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Crosstalk Among Obesity, Chronic Inflammation and Insulin Resistance Pathways

by

Mahtab Zafar

A thesis submitted in partial fulfillment for the
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I dedicate this thesis to my parents and my teachers.

CERTIFICATE OF APPROVAL

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(Mahtab Zafar)

Abstract

Obesity is considered as mother of diseases as it triggers the onset of various co-morbidities, very common among which are cardiovascular disease, nephropathies, diabetes and digestive issues. Similarly, association between obesity, Insulin resistance and chronic inflammation is quite evident, due to rapid increase in onset of obesity owing to life style changes, food, environment, stress with involvement of genetics and hormones, chances of developing chronic inflammation are also turning quite high. Understanding of underlying molecular pathways responsible for onset of obesity and their overlapping/crosstalk with pathways which leads to the onset of insulin resistance and chronic inflammation can help to design an efficient control strategy for management of these diseases. This research was designed with the aim to explore the key pathways involved in onset of obesity and crosswalks of these pathways with the pathways involved in insulin resistance, and disease onset as result of inflammatory pathways including pro inflammatory, insulin signaling and metabolic pathways. For this purpose, key pathways were identified by literature survey. In addition to which, microarray data of obesity, insulin resistance and chronic inflammation of human patients was collected to have a list of differentially expressed genes. Identification of gene ontology and pathway enrichment of these Differentially Expressed Genes had been done for each of three diseases independently, to identify the enriched genes involved. Further, protein protein interactions have been identified and validated through the KEGG database. Finally, cross talks of pathways were generated identifying 45 pathways cross talks among obesity, insulin resistance and chronic inflammation, which were later validated using literature review and along with well-studied pathways few pathways novel for these diseases were identified. Their cross-talks are important for finding and predicting actual potential targets or biomarkers which by further investigations could be the novel approach of diagnosis or treatment.

Keywords: Obesity, Insulin resistance, chronic inflammation, enriched pathways, DEGs, Crosstalk.

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Abbreviations

BAT	Brown adipose tissues
BMI	Body mass index
DAMPs	Danger associated molecular patterns
DEGs	Differentially Expressed Genes
FFA	Free floating fatty acid
GEO	Gene expression omnibus
GO	Gene Ontology
ILs	Interleukins
KEGG	Kyoto Encyclopedia of Genes and Genome
PAMPs	pathogen associated molecular patterns
PRRs	pattern recognition receptors
WAT	White adipose tissues

Chapter 1

Introduction

1.1 Background

Obesity is one of the major health problems faced by various societies and countries, as a huge ratio of people is suffering with this. Over past few decades, incidence and prevalence of obesity has increased exponentially. Obesity is also referred as mother of all diseases as it can lead to various other health conditions such as, hypertension, stroke, diabetes, gallbladder diseases, fatty liver diseases and arthritis. [1]. It has appeared as global pandemic and all parts of the world are suffering with its impacts. According to WHO over 1 billion people are suffering from obesity [2]. It is also reported that among various factors contributing to obesity, sedentary life style, more dependence on machines and technology are two major factors contributing to obesity as excess calories starts accumulating in the tissues (adipose tissues). Body mass index (BMI) is a common measurement used to indicate weight gain, [3]. as it is used to classify degrees of fat accumulation in the adipose tissues. It is calculated by dividing the weight of an individual in kilograms by the square of their height in meters (kg/m^2) [4]. For measuring the obesity World Health Organization (WHO) recommended the BMI in 1997. The risk for other diseases as an outcome of obesity are directly proportional to the increase in body mass index (BMI). Weight gain classified between various classes i.e. overweight to obese [5]. Not only BMI but, waist circumference is also

measured to check the fat accumulation in the abdominal area. Abdominal area is most common area of fat accumulation [4]. These attributes of BMI were defined by Europeans and North Americans [2].

TABLE 1.1: WHO BMI classification for Normal, Overweight and Obese individuals [2]

BMI (Asian population)	**BMI (Non-Asian population)**	**Condition**
<18.5 Kg/m^2	<18.5 Kg/m^2	Underweight
18.5-22.9 Kg/m^2	18-24.9 Kg/m^2	Normal weight
23.0-27.5 Kg/m^2	25-29.9 Kg/m^2	Overweight
>27.5 Kg/m^2	>30 Kg/m^2	Obese

Adipose tissues where the fat is accumulated can produce cytokines referred as obesinogens, which trigger low grade chronic and localized inflammation that connects ultimately to insulin resistance [6]. Chronic inflammation is found in various organs involved in homeostasis and metabolism; organs include liver, adipose tissues, pancreas, hypothalamus and skeletal muscles. Chronic inflammation caused by the excess intake of the nutrients is referred as metabolic inflammation. Nutrients include fatty acids and glucose, can trigger chronic inflammation. Change in the diversity of gut microbiota as a result of high sugar and fat intake can also trigger of the metabolic inflammation involving adipocytes and also changes population of immune cells in metabolic tissues [7].

Although inflammation is a defensive mechanism performed by the body to remove injurious stimuli which include various elements such as pathogens, toxic compounds, damaged cells etc [15]. therefore we can say that infectious as well as non-infectious agents even cell damage can activate inflammatory cells and trigger inflammatory signaling pathways [16]. Obesogens or cytokines from adipose tissues can also trigger inflammation which is not involved in healing rather can damage tissues and cells. Chronic Inflammation can play a key role in the development of insulin resistance and obesity [17]. This critical role is played by adipose tissue macrophages (ATMs). ATMs are source of cytokines; mainly pro-inflammatory

cytokines such as TNF-α and IL-6 which blocks insulin action in the adipose tissue, skeletal muscles and also signaling of liver autocrine/paracrine which results in insulin resistance [18].Insulin, a major hormone, is proteinaceous in nature and involved in metabolism. It is produced in the islets of Langerhans. These islets are actually group of pancreatic cells of different type alpha, beta, delta, epsilon and PP out of which β-cells are mainly involved in insulin production. It is made of two chains A and B which are attached together by disulphide bridges. It is responsible for the movement of the blood glucose into liver, adipose tissues and skeletal muscles for its metabolism.

Insulin resistance is defined as the impaired working of glucose transporters, which restricts the transport of glucose from Blood (where the level is elevated) to the cells (Mucles, Liver, Adipose etc) for metabolism. This resultant elevated level in blood glucose is later diagnosed as diabetes [8]. Insulin resistance is a key factor of developing obesity, heart diseases and metabolism related diseases. Diabetes Mellitus type 2 is prevailing disease mainly caused by insulin resistance. The prevalence of metabolic syndrome and the insulin resistance is increasing, particularly in developing countries in younger people with estimated prevalence ranging from 20 to 40% in different populations [9]. Insulin resistance is directly or indirectly associated with the obesity. But the association is still unclear that whether abdominal fat is the risk factor or causing metabolic syndrome i.e. insulin resistance or it is just indicator and biomarker [12].

Pathway crosstalk describes that signal from two or more inputs within networks effects a common biological output. Signaling pathways involves cascades. Crosstalk is said to be present in two pathways when; combined signals from both pathways produce different responses than that produced by each pathway alone or both the pathways must be directly or indirectly connected. Crosstalk is direct or indirect. When two signaling pathways share some common components, the crosstalk is direct. On the other hand only sharing components is not sufficient to describe the crosstalk between two pathways until or unless when one signaling pathway exerts an effect on another different signaling pathway [20]. Due to the link between onsets of various comorbidities, it is somehow possible that obesity,

chronic inflammation and insulin resistance are interrelated or all of these have some common components, signals and factors [19].

