Bioinformatic Analysis of Functional Proteins Involved in Obesity Associated with Diabetes

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ABSTRACT

The twin epidemic of diabetes and obesity pose daunting challenges worldwide. The dramatic rise in obesity-associated diabetes resulted in an alarming increase in the incidence and prevalence of obesity an important complication of diabetes. Differences among individuals in their susceptibility to both these conditions probably reflect their genetic constitutions. The dramatic improvements in genomic and bioinformatic resources are accelerating the pace of gene discovery. It is tempting to speculate the key susceptible genes/proteins that bridges diabetes mellitus and obesity. In this regard, we evaluated the role of several genes/proteins that are believed to be involved in the evolution of obesity associated diabetes by employing multiple sequence alignment using ClustalW tool and constructed a phylogram tree using functional protein sequences extracted from NCBI. Phylogram was constructed using Neighbor-Joining Algorithm a bioinformatic tool. Our bioinformatic analysis reports resistin gene as ominous link with obesity associated diabetes. This bioinformatic study will be useful for future studies towards therapeutic inventions of obesity associated type 2 diabetes. (Int J Biomed Sci 2008; 4 (1): 70-73)

Keywords: bioinformatics; resistin; obesity and type two diabetes

INTRODUCTION

Diabetes Mellitus continues to be a devastating and daunting health scourge spreading across geographical and genetic boundaries. The growing incidence of type 2 diabetes with increasing obesity reflects that obesity is an emerging risk factor for the progression of insulin resistance and subsequently to overt type 2 diabetes. Both in normoglycemic and hyperglycemic states, obese people exhibit a higher degree of hyperinsulinemia that correlates with the degree of insulin resistance, in order to maintain normal glucose tolerance (2). Following attainment of certain point, the progressive deterioration of the metabolic milieu leads to eventual failure of hyperinsulinemia to compensate fully for the insulin resistance and thereby produces impaired glucose tolerance that progress to overt diabetes (5, 6). It has been presumed from genetic studies that there could be subset of genes whose expression changes with obesity and those genes whose expression further changes in the progression to type 2 diabetes. However, the molecular basis that links obesity and diabetes is still largely unknown.
Despite multiple efforts being made to dampen their impact on the quality of life of affected patients, there remains a lot of complexity in obesity mediated type 2 diabetes. By virtue of endocrinal role of adipose tissue, it is known to produce a vast array of adipocyte derived factors such as tumor necrosis factor alpha, interleukin-6, leptin, adiponectin and resistin. Since many of these adipokines profoundly influence insulin sensitivity and glucose metabolism, they form a fundamental bridge between increased adiposity and impaired insulin sensitivity (7). Although adipocytes are critical in obesity, their role in diabetes has been recognized.

Recently Gerken T et al (8) performed bioinformatic analysis and reported that the variants in the fat mass and obesity associated gene are associated with increased body mass index in humans. Barcelo-Batlloví S et al (1) utilizes the DIGE and Bioinformatic analysis for identification of potential drug targets of tungstate, DIGE analysis identified 20 proteins as tungstate obesity-direct targets, involved in: Krebs cycle, glycolysis, lipolysis and fatty acid oxidation, electron transport and redox. Protein oxidation was decreased by tungstate treatment, which confirmed a role in redox processes; however palmitate oxidation, as a measure of fatty acid beta-oxidation, was not altered by tungstate, thus questioning its putative function on fatty acid oxidation. Bioinformatic analyses using Ingenuity pathways highlighted peroxisome proliferator activated receptor coactivator 1 alpha (PGC-1 alpha) as a potential target. Elbers CC et al (3) identified five overlapping chromosomal regions for obesity and diabetes. These results illustrate the importance of proteomics and bioinformatics approaches for identifying new therapeutic invention of obesity is a challenging subject.

Bioinformatics has been in the focus since recent years for unraveling the structure and function of complex biological mechanisms. The analysis of primary gene products has further been considered as diagnostic and screening tool for disease recognition. Such strategies aim at investigating all gene products simultaneously in order to get a better overview about disease mechanisms and to find suitable therapeutic targets. This paper will therefore focus on potential implications of bioinformatics as a tool to identify novel metabolic patterns or markers associated with disease status. We will exemplify the potential of this method using the association between specific fats and development of obesity associated diabetes as a test case. In the present in silico study we have employed clustalW online bioinformatics tool for the analysis of seventeen genes, which are expected to be play major role in obesity and diabetes, we sought to identify the common central gene/protein that connects both the metabolic disorders such as obesity and diabetes.

**METHODOLOGY**

The present research aims at finding the proteins responsible for obesity associated diabetes in two phases. The first phase of the research aims to identify the candidate proteins/genes which are involved in these disorders through thorough literature search. The data pertaining to these proteins is extracted from the databases that are available online for free access. The functional protein sequences of these proteins in FASTA are extracted from (National Center for Biotechnology Information (NCBI), (http://www.ncbi.nlm.nih.gov).

The second phase of the research analyzes the data by employing Multiple Sequence Alignment using ClustalW online tool. These alignments produce a Phylogram tree along with the alignment scores. ClustalW adds sequences one by one to the existing alignment to build a new alignment because of its progressive nature. Progressive in this context means, it starts with using pairwise method to determine the most related sequences and then progressively adding less related sequences initial alignment.

