ORIGINAL ARTICLE Open Access

ISSN 2278-1404

International Journal of Fundamental & Applied Sciences

The interactions between variants of GALT in Galactosemia: *In-silico* Approach

Pallavi Surana[1], Shruti Singh[2], Sumathra Manokaran[3] and AH Manjunatha Reddy[4*]
Department of Biotechnology, RV College of Engineering, Mysuru Road, Bengaluru- 560059

Manuscript: received 02 Nov, 2018 revised 06 Nov, 2018 accepted 09 Nov, 2018

Abstract

Background: The In-silico studies encourages design of the drug molecules, understanding and development of the pathways, protein-protein interactions, stability of the molecules etc. these studies also help in identifying the disorders and in turn to derive the new drug molecules. Galactosemia is one of the metabolic disorders that affect the metabolism of the sugar galactose. It leads to accumulation of the galactose in the blood leading to quite morbidity and genetic disorders. **Objective**: The current paper discuss about the interactions between the variants of Galactose-1 -phosphate uridylyltransferase (GALT). **Methodology:** Using the Mutater software, the mutations N314D, S135L K285N and Q188R were incorporated in the sequence of the unmutated GALT. **Results:** The results obtained from webserver PROVEAN classified the mutation N314D as neutral compared to the other three, which were deleterious. The docking studies on Autodock Vina revealed that some mutated forms did not show 2-D interaction diagrams despite having a good bonding interaction. It was found that the mutated forms and unmutated forms interact largely with N314D mutated structures, which are a common incidence in galactosemia disorders.

***Keywords*:** Galactosemia; In-silico; mutations; GALT

1. Introduction

Galactosemia is a metabolic disorder due to absence of enzymes involved in galactose metabolism. There are three enzymes which are primarily involved in Leloir pathway Galactose-1-phosphate uridyl transferase (GALT), mutarotase and Galactokinase (GALK1) [1]. The various pathways involved in galactose metabolism are elucidated in Figure 1. It is an autosomal recessive disorder and the most common mutation which has been detected in GALT gene is Q188R and N314D. It occurs closest to the active site [2]. Galactosemia is of three types based on the affected enzyme of the Leloir Pathway. In this paper we are primarily going to discuss about Classical galactosemia which is due to the mutation of GALT enzyme. Due to lack of GALT enzyme, aldose reductase enzyme acts on galactose to convert it to galactitol. High levels of galactitol can act as metabotoxin, neurotoxin and hepatotoxin.The common mutations in GALT gene as confirmatory test for diagnosis of galactosemia is mostly S135L,Q188R,K285N and N314D variant [3].

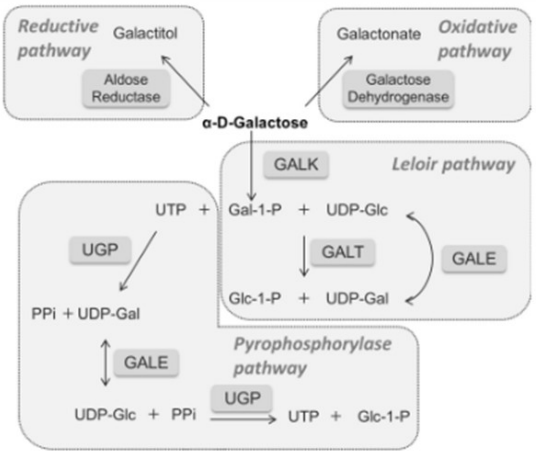


Figure 1: These are the different pathways of Galactose metabolism. Galactose is involved in Leloir pathway in the presence of GALT enzyme. In the absence of GALT enzyme galactose is converted to galactitol by the action of Aldose Reductase enzyme [1]

Corresponding author
Full Address :
Department of Biotechnology, RV College of Engineering, Mysuru Road, Bengaluru- 560059
E-mail: *ahmreddy@gmail.com*

2. Materials and methods

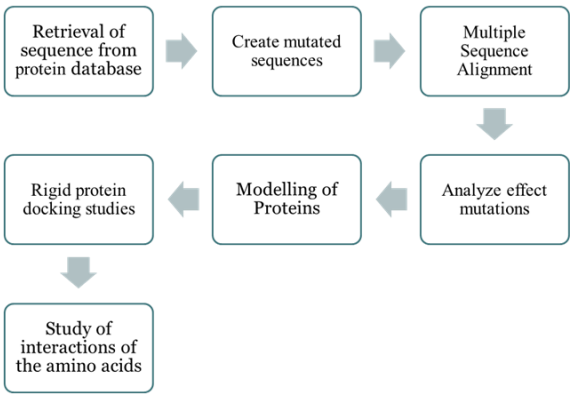


Figure 2: Workflow followed in this study

2.1 Data Retrieval

The data was retrieved in FASTA format from UniProt. The accession number of the GALT sequence is P07902 [4]. The most incidences of mutations were observed in GALT gene with over 300 mutations [5]. The most frequently observed point mutations are Q188R, K285N, S135L, and N314D [6].