1.2 Aim and Objectives

It is quite evident and doubtless to say that obesity, chronic inflammation and insulin resistance share a common trigger, all these diseases usually occur as co-morbidity or the onset of one increases the risk to develop others. The suggestive evidence that there are chances of overlap between the molecular mechanisms or triggers that are same or interdependent. To identify these triggers pathway cross talk could be exploited, which enables the identification of pathway interactions to identify key genes/proteins. These proteins could be targeted in drug discovery, biomarker identification and therapeutic strategies. This study is design with an aim to analyze the key pathways associated with obesity, chronic inflammation and insulin resistance for identification of possible cross talks between these pathways. In order to achieve the aim, study is divided in following objectives.

1. To identify key pathways reported in literature to be involved in obesity, insulin resistance, and inflammation.
2. To identify Hub genes involved in the pathways of insulin resistance, obesity and inflammation.
3. To identify Key triggers for pathway crosstalk.

Chapter 2

Literature Review

2.1 Obesity

Obesity is described as the increased fat deposit in adipose tissues/ adipocytes (fat storage cell) [21]. Body weight increase is directly dependent on the fat accumulation in adipocytes, as proteins and glycogen is readily utilized by the body [22]. Obesity occurs due to an imbalance in calorie intake and consumption as energy [23]. Sedentary lifestyle, unhealthy diet, stress, hormones etc. are major factors that play a key role in the onset of obesity [24]. A study in 1947 conducted by the Vague, women having obesity in upper portion of body are more prone to develop insulin resistance as compared to those with lower body obesity [10]. Nations with highest weight of obesity pandemic includes Papua New Guinea; 79–80 percent obese general population, Qatar 34–45 percent, Lebanon 36–38 percent obese population and United States 32–35 percent of obese population generally. Obesity is a worldwide problem now. It is highly raised in South Asian countries. Prevalence of obesity is highest in Thailand and lowest in India among Asian countries. China was once among the low weight population country, now rapidly catching up status (in term of obesity) like western countries. Prevalence of obesity is increasing drastically and Asian countries are at different stages of development of obesity [25]. Pakistan is unfortunately also included in the higher obese countries. In a research it is revealed that among the "fattest countries" of

the world, Pakistan is at 9th number out of 194 countries [26]. One-out of every four Pakistani adults is overweight. Risk factors associated with obesity are shown in figure 2.1;

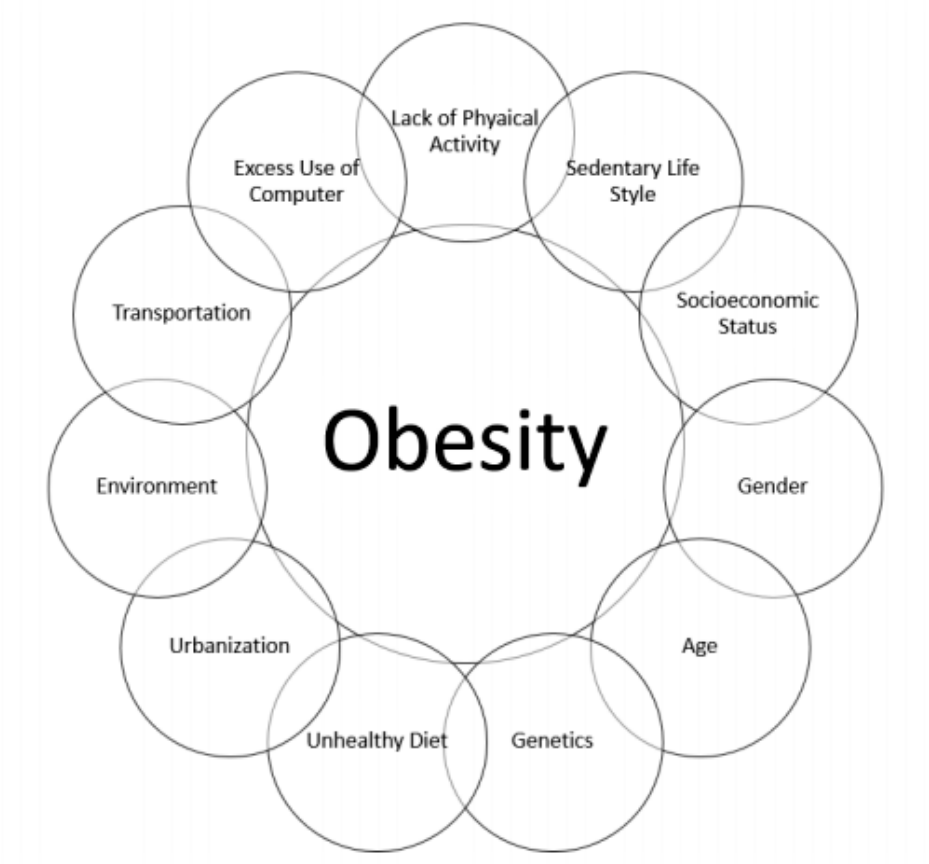

FIGURE 2.1: Risks Factors of Obesity [27] [28].

2.2 Inflammation

Inflammation is the response of body to harmful stimuli like pathogens, toxic substances, injury etc. This response is initiated by immune system to remove the triggering agent and to start healing. In acute inflammatory action cellular and molecular events takes place, that interacts to eliminate infection. After these events cells and tissues restore their identity and homeostasis. Inflammation could be acute or chronic. Acute inflammation is defined as short term, fast inflammatory response that may last for short time period, for minutes or days. It basically aid in healing and repair of injury [29].

2.2.1 Chronic Inflammation

In some cases, acute inflammatory response may not stop and become chronic. This long term chronic inflammatory response contributes to the variety of diseases. At tissue level, local immune cells responses to the infection. Some important cellular events occurring at the site of injury or infection includes vascular permeability changes, immune cell assimilation at the site of injury and also release of some inflammatory cytokines.

Inflammation could be infectious or non- infectious [30]. Chronic inflammation is actually slow process that may persists for prolonged time. It may last for months and even years. It is long-term process may occur after a very minute and rare inflammatory response. Extent and effects of the inflammation directly depends on the cause of inflammation and also on the condition and ability of the tissues and body to cope with the damage [31]. Adipose tissues are the main site where chronic inflammatory response takes place. Adipocyte when increases in size due to large fat deposit, it protrudes out and blocks blood supply. No oxygen reaches to the adipocyte and it dies.

Macrophages and immune cells process to eliminate dead cells. Adipose tissues and resident macrophages of adipose tissue produce pro-inflammatory cytokines like tumor necrosis factor (TNFα) and interleukin-6 (IL6) that may also induce inflammatory responses in other tissues. These pro-inflammatory signaling molecules may play an important role in the insulin resistance in peripheral insulin sensitive cells [32].

2.2.2 Mechanism of Inflammatory Response

Inflammatory response leads to the activation of inflammatory pathways. Activation of these pathways regulates and elevates the level of inflammatory mediators at the site of the resident cell. Inflammatory cells from the blood start accumulating at the site of injury [33]. There are some common mechanisms in inflammatory response, regardless of nature and initial site of injury or infection, these are:

1. Cell surface pattern receptor recognizes detrimental stimuli
2. Signaling pathway activation
3. Inflammatory markers are released
4. Inflammatory cells are recruited

2.2.3 Pattern Recognition Receptor Activation

Etiology of inflammation could be pathogenic or non-infectious. In microbial or pathogenic inflammation, pathogen associated molecular patterns (PAMPs) binds to the pattern recognition receptors (PRRs) [34]. These receptors are also expressed in non-immune cells. Danger associated molecular patterns (DAMPs) are non-pathogenic signals that are recognized by some of the pattern receptors.

