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Bioinformatics assessment of Functional Genes/Proteins Involved in Obesity-Induced Type 2 Diabetes

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BACKGROUND & OBJECTIVE: Worldwide, the incidence of type-2 diabetes is rising rapidly, mainly because of the increase in the incidence of obesity, which is an imporant risk factor for this condition. Both obesity and type-2 diabetes are complex genetic traits but they also share some nongenetic risk factors. 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. **METHODOLOGY:** In this regard, we evaluated the role of several genes/proteins that are believed to be involved in the evolution of obesity associated diabetes through thorough literature search. Also we analyzed the data pertaining to genes of these proteins extracted from the databases that are available online for free access. **RESULTS:** The functional cDNA sequences of these genes/proteins are extracted from National Center for Biotechnology Information (NCBI) and Ensembl Genome Browser. Our bioinformatic analysis reports 21 genes as ominous link with obesity associated diabetes. Also this study indicated that, adipose tissue is now known to express and secrete a variety of metabolites, hormones and cytokines that have been implicated in the development of insulin resistance. **CONCLUSION:** This bioinformatic study will be useful for future studies towards therapeutic inventions of obesity associated type-2 diabetes.

Key words: *Bioinformatics tools; functional genes; obesity and type 2 diabetes.*

1. Introduction

Many chronic diseases like type 2 diabetes and its complications may be preventable by avoiding factors that trigger the disease process (primary prevention) or by use of therapies that modulate the disease process before the onset of clinical symptoms (secondary prevention). Accurate prediction and identification using biomarkers will be useful for disease prevention and initiation of proactive therapies to those individuals who are most likely to develop the disease. Recent technological advances in genetics, genomics, proteomics and bioinformatics offer great opportunities for biomarker discovery[1].

Obesity and its pathological complications, including atherosclerosis, hypertension and insulin resistance, have increased to reach epidemic dimensions nowadays[2]. Some important factors for the development of these disorders are excessive accumulation of abdominal fat, which is known to play an important role in development of chronic inflammation; deposition of lipids into non-adipose tissues such as liver and muscles; atherosclerosis and chronic inflammation that increase risk in cardiovascular disorders and diabetes[3].

Adipose tissue is not just a site of energy storage but also behaves as a dynamic endocrine organ[4]. It also plays an important role in energy expenditure, both as depot for energy-rich triglycerides and as a source for metabolic hormones as well[5,6]. Adipocytes produce a large number of so-called adipokines, such as leptin, adiponectin, interleukin (IL)-1b, IL-6 and tumor necrosis factor-alpha (TNF-a). Some of these molecules affect energy metabolism and insulin sensitivity in other tissues such as muscle and liver[7]. During obesity, lipid storage in adipocytes is increased, which triggers the release of adipokines[8,9]. During inflammation, the mature adipocytes of the adipose tissue are responsible for increasing production of pro-inflammatory adipokines[10], including mentioned TNF-a, IL -1b, IL-6. That disregulation contributes to obesity and chronic inflammation[11]. The local increase of these adipokines have been directly related to insulin resistance, increasing lypolisis and increasing leptin levels[6].

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[12]. 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[13]. 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[14,15,16]. However, the molecular basis that links obesity and diabetes is still largely unknown.

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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. Recently Gerken *et al.*[17] performed bioinformatics analysis and reported that the variants in the fat mass and obesity associated gene are associated with increased body mass index in humans. Although Elbers *et al.*[14] identified five overlapping chromosomal regions for obesity and diabetes. These results illustrate the importance of proteomics and bioinformatics approaches for identify new therapeutic invention of obesity is a challenging subject.

This study 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 study we have employed online bioinformatics tools for the analysis of 21 genes, which are expected to 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.