2.2 Generation of mutations patterns

Software called Mutater was used to create mutated sequences of the protein sequence. These were then stored in .txt format [7]. Protein Variation Effect Analyzer (PROVEAN) was used in order to understand the nature of the mutations and to predict the effect of a mutation on the amino acid sequence [8]. Its prediction cut-off value is -2.5.

2.3 Multiple sequence alignment (MSA)

Multiple sequence alignment was done to find the similarity in unaltered sequence point-mutated sequences [9]. It also helped in understanding the evolutionary relationship via the cladogram construct. In Clustal omega the alignments are finally compiled by the alignment of two profile Hidden Markov Models (HMM) [10]. The output of the MSA was viewed on MView. It gives the results in a color-coded manner which is easier to interpret [11].

2.4 Homology modelling

Phyre^2 was used for homology modelling [12]. Phyre^2 is an updated version of Phyre. It has functionality to predict the 3D protein model by phenotypic expression of the point mutation [13]. Hence, this was well-suited for our study.

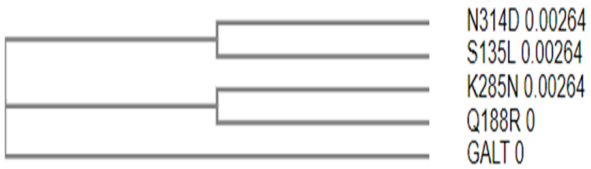
2.5 Docking studies

Molecular docking studies were performed using PatchDock [14, 15, 16, 17] and refinement of energy of the models using Firedock [18, 19]. The clustering RMSD was a default value of 4.0. The Patchdock server does rigid protein-protein docking. The top 10 models generated were refined on the basis of their binding energy or Gibbs free energy and the best output was chosen from Firedock. The 2D interaction diagram was viewed and enumerated using Discovery Studio [20].

3. Results and discussion

The software Mutater gave the mutated sequences off which the 3D models were generated using Phyre^2. The MSA was done on Clustal Omega. The mutations N314D and S135L belong to the same branch like K285N and Q188R. The cladogram construct has been illustrated in Figure 3.

Figure 3: Cladogram construct via Clustal Omega



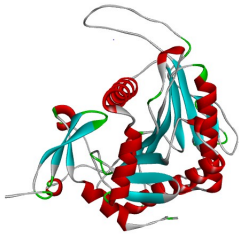
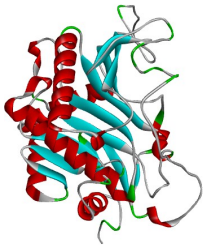
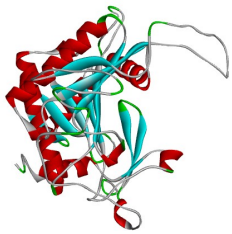
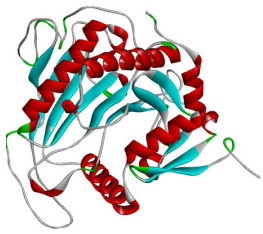
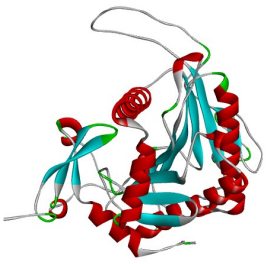
PROVEAN is a web-server used to predict the result of the mutation on a sequence and classifies them as either neutral or deleterious. The cut off score is -2.5. Any value above -2.5is considered as a neutral mutation. As concluded in Table 1, out of the 4 mutations considered only the substitution of N314D produced a neutral effect. This might suggest that K285N, Q188R and S135L are the ones that might be linked to causing a perturbation in galactose metabolism in galactosemia.

