JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2016. Vol. **10**(2), p. 1167-1174

Meta-Analysis of Microarray Data and Integrated Pathway Enrichment Analysis Hints Novel Gene Signatures Associated with Pathogenesis of Obesity in *Rattus norvegicus*

P. Onkara Perumal[1]* and Y. Pydi Setty[2]

[1]Department of Biotechnology; [2]Department of Chemical Engineering;
National Institute of Technology Warangal, Telangana, India.

(Received: 18 February 2016; accepted: 21 April 2016)

Obesity is a worldwide community health hazard that has direct implications on reduced lifespan. Obesity induced T2DM is an inherently complex and multi-factored disease, for that reason the intricate mechanistic links remain unclear. An improved understanding of the molecular mechanisms of obesity induced T2DM would greatly help in the appropriate selection and implementation of therapeutic interventions and also lifestyle changes in susceptible individuals. The purpose of this work was primarily to address two objectives. First, to understand the perturbations induced by internal factors in obesity by performing meta-analysis of published microarray data from different, but similar studies to identify meta-gene expression signatures that were not observable in individual studies. Second, perform an integrated pathway enrichment analysis of meta-gene expression signatures to investigate the combined characteristics of the meta-gene expression signature in the pathogenesis of obesity induced T2DM. Our meta-analysis study have identified an improved set of dysregulated gene signatures for obesity that were not identified in individual studies. Further, an integrated pathway enrichment analysis had revealed a significant enrichment for pathways involving Il1rl1, Snap25, Ccl11, Cxcl13 and Fmo1, indicating a probable macrophage infiltration in rat visceral white adipose tissues. However, further investigation with a more comprehensive dataset is needed to draw better conclusions.

Keywords: Obesity, meta-analysis, microarray, integrated pathway enrichment analysis, *Rattus.*

Obesity is a pathologically significant risk factor of vascular disease in individuals with type 2 diabetes mellitus (T2DM) [1, 2]. The increasing incidence of T2DM in non-obese, normal BMI and lean individuals is also a related matter of concern[3]. Mostly obesity arises as a consequence of complex interactions between genetic, dietary, and metabolic factors. A more exhaustive assessment on causes of obesity have been reviewed elsewhere [4, 5]. Obesity definitions essentially attempt to categorize susceptible individuals carrying risk factors for additional interrelated comorbidities. Conventionally, obesity is defined as a body mass index (BMI) of 30 kg/m^2 and higher, while overweight is defined as a BMI between 25 and 30 kg/m^2. However, this index could vary considerably across geographical locations. Among several other definitions of obesity waist circumference (WC), adiposopathy (''sick fat''), body fat percentage (BF %) have also been proposed to categorise metabolic subtype of obese individuals [6, 7, 8]. Coincidently, the pathogenic role of adipose tissue in obesity is the pivotal measure for defining these metabolic subtypes. In this context, we set our focus on the profound influence of body fat (visceral/ abdominal adipose tissue) in the pathogenesis of obesity.

* To whom all correspondence should be addressed.
Tel.: 0870-2462882;
E-mail: popomal@nitw.ac.in

Animal models shares several common characteristics of human obesity and related co-morbidities. So, these models could be extremely useful in research studies focused on the development of novel prevention and/or treatment methods for obesity. Recently, a concise report on animal models of obesity segregated into different categories based on mutations or manipulations of one or more individual genes and genetically intact animal models exposed to obesogenic environments have been reported [9]. Diet-induced obesity models are particularly useful in studying pathogenesis of polygenic obesity. Differential gene expression studies in diet-induced obesity rat models had revealed up regulation of lipid metabolism genes and downregulation of genes related to redox and stress proteins[10]. Cross talks between the visceral-pancreatic adipose tissue and beta-cells that contributed to beta-cell plasticity and a novel obesity pathway facilitating the communication between adipose tissue and pancreatic islets have also been reported in rat animal models [11, 12].

