NC."Familial hypothyroidism caused by a nonsense mutation in the thyroid-stimulating hormone beta-subunit gene," Am J Hum Genet 46: 988-993,1990.

[7] Oetting, A. & Yen, P. M. "New insights into thyroid hormone action," Endocrinol. Metab, 21, 193–208 ,2007.

[8] Roberts CG, Ladenson PW. Hypothyroidism *Lancet* ;363:793–803,2004.

[9] Mariusz w et al. "Thyroid-Stimulating Hormone and Thyroid-Stimulating Hormone Receptor Structure-Function Relationships," *University of Maryland Biotechnology Institute, Baltimore, Maryland; and Centre National.* 82: 473–502, 2002.

[10] Bianco, A. C. Minireview: "cracking the metabolic code for thyroid hormone signalling,". Endocrinology 152, 3306–3311,2011.

[11] Ross DS. "Thyroid hormone synthesis and physiology," Cooper JS, Mulder JE, editors. *www.uptodate.com* 2012.

[12] Ambika Gopalakrishnan Unnikrishnan, Sanjay Kalra, Rakesh Kumar Sahay, Ganapathi Bantwal, Mathew John, Neeraj Tewari "Prevalence of hypothyroidism in adults: An epidemiological study in eight cities of India", Indian Journal of Endocrinology and Metabolism, Volume : 17 , Issue : 4, 2013.

[13] Cheng, S. Y., Leonard, J. L. & Davis, P. J. "Molecular aspects of thyroid hormone actions,". Endocr. Rev. 31, 139–170, 2010.

[14] Brent, G. A. "Mechanisms of thyroid hormone action," J. Clin. Invest. 122, 3035–3043, 2012.

[15] Karges B, LeHeup B, Schoenle E, Castro-Correia C, Fontoura M, et al. "Compound heterozygous and homozygous mutations of the TSHbeta gene as a cause of congenital central hypothyroidism in Europe,". Horm Res 62: 149-155, 2004.

[16] Medeiros-Neto G, Herodotou DT, Rajan S, Kommareddi S, de Lacerda L, et al. "A circulating, biologically inactive thyrotropin caused by a mutation in the beta subunit gene," J Clin Invest 97: 1250-1256, 1996.

[17] Varjosalo M, Taipale J. Hedgehog: functions and mechanisms. Genes Dev 22: 2454-2472, 2008.

[18] Dracopoli NC, Rettig WJ, Whitfield GK, Darlington GJ, Spengler BA, et al. "Assignment of the gene for the beta subunit of thyroid-stimulating hormone to the short arm of human chromosome 1," Proc Natl Acad Sci USA 83: 1822-1826, 1986.

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