2. Materials and methods

The present research aims at finding the genes/proteins responsible for obesity associated diabetes in two phases. The first phase of the research attempts to identify the candidate genes/proteins which are involved in these disorders through thorough literature search. The second phase of the research analyzes the data pertaining to genes of these proteins obtained from the databases that are available online for free access. The functional cDNA sequences of these genes/proteins are extracted from: (1) National Center for Biotechnology Information (NCBI), (http\\www. ncbi.nih.nlm.gov), (2) Rat Genome Database (RGD) (<*http://rgd.mcw.edu/rgdweb /search/search.html*>), (3) Online Mendelian Inheritance in Man (OMIM), which can be accessed with the Entrez database searcher of the National Library of Medicine, Ensembl Genome Genome Informatics (MGI) website is hosted by The Jackson Laboratory, (5) HomoloGene, a tool of the NCBI.

Table I: Showing comparative gene map data of the genes/proteins that have been studied in the present study, which are believed to be involved in type-2 diabetics and obesity

	Rattus norvegicus				*Mus musculus*				*Homo sapiens*			
Gene	**Map name**	**Map position**	**Chr. number**	**Chr. position**	**Map name**	**Map position**	**Chr. number**	**Chr. position**	**Map name**	**Map position**	**Chr. number**	**Chr. position**
Adiponectin	genome 188043164 assembly 3.1	genome 3	79965888 q27		11 assembly 36.1	q23	Mouse genome		23146609 assembly	16	16	B3–B4 human
Resistin	Rat Celera Assembly	3566836	12	p12	Mouse Celera Assembly	3886118	8	8 A1	Human Celera Assembly	7605160	19	p13.2
Leptin	Rat Celera Assembly	52779315	4	q22	Mouse Celera Assembly	29063769	6	6 A3.3	Human Celera Assembly	122684619	7	q31.3
TNF-α	genome assembly 3.1	64647455	10	q25	Mouse Genome Assembly 36.1	78336352	11	11 B5	human genome assembly	23686912	17	q22-q23
IL-6	genome assembly 3.1	456798	4	q11	Mouse genome assembly 36.1	30339701	5	5 B1	Human Celera Assembly	22752396	7	
RBP-4	Rat Celera Assembly	2.33E+08	1	q53	Mouse Celera Assembly	38908311	19	19 D1	human genome assembly	95341583	10	q23-q24
Adipsin	genome assembly 3.1	11325546	7	q11	Mouse Celera Assembly	80905663	10	10 C1	Human Celera Assembly	784591	19	p13.3
LPL	genome assembly 3.1	22532512	16	p14	Mouse Celera Assembly	71423790	8	8 B3.3	human genome assembly	19841057	8	p22
Ghrelin	genome assembly 3.1	1.5E+08	4	q42	Mouse genome assembly 36.1	113666113	6	6 E3	human genome assembly	10302433	3	p26-p25
Chemerin	Rat Celera Assembly	72460060	4	q24	Mouse Celera Assembly	49080699	6	6 B2.3	Human Celera Assembly	144592497	7	
Visfatin	genome assembly 3.1	51132285	6	q16	Mouse genome assembly 36.1	33505340	12	12 B1	human genome assembly	105495899	7	q22.3
Omentin	Genome Assembly 3.4	87445119	13	q24	Mouse genome assembly 36.1	173448254	1	1 H2	human genome assembly	157659404	1	q21.3
PAI-1	Rat Celera Assembly	21377028	12	q11-q12	Mouse Celera Assembly	134078340	5	5 G2	Human Celera Assembly	95778744	7	q21.3-q22
FABP2	genome assembly 3.1	219554563	2	q42	Mouse Genome Assembly 36.1	122598310	3	3 G1	human genome assembly	120596008	4	q28-q31
PPARγ	Rat Celera Assembly	137316681	4	q42	Mouse Celera Assembly	117199637	6	6 F3-F1	Human Celera Assembly	12266744	3	p25
Aguti (AgRP)	genome assembly 3.1	35391672	19	q11	Mouse Genome Assembly 36.1	108090598	8	8 D1-D2	human genome assembly	66073974	16	q22
nSREBF-1	Rat Celera Assembly	44264875	10	q22	Mouse Genome Assembly 36.1	60012591	11	11 B2	human genome assembly	17656110	17	p11.2
FOXO1	Rat Celera Assembly	130806706	2	q26	Mouse Celera Assembly	52001471	3	3 C	Human Celera Assembly	22187272	13	q14.1
11β-HSD1	genome assembly 3.1	109252609	13	q27	Mouse Genome Assembly 36.1	195047834	1	1 H6	human genome assembly	206266585	1	q32-q41
Apelin	genome assembly 3.1	134460719	X	q35	Mouse Genome Assembly 36.1	45378323	X	X A3.2	Human Celera Assembly	129165798	X	q25
Vaspin (Serpina12)	Rat Celera Assembly	120432630	6	q32	Mouse Genome Assembly 36.1	105266979	12	12 F1	human genome assembly	94023372	14	q32.13

