

Article

In-silico study of the immune systems associated genes in case of type-2 diabetes with Insulin action and resistance and in obesity

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Abstract: Type-2 diabetes and obesity are among the leading human diseases and highly complex in terms of diagnostic and therapeutic approaches and are among the most frequent and highly complex and heterogeneous in nature. Based on epidemiological evidence, it is known that the patients suffering from obesity are considered to be significantly at higher risk for type-2 diabetes. There are a number of evidences which support the hypothesis that these diseases interlinked and obesity may aggravate the risk(s) of type-2 diabetes. Multi-level unwanted alterations such as (epi-) genetic alterations, changes at the transcriptional level, and altered signaling pathways (receptor, cytoplasmic, and nuclear level) are the major source which promotes a number of complex diseases and such heterogeneous level of complexities are considered as the major barrier in the development of therapeutic. With so many known challenges, it is critical to understand the relationships and the common shared causes between type-2 diabetes and obesity which is difficult to unravel and understand. For this purpose, we have selected publicly available datasets of gene expression for obesity and type-2 diabetes and unraveled the genes and the pathways associated with immune system and have also focused on T cell signaling pathway and its components. We have applied simplified computational approach to understand differential gene expression and patterns and the enriched pathways for obesity and type-2 diabetes. Furthermore, we have also analyzed the genes by using network-level understanding. In the analysis, we observe that there are less number of genes which are commonly differentially expressed while comparatively higher number of pathways are shared between them. There are only four pathways which are associated with immune system in case of obesity and ten immune-associated pathways in case of type-2 diabetes and among them only two pathways are commonly altered.

Keywords: Type-2 diabetes; obesity, insulin action and resistance; immune system; enriched pathways, differentially expressed genes; TCR network; network-level understanding.

1. Introduction

Type 2 diabetes (T2D) is a global epidemic, strongly correlated which is more common among the obese[1,2]. Adipose tissue inflammation associated with obesity is known as a major cause for the decreased insulin sensitivity in case of T2D[1,3,4]. There are a number of works where the crosstalk between the immune system and metabolism have been presented and a number of evidences which

support the hypothesis that these diseases interlinked and obesity may aggravate the risk(s) of T2D[5-8]. In such cases, it is critical to understand multi-level unwanted alterations such as (epi-)genetic alterations, changes at the transcriptional level, and altered signaling pathways (receptor, cytoplasmic, and nuclear level). These are the major source which promotes a number of complex diseases and such heterogeneous level of complexities are considered as the major barrier in the development of therapeutic approach(es). With such diverse challenges, it is crucial to understand and unravel the relationships and the common shared causes between type-2 diabetes and obesity[9-13].

So far, there are a number of previous study where a number of factors have been investigated to understand the biological mechanism of obesity and T2D and the relationship between each other[1,2,14-16]. T2D is mainly characterized by a progressive status of chronic and inflammation of low grade and inflammatory responses are triggered by many factors. The well-known factors contributing to T2D are age, metabolic syndrome, systematic low-grade inflammation, insulin resistance, islet cell auto-antibodies, beta cell dysfunction, and C-peptide. Some of the factors which promotes inflammation are altered function of specific T lymphocyte cells, B lymphocytes, Th1, and Th17[17-21]. As mentioned above that T2D is also linked with obesity and these diseases are also the case of metabolic disorder and there exist strong evidences of immune—metabolic connection[10,22,23].

For this purpose, we have selected publicly available datasets of gene expression for obesity and T2D and unraveled the genes and the pathways associated with immune system and have also focused on T cell signaling pathway and its components. We have applied simplified computational approach to understand differential gene expression and patterns and the enriched pathways for obesity and T2D. Furthermore, we have also analyzed the genes by using network-level understanding. In the analysis, we observe that there are less number of genes which are commonly differentially expressed while comparatively higher number of pathways are shared between them. There are only four pathways which are associated with immune system in case of obesity and ten immune-associated pathways in case of T2D and among them only two pathways are commonly altered. Here, we have studied the differentially expressed genes (DEGs), enriched pathways, and the connection between immune signaling pathways via their components between obesity and T2D and with the major focus on the roles TCR signaling components.

2. Results

As mentioned above that we have compared immune signaling genes by using gene expression profiling of T2D and obese for which the datasets have been collected from publicly available data. Here, the results have been presented from work from generalized analysis (i.e., DEGs, enriched pathways) to precise analysis i.e., TCR signaling.

