Original Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN OMENTAL ADIPOSE TISSUES OF OBESITY AND TYPE2DIABETES: A META-ANALYSIS OF MICROARRAY DATASETS

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ABSTRACT

The Prevalence of metabolic disorders such as obesity and diabetes has been increased throughout the world. Several studies have established the association of obesity with diabetes in terms of causative agent for insulin resistance. The role of immune system in causing adipocyte inflammation is the new area of research in identifying obesity associated insulin resistance leading to type 2 diabetes. Thus understanding the differential gene expression profile with array data in comparison of both the conditions aids in significant outcomes in clinical research. With only limited meta-analysis research performed on the estimate of obesity and type 2 diabetes, we aimed to analyze the microarray datasets of healthy obese and diabetic obese subjects to identify the differentially expressed genes and further subjected to gene ontology studies. The meta-analysis of four different microarray datasets resulted in 145 genes with differential pattern of expression. The significantly expressed genes were clustered hierarchically by comparing obese and diabetic obese individuals. The genetic ontology of the differential genes was identified in terms of functional and biological annotations showing significant mechanisms involved in inflammation. The differential genes in KEGG enriched categories of different immune response pathways were observed. In conclusion, this meta-analysis study has successfully examined the differential expression profile of microarray datasets in conditions comparing obese and diabetic obese individuals. It has shown the significance of inflammatory mediators of adipose tissue in terms of progression of insulin resistance with strong interferences linking obesity with insulin resistance. Of note, inflammation formation in adipose tissue of obese individuals with contributory roles to the etiology of diabetes has been clearly established. The contemporary data and evidence showing the incidence of type 2 diabetes in obese individuals have created hype in understanding the expression profile of both the conditions. With only limited meta-analysis research performed on the estimate of obesity and type 2 diabetes, we aimed to analyze the microarray datasets of healthy obese and diabetic obese subjects to identify the differentially expressed genes and further subjected to gene ontology studies. The results have given strong inferences showing strong associations linking obesity with insulin-resistant diabetes mediated by inflammatory responses. Of note, inflammation formation in adipose tissue of obese individuals with its contributory roles to the etiology of diabetes has been clearly established.

KEYWORDS: ***Meta-analysis, obesity, type2diabetes, differentially expressed genes, microarray.***

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Received on: 16-08-2018
Revised and Accepted on: 08-10-2018
DOI: http://dx.doi.org/10.22376/ijpbs.2018.9.4.p135-144

INTRODUCTION

Over the past decades, there has been a significant increase in the prevalence of metabolic disorders such as obesity and diabetes in both developed and developing countries. Diabetes is a group of diseases characterized by chronic high blood glucose levels (hyperglycemia) due to the body's inability to produce sufficient insulin, the hormone that regulates blood glucose levels.[1] Equally challenging is the problem of weight gain, associated in part with major worldwide changes in caloric intake and dietary composition. Recent studies have established that obesity is associated with chronic systemic inflammation and that this low-grade inflammation may play a vital role in obesity-associated insulin resistance leading to type 2 diabetes[2]. Adipose tissue secretes a variety of substance like inflammatory mediators for immune response and also other hormones that help in metabolic regulation. The elevated inflammatory status appears to originate from infiltrated macrophages or other emerging immune cells in adipose tissue[3]. The altered elevatory levels of proinflammatory molecules in adipose tissues can owe to metabolic complications of obesity. Thus indeed, the role of the immune system in adipose tissues has become an exciting new area of research in the field of obesity and metabolic regulation owing to the mediation of pathogenesis causing the development of obesity-induced insulin resistance[4]. High throughput sequencing has improved rapidly as one of the important strategies for quantifying RNA expression levels, and along with sequencing technologies it has adopted a vital space in molecular biology research. Microarrays are one of the successful techniques in the field of molecular biology to monitor the expression levels of even ten thousand of genes simultaneously. Microarrays have been applied to studies in gene expression, genetic mapping, and discrimination of SNP, determining transcription factor activity and toxicity, pathogen identification and many other applications[5, 6].A meta-analysis is a statistical approach to quantitative, formal and epidemiological study design that accesses the reports of primary research to derive conclusions on the body of research. The outcomes of meta-analysis may include more precise estimates of the effect of a particular disease, risk factors of it or other consequences that relate the pooled analysis. The key requirements are the stated objectives with defined eligibility criteria, reproducible methodology, a search to identify the eligible studies relating the goal, assessment of the validity of the research findings and systematic presentation of the conclusion of the study[7]. And thus with the advent of these technologies for understanding pathological conditions at the molecular level, the meta-analysis of microarray data serves as a useful tool for interpreting the underlying differential gene expressions and variations in different samples. So the present study was carried out to identify the differentially expressed genes in healthy and diabetic obese to get a clear insight into the genetics of diabetes linked to obesity.