Table 1: PROVEAN results which classify mutations as deleterious or neutral

Sl No	Variant	PROVEAN Score	Predic-tion (cutoff = -2.5)
1	K285N	-4.075	Delete-rious
2	N314D	0.590	Neutral
3	Q188R	-3.901	Delete-rious
4	S135L	-4.701	Delete-rious

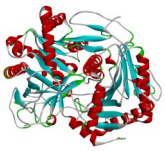
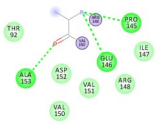
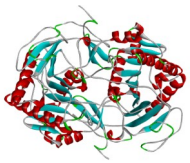
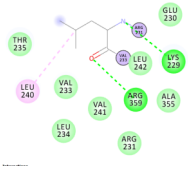
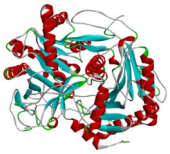
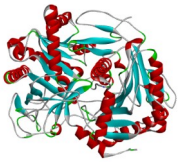
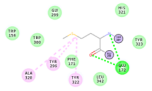
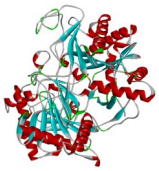
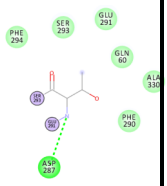
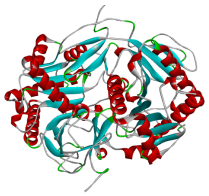
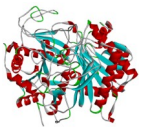
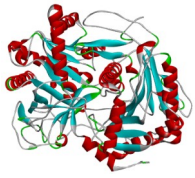
The 3D model of the sequences was constructed using Phyre^2. The best model with highest percentage similarity and best resolution were chosen for further studies. The results obtained from Phyre^2 are summarised in Table 2. The percentage identity of the template and the amino acid sequence for a good accuracy model is

Table 2: Homology modelling results from Phyre[2]

Name of protein	3D structure	Percentage identity (between template and sequence)	Secondary structure and Disorder Prediction curated results
Unmutated GALT		56%	Disordered (20%) Alpha helix (23%) Beta strand (19%)
K285N		56%	Disordered (20%) Alpha helix (23%) Beta strand (19%)
N314D		56%	Disordered (20%) Alpha helix (24%) Beta strand (18%)
Q188R		56%	Disordered (21%) Alpha helix (23%) Beta strand (19%)
S135L		56%	Disordered (21%) Alpha helix (24%) Beta strand (19%)

supposed to be in the 30-40% range. The secondary structure and disorder prediction of the template has been summarised. The alpha helixes have been represented by green helixes, beta sheets are indicated by the blue arrows and the faint lines signify the coils. The SS confidence indicates the confidence in the alignment; red for high accuracy and blue for least. The secondary structure and disorder results are cumulated and the percentage of the alpha helixes, beta sheets and the disordered amino acids are accounted. This also infers that the single point mutation doesn't contribute much to the overall identity of the resulting structure. Further, we estimated the interactions of the mutant and mutant; unmutated and mutant protein structures. The docking interactions are summarised as explicated in Table 3. The docking interactions were predicted using Patchdock. The refinement of the top 10 structures generated was then sent to Firedock for further energy minimisation and structure refinements. Out of the 10 combinations considered, the good interaction

Table 3: The interpretation of docking results from Patchdock and Firedock

Proteins involved	Docked Model	2D interaction diagram	Ligand and Protein atom involved in HB	Global Energy-GE (Kcal/mol)	HB contribution to GE (Kcal/mol)
N314D and S135L			Ala 153 with reactive O; Glu 146, Pro 145 with Nitro group	-202.44	-7.57
N314D and Q188R			Arg 359 with reactive O; Lys 229 with Nitro group	-187.81	-5.74
N314D and K285N		No diagram observed	None	-194.28	-7.91
GALT non-mutated (NM) and Q188R			Glu 174 with reactive O and N groups	-180.74	-8.94
K285N and Q188R		No diagram observed	None	-167.14	-8.01
N314D and GALT NM			Asp 287 with reactive Nitro group	-204.38	-7.71
GALT NM and K285N		No diagram observed	None	-198.90	-6.83
GALT NM and S135L		No diagram observed	None	-206.97	-7.88
Q188R and S135L		No diagram observed	None	-191.27	-4.63
K285N and S135L		No diagram observed	None	-196.2	-6.67

The interactions between neutral mutation N314D was seen with S135L, Q188R and the unmutated GALT protein with very good hydrogen bonding interactions. N314D on the other hand showed no interactions with K285N even though there was a good binding interaction of -194.28 Kcal/mol predicted using Firedock. None of the other combinations showed any noticeable interactions. The neutral effect mutation N314D shows interaction with other subtypes except K285N. This study helps us to conclude that the mutated forms and unmutated forms interact predominantly with the N314D mutated structures. We can predict that N314D mutated structures will be present in most galactosemia incidents for most patients. Further studies would be required to elucidate the interactions at the molecular level in the model organisms like Mus musculus and then conclude these preliminary findings.

Conflict of interest

The author's declares none.