2.1. Gene expression profiling for type-2 diabetes and obesity

For diabetes and kidney cancer collected data, we have performed comparative analysis in terms of altered gene expression pattern and their respective functions. Here, we have presented the DEGs for both the cases T2D and obesity (Figure 1a) and we observe that there are only 12 genes which are differentially expressed in both the cases while T2D-specific genes are 456 and 580 genes are obesity-specific genes while in terms of the altered functions they share 14 pathways and the T2D and obesity specific pathways are 17 and 18 (Figure 1b and Table 1), respectively. Based on this it appears that there are only few genes which differentially expressed in both the cases while in terms of biological functions, a large number of pathways are affected which means that T2D and obesity share more biological functions in terms of alterations and their source of alterations i.e., DEGs are not shared at large scale. The green color nodes are the DEGs which either belong to T2D or obesity case. For this reason, we have mapped out the network for common DEGs and highlighted common DEGs in red color (Figure 1c). Among these common DEGs, FN1, SFRP1, and SNX5 appears to be the major source of alteration. FN1 appears to be associated with a large number of DEGs leading to the conclusion that either FN1 is overexpressed by those genes or it promotes the over expression of the directly associated genes. For understanding the alteration in biological function, a heatmap has been displayed in Figure 1d for those pathways which are commonly enriched. For this plot, the p-values in both the conditions have been used. Cell adhesion molecules, PI3K–AKT, and MAPK pathways appear highly enriched in both the conditions, regulation of actin cytoskeleton, cAMP, phospholipase D, and Focal adhesion are exclusively and highly enriched in case of T2D, and hematopoietic cell lineage and phagosome are potentially enriched mainly in case of obesity. There are additional pathways which are enriched in both the cases but have comparatively higher p-values and these pathways are Rap1, tight junction, leukocyte transendothelial migration, ECM-receptor interaction, and Ras signaling pathways. The complete details about the fold changes of the genes have been presented in supplementary data S1 and S2.