MATERIALS AND METHODS

The workflow for the analysis of differential gene expression consists of multiple components: retrieval of datasets, a meta-analysis of microarray datasets, processing of raw data, differential gene expression analysis, cluster analysis, gene ontology and pathway enrichment analysis (Figure 1).

Retrieval of datasets for the study

Microarray data repositories are the large collections of data that are implemented from different array experiments to serve the research community. The databases GEO (Gene Expression Omnibus) (https://www.ncbi.nlm.nih.gov/geo/) and Array Express (https://www.ebi.ac.uk/arrayexpress/) were utilized to obtain gene expression profile from microarray experiments. A search was conducted for microarray datasets using the keyword "Obesity and Diabetes". Among the list of 2259 hits obtained, 392 datasets were selected based on the organism type - *Homo sapiens*. Information based on age, clinical condition, pathology, tissue type was collected, and the data was further scrutinized based on several inclusion and exclusion criteria.

Inclusion criteria

Among all the datasets from *Homo sapiens,* experiments performed only on obese subjects with/without diabetes were included in this study. Studies carried only on omental adipose tissue origin were considered.

Exclusion criteria

Experiments carried out on obese subjects under pathological conditions other than diabetes and patients undergoing drug treatment were excluded. Further, datasets related to visceral and liver tissues, stem cells and blood cells and children were excluded. In this simplest experimental situation, a total of four datasets with the common array platform (Affymetrix) with the annotation type hgu133plus2 array was selected and downloaded from the Gene Expression Omnibus (Table1).

Table 1
Datasets selected for the study

S.No	Accession No	No of samples	Conditions	
			Obese	Diabetic Obese
1	GSE15773 (Hardy *et al.,*2011)	10	5	5
2	GSE29410 (Hoggard *et al.,*2012)	3	3	-
3	GSE20950 (Hardy *et al.,* 2011)	20	10	10
4	GSE71416 (Documatey *et al.,* 2015)	20	6	14
		Total		53

References of each datasets obtained for the study are mentioned in brackets

Meta-analysis of microarray datasets

A total of 53 samples from the selected datasets were pooled, and the open source software AltAnalyze and R programming environment were used to carry out the different layers of microarray data analysis (Figure 1).

Processing of raw data and normalization

To improve the ability to detect outliers and defects in the sample, it is essential to measure the quality of the datasets before subjecting it to any analysis. It starts with the processing of the raw data regarding quality control and normalization. The raw, CEL files of the datasets obtained for the study were processed using AltAnalyze to remove all the outliers. The preprocessing of microarray data includes the normalization of the data in which the expression ratios are log-transformed before the detection of differentially expressed genes[8].

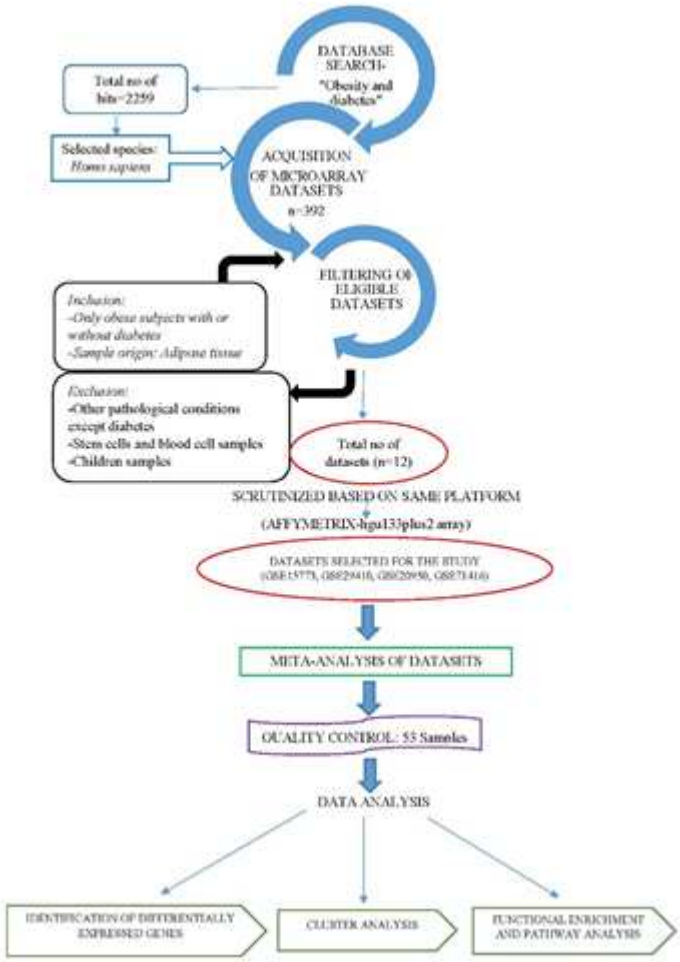

Figure 1
Study Overview

Differential gene expression analysis

Alt Analyze and the database version EnsMart72 were used to find the differentially expressed (DE) genes between the healthy obese and diabetic obese. The two groups were compared using moderated t-test and the linear model for microarray data (LIMMA) analysis with the cut-off of minimum fold change of 2.0 and a maximum p-value of 0.05 (fold change>2.0 and p-value<0.05).