MATERIALS AND METHODS

Probing differentially expressed genes is a common practice in identifying biomarkers or signatures of phenotypic states such as diseases or multifaceted treatments. Meta-analysis of microarray data from public repositories have also been proven as a viable strategy to identify key mediating factors in complex diseases. "Meta-analysis of microarray data is a statistical approach that combines microarray dataset results from independent but related studies. This approach is comparatively an inexpensive alternative that has the potential to increase equally the statistical power and generalizability of individual-study analysis."[13]. Our objectives were to first identify genes expressed differentially between two groups and subsequently perform meta-analysis of the selected microarray datasets to identify the meta-gene expression signature between samples from heterologous datasets and in turn use the meta-gene expression signature data for integrated pathway enrichment analysis and construct a transient gene interactions network.

Selection of suitable data sets for the study

Microarray datasets were searched in the open source repository National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) [14] Microarray datasets containing supplementary data (.CEL) files were only considered for the study. The search filters were set for "Expression profiling by array" and search terms included "cel [Supplementary Files]","affymetrix" "obesity", "adipose" "*Rattus*". "*norvegicus*". Based on the search two datasets GSE36935 and GSE44372 were retrieved and processed for the meta-analysis study. GSE36935 included five samples obtained from rat adipose tissues from the mesenteric surrounding the pancreas (pMES) from two obese and three non-obese animal subjects. GSE44372 included six samples obtained from rat visceral pancreatic white adipose tissues from three obese, and three non-obese animal subjects. All sample data were considered for the meta-analysis study. The characteristics of selected data sets are shown in Table 1

Individual Microarray Dataset Processing

Prior to meta-analysis study, we have performed individual analysis of the two microarray datasets from adipose tissue samples from obese rats (*Rattus norvegicus)*. Individual microarray datasets (raw files) that were retrieved from GEO were processed using Microarray ꟻ US [15]. Featuring a user-friendly graphic interface, Microarray ꟻ US is an R based program that integrates functions from most widely used Bioconductor[16] packages. The affy package was employed for initial data analysis. Pre-processing of the datasets were carried out with robust multi-array average (RMA). The presence of technical artefacts and variance in microarray experiments were identified by quality control analysis using QC report Hierarchical clustering analysis was performed with Euclidean distance. Statistical analyses of differentially expressed genes were performed using Bioconductor's RankProd package[17] The non-parametric statistical method based on the rankings of the fold changes of genes was applied to evaluate differences in gene expression between individual samples from case and controls.

Meta-analysis of microarray datasets

The pre-processed datasets were used for

the meta-analysis using combined rank product method based on the RankProd package. Genes were ranked based on the combined rank product (combinedRP). The p-value for meta-analysis was $p < 0.05$. The top 20 (up & down regulated) genes are shown in Table 2. The heat maps for the top 20 up and down regulated genes based on the combined rank product are is shown in Figure 1 (c). & Figure 1 (d).

Integrated pathway enrichment analysis

Genes involved in the same biological processes, functions, or localizations present correlated behaviors in terms of expression levels and signal intensities, therefore statistical tests could be applied to identify perturbed pathways. Integrated pathway enrichment analysis is one of such techniques. To improve our understanding of the altered biological coordination involved in the meta-gene expression signature, an integrated pathway enrichment analysis was carried out to explore the role of obesity factors observed in these meta-gene expression signature data.

Integrated pathway enrichment analysis was carried out with INMEX (INtegrative Meta-analysis of EXpression data) [18] and ClueGO[19] a plug-in application in Cytoscape[20]. INMEX pathway analysis includes Gene Ontology (GO) [21] terms and pathways while ClueGO includes a comprehensive data of all known Gene Ontology (GO) terms and pathways, Kyoto Encyclopedia of Genes and Genomes (KEGG) [22], REACTOME[23] and consolidates them in to a functionally unified network, which represents the biological link in relation to the pathways and the Gene Ontologies. For the analysis, we have used the two-sided (enrichment/depletion) hyper-geometric distribution tests. The p-value significance was set to ≤ 0.05. The kappa-statistics score was set to 0.3. The enriched pathways are shown in Figure 2. The list of enriched pathways along with their associated genes are shown in Table 2.

RESULTS AND DISCUSSION

Individual microarray data studies

A list of 106 genes for GSE36935 and 173 genes for GSE44372 were considered dysregulated genes with a $p \leq 0.05$, adjusted $Pfp \leq 0.05$ and fold change of 1.5. After removing probes for which no information were available 69 genes for GSE36935 and 118 genes for GSE44372 were considered to be statistically significant and differentially expressed between the case and the control. The differentially expressed gene list from the two individual data sets are given in *(Supplementary Table ST-1, ST-2)*. The heat-maps displaying the significant differentially expressed (up-down regulated) genes are shown in figure 1 (a) and 1 (b).