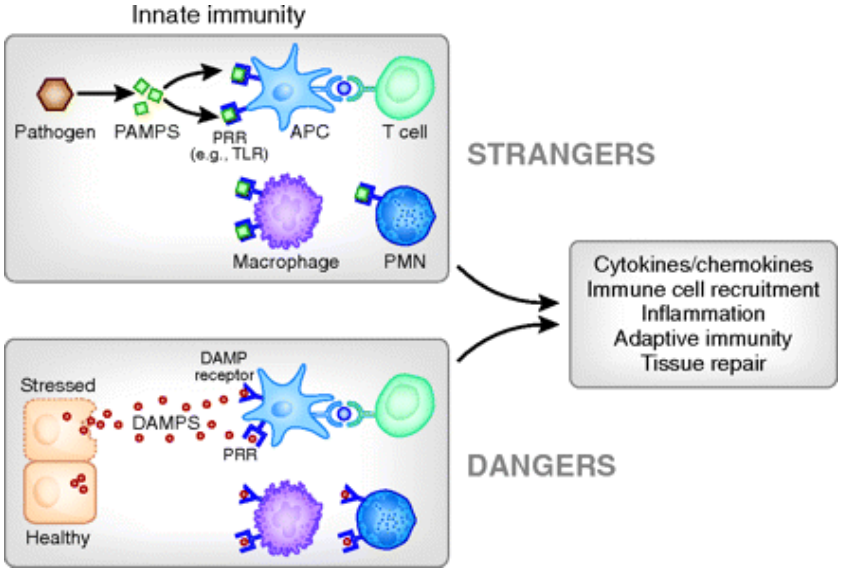

FIGURE 2.2: PAMPs are released as a result of attack by disease causing microorganisms. PAMPs when released, binds to PRR present on the immune cells to initiate innate and adaptive immunity along with inflammatory response to overcome the infection and start healing and repair [37]

DAMPs are host mediated biomolecule, responsible for the non-pathogenic inflammatory response (as shown in figure 2.2) [35]. Pattern recognition families have many classes, Toll-like receptors (TLRs) are most important and highly conserved families found in mammals and involved in inflammatory response [36].

2.2.4 Inflammatory Pathway Activation

Inflammatory agents when triggers the cell, it activates signaling pathways that are responsible for the production of mediators [38]. Inflammatory stimuli including microbial products and different cytokines (interleukin-6, TNFα etc.) interacts with Toll-like receptors (TLRs) and tumor necrosis factor receptor (TNFR) to mediate inflammation. Upon interacting with these receptors, inflammatory pathways like mitogen-activated protein kinase (MAPK or MAP kinase), NF-κB, JAK-STAT etc. gets activated [39].

2.2.4.1 NF- κB Pathway

NF-κB pathway is activated by microbial products or range of many cytokines and enzymes. IKB is a protein present in cell cytosol. NF-κB is a dimer of two components, RelA and p50. Initially IKB blocks the activity of NF-κB and pathway remains inactive [40]. I

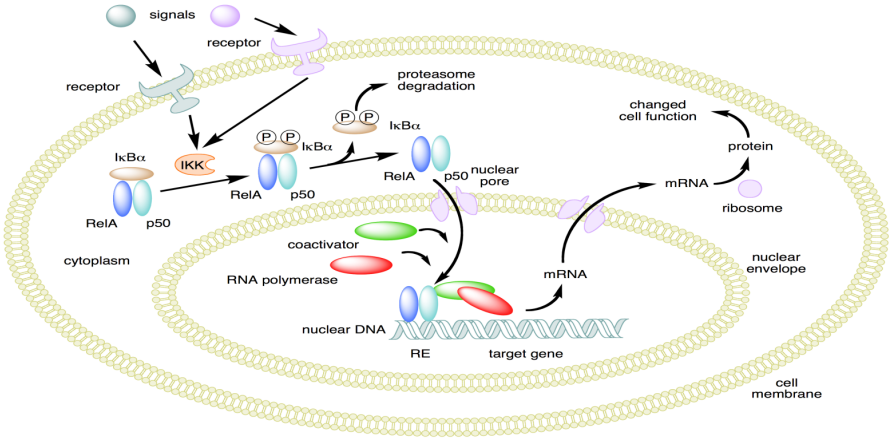

FIGURE 2.3: NF-κB is a complex of two subunits, RelA and p50. Normally in inactive form it remains in cytosol when is blocked by IKB. As a result of external stimuli, IKK (enzyme) gets activated and degenerate the IKB from NF-kB complex. NF-kB complex translocate from cytosol to nucleus and bind to specific target genes, following transcription and translation, final product (protein) change cell function [43].

KB kinase (IKK) phosphorylates IKB and proteasomes degrade and release NF-κB components [41]. In this way pathway gets activated. NF-κB pathway regulates

pro-inflammatory cytokine production and also accumulates inflammatory cells to initiate inflammatory response as shown in figure 2.3 [42].

2.2.4.2 MAPK Pathway

MAPK is activated by several intra and extracellular stimuli, like growth factors, polypeptides (hormones) and cytokines [44]. In MAPK pathway there are three main components involved a MAPK, a MAPKK (having two kinases) and a MAPKKK (having three kinases). MAPK kinase kinase phosphorylates MAPK kinase, which then activates MAPKs which is involved in P38 phosphorylation. P38 is a transcriptional factor, it starts inflammatory response. From evidences, it is revealed that p38 is not only involved in inflammatory response, but proliferation and apoptosis; which involved in etiology of cancers [45]. P38 can serve as a target

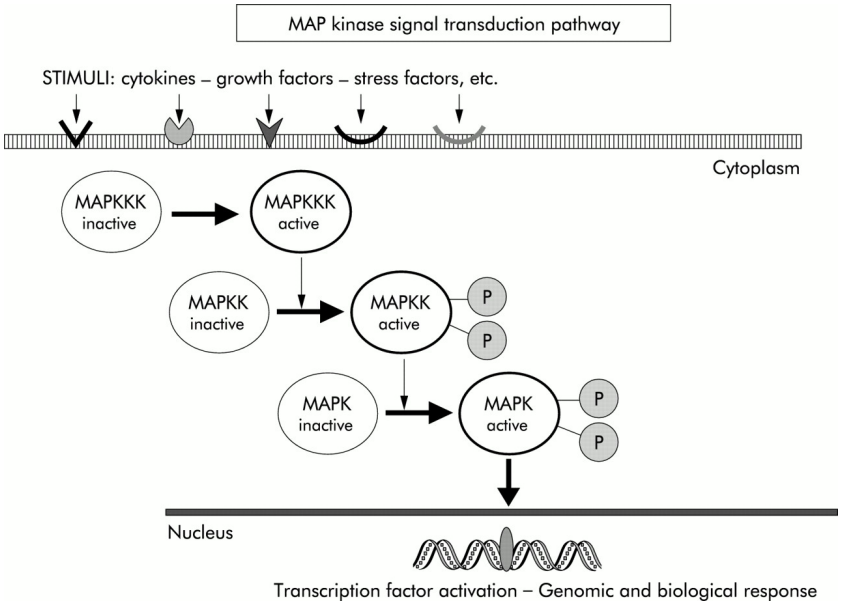

FIGURE 2.4: External stimuli either stress or messengers, initiates the signaling cascade followed by internal stimuli. At every step phosphorylation take place cause of kinases. MAPKKK when gets phosphorylated activates MAPKK which in return finally activate MAPK [47].

for drug discovery for the treatment of inflammatory disorders and cancers [46]. Along with p38, ERK and JNK are also considered as the key regulators in the development of cellular pathogenicity. P38 and JNK are involved in apoptosis, while ERK are involved in cell proliferation. MAPK subfamilies, JNK and P38 are activated by the trigger of cytokines, majorly IL-1β and TNF-α [44].

2.2.5 Inflammatory Cytokines

Cytokines are chemicals and immune - modulating agents, proteinaceous in nature. Cytokines are involved in immunity (regulated by antibody) of the body [48]. Pro-inflammatory cytokine are present even before inflammation. Pro-inflammatory cytokine includes Interferon gamma (IFNγ), Tumor necrosis factor α (TNF α), IL-6, IL-8, I-L12, p40 etc [49]. Anti-inflammatory cytokines on the other hand inhibits inflammatory responses. Both Pro and anti-inflammatory cytokines helps the body to maintain homeostasis by starting inflammatory response when cell or tissue is targeted and to stop when tissue is healed. Production of excessive cytokines when they are not needed causes serious physiological problems like tissue damage even organ failure, ultimately death [50].