Cluster analysis

The differentially expressed genes among the groups were clustered using "ggplot" package in R, and the heat map was constructed. In this heat map, the expressed genes were represented graphically by coloring each cell from measured fluorescence ratio with fold change and p-value.

Gene ontology and pathway enrichment analysis

Genecodis (www.genecodis.cnb.csic.es) was used to find the gene ontology of the differentially expressed genes. Gene ontology studies provide a brief descriptive framework on the functional annotation and biological classification of the gene sets in three different categories: biological process, cellular component and molecular functions. The pathway analysis was carried out using the program Web Gestalt (www.webgestalt.org) to identify the significant positive and negative regulating pathways of the DE genes.

RESULTS

Processing of raw data and normalization

The average raw intensity signals in each array samples of the dataset against their prominent intensity based on the mean quartile variations were compared before and after normalization (Figure 2 and 3). The probe distribution of each array based on the log2 expressions were identified and measured (Figure 4).

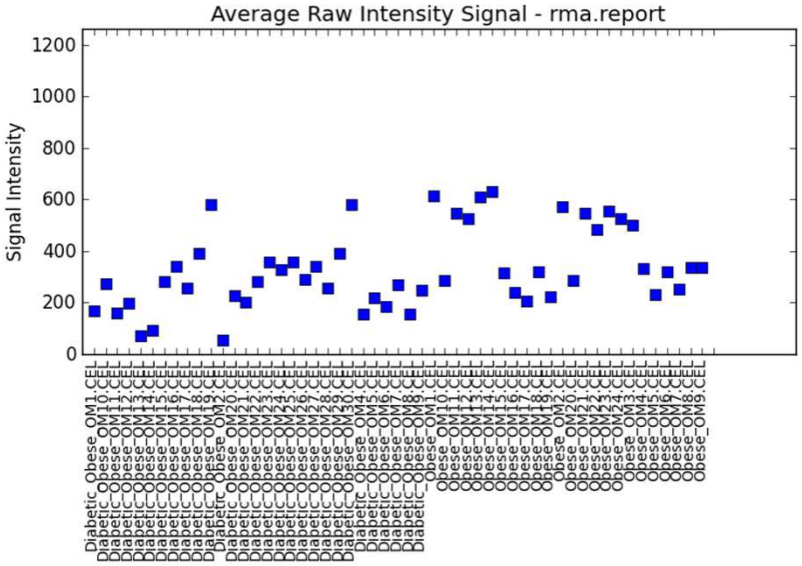

Figure 2
Box Plot of each sample before normalization

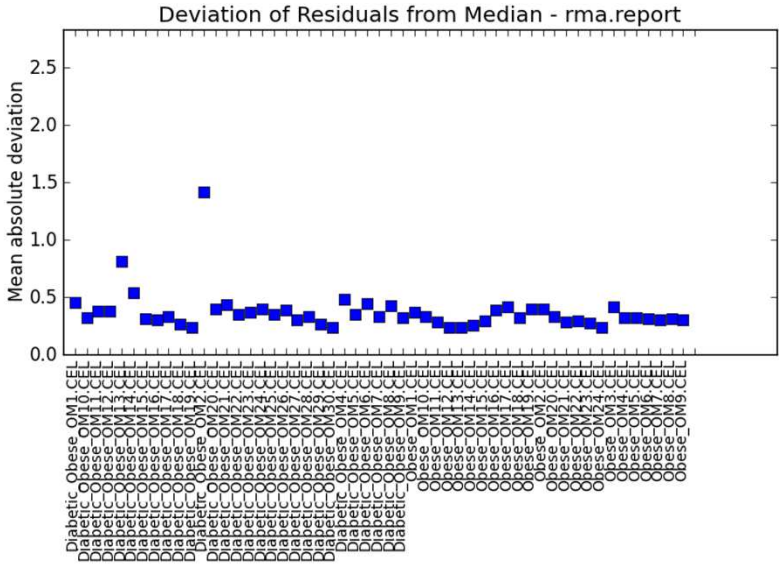

Figure 3
Box Plot of each sample after normalization

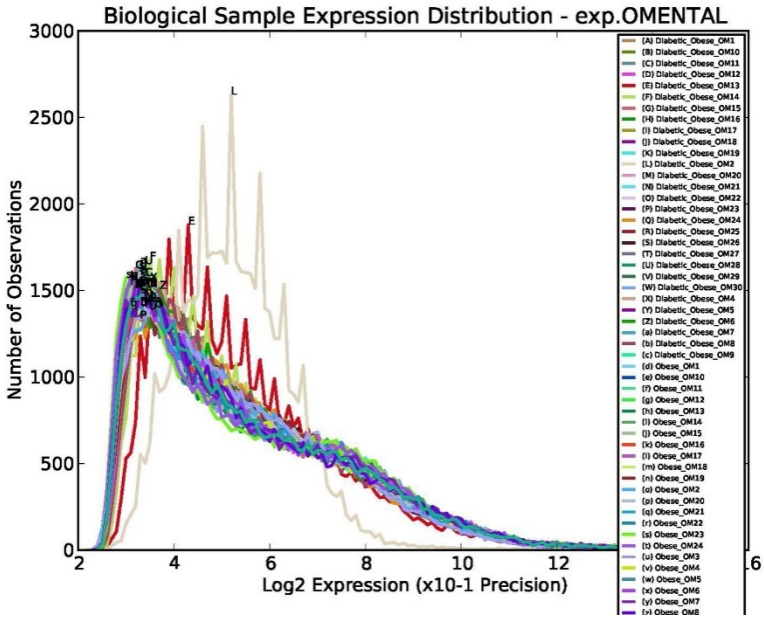

Figure 4
Expression level of each sample based on log transformation

Differential gene expression analysis

A total of 145 genes were found to show altered expression in samples of diabetic obese with healthy obese controls with the p-value < 0.05 and log fold change 2.0. Among which, 20 DE genes were found to be up-regulated and the remaining 125 genes were found to be down-regulated. A list of top 15 most significantly up and down-regulated genes are presented in Table 2 and 3.

Cluster analysis