The significant gene signatures from individual datasets were used to classify the associated Gene Ontology (GO) terms and their associated pathways. Analysis of GSE36935 and GSE44372 datasets revealed pathways and Gene Ontology terms primarily associated with glycero-lipid metabolism, T-cell receptor signalling pathway, cytokine-cytokine receptor interaction, Jak-STAT signalling pathway, chemokine signalling pathway, pyruvate metabolism, adipo-cytokine signalling pathway, beta cell receptor signalling pathway, and glyoxylate and di-carboxylate metabolism. The complete list of pathways and associated terms are given in (Supplementary Table ST-5, ST-6).

Meta -analysis of microarray datasets

The meta-gene expression signature containing the list of top 20 up and down regulated genes from the meta-analysis of GSE36935 and GSE44372 datasets are shown in Figure 1 (c). & Figure 1 (d). The statistically significant list of DE

Table 1. Characteristics of selected the data sets

GEO Accession No.	Size (Case: Control)	Source	Platform
GSE36935	3:2	Adipose tissue from the mesenteric surrounding the pancreas (pMES)	Affymetrix Rat Genome 230 2.0 Array
GSE44372	3:3	Visceral pancreatic white adipose tissue	Affymetrix Rat Genome 230 2.0 Array

Table 2. Top 20 dysregulated genes identified in meta-analysis (ranked by combined-rank product RP)