2.2.5.1 Adipokines

It is now clear that, adipose tissues mainly WAT not only involved in storage but also works as an endocrine organ. Adipose tissue secretes number of cells like adipocytes, ATMs, immune cells etc. So adipose tissue releases number of polypeptides known as adipokines into systemic circulation. Adipose tissues present in visceral cavity and subcutaneous skin, release unique type of adipokines [51]. This is how inflammation and insulin resistance initiates in obese mice and human. Adipsin, IL-6, TNF-α and leptin are genuine adipokines. Adipokines also function positively when involved in some metabolic activities, but they are mainly involved in inflammatory response. Adipokines are classified as pro and anti-inflammatory cytokines. But most of the adipokines has pro-inflammatory response except adiponectin. Adiponectin is an anti-inflammatory adipokine which stops inflammatory response. Adiponectin is more prone to work and support insulin sensitivity of tissues and organs [52]. Obesity plays a central role in the resistance of tissues to leptin. Leptin is known as satiety hormone, as it works in the energy expenditure and balanced metabolism of the body. It inhibits hunger pangs in the hours when energy is not required. It stabilizes the body by preventing excess accumulation of fats in adipose as well as non-adipose tissues [53].

TABLE 2.1: Outline of cytokines names along with their function and source of their origin [16]

Cytokine	**Main source**	**Function**
IL-1β	Macrophages, monocytes	Pro-inflammatory, proliferation, apoptosis, differentiation
IL-4	TH cells	Anti-inflammation, T,B cell proliferation
IL-6	Macrophages, T-cells, adipocytes	Pro-inflammation, differentiation, Cytokine production
IL-10	Monocytes, T-cells, B-cells	Anti-inflammation, inhibition of the pro-inflammatory cytokines
TNF-α	Macrophages, NK cells, CD4+ lymphocytes, adipocyte	Pro-inflammation, cytokine production, cell proliferation, apoptosis, anti-infection
IFN-γ	T-cells, NK cells, NKT cells	Pro-inflammation, innate, adaptive immunity anti-viral
TGF-β	Macrophages, T cells	Anti-inflammation, inhibition of pro-inflammatory cytokine production

2.2.6 Obesity Induced Inflammation

Obesity is a metabolic disorder that is developed by over nutrition and it is a major cause for inflammation, insulin resistance and cardiovascular events [54]. Adipose tissue secretes inflammatory adipokines that contributes to chronic inflammation and other metabolic dysfunction. It has hypothesized that angiotensinogen (Agt) play role in the adipogenesis or lipogenesis and also inflammation. It is not yet

clearly understood how Agt actually play its role. Currently we used shRNA to successfully silence the Agt gene in 3T3-L1 adipocytes, lowering the intracellular level of Agt and allowing us to investigate the metabolic effects of adipose Agt. We confirmed the direct contribution of adipose tissue-derived Agt to lipogenesis, preadipocyte differentiation, and adipose tissue inflammation using this laboratory model, as evidenced by less triglyceride accumulation, production of pro-inflammatory adipokines, and down-regulation of several adipogenic and inflammatory genes [55]. According to a population study conducted in 1960s, obese individuals have elevated concentration of inflammatory markers. Beside them, pro-inflammatory cytokines and pro-coagulant proteins are also present in obese people [56]. N.M Kaplan in 1989 also suggested that obesity, especially abdominal obesity is a key factor to induce insulin resistance and ultimately metabolic syndrome [11] Adipocytes are the fat cells. Macrophages are the type of blood cells that work as natural cleaner. They surround and kill microorganisms and any foreign invader. Macrophages act as immune cells [57]. Adipose tissue has own microphages known as adipose tissue macrophages (ATM). Adipocytes are actually endocrine glands, which releases different adipokines into the blood stream. These adipokines include hormones and cytokines that regulate inflammation [58]. In obese individual, fat cells enlarge gradually and secrete pro-inflammatory cytokines like TNF-α and IL-6. Adipocyte stores triglycerides. As the lipid droplet in obese adipocyte gets bigger, cell expands and outgrows the blood supply and blocks it. As a result cell starved of oxygen and die [59]. Macrophages migrate through blood stream and join resident macrophages of adipose tissue (ATM) to get rid of dead fat cell. Both types of macrophages surround dead fat cells. ATMs are source of inflammatory cytokines like TNF-α and IL-6 [60]. These cytokines are responsible for the chronic inflammation in body and promotes problems like tumor formation, diabetes and Alzheimer's dementia. TNF-α also down regulate adiponectin, which promote insulin sensitivity [61]. Another factor is that lipid does not accumulate only in adipose tissues, but also in non-adipocytes. Spill of FFAs from adipose tissues leads to the storage of lipid in the tissue not suited for storage function [62][63]. Increased level of fats leads to the initiation of cytokine production which activates immune receptors and stress signaling pathway that

ultimately interferes with insulin signaling pathways of muscle and live [54]. Hypoxia, a condition of dropped level of oxygen when develops in adipose tissues is responsible for both inflammation and insulin resistance of adipose tissue cells.

2.2.7 Major Histocompatibility Complex (MHC)

MHC major histocompatibility complex is collection of genes present on the chromosome 6 and encode three classes of MHC molecules. A Complex of proteins present on the cell surface helps to communicate the cells with one another and allows leukocyte to distinguish healthy and infected cells [64]. MHC has three different classes 1 2 and 3. MHC 1 has four polypeptide units $\alpha 1$ α 2 α 3 and $\beta 2$. MHC is connected to cell membrane through $\alpha 3$ subunit. MHC 1 has a cleft. Healthy cells produce some polypeptides which come out of the cell to bind the cleft portion of MHC1 [65]. Leukocyte upon approaching normal cell recognizes normal self-antigen attached at the MHC1 cleft portion and leaves it. Infected cells has pathogenic antigen which comes out and binds at MHC1 cleft. Leukocyte recognizes pathogenic antigen and binds to the infected cells to initiate immune response [66].

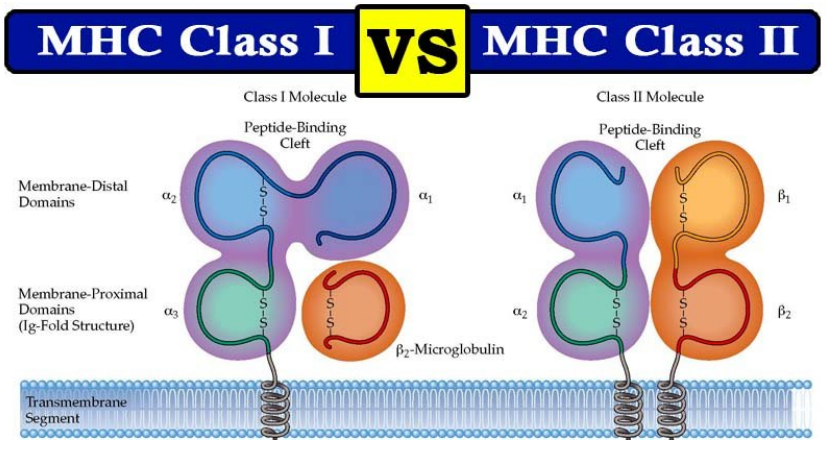

FIGURE 2.5: MHC class 1 and 2 structure and difference in their binding domains with the transmembrane [70]

MHC2 are present on the specialized antigen presenting cells (APCs). It is made up of 4 subunits $\alpha 1$, $\alpha 2$, $\beta 1$ and $\beta 2$ and attached through $\alpha 2$ and $\beta 2$ subunit to the cell membrane [67]. This class also has a cleft portion for antigen binding.