The results of clustering of each gene were displayed using a heat map (Figure 5) with all 145 differentially expressed genes in obese compared with diabetic obese individuals.

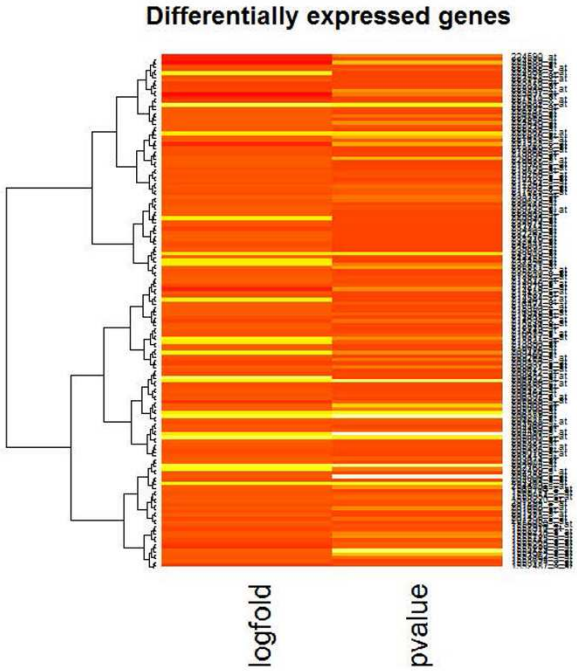

Figure5

Heat map of the Differentially Expressed genes between obese And diabetic obese individuals

Gene ontology and pathway enrichment analysis

The initial process in functional enrichment analysis is the biological process of each differentially expressed genes. Figure 6 shows the more significantly enriched biological processes were mainly associated with Cell adhesion (GO: 0007155, P=5.59749e-05) and negative regulation of transcription from RNA polymerase II promoter (GO: 0000122, P=0.000992776). The significantly enriched GO terms for cellular component (Figure 7) was higher in the cytoplasm (GO: 0005737, P=1.97097e-09) with the maximum number of genes-45 and in membranes (GO: 00160020, P=3.28193e-08) with 35 genes. The molecular functional characterization of the significant differentially expressed genes was carried out to elaborate the study concerning functional categories further. Figure 8 shows a list of 44 genes were significantly enriched with molecular function of protein binding (GO: 0005515, P=3.9281E-11) followed by calcium binding (8 genes) and RNA binding (8 genes).

Table 2

List of top 15 most significantly up-regulated DE genes

S.no	Gene ID	Gene Symbol	p-value	Fold change
1	205000_at	DDX3Y	0.025540518	1.666085603
2	202499_s_at	SLC2A3	0.008501342	1.317940144
3	222830_at	GRHL1	0.022761324	1.019128764
4	206211_at	SELE	0.03570443	1.517881753
5	206359_at	SOCS3	0.026838617	1.012713204
6	206932_at	CH25H	0.000321919	1.753047974
7	209480_at	HLA-DQB1	0.009978465	1.311464052
8	224321_at	TMEFF2	0.000136017	1.257781164
9	213350_at	RPS11	0.003544219	1.047089555
10	214461_at	LBP	3.66E-07	1.201252629
11	204409_s_at	EIF1AY	0.027896892	1.280033635
12	206700_s_at	KDM5D	0.031911492	1.108347414
13	202768_at	FOSB	0.033702334	1.546339468
14	230040_at	ADAMTS18	0.000628552	1.129118161
15	213831_at	HLA-DQA1	0.011036303	1.376856451