Probeid	Symbol	Genename	gene. index	Combined RP	FC(class1 /class2)	PfP	P. value
		Up-regulated genes					
1398276_at	Dlg2	discs, large homolog 2 (Drosophila)	30162	11.9826	0.2369	<0.05	<0.05
1371293_at	LOC688228	similar to Myosin light polypeptide 4 (Myosin light chain 1, atrial isoform)	3842	17.1205	0.2796	<0.05	<0.05
1386936_at	Grifin	galectin-related inter-fiber protein	19004	22.4893	0.2997	<0.05	<0.05
1367660_at	Fabp3	fatty acid binding protein 3, muscle and heart	209	26.0948	0.3201	<0.05	<0.05
1371895_at	Krt14	keratin 14	4444	34.2286	0.3437	<0.05	<0.05
1373684_at	Klhl31	kelch-like 31 (Drosophila)	6233	53.5796	0.3737	<0.05	<0.05
1371959_at	LOC690131	similar to H2A histone family , member O	4508	62.9186	0.3948	<0.05	<0.05
1384487_at	Dmrt2	doublesex and mab-3 related transcription factor 2	17022	80.2733	0.4082	<0.05	<0.05
1370568_at	Adra2c	adrenergic, alpha-2C-, receptor	3117	82.2757	0.4256	<0.05	<0.05
1383599_at	Rbbp4	retinoblastoma binding protein 4	16146	89.9704	0.3763	<0.05	<0.05
1375303_at	Ldb3	LIM domain binding 3	7852	93.4723	0.4477	<0.05	<0.05
1370026_at	Cryab	crystallin, alpha B	2575	110.7841	0.4244	<0.05	<0.05
1387748_at	Lep	leptin	19816	111.0101	0.4096	<0.05	<0.05
1377163_at	Inhbb	inhibin beta-B	9712	121.0292	0.4849	<0.05	<0.05
1368618_at	Grb14	growth factor receptor bound protein 14	1167	128.8616	0.4744	<0.05	<0.05
1384147_at	Eif1a	eukaryotic translation initiation factor 1A	16690	144.04	0.473	<0.05	<0.05
1383624_at	RGD1565033	similar to hypothetical protein LOC284018 isoform b	16171	149.4794	0.4906	<0.05	<0.05
1389809_at	Pmepa1	prostate transmembrane protein, androgen induced 1	21877	159.4288	0.5105	<0.05	<0.05
1368406_at	Star	steroidogenic acute regulatory protein	955	159.7914	0.488	<0.05	<0.05
1368290_at	Cyr61	cysteine-rich, angiogenic inducer, 61	839	161.2961	0.4154	<0.05	<0.05
		Down-regulated genes					
1387273_at	Il1rl1	interleukin 1 receptor-like 1	19341	29.4087	5.7452	<0.05	<0.05
1388272_at	Igh-1a	immunoglobulin heavy chain 1a (serum IgG2a)	20340	33.1987	11.309	<0.05	<0.05
1387319_at	Ccl11	chemokine (C-C motif) ligand 11	19387	39.794	4.192	<0.05	<0.05
1368610_at	Mca32	mast cell antigen 32	1159	48.9714	3.8767	<0.05	<0.05
1398390_at	Cxcl13	chemokine (C-X-C motif) ligand 13	30276	79.0808	4.4979	<0.05	<0.05
1385682_at	Vit	vitrin	18214	99.6759	2.8679	<0.05	<0.05
1393347_at	Itgal	integrin, alpha L	25414	119.7804	2.7871	<0.05	<0.05
1387053_at	Fmo1	flavin containing monooxygenase 1	19121	128.4255	2.7585	<0.05	<0.05
1386869_at	Actg2	actin, gamma 2, smooth muscle, enteric	18937	131.0052	3.0089	<0.05	<0.05
1368420_at	Cp	ceruloplasmin	969	132.172	2.7239	<0.05	<0.05
1387902_a_at	RGD1562855	similar to Ig kappa chain	19970	134.5786	4.2169	<0.05	<0.05
1387073_at	Snap25	synaptosomal-associated protein 25	19141	139.0631	2.5511	<0.05	<0.05
1387134_at	Slfn3	schlafen 3	19202	140.6522	2.5917	<0.05	<0.05
1367785_at	Cnn1	calponin 1, basic, smooth muscle	334	169.853	2.9184	<0.05	<0.05
1369664_at	Avpr1a	arginine vasopressin receptor 1A	2213	172.7489	2.3955	<0.05	<0.05
1367786_at	Psmb8	proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional peptidase 7)	335	180.4497	2.3452	<0.05	<0.05
1387157_at	Pmfbp1	polyamine modulated factor 1 binding protein 1	19225	185.1379	2.664	<0.05	<0.05
1367652_at	Igfbp3	insulin-like growth factor binding protein 3	201	192.4172	2.4245	<0.05	<0.05
1380063_at	Ch25h	cholesterol 25-hydroxylase	12610	205.1111	2.2745	<0.05	<0.05
1394401_at	Elovl6	ELOVL family member 6, elongation of long chain fatty acids (yeast)	26287	207.6419	2.5183	<0.05	<0.05

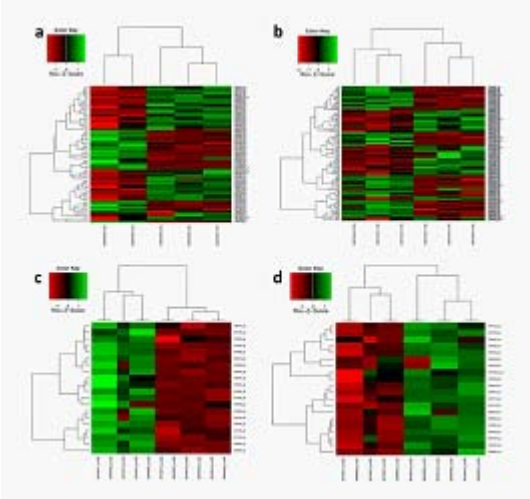

Fig.1. (a) Differentially expressed genes (DEGs) in GSE36935 (n=69), (b). DEGs in GSE44372 (n=118) (c) Meta-gene expression signature heat map constructed with the top 20 up regulated genes (n=20) from GSE36935 and GSE44372. (d) Meta-gene expression signature heat map constructed with the top 20 down regulated genes (n=20) from GSE36935 and GSE44372