MHC2 is used for the communication between different types of leukocytes. For example, macrophage, the major immune cells when encounter a foreign particle, engulfs it making a phagosome in the cytoplasm [68]. Lysozymes start breaking phagosome. Antigen releases from the phagosome and binds in the MHC2 cleft portion. Macrophage releases interleukin-1 which then recruits helper T cells. TH have a receptor on its membrane, it recognizes foreign antigen on MHC2 of macrophage and binds on the antigen. Once TH binds it releases its own chemical, IL-2 [69].

2.3 Insulin Resistance

Pancreas is a dual function organ. It works as an exocrine organ as it is associated with the secretion of digestive enzyme through its duct connected with the gastrointestinal lumen [71]. As an endocrine gland, pancreas plays vital role in the secretion of important hormones like insulin and glucagon. These hormones are secreted by the β cells of pancreas in the blood stream. Amount of insulin secreted is dependent on the level of glucose in the blood [72]. More glucose in blood circulation, more insulin would be secreted for the uptake of glucose by skeletal tissues and adipocytes. Thus insulin resistance is actually a condition where insulin signaling pathways gets disrupted and glucose level increases in blood stream [73]. After each meal, insulin level increases in body. It is produced by the β-cells. When glucose level increases after every meal it enters β-cells of pancreas (islets of Langerhans) through Glucose transporter GLUT2. After entering into β-cells, Glucose moves along different pathways like glycolysis, citric acid cycle and oxidative phosphorylation to form ATP. There are some ATP gated K+ channels in the membrane. Elevated level of ATP inhibits these K+ channels, it causes low efflux of K+ from the channels. It makes membrane depolarized. This depolarization along the membrane of β-cells activates voltage gated calcium channels by opening them and calcium enters β-cells. Elevated calcium level causes exocytosis of pre synthesized insulin and amylin vesicles. In this way insulin enters from β-cells to blood stream [13]. These transporters when fails to perform function sugar

remains within in the blood for a long time and level of insulin elevates. Glucose when remains in the blood for a long time it destroys liver cells and results in the T2D [14].

2.3.1 Insulin Signaling Pathway

Insulin binds on the insulin receptor present on the plasma membrane of cells (adipose, skeletal etc.). These receptors are tyrosine kinases made up of 4 subunits, 2 alphas and 2 betas. Insulin binding subunits are alphas while beta subunits are transmembrane [74]. Tyrosine kinase is parts of β-subunits. When insulin binds with the alpha subunit, it activates transmembrane β-subunits. The β-subunits ultimately activate tyrosine kinase residues and auto phosphorylation occurs [75]. Phosphorylated tyrosine kinase further phosphorylates and activates insulin receptor substrate IRS, activated IRS activates cascade of cellular responses. IRS phosphorylates another kinase known as phosphoinositide-3-kinase (PI3K) which converts membrane phospholipid (PIP2) into PIP3. PIP2 and PIP3 act as second messenger and important signaling molecules [76]. PIP3 signals PDK1 (PIP3 dependent kinase 1). It phosphorylates and sends signals to AKT [77]. Activation of AKT phosphorylates and up regulates GLUT4. This GLUT4 is actually glucose transporter and moves from cytoplasm to the membrane where it opens and transport glucose from blood to the cell [78].

The signaling cascade IRS/PDK1/AKT2/, which ultimately acts on the GTPase-activating protein AS160, involved in the activation of small G protein RAB, which translocate GLUT4 from storage vesicles to the plasma membrane [79]. Atypical PKCs play a role as well. AS160 is directly involved in the activation of G proteins (RAB) and is involved in membrane blocking, by blocking the phosphorylation of GTP to convert into GDP [80]. PKCs isoforms that are atypical Protein Kinases C (PKCs) tend to be involved downstream of PDK1, but not by AKT [81]. The connection between beta cell insulin release and blood glucose concentration involves a set of proxy molecules whose concentrations rise and fall with that of blood glucose proportionally. The net effect of this mechanism is that when increased

level of blood glucose is present result in increased levels of insulin secretion by beta cells and vice versa [82].

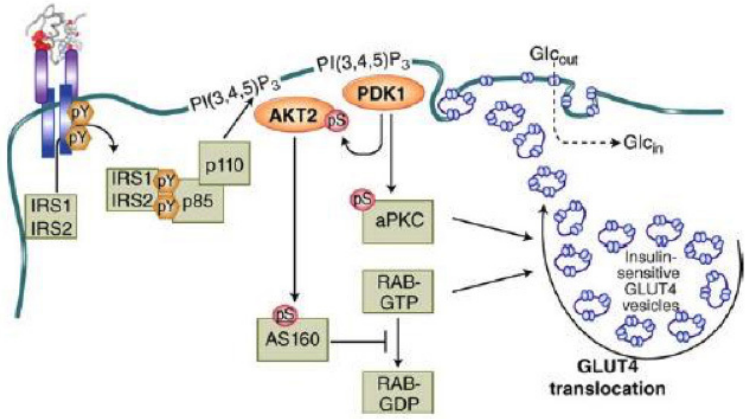

FIGURE 2.6: Mechanism of glucose transport and Glucose transporter 4 translocation [83].

2.3.2 Mechanism of Insulin Resistance

In insulin resistance pro inflammatory cytokines like IkB kinase (IKK) and JNK are involved. These kinases move and inhibit insulin action by promoting phosphorylation of series residues of insulin signaling pathway. It phosphorylates insulin receptor substrate 1 (IRS-1). Actually serine phosphorylation replaces normal tyrosine phosphorylation which impairs normal insulin signaling [84]. In humans and rodents, inflammation is characterized by macrophage penetration in adipose tissues or the liver during obesity, and immune cell activation is closely related to insulin sensitivity [85]. Macrophages are of two types M1 and M2; M1 supports microbicidal activity and M2 support allergic and ant parasitic responses. In normal conditions M2 macrophages produces IL-4 and IL-10 that supports normal insulin function and insulin sensitivity [86]. While M1 macrophages secrete pro-inflammatory cytokines, such as TNFα, and induce insulin resistance. In normal state M2 activates by the action of TH2 cells, eosinophil and natural killer cells, as a result IL-4 and IL-10 level increases [87]. While obesity causes alteration, TH1, B-cells, neutrophils or mast cell induces M1 macrophages and TNF-α and INFγ [88].

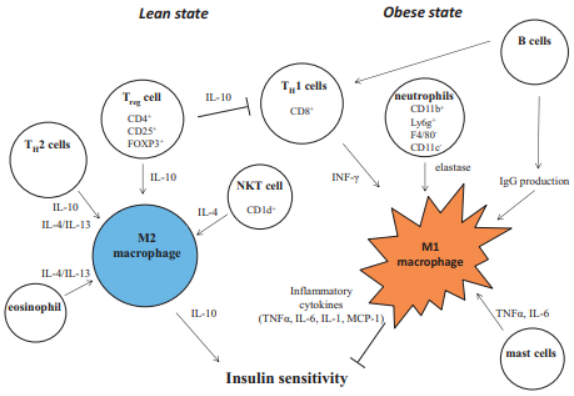

FIGURE 2.7: M1 and M2 polarization by immune cells. In normal state, T-cells mainly TH2 and Treg along with the eosinophil activate M2 through the production and secretion of Interleukins. But in obese state, T.cells like TH2 and Treg level declines and TH1 and B-cell level increases. As a result M1 macrophage gets activated .

