Insilico Methodolgy - Resistin Involved In Diabesity
M Nimmala, A Rao, A Kumar

Abstract
In the present study, the role of several genes/proteins involved in the evolution of diabesity have been studied by several bioinformatics tools and software. Sequence and Phylogenetic Analysis was done using SDSC Biology workbench, a web based bioinformatics toolkit. Our bioinformatics analysis reports resistin gene as ominous link with diabesity. This bioinformatics study will be useful for future studies towards therapeutic inventions of diabesity. A multiple sequence alignment approach is used followed by construction of phylogenetic tree to compare the homology of functional proteins in one species and between other species. Sequences are analyzed to find mutations, evolutionary relationships, predicting structure, function and its abnormalities.

INTRODUCTION
Diabetes associated with obesity (diabesity) is common and is a life-long disease. In view of this, it calls for effective co-ordination among doctors, nurses, dietitians that requires reliable documentation and exchange of information[1]. The management of patients with type 1 diabetes mellitus requires regular insulin injections and monitoring of the metabolic status of the patient Diabetes mellitus is a chronic disease[2]. In this article, we briefly reviewed the computer assisted case interventions and current state of bioinformatics in the understanding of diabetes associated with obesity. The diabesity epidemic-i) Prevalence of diabetes 135 million people in 1995[3]. ii) Projected to be over 300 million by 2025[6]. iii)Over 80% of type 2 diabetic patients are overweight .The adipocyte is a metabolically active source of multiple proteins and cytokines that act via autocrine, paracrine and endocrine means- The adipocyte, gut and brain communicate regarding the body’s state of energy balance and set the “satiety” thermostat [4,5]
In view of this increasing incidence of diabetes mellitus, it is imperative that more sophisticated, fast, reliable and robust methods need to be devised to develop the best use of information science and technology in relation to diabetes, decision support and clinical management [16-22].

Over the years a wide range of comparative modeling-based methods have been developed for predicting the structure of a protein (target) from its amino acid sequence. The central idea behind these techniques is to align the sequence of the target protein to one or more template proteins and then construct the target’s structure from the structure of the template(s) using the alignment(s) as reference. The overall performance of comparative modeling approaches depends on how well the tree, constructed by considering sequence and sequence-derived information, agrees with the structure between the target and the template proteins. This can be quite challenging, as two proteins can have high structural similarity even though there exists very little sequence identity between them [23-26]. This led to the development of sophisticated profile-based methods and scoring functions [7] that allowed high-quality alignments between protein pairs whose sequence identities are as low as 20%. However, these profile-based methods become less effective for protein pairs with lower similarities. As a result, researchers are increasingly relying on scoring methods that also incorporate various predicted structural information such as secondary structure, backbone angles, and protein blocks [7,12,13,20].

Recently developed methods [21] that can accurately estimate the various parameters of resistin protein w.r.t to other proteins. For the first time, we're starting to understand why it is that being obese, diabetic and insulin resistant increases risk of atherosclerotic disease two-to-five-fold. Patients with diabetes have increased risk of coronary artery disease, stroke, and peripheral vascular disease [27-33]. Advances in scientific knowledge have provided the medical community with information and disease management strategies designed to tackle diabetes and its related complications. These strategies include behavioral and pharmaceutical methods for maintaining tight glycemic control, reducing hypertension, and reducing plasma lipids, which can help to prevent complications of the disease [34-39]. Because patient behavioral regimens can have a salient impact on the course of disease in diabetes, a patient-centered approach is particularly important. Accordingly, there is evidence to suggest that assessment of quality of life can be can be used in a clinical setting to improve communication among physician, diabetologist and patient to ensure that the clinical encounter focuses on topics that enhance well-being, that include effective symptom management, referral to relevant sub-specialty clinician , and good diet control and effective medication [40-42].

Through our research, we will look for the mechanisms involved. We hope that the knowledge we gain will help in preventing and treat Diabesity is classed as a metabolism disorder.

This is an exciting area of research for researchers to pinpoint relationship between obesity-associated diabetes and heart disease. The rise in diabesity, now at epidemic levels in the United States, a deadly combination that increases heart disease risk by two to five times. Research has shown an association between obesity and diabetes (diabesity) [43-48].

**MATERIALS AND METHODS**

Analysis encompasses a wide variety of methods used to find recurrent trends in data. In Bioinformatics the predominant applications are sequence analyses and microarray data analyses.

For the functional protein i.e resistin (receptor) for diabesity

**Sequence analyses – BLASTP**

Basic Local Alignment Search Tool---BLAST is one of the most widely used bioinformatics programs. Blast enables to compare a query sequence with a library or database of sequences. It identifies library sequences resembling the query sequence above a certain threshold which is a heuristic method. Performs local alignments through searches of high scoring segment pairs (HSP’s) before fast algorithms such as BLAST and FASTA were developed Dynamic programming was used. Database searches for the protein or a nucleic sequence was very time consuming by using a full alignment program like dynamic programming. BLAST is about 50 times faster than FASTA by searching only for the more significant patterns in the sequences, but with comparative sensitivity. When we want to analyze a sequence, the best thing to do is to search for homologous sequences in a database. Sequence alignment is an arrangement of primary sequence of DNA, RNA, or protein. Identification of regions of similarity. Consequence of functional, structural, or evolutionary relationships between the sequences.
can perform both Global and Local alignment. Tips to improve BLAST searches Don't always use the default parameters View reports