Table II: Showing gene ontology data of the genes/proteins that have been studied in the present study, which are believed to be involved in type-2 diabetics and obesity

Gene		Identifiers				Gene ontology	
	Secreted tissue(s)	MGD	OMIM	Homo-logene	Array IDs	Molecular function	Biological activities
Adiponectin	Adipose tissue	106675	605441	3525	rc_AI176736_at	Hormone activity	Positive regulation of I-kappaB kinase/NF-kappaB cascade. Negative regulation of gluconeogenesis. Positive regulation of fatty acid metabolic process. Positive regulation of glucose import.
Resistin	Brain, cerebral cortex, lung	1888506	605565	10703	rc_AA819348_at	Hormone activity	Increase transcriptional events leading to an increased expression of several pro-inflammatory cytokines.
Leptin	Adipocytes	104663	164160	193	D49653_s_at	Growth factor activity	Serve as a link between obesity and T2DM. Regulation of insulin secretion. Regulation of intestinal cholesterol absorption. Negative regulation of appetite.
TNF-α	Numerous cells, but mainly macrophages and lymphocytes	104798	191160	496	rc_AA943494_at	Cytokine activity	Induction of apoptosis via death domain receptors. Regulation of cell proliferation. Positive regulation of I-kappaB kinase/NF-kappaB cascade. Negative regulation of glucose import.
IL-6	Fibroblasts, lympho-cytes, adipose tissue	96559	147620	502	M26745cds_s_at	Cytokine activity	Cell-cell signaling Positive regulation of cell proliferation Negative regulation of apoptosis
RBP-4	Adipocyte tissue	97879	180250	4908	K03045cds_r_at	Transporter activity	Transport, visual perception and response to stimulus
Adipsin	White fat adipocytes		134350		GE1112269	Stimulates glucose transport in fat cells and inhibit lipolysis	The encoded protein is a component of the alternative complement pathway best known for its role in humoral suppression of infectious agents and the encoded protein has a high level of expression in fat, suggesting a role for adipose tissue in immune system biology.
LPL	Adipose tissue	96820	238600	200	L03294_g_at	Lipid transporter activity	Regulate fatty acid metabolic process Regulate lipid catabolic process
Ghrelin	Produced by P/D1 cells lining the fundus of stomach	1930008	605353	9487	A_44_P420046	Hormone activity	Positive regulation of appetite. Positive regulation of body size.
Chemerin	Hepatocytes, white adipose tissue	1918910	601973	2167	rc_AI176061_at	Cell differentiation activities	Retinoid metabolic process
Visfatin	Visceral adipose tissue	1929865	608764	4201	rc_AI177755_at	Cytokine activity	Cell-cell signaling, Positive regulation of cell proliferation
Omentin	Visceral adipose tissue	3057189	609873	79454		Sugar binding activity	Signal transduction
PAI-1	Endothelial cells, adiocytes	97608	173360	68070	GE1137951	Endopeptidase inhibitor activity and plasminogen activator activity	Glucose homeostasis Many other biological processes; associated with diabetes Mellitus
FABP2	Adipose tissue	95478	134640	107	A_43_P11691	Lipid transporter activity and fatty acid binding	Increase partitioning of glucose to triacylglycerols and enhance insulin resistance
PPARγ	Vascular smooth muscle cells, endo-thelial cells, adipo-cytes	97747	601487	7899	A_42_P462474	Transcription factor activity	Regulate lipid metabolic process Epithelial cell differentiation Regulation of fat cell differentiation Positive regulation of transcription
Aguti (AgRP)	Macrophages	892013	602311	7184	A_44_P257522	Receptor binding, neuro-peptide and hormone activity	Adult feeding behavior, neuro-peptide signaling pathway and hormone-mediated signaling
nSREBP-1	Adipose tissue	107606	184756	3079	rc_AI013042_at	Transcription regulator activity	Regulation of lipid metabolic process, steroid metabolic process, cholesterol metabolic process and regulation of transcription
FOXO1	Adipose tissue	1890077	136533	1527	rc_AA893671_at	Transcription factor activity	Regulation of transcription Regulation of cell proliferation
11β-HSD-1	Visceral adipose tissue	103562	600713	68471		Dehydrogenase activity and oxidoreductase activity	Lipid metabolic process
Apelin	Adipocytes	1353624	300297	8498	A_43_P12613	Hormone activity	Plays a role in regulation of blood pressure Stimulate gastric cell proliferation
Vaspin	Visceral adipose tissue	891971	107400	20103	A_44_P288224	Hormone activity	Regulates glucose tolerance and insulin sensitization