Table 3
List of top 15 most significantly down-regulated DE genes

S.no	Gene ID	Gene Symbol	p-value	Fold change
1	204560_at	FKBP5	0.002686374	-1.279602126
2	215990_s_at	BCL6	0.000164942	-1.055963606
3	203074_at	ANXA8	0.004389937	-1.049428951
4	217202_s_at	GLUL	0.005905253	-1.090647399
5	214587_at	COL8A1	0.00011324	-1.063075115
6	217023_x_at	TPSAB1	0.002854742	-1.18114921
7	210317_s_at	YWHAE	0.003708975	-1.177635876
8	1565717_s_at	FUS	0.000220036	-1.129446408
9	214218_s_at	XIST	0.010493486	-1.901237471
10	214693_x_at	NBPF10	3.34E-05	-1.157864095
11	205960_at	PDK4	0.003334388	-1.603200115
12	230165_at	SGO2	0.000729407	-1.126428319
13	204004_at	PAWR	0.000445108	-1.020047701
14	233314_at	PTEN	5.10E-05	-1.135778793
15	219529_at	CLIC3	3.61E-05	-1.210208836

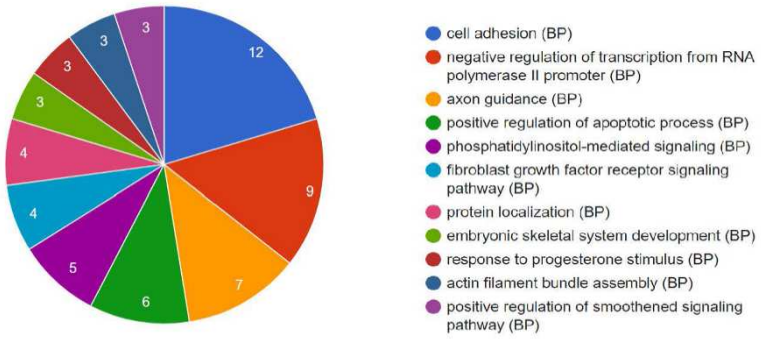

Figure 6
The hub genes representing the number of biological process

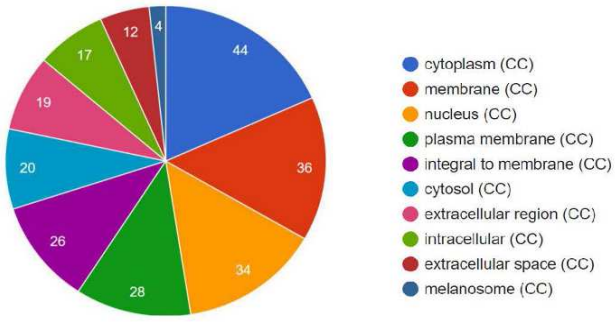

Figure 7
The hub genes representing number of cellular component

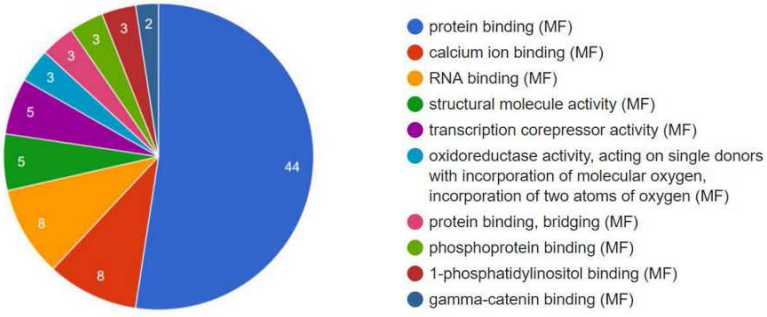

Figure 8
The hub genes representing number of molecular function

Pathway analysis

The evaluation of biological significance for the differentially expressed genes was made by the KEGG pathway enrichment analysis. Hypergeometric test with the p-value <0.05 was used as the criteria for pathway detection. The DE genes were found to be significantly enriched in two categories with 10 positively regulated and 10 negatively regulated pathways (Table 4).