**RESULTS & DISCUSSION**

From thorough literature search seventeen proteins (Table 1) were collected and constructed phylogram as shown in Figure 1. From the close identification of the figure it has came to know that resistin is an important protein of obesity-associated diabetes.

Numerous factors in obesity such as elevated free fatty acid levels, decreased adiponectin and increased adipocytokines are majorly responsible for evolution of insulin resistance (13). Resistin is a one such novel putative adipocyte derived signaling molecule induced during adipogenesis (15). It was discovered by virtue of its altered gene expression in mouse adipocytes in response to insulin sensitizers such as thiazolidinediones (TZD’s) resistin was originally named for its resistance to insulin resistin circulates as trimer and hexamer with intertrimer disulfide bond and processing of these bonds may be crucial to resistin activation (15). It is a peptide hormone that belongs to a family of tissue specific resistin like molecules (16). Since the discovery of resistin, there remains a lot of ambiguity with regard to the functional significance of resistin.
Plasma resistin levels are increased in ob/ob, db/db and diet induced obese mice (15). Concomitantly resistin m-RNA levels in obese rodents are often found to be decreased (12). There is often a discrepancy between circulating protein levels of resistin and m-RNA content in adipocytes (9).

In animals, resistin has been shown to be secreted by

| S. no | Gene name | Accession number | Length | Tissue |
|-------|-----------|------------------|--------|--------|
| 1     | *ADIPOQ*  | AAH54496         | 244 aa | Peripheral Nervous System, sympathetic |
| 2     | *CETP*    | AAB59388         | 425 aa | Liver |
| 3     | *HTR2C*   | CAI41335         | 458 aa | no |
| 4     | *IAPP*    | CAA39504         | 89 aa  | no |
| 5     | *ICAM1*   | AAH15969         | 532 aa | Kidney, renal cell adenocarcinoma |
| 6     | *IL6*     | CAG29292         | 212 aa | no |
| 7     | *LEPR*    | CAI31780         | 232 aa | PCR rescued clones |
| 8     | *LMNA*    | CAI15523         | 614 aa | no |
| 9     | *MAPK8*   | AA130571         | 427 aa | Pooled, cerebellum, kidney, placenta, testis, lung, colon, liver, heart, thyroid, bladder, uterus, PCR rescued clones |
| 10    | *PPARG*   | AAH06811         | 477 aa | Placenta, choriocarcinoma |
| 11    | *PPARGC1A*| NP_037393        | 798 aa | no |
| 12    | *RETN*    | AA101561         | 108 aa | Brain, cerebral cortex and lung, PCR rescued clones” |
| 13    | *SELE*    | CAI19360         | 484 aa | no |
| 14    | *SLC2A4*  | AAH34387         | 415 aa | Colon, Kidney, Stomach, adult, whole pooled |
| 15    | *SOCS3*   | CAG46495         | 225 aa | no |
| 16    | *UCP2*    | AAC51336         | 309 aa | skeletal muscle |
| 17    | *RBP4*    | CAI72328         | 201 aa | |

Figure 1. Phylogenetic tree that was constructed based on the alignment scores of all the protein sequences involved in obesity associated with diabetes.
adipocytes and to impair glucose tolerance and insulin action when infused into mice. A study has also reported increased resistin expression in human abdominal tissue. Several studies, however, have reported reduced resistin expression in human and rat obesity. Insulin, FFAs, and TNF-a have all been shown to inhibit resistin expression and all of these factors are elevated in obesity. Therefore, contrasting results obtained from both human and a rodent study made the role of resistin in obesity-induced diabetes is more and more controversial. The human resistin is a dimeric protein with 108 amino acids as compared to the murine resistin which comprises 114 amino acids. It raises blood glucose and insulin concentration and reduces hypoglycemic response to insulin infusion (18). Thus it was proposed to be an important link between obesity and insulin resistance. But in human its physiological function is still debatable. This is also produced by peripheral monocytes and its level correlate with IL-6 concentration raising the possibilities that it is probably associated with inflammation induced insulin resistance.

Recently List Eo et al (10) performed proteomic analysis using MALDI-MS/MS and reported that 17 proteins out of 28 proteins are involved in the energy metabolism. Smith et al (14) study reported that a polymorphism in the promoter region was associated with resistin mRNA levels in abdominal subcutaneous fat. Associations between resistin polymorphisms and type 2 diabetes have been reported in few studies (17). On the contrary, few other studies reported no such association between resistin polymorphisms and type 2 diabetes (11). Variation in the resistin gene is associated with obesity and insulin related phenotypes in Finnish human population. The variation in the resistin gene is not directly involved in the beta cell dysfunction but it may play crucial role in the pathobiology of obesity and insulin resistance that resulted in type 2 diabetes (4). Therefore, for the first time, this bioinformatics study reinforces the role of resistin in the pathophisiology of obesity mediated insulin resistance and type 2 diabetes.

CONCLUSION

Any rigid assessment of disease patterns will need support from well documented and curated databases. However, there are also severe practical and theoretical constraints known if applying bioinformatics as a tool for improved understanding and diagnostics of disease patterns. Though lot of controversies exist with regard to the role of resistin in metabolic disorders such as obesity and diabetes mellitus, it’s role is not completely excluded. Our Bioinformatics analysis once again heightens the possible role of Resistin gene that connects obesity and diabetes mellitus. In future studies like this may pave way for new therapeutic inventions of obesity associated diabetes.

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