NCBI is a system for automated detection of homologs (similarity attributable to descent from a common ancestor) among the annotated genes of several completely sequenced eukaryotic genomes and (6) GeneCards is a database of human genes that provides genomic, proteomic, transcriptomic, genetic and functional information on all known and predicted human genes. GeneCards is being developed and maintained by the Crown Human Genome Center at the Weizmann Institute of Science.

3. Results

3.1 First phase (literature search)

From literature search several adipocyte-secreted factors has been demonstrated to potentially link obesity, insulin resistance and type 2 diabetes mellitus. These adipocytokines comprise mediators (Supplementary Table, S I) such as adiponectin, resistin, leptin (obesity factor), tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), retinol binding protein-4 (RBP-4), adipsin, lipoprotein lipase (LPL), ghrelin, chemerin,

plasminogen activator inhibitor-1 (PAI-1), fatty acid binding protein-2 (FABP2), peroxisome proliferators-activated receptor-g (PPARγ), Aguti (AgRP), nuclear sterol regulatory element-binding proteins-1c (nSREBP-1), winged-helix-forkhead box class O-1 (FOXO-1), 11b-hydroxysteroid dehydrogenase type-1 (11b-HSD-1), apelin and vaspin. These adipose derived factors are presently subjected to intensive research concerning their involvement in the regulation of adipose tissue physiology and in particular, their potential implication in insulin resistance, obesity and diabetes. In addition, most of these mediators may directly or indirectly interact with insulin receptors and/or insulin signaling, leading to insulin resistance in liver and peripheral tissues, especially in visceral obesity. The roles and mechanisms of some of the most important adipokines were suggested by some publications illustrated in S I.

3.2 Second phase (databases analysis)

The second phase of the research analyzes the gene orthologs and the gene ontology (***Tables I*** and ***II*** respectively) of the 21 detected genes. The data pertaining to these genes/proteins obtained from the databases that are available online for free access.

4. Discussion

The emerging epidemic of diabetes in Egypt and around the world cannot be ignored. According to the World Health Organization, over 180 billion people now have diabetes worldwide and this number is expected to double by the year 2030. Similarly alarming is the high prevalence of two factors closely linked with increased risk for diabetes: Metabolic Syndrome (MetS) and obesity[68]. Several recent studies investigated that, a number of common factors including genetic predisposition, poor dietary patterns, increased physical inactivity and longer life expectancy contribute to the rising prevalence of these disorders; subclinical inflammation may represent an additional novel risk factor. In this regard, epidemiologic data suggest that inflammatory biomarkers may serve as important risk indicators for the future development of diabetes[16,69,70,71,72,73].

Also, there is growing evidence that the insulin-resistance syndrome associated to obesity is mainly caused by excessive accumulation of fat in intra-abdominal adipocytes[22,74]. It has been observed that the surgical removal of visceral fat improves insulin effect on hepatic glucose production in animal models of obesity[75]. Adipose cells from visceral or subcutaneous depots largely differ concerning their metabolic characteristics as the control of lipolysis and the sensitivity to insulin[76]. Therefore, it would be interesting to define the regional adipose differences in the expression of the recently discovered proteins, which are candidate links between fat accumulation and insulin resistance.