Table 4

List of top 20 enriched KEGG pathway of differentially expressed genes

KEGG ID	KEGG name	No of genes	FDR value
	Positively regulated		
hsa05164	Influenza A	5	2.49E-02
hsa04940	Type I diabetes mellitus	2	6.08E-02
hsa05152	Tuberculosis	4	6.27E-02
hsa05321	Inflammatory bowel disease (IBD)	2	6.41E-02
hsa05310	Asthma	2	6.57E-02
hsa04668	TNF signaling pathway	3	6.83E-02
hsa04672	Intestinal immune network for IgA production	2	6.88E-02
hsa05166	HTLV-I infection	3	7.06E-02
hsa05320	Autoimmune thyroid disease	2	7.11E-02
hsa05145	Toxoplasmosis	3	7.17E-02
	Negatively regulated		
hsa04610	Complement and coagulation cascades	2	4.70E-01
hsa04390	Hippo signaling pathway	3	5.45E-01
hsa04621	NOD-like receptor signaling pathway	2	8.05E-01
hsa05133	Pertussis	2	9.49E-01
hsa01230	Biosynthesis of amino acids	1	9.50E-01
hsa05100	Bacterial invasion of epithelial cells	2	9.55E-01
hsa05200	Pathways in cancer	4	9.67E-01
hsa04915	Estrogen signaling pathway	3	9.70E-01
hsa04152	AMPK signaling pathway	3	9.73E-01
hsa01100	Metabolic pathways	5	9.74E-01

DISCUSSION

The findings revealed the differential gene expression pattern among the two adopted conditions: obese and obese diabetic obtained from omental adipose tissues. Since the selection of data was solely based on obesity and adipose tissue (mental), there was much exclusion incorporated in retrieving the datasets for the study. But in spite of all limits, the study reveals novel features of obesity and its associated contribution to diabetes. Primarily, the differential gene expression analysis was carried out by comparing the two conditions: obese and obese diabetic samples. The results obtained from the expression analysis suggested a list of 145 genes that were differentially expressed between the conditions with the regulation of fold change value >2.0 and p-value <0.05. The top 15 significantly up- and down-regulated genes showed relatedness with obesity-induced diabetes in adipose tissue (Table 2 and 3).

Differentially expressed genes involved in the mechanism of inflammation and its pathways

The DE genes in the healthy obese and diabetic obese have shown a significant response in relating the secretion of the adipocytes contributing to inflammation leading to resistant insulin action or inactivation of important insulin signaling pathways. The significant relation of the up-regulated genes with inflammation and immune responses in adipose tissues that connect obesity-mediated diabetes. The up-regulated gene CH25H cholesterol 5-hydroxylase acts as a corepressor in lipid metabolism blocking sterol regulatory element binding protein. And it also has a broad activity in pathogenesis playing a beneficial role in promoting host immunity; activated macrophage that lacks CH25H were unable to produce cytokine during the type I IFN inflammatory responses[9]. Interestingly the LBP gene for the protein lipopolysaccharide binding protein plays an acute-phase immunological response binding to the bacterial lipopolysaccharide and LPS interacts with LBP which activates inflammatory changes through NF-kappa-B cells and MAPK signaling[10].With consideration of the FDR value of the significant pathway, the most significant positively related is the Influenza-A pathway that is initiated via infection by the immune system. The infected pathway will lead to intracellular signaling cascades such as P13K pathway as well as MAPK signaling which plays an essential role in maintaining the insulin signaling[11].Significant insight was found in the pathway of TNF signaling which is a multi-functional cytokine that regulates cellular and molecular function in immune cells and also in energy metabolism. And also it has been stated that TNF-α is highly associated with obesity-induced insulin resistance by its converging effects on inhibition of insulin signaling and blunting the insulin action in target tissues[4].

Differentially expressed genes involved in the mechanism of glucose homeostasis

The up-regulated gene SLCA3 coding the protein solute carrier family 2 member 3 has significant pathway relation with the mechanism of glucose transport facilitating the transport of glucose that mediates the uptake of monosaccharides across the cell membrane. The exaggerated expression of SOCS3 genes has shown a marked increase in the muscle of type 2

diabetic patients with reduced insulin resistance.SOCS3 is strongly related to inhibition of insulin signaling suggesting that elevated expression of SOCS3 in the muscle may contribute to insulin resistance[12].The complement and coagulation cascades are the most significant negatively-regulated pathway with the FDR score of 4.70E-01 is the main column of innate immunity and the coagulation system also a major column for massive activation of immune responses at early injury stages. In spite of the immune responses, these cascades also result in dysfunction of metabolic and endocrine organs due to certain inflammasome formation. The significant role of metabolic dysfunction is seen in insulin target organs such as the adipose tissue or even liver in the cases of obesity. On the other hand, the inflammasome at pancreas due to macrophages and IL-1 contributes directly to the islets dysfunction in the course of type 2 diabetes pathogenesis[13].The pathway of the NOD-like receptor has shown vital role in inflammasome. These NLRp3 (NOD-like receptors) causing inflammasome might actually inhibit glycolysis. The TLR (Toll-like receptor) and NLPr3 produce the pro-inflammatory cytokine IL-1β. Hyperlipidemia will generate lipids such as palmitate and ceramides which activated NLRP3 that causes deposition of IAPP (islet amyloid polypeptide precursor) in the pancreas of type 2 diabetes. The activated NLRP3 caspase-1 that leads to the production of IL-1β, has negative feedback in blocking insulin and glucose uptake which are critically identified in the pathogenesis of type 2 diabetes[14].The major up-regulated gene has implications in causing inflammatory responses. Each gene associated with adipose inflammation links the suppression of secretion of insulin or increased resistance to insulin hormone via subsequent pathways. For instance, LBP gene has shown interactions with protein STAT3 (Signal transducer and activator of transcription 3) (Figure 9a) which is a transcription factor that mediates cytokine signaling playing a critical role in the pathogenesis of diabetes[15]. The signal transducer and the activation of transcription 3 (STAT3) involved in cytokine-induced insulin resistance and leads to the development of insulin resistance and type 2 diabetes are completely defined by Mashili et al[16]. Interestingly, the interaction of SOCS3 has also resulted in the association of it with STAT3 (Figure 9b) inducing cytokine leading to leptin resistance[17].