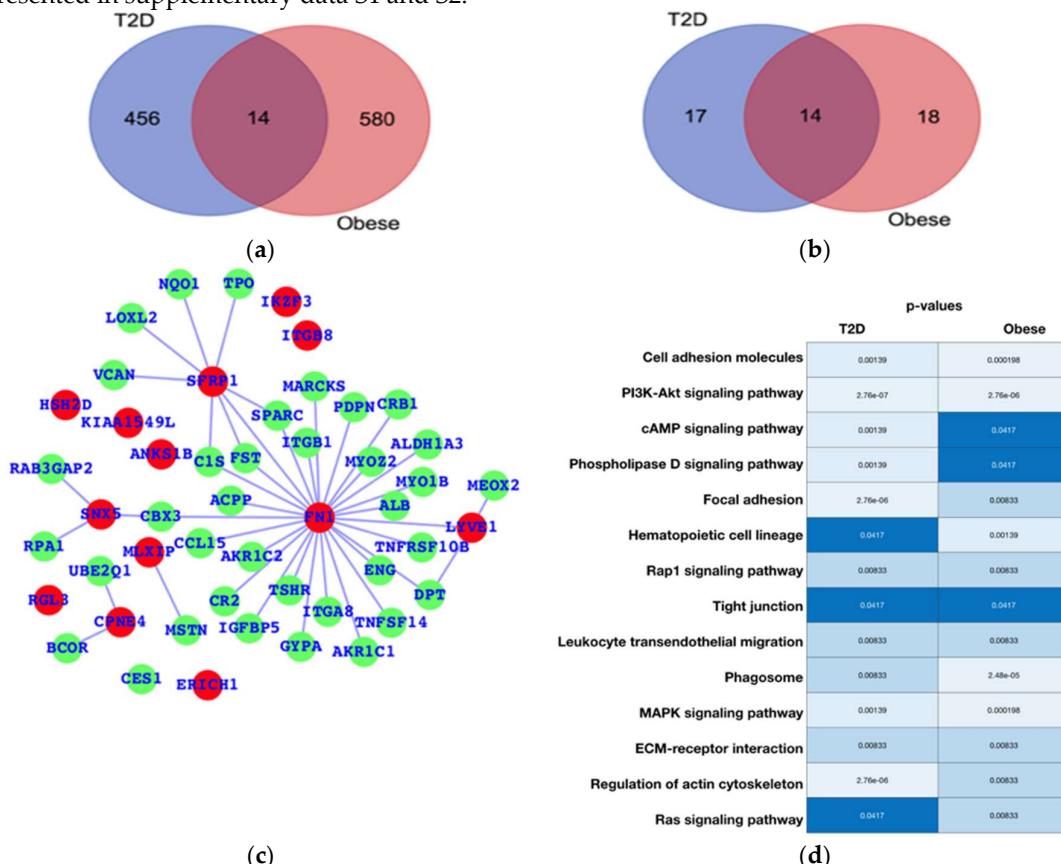


Figure 1. Gene expression profiling. (a) Venn diagram for differentially expressed genes. (b) Venn diagram for enriched pathways. (c) Network representing the DEGs common between T2D and obesity. (d) Commonly enriched pathways.

Table 1. Pathways common between T2D and obesity and specific to T2D and obesity (venn diagram outcome).

Name	Total	Pathways
T2D and Obese	14	Cell adhesion molecules (CAMs); PI3K-Akt signaling pathway; cAMP signaling pathway; Phospholipase D signaling pathway; Focal adhesion; Hematopoietic cell lineage; Rap1 signaling pathway; Tight junction; Leukocyte transendothelial migration; Phagosome; MAPK signaling pathway; ECM-receptor interaction; Regulation of actin cytoskeleton; Ras signaling pathway
T2D	17	Neurotrophin signaling pathway; Insulin signaling pathway; Vascular smooth muscle contraction; Sphingolipid signaling pathway; cGMP-PKG signaling pathway; mTOR signaling pathway; T cell receptor signaling pathway; Natural killer cell mediated cytotoxicity; Jak-STAT signaling pathway; Progesterone-mediated oocyte maturation; B cell receptor signaling pathway; Oxytocin signaling pathway; Platelet activation; Thyroid hormone signaling pathway; Wnt signaling pathway; ErbB signaling pathway; Ubiquitin mediated proteolysis
Obese	18	Fatty acid metabolism; Cell cycle; Tyrosine metabolism; Thyroid hormone synthesis; Starch and sucrose metabolism; Purine metabolism; Cytokine-cytokine receptor interaction; Metabolism of xenobiotics by cytochrome P450; Retinol metabolism; Drug metabolism - cytochrome P450; Neuroactive ligand-receptor interaction; Tryptophan metabolism; p53 signaling pathway; Complement and coagulation cascades; Antigen processing and presentation; Arachidonic acid metabolism; Axon guidance; Retrograde endocannabinoid signaling

2.2. Genes associated with immune system

After analyzing the overall DEGs and the all the enriched pathways, we have performed immune system specific analysis for the genes and the pathways. Here, we observe that there are 10 pathways associated with immune system in case of T2D while there are four pathways in case of obese which are associated with immune system (Figure 2). Hematopoietic cell lineage and leukocyte transendothelial migration pathways are commonly altered in both the cases T2D and obese and these two pathways are known to play roles blood cell development and immune surveillance and inflammation leading to the conclusion that in both the cases T2D and obesity, the blood cell development, immune surveillance, and the inflammation process are affected. Obesity-specific immune signaling pathways are oxytocin and antigen processing and presentation (APP). Oxytocin signaling pathway is known to exert a wide range of effects which could be central or peripheral effects and this pathway itself is known to crosstalk with a number of critical pathways such as RhoA, GPCR, MAPK, ANP-cGMP, and NO-cGMP. APP is one of the most important part of entire immune system and is the process by which protein antigen is ingested by an antigen-presenting cell (APC), partially digested into peptide fragments and then displayed on the surface of the APC associated with an antigen-presenting molecule (MHC class I or MHC class II), for recognition by specific lymphocytes (T cells). While there are eight immune system specific pathways in case of T2D which are NK cell-mediated cytotoxicity, insulin signaling, TCR signaling, ubiquitin-mediated proteolysis, platelet activation BCR signaling, neurotrophin, and oxytocin signaling pathways. Almost all these pathways are major part of immune system. Based on this, we could conclude that in case of T2D, there is more alterations in the gene expression pattern of those genes which are known to control major part of immune system while in case of obesity there is less effect than T2D but these few pathways are also known to be associated with controlling critical roles of immune system.