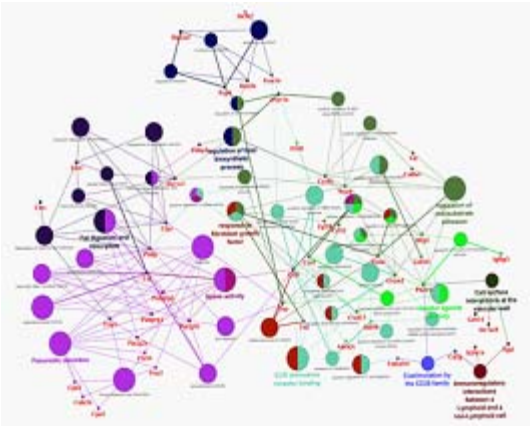

Fig. 2. Enriched gene pathways identified using ClueGO. The network represents topmost enriched biological processes that were predicted from the meta-gene signatures from the meta-analysis. ClueGO software was used to group the genes in to functional clusters. (Kappa score=0.3) The nodes represent distinct biological process. The genes associated with the node are represented as dots. Same colour nodes and dots belong to one functional group. Mixed colour belong to multiple groups. Lines/edges represent interactions, thicker the lines more significant the interaction. The label of the most significant network term per group is shown in colour

genes are given in *(Supplementary Table ST-3 and ST-4).* The meta-gene expression signature were subsequently used for integrated pathway enrichment analysis. The completed list of enriched Gene Ontology (GO) and REACTOME pathways identified is given in *(Supplementary Table ST-7)*

Integrated Pathway Enrichment Analysis

A comprehensive list of enriched pathways that were identified in the meta-analysis are shown in Table 2. It was not unexpected to find few hits that were up-regulated and had functions that overlapped with additional cellular pathways, for at times relying on chance drives to increase the obvious number of significant associations for those study characteristics that have no real relations with the study out comes. However, most hits were found relevant and of particular note were Discs large homolog 2 (Dlg2), Myosin, light chain 4 (Myl4) and Fatty acid binding protein 3 (Fabp3). Dlg2 a membrane linked guanylate kinase restricted to the post-synaptic density (PSD); had a key function in the maintenance of the structure of PSD by concentrating its components to the membrane area.[24]. Myl4 gene encodes a myosin alkali light chain in embryonic muscle and adult atria. Two alternatively spliced transcript variants have been found for this gene. In addition to the role in heart muscle contraction, it is also found to play a crucial role in regulation of ATPase activity. The fabp3 also named mammary derived growth inhibitor (MDGI) is primarily expressed in the heart and also in the skeletal muscle, brain, kidney, lung, stomach, testis, aorta, adrenal gland, mammary gland, placenta, ovary, and brown adipose tissue[25].

Among the genes that were significantly down regulated in the meta-gene expression signature, Interleukin 1 receptor-like 1 (Il1rl1), Synaptosomal-associated protein 25 (Snap25), Chemokine (C-C motif) ligand 11 (Ccl11), Chemokine (C-X-C motif) ligand 13 (Cxcl13), Flavin containing monooxygenase 1 (Fmo1) were found to be important. Interleukin-1 receptor-like 1 (Il1rl1) has a negative regulation on cell proliferation. As reported in a recent ingenuity pathway analysis study on high-Feed Efficiency (FE) chickens, Chemokine (C-C motif) receptor 2 (CCR2) and Il1rl1 were found highly expressed in infiltrating macrophages and played a crucial role in muscle regeneration [26]. Snap25 polymorphisms were

associated with glycaemic parameters in type 2 diabetes patients indicating a likely role in the pathogenesis of obesity induced T2DM [27]. Ccl11 had shown inflammatory role mainly interacting with toll-like receptors in human and murine adipose tissue[28]. In support of our findings, adipocytes had dominantly enhanced Cxcl13 expression when compared with pre-adipocytes. Chemokine (C-C motif) ligand 6 (Ccl6) a rodent-specific chemokine and Cxcl13 were found to be considerably amplified in adipocytes[29.] However, the precise roles of Ccl6 and Cxcl13 in obesity have not been yet been clarified. Beta cells from ob/ob mice have shown a higher tendency to migrate to the liver over a Cxcl13 mediated signalling pathway[30] indicating Cxcl13 may be associated with macrophage infiltration in obesity, consequently to chronic inflammation[29]. Fmo1 catalyses thiobenzamide S-oxidation and commonly is identified as a main hepatic microsomal enzyme but recently was found to play a vital role in the mammalian brain[31]. The expression and actions of Fmo1 may be influenced by hyperosmotic settings in the kidney of rats [32] Fmo1is also highly expressed in metabolic tissues, including liver, kidney, white and brown adipose tissue and has an active role in mammalian endogenous metabolism[33].