2.3.3 Nutritional Imbalance Link to Chronic Inflammation and Insulin Resistance

Toll-like receptors (TLR) play an important role in the innate immunity, the detection of pathogens is done through pathogen associated molecular pattern (PAMPs), and another pattern known as DAMPs is used for the detection of injury [89]. Toll-like receptor gets stimulated by the disease causing agents (pathogens) through a pathway known as MYD88, it leads to the production of inflammatory cytokines and activation of some transcriptional factors [90]. Mainly TLR2 and TLR4 are involved and plays role in obesity induced inflammation. TLR4 is increased in obese mice and diabetic human patients. In recent years it is also proposed that in obese people metabolic endotoxemia occurs [91]. Endotoxemia is increased level of circulating lipopolysaccharides (LPS) which is due to micro organismic invasion, gram negative bacteria is usually causing agent of increased LPS; it also alters normal gut micro biota. As a result TLR4 is get activated in metabolic tissues, which causes inflammation and metabolic disorders [92].

Lack of balanced diet and enriched fat containing diet, migrates bacteria (gram negative bacteria) from the digestive tract to the adipocytes. CD14 also helps TLR4 to sense LPS [93]. Evidences indicate that obesity is linked to low- grade inflammation, TNF-α which was expressed in the adipose tissue of obese mice,

was linked to insulin resistance [94]. Obesity is also linked with the immune cell infiltration, disrupted tissue function that are involved in glucose homeostasis, impaired lipid metabolism leads to obesity by impairing insulin signaling . Free fatty acids (FFAs) circulate in the blood and have negative impact on insulin target tissues as it activates the inflammatory pathways [95]. Lipid derivatives like diacyl glycerols (DAG) could also move freely due to poor lipid metabolism and negatively regulates insulin action [96].

Adipose tissues are of two types mainly, white adipose tissue and brown adipose tissue. BAT is associated with the thermogenesis, as it breaks down in the presence of oxygen involving aerobic respiration and yields ATPs along with generation of heat, which is used by the muscles to work out. WAT functions in the lipid storage that is used by the body in nutrient deprivation [97]. With obesity and insulin resistance, more lipids are breakdown so more TAG is released leading to the activation of inflammatory pathways and impaired insulin signaling. WAT releases some adipokines, include interleukin (IL)-6, IL-1β, TNFα, leptin and adiponectin [98].

2.4 Pathway Analysis

Pathway analysis plays an important role in drug discovery. National Human Genome Research institute describe biological pathway as a series of action among molecules in a cell that leads to certain changes in the cell. In terms of function the biological pathway are classified in three categories metabolic pathway which is involve in many chemical reaction in biosynthesis or decomposition of many metabolites. Gene regulation which is responsible for on and off genetic information flow that predicts the protein expression and signal transduction pathway which are responsible for carrying signal from external environment to interior cellular compartments [99]. In this study KEGG (Kyoto Encyclopedia of Genes and Genome) was used for Pathway analysis. This is widely used database for pathway analysis. It consists of manually drawn reference pathway along with

organism-specific pathway that is computationally generated by matching KO assignments in the genome with references pathway maps. It is quite user friendly and much reliable database in term for pathway analysis [100].

2.5 Pathways Crosstalk

Current studies on obesity-induced inflammation and insulin resistance explained many therapeutic targets to overcome the development of diseases. Moreover, pathways can affect each other's P-value which is termed as cross-talks. These cross talks occur due to interactions of biomolecules, or gene overlapping among the pathways [101]. Pathway cross talk describes communication between the pathways because biomolecules involved perform more than one part in particular pathway so it may be involved in more than one biological function. Their cross-talks are also important for finding and predicting actual potential targets or biomarkers which by further investigations could be the novel approach of diagnosis or treatment [102].

Chapter 3

Material and Methods

3.1 Methodology

This research is designed to analyze pathway crosstalk involved in obesity, chronic inflammation and insulin resistance. To establish a crosstalk among major pathways following methodology (Figure 3.1) used is summarized below.

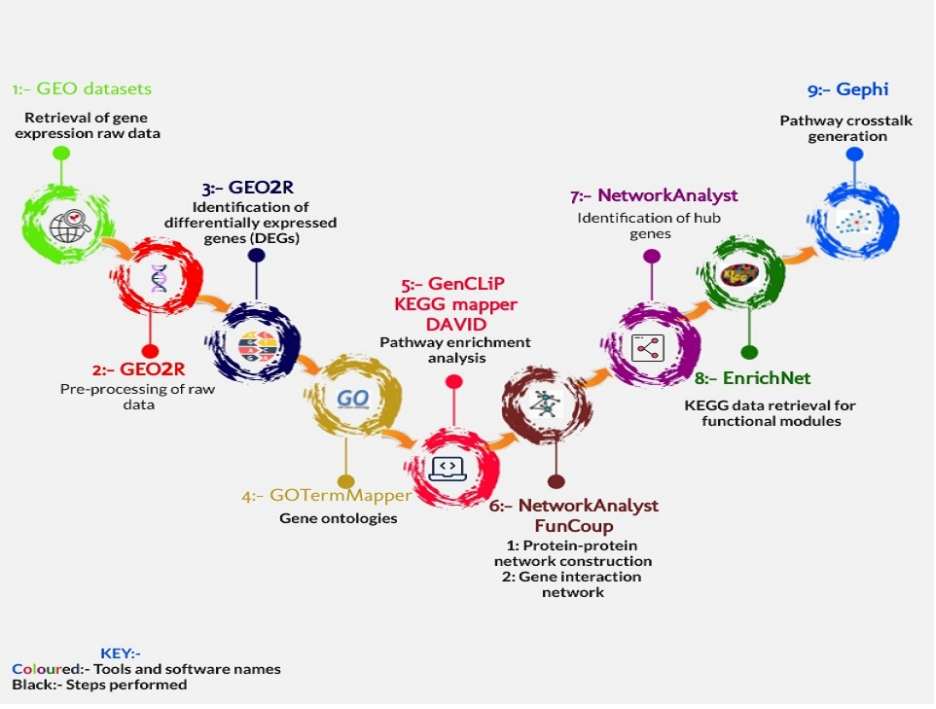

FIGURE 3.1: Flow chart methodology used for generating crosstalk among key pathways of Obesity, insulin resistance and chronic inflammation.

Obesity, chronic inflammation and insulin resistance are multifactorial diseases occurred due to the alteration in inflammatory, immune and metabolic pathways. These pathways are linked and their components show crosstalk among them. These components and links would be new therapeutic target in future. The microarray data is retrieved and differentially expressed genes involved in obesity, chronic inflammation and insulin resistance are identified, after identification of these DEGs, gene ontology and pathway enrichment were analyzed. Protein-protein networks were constructed which would help to identify the networks of DEGs. A crosstalk among these pathways would help to identify genes that are majorly involved in development of these diseases. Each disease was reviewed separately.

3.2 Prioritizing of Key Pathways Mentioned in Literature Involved in Obesity, Chronic Inflammation and Insulin Resistance

The first objective of the study is to prioritize the key pathways which are involved in obesity, chronic inflammation and insulin resistance from the literature. For this purpose literature was thoroughly and keenly reviewed. However few pathways were present and the selection of pathways were done according to the crosstalk present among the pathways involved. These pathways were identified as immune pathways, metabolic pathways, pro inflammatory and cytokines pathways for each disease (obesity, chronic inflammation and insulin resistance). However few pathways were present and the selection of pathways were done according to the crosstalk present among the pathways involved. These pathways were identified as immune pathways, metabolic pathways, pro inflammatory and cytokines pathways for each disease. However few pathways were present and the selection of pathways were done according to the crosstalk present among the pathways involved. These pathways were identified as immune pathways, metabolic pathways, pro inflammatory and cytokines pathways for each disease

3.3 Elucidation of Therapeutic Targets Based on Pathway Crosstalk

The third and main objective of the study is to define new therapeutic targets based on pathways crosstalk.

3.3.1 Pathway Cross Talk Generation

For pathway crosstalk generation, Gephi is open source network visualization, analysis and exploration software is used. The pathways which showed the cross talks were analyze and visualize with the gephi. It uses statistical value and used for visualization and manipulation of large graphs.