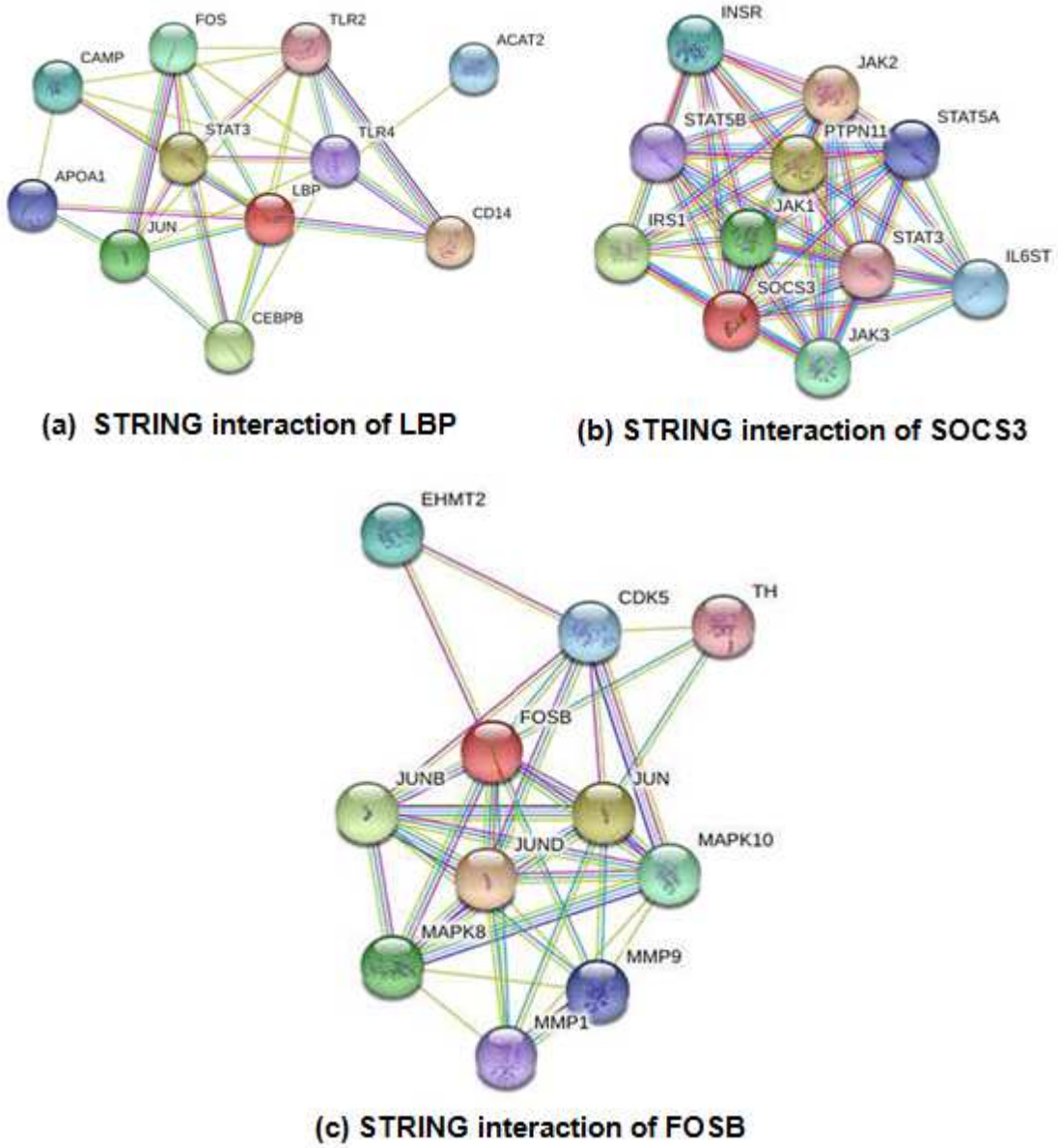

Figure 9

STRING interaction diagrams of most significantly genes involved in the inflammatory/immune Responses and insulin resistant pathways.