Complex traits such as obesity and type-2 diabetes pose special challenges for genetic analyses because of gene–gene and gene-environment interactions, genetic heterogeneity and low penetrance of the individual genes. The heterogeneity means that it is difficult to generalize genome scan results over different populations and ethnicities. In addition, the exponential and alarming growth of the obesity epidemic has led scientists to begin to take advantage of proteomics to identify obesity molecular targets and to study the mechanisms of action of potential obesity therapies. Proteomics analyses have been proven useful in the characterization of the adipocyte proteome[77], in the identification of obesity targets in different models of experimental obesity and to characterize targets of several agents such as the insulin sensitizer rosiglitazone[78]. Although they are highly informative, these strategies often generate large amounts of data and long lists of proteins that are difficult to analyze and understand their biological importance.

The approach in this article is similar to the one in Rao *et al.*[79] and Park *et al.*[80], but it is more robust to the data here, which are more heterogeneous and encompassing the bioinformatic gene analysis of human, mouse and rat models in addition to other variables.

The present bioinformatic analysis showed significant relationships between metabolic and obesity type 2 diabetes disease risk factors and abdominal subcutaneous adipose tissue gene expression. Recently, You *et al.*[81] investigated that, the quantity of visceral fat was negatively related to leptin and adiponectin abdominal adipose tissue gene expression. In addition, hyperinsulinemia, as indicated by fasting insulin and 2 h insulin during the Oral Glucose Tolerance Test, was positively associated with adipose TNF-α and IL-6 gene expression. Also, Elbers *et al.*[14] yielded an interesting list of candidate genes by investigating the overlapping chromosomal linkage regions for type 2 diabetes and obesity, using a combination of six computational disease gene identification methods. Many of these identified genes are excellent candidates to study further for their role in the shared disease etiology between obesity and type 2 diabetes and a few have already been genetically or functionally associated with both disorders.

Current evidence supports that metabolic risk factors, including dyslipidemia, glucose intolerance and hyperinsulinemia, are linked with circulating levels of inflammatory and thrombotic cytokines[82,83]. Relationships between cytokine gene expression in adipose tissue and metabolic risk and insulin resistance have been reported as well[84,85]. Abdominal adipose gene expression levels of TNF-α[86], IL-6[87] and PAI-1[86] are positively linked with insulin resistance and other cardiovascular risk factors, whereas adiponectin gene expression is negatively associated with metabolic variables[85]. Our results were consistent with these previous findings and demonstrated that hyperinsulinemia was positively linked to adipose TNF-α and IL-6 gene expression and hyperinsulinemia and glucose intolerance were negatively linked to adipose adiponectin expression. Although these adipose-derived cytokines are traditionally viewed as the causes of the insulin resistance and metabolic risk[87], recent evidence suggests that an elevated TNF-α and IL-6 expression[88] and a decreased adiponectin expression[88] may also be a consequence of hyperinsulinemia. However, insulin infusion did not affect adiponectin gene expression in either healthy or type 2 diabetic individuals[89]. Therefore, this study provides information from previous literatures and genome databases of different websites and act as a material for future studies to clarify the underlying mechanisms of these associations and finding of new therapies of obesity associated type2 diabetes mellitus.

Conclusion

In conclusion, any rigid assessment of disease patterns will need support from well documented and curated databases. However, there are also several practical and theoretical constraints known if applying bioinformatics as a tool for improved understanding and diagnostics of disease patterns. So that, the current study provides evidence that the quantity of visceral fat and glucose/insulin complications of obesity is related to abdominal subcutaneous adipose tissue cytokine gene expression. Moreover, additional research is needed to discern whether abdominal subcutaneous adipocyte gene expression is causative for these risk factors or whether there is compensatory regulation of adipose tissue gene expression as a result of elevated visceral fat and/or insulin resistance.

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