3.4 Identification of Key Genes Involved in Obesity, Insulin Resistance and Chronic Inflammation using Pathway Crosstalk

Second objective of this study is to identify those genes which are involved in the pathways of obesity, chronic inflammation and insulin resistance, for this purpose following steps are performed:

3.4.1 Retrieval of Data Profiles

For data retrieval, National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/gds) was used. NCBI was established in 1988 for providing information about different biological aspects especially molecular biology. Amount of data maintained in NCBI increased and grouped in 6 categories; literature, health, genomes, genes, proteins and chemicals [103]. NCBI provides a huge set of data. Over millions of genes from almost 10,000 species are achieved

in NCBI database. There are in-house employees working on the correction and maintenance of the microarray data. Many international partnerships also coordinate in the accumulation and rectification of data. For microarray data retrieval, GEO datasets database can be used [104].

3.4.2 Pre Processing of DEGs

Raw data includes whole DNA stretch and genes. If a change or discrepancy is found in expression of a gene in two different physiological conditions, that gene would be known as differentially expressed gene. DEGs were extracted and pre-processed using bioinformatics tool. GEO2R is an automated tool of NCBI used to fetch out DEGs from raw data. To check gene expression some statistical methods are available. To label genes as differentially expressed genes, threshold criteria for change in gene expression should be present. To retrieve DEGs, statistical modeling is used [105].

The Gene Expression Omnibus (GEO) database is an international repository that stores gene expression and other functional genomics datasets. GEO has been evolved with rapid increase in its repository and techniques. This database provides access to data for millions of studies, and also an approach to different web-based tools and techniques to facilitate the users to retrieve and analyze data of their interest.

3.4.3 Identification of Differentially Expressed Genes

For the identification of DEGs, GEO2R was used. It is an interactive instrument that allows approximately 90 percent of the GEO Sequence to be analyzed. Disease name is in input bar of NCBI GEO2R, by selecting “Analyze with GEO2R” clicking ”Value Distribution” tab for verification of distribution of the sample values. Groups are defined as diseased or control. Parameters were set as default and only selected top 250 from home page. DEGs along with their P-values were presented in new window tab [106].

3.4.4 Gene Ontology and Pathways Enrichment Analysis

Pathway enrichment analysis gives us information about the gene list extracted from experimental or research data. It identifies pathways that are enriched in our query gene list and also which gene is over or under represented. For pathway enrichment analysis, Gene Ontology (GO) is performed using the tool Go Term Mapper (https://go.princeton.edu/cgibin/GOTermMapper). GO term provides us explanation about the gene products of all the organisms. In gene ontology (GO), the molecular function (MF) means direct interaction of molecular products with single macromolecular machine. Function of a gene is described as any process or action carried out by the product of particular genes. Action or activity involves biochemical activity that can take part in a larger process. While biological process (BP) is a particular target that can be achieved through genetic program of organism. For instance, cell division is a biological process in which a single cell undergoes phase of division and as a result two daughter cells forms. The final outcome of this process would define its BP. Every BP is achieved through a proper network, molecular components and pathway regulation carried out by specific gene product [107]. In this study the GO term mapper input windows has uploading genes list tab, choosing ontologies, of MF, BP and cellular components, an ontology option (generic slim, GOA slim), organism selection and results in form of plain text or HTML. Here GO of MF and BP genes were collected for Homo sapiens genes, in HTML table format.

3.4.4.1 Pathway Enrichment Analysis

Pathway enrichment analysis provides the information about enriched pathways in a particular gene list [108]. PEA were performed by using Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) tool that is used for functional annotation, gene id conversion and classification of gene function. It is an efficient tool which can give results within few seconds of a large stretch of genes (up to 3000) in one go. It also allows one gene to be involved in many pathways and activities, which describes that a single gene could

play many roles in different processes [109]. Using DAVID gene functional classification tools genes clusters were retrieved, in which the pathways having these genes were noted. Gene list were uploaded separately for each disease.

3.4.4.2 Malacards Database

A database named malacards (https://www.malacards.org/) is human disease database. It is used for the verification of the pathways with enriched genes by comparing them with already existing pathways in database. In malacard database, data of more than 26,000 diseases affecting human organs is stored [110]. Pathways related to obesity, chronic inflammation and insulin resistance according to KEGG, GO terms (cellular components, MF and BP) had been compare with the pathways retrieved from DAVID. Those pathways which were matched, there genes were saved in tabular form.

3.4.4.3 GenCLiP 2.0

For network analysis of differentially expressed genes, GenCLip had been used. It is a web based server for human gene functions and network analysis, used for the identification of functions and networks of the query gene list [111]. A default cutoff criteria to perform its GO clustering is p value $<$ 1e-4 and hit $>$ 3. Two out of three modules have been used, Gene cluster with literature profiles, and literature mining gene networks. Clusters of genes were identified with literature survey and genes, which graphically represent a heatmap. In gene network analysis, a network is generated with their respective genes.

3.4.4.4 KEGG Mapper

KEGG mapper is a database used for KEGG mapping, three databases integrated are pathways, (brite, module, network and diseases) it contains data from publications and experiments [112]. In Previous version the tools were available

separately for each database, but in new version they have been merged. In new version, three mapping tools are available, each of them allows mapping against multiple databases in one time. For searching pathways, default parameters were selected entering DEGs for obesity, chronic inflammation and insulin resistance separately. Selected species was Homo sapiens. Hit genes from GenCLip were used as an input for KEGG mapper to cross check the results.

3.4.5 Analysis of Functional Modules in Protein-Protein Interactions

Network Analyst is a web based tool which helps in meta-analysis of data by showing protein-protein interactions (PPIs) of gene expression data. It calculates the values based on centrality. It is an important point because it shows the nodes which holds main central position in a network. Betweenness centrality is to measure mediation of a particular node in network. If nodes, like contract, link, transaction etc. go through a single node present in a hub, then this one node is more essential having high centrality of betweenness [113].

In this research PPI of two modules were selected, generic PPI and tissue specific PPI. In these modules, PPI were visible along with data in tabular form. Those genes which betweenness centrality occurs above 0 for sub networks were selected. Hence final 41 DEGs of obesity, 46 DEGs of insulin resistance and 36 DEGs of inflammation were retrieved from network analyst.

3.4.6 Gene Interaction Network

For cross checking of the gene functional association network, FunCoup (https://funcoup.sbc.su.se) is used. It is a web-based framework having data of 22 model organisms [funcoup]. In search bar, gene list retrieved from KEGG pathways was uploaded. Selected species was Homo sapiens. Network, interactors and interactions were fetched out.

3.4.7 Retrieval of KEGG Data for Functional Modules

EnrichNet is a tool used for enrichment analysis for evaluation of functional association of DEGs. It shows new statistical values, which explore the molecular networks connecting two genes set with new visualization of sub networks structure [114].

DEGs of obesity, chronic inflammation and insulin resistance were given in enrichnet tool query gene tab. We retrieved three different annotated databases results, and compare all three database results seperately with KEGG pathways. Those pathways were selected which were common in all three databases and show cross talks with same DEGs.