CONCLUSIONS

A list of statistically significant meta-gene expression signature have been identified from the meta-analysis studies. Integrated pathway enrichment analysis revealed significant pathways involving Il1rl1, Snap25, Ccl11, Cxcl13 and Fmo1in rat visceral white adipose tissues and marks a possible macrophage infiltration and further complement the dysregulated meta-gene expression signatures role in obesity related pathways as compared between obese and control groups. Our results show meta-gene expression signatures and their related networks that might be associated in the pathogenesis of obesity induced T2DM by providing insights that is consistently observed at the transcriptomic level across independent studies. However, further investigation with more comprehensive and large datasets are needed to draw better conclusions.

ACKNOWLEDGEMENTS

The authors would like to thank the Director, National Institute of Technology Warangal. Telangana, INDIA for providing the necessary facilities to carry out this research work.

REFERENCES

1. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Physical therapy.* 2008; **88**(11):1322-35.
2. Tomic M, Poljicanin-Filipovic T, Pavlic-Renar I. Obesity–risk factor for microvascular and neuropathic complications in diabetes. InHrvatski dijabetološki kongres. 2001, E2.
3. Ip B, Cilfone NA, Belkina AC, DeFuria J, Jagannathan Bogdan M, Zhu M, Kuchibhatla R, McDonnell ME, Xiao Q, Kepler TB, Apovian CM. Th17 cytokines differentiate obesity from obesity associated type 2 diabetes and promote TNF± production. *Obesity.* 2016; (1):102-12.
4. Kaiyala KJ, Schwartz MW. Toward a more complete (and less controversial) understanding of energy expenditure and its role in obesity pathogenesis. *Diabetes.* 2011; **60**(1):17-23.
5. Wright SM, Aronne LJ. Causes of obesity. *Abdominal imaging*. 2012; **37**(5):730-2.
6. Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity-related health risk. *The American journal of clinical nutrition.* 2004; **79**(3):379-84.
7. De Lorenzo A, Soldati L, Sarlo F, Calvani M, Di Lorenzo N, Di Renzo L. New obesity classification criteria as a tool for bariatric surgery indication. *World J Gastroenterol.* 2016; **22**(2):681-703.
8. Phillips CM, Tierney AC, Perez Martinez P, Defoort C, Blaak EE, Gjelstad IM, Lopez Miranda J, Kiec Klimczak M, Malczewska Malec M, Drevon CA, Hall W. Obesity and body fat classification in the metabolic syndrome: impact on cardiometabolic risk metabotype. *Obesity.* 2013; **21**(1):E154-61.
9. Lutz TA, Woods SC. Overview of animal models of obesity. *Current Protocols in Pharmacology*. 2012:5-61.
10. López IP, Marti A, Milagro FI, Zulet MD, Moreno Aliaga MJ, Martinez JA, Miguel C. DNA Microarray Analysis of Genes

Differentially Expressed in Diet Induced (Cafeteria) Obese Rats. *Obesity research.* 2003;**11**(2):188-94.