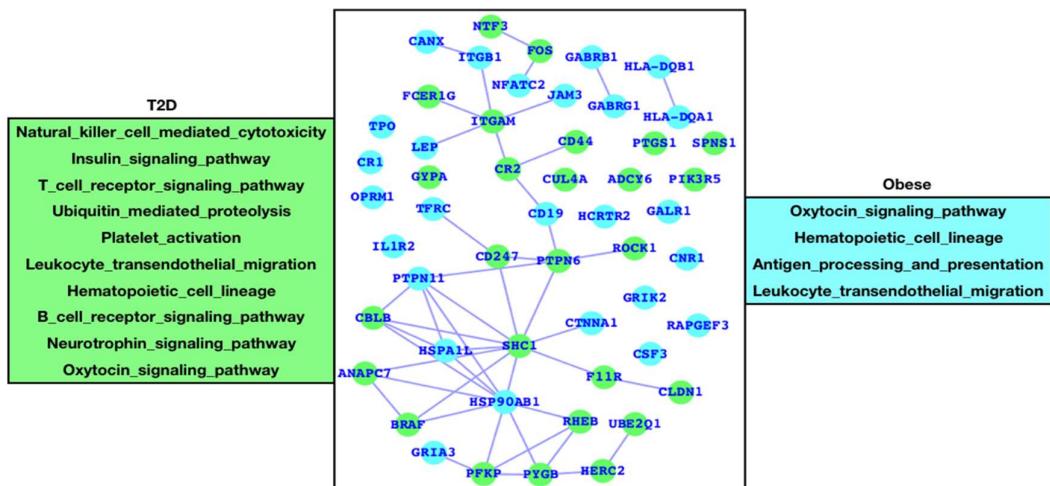


Figure 2. Genes associated with immune systems. Green color genes belong to the immune system when the patient is suffering from T2D and cyan color genes belong to the immune system in case of obese.

2.3. DEGs of T2D and TCR signaling network

As mentioned above that there are a number of pathways which are affected as a result of T2D and obesity while in comparison to T2D, because of obesity there are only four pathways which are mainly affected and TCR signaling pathway is not affected here. TCR signaling pathway is affected in case of T2D. Therefore, we have now mapped out the individual genes which are associated with TCR signaling pathway and presented the networks for all the five genes (SPNS1, PTPN6, CD247, FOS, and PIK3R5) which are the component of TCR signaling pathway and their association with the other TCR genes (Figure 3a–3e). In addition, we have also presented the TCR network (Figure 3f).

SPNS1 gene is the direct component of TCR, NK cell mediated cytotoxicity, and FC-epsilon RI signaling pathways, PTPN6 belongs to TCR, BCR, NK cell-mediated cytotoxicity, JAK-STAT, and adherens junction, CD247 belongs to TCR and NK cell-mediated cytotoxicity, and FOS belongs to TCR, BCR, TLR, MAPK, TNF, cAMP, estrogen, oxytocin, prolactin, circadian entrainment, and osteoclast differentiation. PIK3R5 alone belongs to a large number of critical pathways such as TCR, BCR, NK cell-mediated cytotoxicity, JAK-STAT, ErbB, chemokine, phosphatidylinositol, mTOR, apoptosis, VEGF, focal adhesion, regulation action cytoskeleton, etc., and the complete details of the list of pathways for all these five genes have been presented in Table 2.