Sequence analyses is done using BLASTP for query sequence i.e Resistin protein with the following parameters for given organisms for Resistin is a recently discovered signal molecule, which could help elucidation of the pathophysiology of the insulin resistance and its correlation with obesity [49-51] As little information was available about resistin determination in venous blood at the time of our study, we focused on the question whether any correlation exists between persons with type 2 diabetes mellitus, with systemic inflammation, healthy persons and resistin concentrations and laboratory markers of inflammation, peptone, BMI. Differences of resistin values in these types of volunteers were studied as well.[52-55]

In this paper we focus mainly on tree representation of the family history of set of sequences that share a common ancestor is called a PhylogeneticTree. A phylogeny tree shows the connection among various organisms and weight of the branches in the tree indicates time between evolutions of different organisms. Uses of Phylogenetic Tree

- Determining the relatives of the organisms and interested.
- Identify the functionality of a gene
- Trace the origin of a gene

1. Retrieving Required Sequence (Protein/DNA) from Major Databases.
2. Using BLAST to Compare query sequence (Protein/DNA) to other Sequences
3. Preparing our session

1. Retrieving Protein sequence using major Protein databases.

Protein Information Resource (PIR)

Example to retrieve required Protein sequence from Swiss-Port

1.1. Open the link at expasy
1.1.2. Present required protein/gene i.e Resistin sequence in the Gene name window, and Submit
1.1.3. Select the required gene from given result

1.1.4. Obtain FASTA format of it.
1.1.5. Save the Sequence as in fig a

2. Retrieving DNA sequence using major Nucleotide Sequence databases

Major Nucleotide Sequence databases are
- European Molecular Biology Laboratory (EMBL)
- GenBank
- DNA databank of Japan (DDBJ)

2. Using BLAST to Compare query sequence (Protein/DNA) to other Sequences

2.1. Open the link at NCBI/BLAST
2.2. Click the Standard Nucleotide-nucleotide BLAST[blastp]
2.3. Paste the saved FASTA-formatted sequence into the BLAST Searching window as shown in fig b
2.4. Deselect the Do CD-Search box.
2.5. If you use Protein Sequence don’t change the Choose Database setting, because the nr (for non redundant) is the default protein database

2.7. Click the BLAST! Button
2.8. Click the Format button
2.9. When the results page appear, scroll down the page until you reach along list of sequences and save all these sequences

3. Preparing our session

To compute your multiple sequence alignment, any of the following can be used

- ClustalW :
- Dialign :
- Biology Workbench:

In my work,
Steps to produce multiple sequence using Biology Workbench server

3.1. Open the link at the Biology Workbench

3.2. Start a new session from session tools and run. Give session description

3.3. Session gets created

3.4 Resume session and run

4. Computing the Tree.

Steps involved in a phylogenetic tree

4.1. Open the link at the Biology Workbench

4.2. In the protein tools of our session add on our protein sequences as listed in the table 1.1

4.3. Select all protein sequences and run ClustalW multiple sequence alignment with required parameters, then submit which results in phylogenetic tree.

4.4. Click the Run Button and view the tree.

Shown in fig g

**Figure 2**

Table 1.1

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>1</td>
<td>ADIPOQ</td>
<td>AAH54496</td>
<td>244 aa</td>
</tr>
<tr>
<td>2</td>
<td>CETP</td>
<td>AAB59388</td>
<td>425 aa</td>
</tr>
<tr>
<td>3</td>
<td>HTR2C</td>
<td>CAI41395</td>
<td>456 aa</td>
</tr>
<tr>
<td>4</td>
<td>ICAM1</td>
<td>CAI39504</td>
<td>89 aa</td>
</tr>
<tr>
<td>5</td>
<td>IL6</td>
<td>CAG29292</td>
<td>212 aa</td>
</tr>
<tr>
<td>6</td>
<td>LEPR</td>
<td>AAI31780</td>
<td>232 aa</td>
</tr>
<tr>
<td>7</td>
<td>LMNA</td>
<td>CAI5523</td>
<td>614 aa</td>
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<tr>
<td>8</td>
<td>MAPK8</td>
<td>AAI30571</td>
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<td>9</td>
<td>PPARC</td>
<td>AAI06811</td>
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<td>PPARG</td>
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<tr>
<td>16</td>
<td>RBP4</td>
<td>CAH72328</td>
<td>201 aa</td>
</tr>
</tbody>
</table>

**Figure 3**

Fig a

**Figure 4**

Fig b

**Figure 5**

Fig c
RESULTS AND DISCUSSION
In this paper, work projected the phylogenetic tree along with the distance matrix result screenshot in fig as well as boxshade results in fig I, fig j. These results are helpful for further studies of this particular protein analysis in diabesity which aid in drug discovery for treating the patients after conducting the experiments i.e.

Invitro methodology

CONCLUSION
For phylogenetic tree construction, the essential performance parameters have been discussed here in detail to provide guidance to computational biologists. In particular, the stepwise discussion strategy is a very realistic and attractive goal, and this methodology, which, if appropriately used, can solve several problems and constitutes a powerful tool in the hands of researchers. The aim of this article, with application to comparison of functional proteins in species and further drug discovery. Phylogenetic tree construction of Resistin has methodology, which, if appropriately used can contribute to a further research towards the Research towards therapeutical aspects.

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Author Information

Manga Thayaru Nimmala  
Associate Professor, VNR Vignana Jyothi Institute Of Engg & Tech

Allam Appa Rao  
Jawahar Lal Nehru Technological University, Kakinada

Amit Kumar  
BioAxis DNA Research Centre