References

1.Ana I. Coelho, Maria Estela Rubio-Gozalbo, Joao Vicente, Isabel Rivera. Sweet and sour: an update on classic galactosemia. Journal of Inherited Metabolic Disease. 2017: 40(3): 325-342.

2.Ramandeep Singh, Babu R. Thapa, Gurjit Kaur, Rajendra Prasad. Frequency Distribution of Q188R, N314D, Duarte 1, and Duarte 2 GALT Variant Alleles in an Indian Galactosemia Population. Biochemical Genetics. 2012: 50(11-12): 871-880.

3.Dobrowolski SF, Banas RA, Suzow JG, Berkley M, Naylor EW. Analysis of common mutations in the galactose-1-phosphate uridyl transferase gene: new assays to increase the sensitivity and specificity of newborn screening for galactosemia. Journal of Molecular Diagnostics. 2003: 5(1): 42-47.

4.Chen C, Huang H, Wu CH, Protein Bioinformatics Databases and Resources, Methods Mol. Biol. 2017: 1558: 3-39.

5.Anderson, Sharon, DNP, NNP-BC, APNG, GALT Deficiency Galactosemia, MCN. The American Journal of Maternal/Child Nursing. 2018: 43(1): 44–51.

6.Tyfield L, Reichardt J, Fridovich-Keil J, Croke DT, Elsas LJ 2nd, Strobl W, Kozak L, Coskun T, Novelli G, Okano Y, Zekanowski C, Shin Y, Boleda MD, Classical galactosemia and mutations at the galactose-1-phosphate uridyl transferase (GALT) gene, Hum Mutat. 1999: 13 (6): 417-430.

7.Butt AM, Ahmed A. MUTATER: Tool for the Introduction of Custom Position Based Mutations in Protein and Nucleotide Sequences. J Proteomics Bioinform. 2009: 2: 344-348.

8.Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the Functional Effect of Amino Acid Substitutions and Indels. PLoS ONE. 2012: 7(10): 1-13.

9.Fabian Sievers, Andreas Wilm, David Dineen, Toby J Gibson, Kevin Karplus, Weizhong Li, Rodrigo Lopez, Hamish McWilliam, Michael Remmert, Johannes Söding, Julie D Thompson, Desmond G Higgins, Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology. 2011: 1-6.

10.Fabian Sievers, Desmond G. Higgins, Clustal Omega for making accurate alignments of many protein sequences, Tools for Protein Science, The Protein Society. 2017: 27: 135-145.

11.Brown, N. P., C Leroy, C Sander; MView: a web-compatible database search or multiple alignment viewer, Bioinformatics, 1998: 14(4): 380-381.

12.Kelley, L.A., Sternberg, M.J.E. Protein structure prediction on the web: a case study using the Phyre server. Nat. Protoc. 2009: 4: 363-371.

13.Lawrence A Kelley, Stefans Mezulis, Christopher M Yates, Mark N Wass, Michael J E Sternberg, The Phyre2 web portal for protein modeling, prediction and analysis, Nature Protocols. 2015: 10: 845–858.

14.Zhang C, Vasmatzis G, Cornette JL, DeLisi C. Determination of atomic desolvation energies from the structures of crystallized proteins. J Mol Biol. 1997: 267 (3):707-26,

15.Duhovny D, Nussinov R, Wolfson HJ. Efficient Unbound Docking of Rigid Molecules. In Gusfield et al., Ed. Proceedings of the 2'nd Workshop on Algorithms in Bioinformatics (WABI) Rome, Italy, Lecture Notes in Computer Science. Springer Verlag, 2002: 2452: 185-200.

16.Schneidman-Duhovny D, Inbar Y, Polak V, Shatsky M, Halperin I, Benyamini H, Barzilai A, Dror O, Haspel N, Nussinov R, Wolfson HJ. Taking geometry to its edge: fast unbound rigid (and hinge-bent) docking. Proteins. 2003: 52(1): 107-112.

17.Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucl. Acids. Res. 2005: 33: W363-367.

18.N. Andrusier, R. Nussinov and H. J. Wolfson. FireDock: Fast Interaction Refinement in Molecular Docking, *Proteins* (2007): 69(1):139-159.

19.E. Mashiach, D. Schneidman-Duhovny, N. Andrusier, R. Nussinov, H. J. Wolfson. Fire Dock: a web server for fast interaction refinement in molecular docking. *Nucleic Acids Res*. 2008: 36(Web Server issue):W229-232.

20.Dassault Systèmes BIOVIA, Discovery Studio 2016, v.16.1.0.15350, San Diego: Dassault Systèmes, 2016.