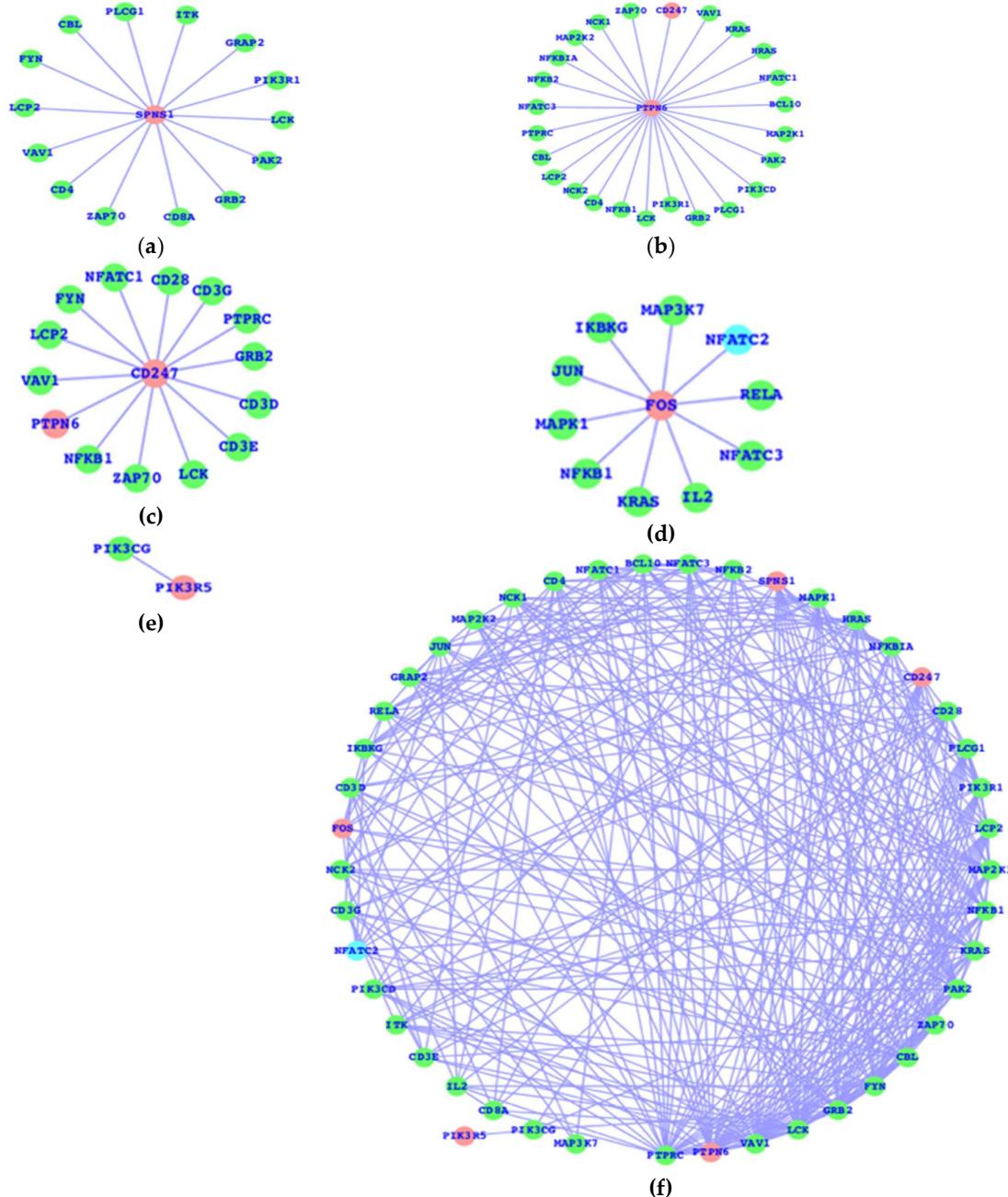


Figure 3. DEGs of T2D and TCR signaling network. (a) SPNS1 network, (b) PTPN6 network, (c) CD247 network, (d) FOS network, (e) PIK3R5 network, and (f) Overall association of these T2D DEGs with the TCR genes and also the intra-associations.

Table 2. Selected gene specific pathways.

Gene	Pathways
SPNS1	T cell receptor signaling pathway Fc epsilon RI signaling pathway Natural killer cell mediated cytotoxicity
PTPN6	Adherens junction Jak-STAT signaling pathway

	Natural killer cell mediated cytotoxicity
	T cell receptor signaling pathway
	B cell receptor signaling pathway
CD247	Natural killer cell mediated cytotoxicity
	T cell receptor signaling pathway
	MAPK signaling pathway
	Toll-like receptor signaling pathway
	T cell receptor signaling pathway
	B cell receptor signaling pathway
	TNF signaling pathway
FOS	cAMP signaling pathway
	Osteoclast differentiation
	Circadian entrainment
	Estrogen signaling pathway
	Prolactin signaling pathway
	Oxytocin signaling pathway
	ErbB signaling pathway
	Chemokine signaling pathway
	Phosphatidylinositol signaling system
	mTOR signaling pathway
	Apoptosis
	VEGF signaling pathway
	Focal adhesion
	Toll-like receptor signaling pathway
	Jak-STAT signaling pathway
	Natural killer cell mediated cytotoxicity
	T cell receptor signaling pathway
PIK3R5	B cell receptor signaling pathway
	Fc epsilon RI signaling pathway
	Fc gamma R-mediated phagocytosis
	Leukocyte transendothelial migration
	Neurotrophin signaling pathway
	Regulation of actin cytoskeleton
	Insulin signaling pathway
	Progesterone-mediated oocyte maturation
	Type II diabetes mellitus
	Aldosterone-regulated sodium reabsorption
	Bacterial invasion of epithelial cells