11. Palau N, Rebuffat SA, Altirriba J, Piquer S, Hanzu FA, Gomis R, Barbera A. Role of IGFBP-3 in the regulation of ²-cell mass during obesity: adipose tissue/²-cell cross talk. *Endocrinology.* 2011; **153**(1):177-87.
12. Malpique R, Figueiredo H, Esteban Y, Rebuffat SA, Hanzu FA, Vinaixa M, Yanes O, Correig X, Barceló-Batllori S, Gasa R, Kalko SG. Integrative analysis reveals novel pathways mediating the interaction between adipose tissue and pancreatic islets in obesity in rats. *Diabetologia*. 2014; **57**(6):1219-31.
13. Ramasamy A, Mondry A, Holmes CC, Altman DG. Key issues in conducting a meta-analysis of gene expression microarray datasets. *PLoS Med*. 2008; **5**(9):e184.
14. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic acids research.* 2002; **30**(1):207-10.
15. Dai Y, Guo L, Li M, Chen YB. Microarray Я US: a user-friendly graphical interface to Bioconductor tools that enables accurate microarray data analysis and expedites comprehensive functional analysis of microarray results. *BMC research notes.* 2012; **5**(1):282.
16. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K. Bioconductor: open software development for computational biology and bioinformatics. *Genome biology.* 2004; **5**(10):R80.
17. Hong F, Breitling R, McEntee CW, Wittner BS, Nemhauser JL, Chory J. RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. *Bioinformatics.* 2006; **22**(22):2825-7.
18. Xia J, Fjell CD, Mayer ML, Pena OM, Wishart DS, Hancock RE. INMEX—a web-based tool for integrative meta-analysis of expression data. *Nucleic acids research.* 2013:gkt338.
19. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pagès F, Trajanoski Z, Galon J. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics.* 2009; **25**(8):1091-3.
20. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome research.* 2003; **13**(11):2498-504.
21. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA. Gene Ontology: tool for the unification of biology. *Nature genetics.* 2000; **25**(1):25-9.
22. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic acids research.* 1999; **27**(1):29-34.
23. Joshi-Tope G, Gillespie M, Vastrik I, D'Eustachio P, Schmidt E, de Bono B, Jassal B, Gopinath GR, Wu GR, Matthews L, Lewis S. Reactome: a knowledgebase of biological pathways. *Nucleic acids research*. 2005; **33**(suppl 1):D428-32.
24. Guo ML, Xue B, Jin DZ, Mao LM, Wang JQ. Interactions and phosphorylation of postsynaptic density 93 (PSD-93) by extracellular signal-regulated kinase (ERK). *Brain research.* 2012; **1465**:18-25.
25. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nature reviews Drug discovery*. 2008; **7**(6):489-503.
26. Zhou N, Lee WR, Abasht B. Messenger RNA sequencing and pathway analysis provide novel insights into the biological basis of chickens' feed efficiency. *BMC genomics.* 2015; **16**(1):1.
27. Al-Daghri NM, Costa AS, Alokail MS, Zanzottera M, Alenad AM, Mohammed AK, Clerici M, Guerini FR. Synaptosomal Protein of 25 kDa (Snap25) Polymorphisms Associated with Glycemic Parameters in Type 2 Diabetes Patients. *Journal of diabetes research.* 2015; 2016.
28. Poulain-Godefroy O, Le Bacquer O, Plancq P, Lecœur C, Pattou F, Frühbeck G, Froguel P. Inflammatory role of Toll-like receptors in human and murine adipose tissue. *Mediators of inflammation.* 2010; 2010.
29. Kabir SM, Lee ES, Son DS. Chemokine network during adipogenesis in 3T3-L1 cells: Differential response between growth and proinflammatory factor in preadipocytes vs. adipocytes. *Adipocyte*. 2014; **3**(2):97-106.
30. Bigorgne AE, Bouchet–Delbos L, Naveau S, Dagher I, Prévot S, Durand–Gasselin I, Couderc J, Valet P, Emilie D, Perlemuter G. Obesity-induced lymphocyte hyperresponsiveness to chemokines: a new mechanism of Fatty liver inflammation in obese mice. *Gastroenterology.* 2008; **134**(5):1459-69.
31. Li B, Yuan Y, Zhang W, He W, Hu J, Chen N. Flavin-containing monooxygenase, a new clue

of pathological proteins in the rotenone model of parkinsonism. *Neuroscience letters.* 2014; **566**: 11-6.

32. Rodríguez-Fuentes G, Coburn C, Currás-Collazo M, Guillén G, Schlenk D. Effect of hyperosmotic conditions on flavin-containing monooxygenase activity, protein and mRNA expression in rat kidney. *Toxicology letters.* 2009; **187**(2):115-8.

33. Veeravalli, S., Omar, B.A., Houseman, L., Hancock, M., Malagon, S.G.G., Scott, F., Janmohamed, A., Phillips, I.R. and Shephard, E.A. The phenotype of a flavin-containing monooyxgenase knockout mouse implicates the drug-metabolizing enzyme FMO1 as a novel regulator of energy balance. *Biochemical pharmacology.* 2014; **90**(1): 88-95.