The interaction study of FOSB gene (Figure 9c) has shown the incidence of its relation with the mitogen-activated kinase (MAPK) signaling pathway. In studies performed in the identification of metabolic stress, models have shown pronounced and transient up-regulation of the transcription factors encoded by FOSB gene. The inference of the identification of differentially expressed genes between healthy obese and diabetic obese subjects of omental adipose tissue gave significant genes involving inflammation/immune response. Also the fact that a larger proportion of inflammation-related genes were upregulated (rather than down-regulated) in adipocytes of the obese individual samples further implies an active role for cells causing tissue inflammation. Some of these inflammation-related genes encode chemokines, cytokines and other up-regulated genes encode for cell adhesion molecules like fibronectin, integrin that helps to retain filtrating macrophages in the tissues leading to inflammation of adipocytes[18].

Comparison of differentially expressed genes between healthy obese vs diabetic obese and their expression in other non-inflammatory pathways

The anti-inflammatory adipokines such as adiponectin, IL-4, IL-10, IL-13, IL-1 receptors antagonist and ILF-β are abundant in the adipose tissue of lean individuals. In contrary, the adipose tissue of obese is dominated by proinflammatory adipokines like leptin, TNF-α, IL-6, IL-18, retinol binding protein 4, angiopoietin-like protein 2, chemokine ligand 2, chemokine ligand 5 and nicotinamide phosphoribosyl transferase. The investigations towards further characterization of genetic contributions to the pathways of abiogenesis, apoptosis and angiogenesis that explains the difference between the adipose tissue in the context of healthy obese individuals. The healthy obese were reported to expand the adipose tissue in a healthier way by increasing the number and size of adipocytes whereas diabetic obese interact with the cellularity of adipose tissue[19]. There are also reports claiming that metabolic healthy obese have higher lipogenic and angiogenic capacity compare to diabetic obese. The gene ontology studies on the DE genes of healthy obese and diabetic obese has shown an interesting insight of higher significance in positive regulation of the apoptotic process. In type 2 diabetes mellitus, insulin resistance with obesity leads to glucose toxicity effect accelerating β-cell death by apoptosis. Specific proinflammatory cytokines cause β-cell death by the induction of mitochondrial stress and other responses because cytokines of immune cells that have infiltrated the pancreas are reported to be crucial mediators of β-cell destruction via the apoptotic pathway[20]. Hyperglycemia exposed chronically may lead to oxidative stress and also inflammation that disrupts the regulation of gene expression which causes impaired insulin secretion and increased apoptosis[21]. The higher oxidative stress levels damage mitochondria and endoplasmic reticulum along with the cellular proteins, lipids and nucleic acids. Among which the endoplasmic stress has shown to be strongly associated with the β-cell apoptosis in type 2 diabetes[22]. The β-cell mass fluctuates according to the body need of insulin, β-cell deficiency correlates with glucose intolerance, β-cell death may directly lead to insulin deficiency when the loss is above 60% or more, it is accompanied by the presence of insulin resistance with obesity. Of note, inflammasome formation in adipose tissue of obese individuals has been clearly established and its contributory roles to the etiology of diabetes have also been inferred significantly.

CONCLUSION

In conclusion, the meta-analysis successfully examined the differential gene expression pattern in omental adipose tissues of healthy obese and diabetic obese subjects that showed a great progress in terms of understanding of obesity-induced inflammation a causative factor for developing insulin resistance. The differentially expressed genes that are involved in inflammatory insulin resistant pathways are CH25H, LBP, FOSB, SOCS3, SELE, HLA-DQA1 and HLA-DQA. In addition, the interaction of three genes namely LBP, FOSB and SOCS3 have shown strong interactions with significant pathways such as MAPK signaling pathway, STAT3 signaling which plays a vital role in insulin signaling cascades. The meta-analysis also further confirms the role of inflammatory mediators like TNF-α and IL-6 interfering with the hormone insulin causing insulin resistance in obese individuals. Thus it is intriguing that an increase in inflammatory mediators predicts the interlink of obesity and diabetes.

AUTHORS CONTRIBUTION STATEMENT

Authors Vetrivel Preethi and Murugesan Rajeswari has contributed to the design and implementation of the research, Authors Natchimuthu santhi, Senthil Kalaiselvi has involved in the planning and supervised the work, and Author Vetrivel Preethi wrote the manuscript with consultation and discussions with Murugesan Rajeswari, Senthil Kalaiselvi and Natchimuthu Santhi.

CONFLICT OF INTEREST

Conflict of interest is declared none.

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