3. Discussion

Diabetes mellitus (DM) and obesity are considered as metabolic disorder related diseases and DM is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1,24,25]. The diabetic chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of various organs such as kidneys, eyes, nerves, blood vessels, and heart[1,17,25-29]. T2D is most often found to be frequent in case of the obese and is characterized by abnormal insulin secretion and/or a decreased sensitivity for insulin which is also known as insulin resistance and results in increased blood glucose levels and T2D is known to be strongly associated with obesity. There are a number of biological processes and mechanisms which are considered as the source of association between these conditions via mediating inflammation in adipose tissue and systemic insulin resistance such as oxidative stress, endoplasmic reticulum stress, hypoxia, amyloid and lipid deposition, lipotoxicity and glucotoxicity.

With such level of complexities, there exist a number of challenges for understanding the mechanism of so we have thoroughly analyzed the DEGs, enriched pathways with major focus on immune signaling pathways and the networks of DEGs belonging to the immune system. Among the top enriched pathways are: cell adhesion molecules, PI3K—AKT, MAPK, regulation of actin cytoskeleton, cAMP, phospholipase D, Focal adhesion, hematopoietic cell lineage, phagosome, Rap1, tight junction, leukocyte transendothelial migration, ECM-receptor interaction, and Ras signaling pathways.

For immune systems—related analysis, we observe that there are 10 pathways associated with immune system in case of T2D while there are four pathways in case of obese which are associated with immune system. Hematopoietic cell lineage and leukocyte transendothelial migration pathways are commonly altered in both the cases T2D and obese and are known to play roles blood cell development and immune surveillance and inflammation. Oxytocin and APP are obesity-specific immune signaling pathways and NK cell-mediated cytotoxicity, insulin signaling, TCR signaling, ubiquitin-mediated proteolysis, platelet activation BCR signaling, neurotrophin, and oxytocin signaling pathways are T2D immune system specific pathways. Almost all these pathways (obese and T2D immune signaling pathways) are major part of immune system known to control major part of immune system. Furthermore, it is also known that altered TCR signaling is associated with T2D. Therefore, we have now mapped out the individual genes which are associated with TCR signaling pathway and presented the networks for all the five genes (SPNS1, PTPN6, CD247, FOS, and PIK3R5) which are the component of TCR signaling pathway and their association with the other TCR genes (Figure 3). These five genes SPNS1, PTPN6, CD247, FOS, and PIK3R5 are the potential components of TCR and also a number of additional immune system associated pathways. Among those pathways are NK cell mediated cytotoxicity, FC-epsilon RI signaling pathways, BCR, JAK-STAT, adherens junction, TLR, MAPK, TNF, cAMP, estrogen, oxytocin, prolactin, circadian entrainment, and osteoclast differentiation. PIK3R5 alone belongs to a large number of critical pathways such as TCR, BCR, NK cell-mediated cytotoxicity, JAK-STAT, ErbB, chemokine, phosphatidylinositol, mTOR, apoptosis, VEGF, focal adhesion, regulation action cytoskeleton, etc[30].

As we can see in the results that PIK3R5 and FOS which are the components of TCR signaling pathway and also control a large set of biological functions most of them part of immune system and CD247, PTPN6, and SPNS1 comparatively controls less number of pathways but in terms of connectivity PTPN6 has the highest connectivity then SPNS1, CD247, FOS, and PIK3R5. It means that PIK3R5 simultaneously controls large number of biological functions but does not impact more genes or in other words the higher connectivity of the gene will have more chance to affect the expression patterns of the gene or vice versa. In terms of future perspective, this study will be helpful for therapeutic approach also for drug targeting.

4. Materials and Methods

For obese, GSE12050[31] dataset used where there are 36 samples out of which nine lean needle biopsy have been compared with 9 obese biopsy samples. For sample details, it has been mentioned that abdominal subcutaneous fat specimens have been collected by using needle biopsy approach

from nine obese patient samples and 10 lean samples. Then nine obese patient samples have been collected from abdominal subcutaneous fat biopsies and nine lean samples from abdominal fat biopsies[31]. For T2D, GSE121 (GPL101 platform)[32] has been used for type-2 diabetes gene expression profiling which contains 5 (insulin resistant (IR)) and 5 samples (Insulin sensitive (IS)) are with type II diabetes while 11 are control samples. The samples were non-diabetic Pima Indians or Tohono O'Odham Indians who were classified as IS or IR[32].

These gene expression datasets were collected from GEO have been processed for expression value calculation, fold change, and p-value calculation by the in-built code at GEO i.e., GEO2R [33,34]. The list of DEGs have been processed for pathway enrichment analysis and understanding[35,36]. KEGG[30] database have been used for calculating enriched pathways and further, own developed code have been used for pathway and network analysis. To retrieve DEGs association as a network, FunCoup2.0 network database[37] has been utilized throughout the work and the network has been visualized with the help of cytoscape[38]. For most of our coding and calculations MATLAB has been used.

Now, we briefly summarize all the steps used are raw file processing, intensity calculation and normalization. Since, the high-throughput data mainly microarray-based gene expression values are calculated as intensity which ranges from lowest values to extremely high so it is needed to have optimized values or scaled down values so we normally convert them to log2 values. For normalization[39-41], GCRMA[42-46], RMA, and EB are the most commonly used approaches. Here, we have used EB for raw intensity normalization. After normalization, we proceed for our goal which is to understand the gene expression patterns[35,47] and its inferred functions[35,36]. For differential gene expression prediction and statistical analysis, MATLAB functions (e.g., mattest) has been used and volcano plot. From mattest, we get the p-values and then we go for p-values corrections and assign the p-value threshold and finally volcano plot has been used for fold change calculation and the selection of genes with p-values less than 0.05 and fold changes within this range $\geq +2.0$ and ≤ -2.0 and this range of fold change as well as the p-value cutoff are commonly used while in our study we have selected the top ranked genes to have strict condition of selection based on fold change so the fold changes are either extremely high or extremely low than the cutoff fold change. For pathway analysis, we used KEGG[30] database and have our own code designed to pathway and network analysis[48-50]. We have described about the fold change and by default for considering genes as DEGs, we have applied the threshold for $FC \geq +2.0$ (up regulated genes) and $FC \leq -2.0$ (down regulated genes) and these DEGs have been considered for pathways enrichment analysis. But for heatmap and network presentations the top 100 DEGs (50 up and 50 down) genes have been presented[51,52].

5. Conclusions

Here, we observe that there are less number of genes which are commonly differentially expressed while comparatively higher number of pathways are shared between them. There are only four pathways which are associated with immune system in case of obesity and ten immune-associated pathways in case of T2D and among them only two pathways are commonly altered. Here, we have studied the differentially expressed genes (DEGs), enriched pathways, and the connection between immune signaling pathways via their components between obesity and T2D and with the major focus on the roles TCR signaling components. We conclude that in case of T2D, there is more alterations in the gene expression pattern of those genes which are known to control major part of immune system while in case of obesity there is less effect than T2D but these few pathways are also known to be associated with controlling critical roles of immune system.

Author Contributions: "Conceptualization, B.M.E., H.A.S., H.C., and M.M.; methodology, B.M.E., H.A.S., H.C., and M.M.; software, M.M.; validation, B.M.E., H.A.S., H.C., and Z.Z.; formal analysis, B.M.E., H.A.S., H.C., and

M.M.; investigation, B.M.E. and M.M.; resources, B.M.E. and M.M.; data curation, B.M.E. and M.M.; writing—original draft preparation, B.M.E., H.A.S., H.C., and M.M.; writing—review and editing, B.M.E., H.A.S., H.C., and M.M.; visualization, B.M.E., H.A.S., H.C., and M.M.; supervision, H.C. and M.M.; project administration, B.M.E. and H.C.; funding acquisition, B.M.E. All authors have read and agreed to the published version of the manuscript.”

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Abbreviations

DEGs	Differentially expressed genes
TCR	T cell receptor
ISPs	Immune signaling pathways
T2D	Type-2 diabetes
APP	Antigen processing and presentation
APC	Antigen-presenting cell

Appendix A

The appendix is an optional section that can contain details and data supplemental to the main text. For example, explanations of experimental details that would disrupt the flow of the main text, but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

Appendix B

All appendix sections must be cited in the main text. In the appendixes, Figures, Tables, etc. should be labeled starting with ‘A’, e.g., Figure A1, Figure A2, etc.

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