Table 3.1: Description of tools, databases and software used in the Crosstalk among Obesity, Chronic Inflammation and Insulin Resistance Pathways

Sr. No	Database/Tools	URL	Description
1	GEO	https://www.ncbi.nlm .nih.gov/geo/	Retrieval of gene expression raw data
2	GEO2R	https://www.ncbi.nlm. nih.gov/geo/geo2r/	Pre-processing of raw data
3	GEO2R	https://www.ncbi.nlm. nih.gov/geo/geo2r/	Identification of differentially expressed genes (DEGs)
4	GOTerm Mapper DAVID	https://go.princeton.edu/ cgi-bin/GOTermMapper 5https://david.ncifcrf.gov/	DEGs ontologies identification
5	Malacards GenCLip KEGG pathway mapper	https://www.malacards.org/ http://ci.smu.edu.cn/ GenCLiP2/analysis.php https://www.genome.jp /KEGG/mapper.html	pathways enrichment analysis of DEGs

Table 3.1 continued from previous page

6	Network Analyst	https://www.networkanalyst.ca/	Protein-protein interaction networks generation
7	FunCoup	https://funcoup5.scilifelab.se/search/	Identification of hub genes
8	Enrichnet	https://lcsb-enrichnet.uni.lu/enrichnet/index.php	Identification of pathways associated with hub genes
9	Gephi	https://gephi.org/	Pathways cross talk generation

Chapter 4

Result and Analysis

In this chapter, each result of this research work is given in detail. This result is compiled in the form of tables, figures and diagrams. Description of tools and databases used are also mentioned with each step. Result of each objective is mentioned separately under main heading. This pathway crosstalk is generated to find out the hub genes that are actually involved in regulation of pathways. These genes could be targeted in future for better therapeutic techniques.

4.1 Prioritizing of Key Pathways Reported in Literature to be Involved in ASD

To achieve first objective of this research study, literature review had been done keenly to extract out key pathways involved in obesity, chronic inflammation and insulin resistance. For better understanding of links among the obesity, chronic inflammation and insulin resistance, pathways involved had been targeted to check out the crosstalk present among them. In this section, pathways involved are briefly discussed. Pathways related to obesity, chronic inflammation and insulin resistance were selected. Obesity related pathways include metabolic pathways like MAPK/ERK and PI3k/Akt signaling pathways. All these pathways are involves series of reactions, where product of one reaction becomes reactant of proceeding

reaction [115]. Chronic inflammation includes many proinflammatory pathways like JAK/STAT and NF-κb signaling pathways. In chronic inflammation, proinflammatory cytokines play vital role. These cytokines helps the body to activate inflammatory response for defense of body. These proinflammatory cytokines conveys the message of existence of inflammation to peripheral cells [116]. Insulin resistance is a multifactorial problem. Pathways selected were insulin signaling pathways, TNF signaling pathways, mTOR and adipocytokine signaling pathway.

4.2 Identification of Key Genes Involved in Obesity, Insulin resistance and Chronic Inflammation using Pathway Crosstalk

This is second step of this research study. Many steps are performed in order to retrieve and filter those genes that are involved in pathways of obesity, insulin resistance and chronic inflammation. Following steps were performed;

4.2.1 Retrieval of Microarray Data

To get microarray data, National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/gds) was used. NCBI was established in 1988 for providing information about different biological aspects especially molecular biology. Volume of data maintained in NCBI increased and grouped in 6 categories; literature, health, genomes, genes, proteins and chemicals [103]. NCBI provides much of data. More than millions of genes data from almost 10,000 species are deposited in NCBI database. There are in-house employees working on the correction and maintenance of the microarray data. Many international coworkers also take part in the collection and rectification of data. For microarray data retrieval, GEO datasets database can be used. NCBI database search bar has different attribute options to search like text, id, keywords, organism, DataSet

type and authors. The input had been given in search bar as disease name. Search for each disease "obesity" or "chronic inflammation" or "insulin resistance" is done separately. In the database selection bar, GEO datasets were selected. Raw data of many sets of genes were displayed in results.

4.2.2 Pre Processing of DEGs

To bring data in proper form, differentially expressed genes (DEGs) were pre-processed by using an automated tool of NCBI, GEO2R. The Gene Expression Omnibus (GEO) database is an international repository that stores gene expression and other functional genomics datasets. For preprocessing of DEGs only four data sets were selected as they show the option for "Analyze with GEO2R". After selection of this preprocessing data a new window showed the GEO accession, and platform in which more than one data were generated separately for different platform and same accession numbers.

4.2.3 Gene Ontology and Pathways Enrichment Analysis

For the identification of enriched functionally associated genes groups, Gene Ontology (GO) is performed using the tool Go Term Mapper. The gene ontologies offer structure and principals to explain the functions of gene products from all species [117]. The GO term mapper were used for generic annotation of DEGs, the input windows contains uploading genes list tab, ontologies aspect (MF, BP), an ontology option (generic slim) and organism selection (Homo sapiens). The results were in form of gene id, gene annotations for the GO term, use of the Go term in the gene list and the frequency of use of the genome. For DEGs of insulin resistance, total of 467 genes were given as an input, in which 31 were duplicated, and about 77 were not found to be annotated. For inflammation total of 238 genes were given as an input, in which 81 were duplicated, and about 34 were not found to be annotated. In case of obesity, total of 468 genes were given as an input, in which 26 were duplicated, and about 46 were not found to be annotated.

4.2.4 Identification of DEGs

GEO2R was used for the identification of DEGs. Groups were defined by giving names as control vs disease. Sample had been selected for each group accordingly from the list. Default parameters were selected from "options" i.e. Benjamini and hochberg method, significance cut-off <0.05 and analyze. As a result, the top DEGs were displayed in tabular form with the columns id, adj P-value, P value, log FC, gene symbol and gene title. This data were retrieved and saved in the forms of spread sheet. For obesity and insulin resistance, two sets of DEGs from two different cases are retrieved separately. For obesity total 468 genes, in case of chronic inflammation 238 and for insulin resistance top 467 genes were retrieved as DEGs.

4.2.4.1 Pathways Enrichment Analysis

Pathway enrichment analysis provides the information about enriched pathways in a particular gene list [108]. PEA were performed by using Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) tool that is used for functional annotation, gene id conversion and classification of gene function. It is an efficient and valuable tool which can give results within few seconds of a large stretch of genes (up to 3000) in just one go. It also allows one gene to be involved in many pathways and activities, which describes that a single gene could play many roles in different processes [109]. Using DAVID gene functional classification tools, genes clusters were retrieved in which the genes involved in functional pathways were noted. For this purpose gene list were uploaded separately for each disease. The DEGs were uploaded in input section of DAVID gene functional classification tool, with the selection of Homo sapiens species. Functional annotation clustering were selected, as a results annotation clusters, E score, P-values, gene counts and benjamini values were displayed in new tab [118]. For checking clusters of obesity, 468 DEGs were given and 70 total clusters were retrieved. 238 DEGs of chronic inflammation were given in search box and 21 clusters were retrieved as a result. In case of insulin resistance 467

DEGs were given in input box and 66 clusters were retrieved. Only genes of those clusters were retrieved which were functional to their respective disease pathways.

4.2.4.2 Malacards Database

Once the pathway with enriched genes had been identified, verification of these pathways with already existing pathways involved in obesity, insulin resistance and chronic inflammation had been performed using a database named malacards [110]. In a search bar each disease name "obesity", "Chronic inflammation" and "insulin resistance" were searched separately, as a result for obesity 4626 types, for insulin resistance 4032 types and for chronic inflammation 6267 types with their respective scores were displayed. Pathways related to obesity, insulin resistance and chronic inflammation according to KEGG, GO terms (cellular components, molecular function and biological process) had been compare with the pathways retrieved from DAVID. The pathways were identified in DAVID enriched pathways. Pathways were compared in both databases and saved as functional clusters. For obesity, 18 pathways in 6 clusters, 14 pathways in 7 clusters for insulin resistance and 7 pathways in 5 clusters for inflammation were saved. Functional genes from both DAVID and malacards were retrieved and saved for each of the disease. Total 116 DEGs of obesity, 161 of insulin resistance and 67 for chronic inflammation were retrieved and given in tables 4.1, 4.2 and 4.3 respectively.

Table 4.1: Table 4.1: Top clusters gene ontologies of DEGs of Obesity.

Categories	**GO term**	**Hits**	**P-value**
Cluster 1 E. score: 2.71	Propanoate metabolism	ACAT2, ACACA, ACSS3, ALDH6A1, PCCB, SUCLG2	3.70E-06
	Fatty acid metabolism	ELOVL5, ACACA, ECI1, HADH, LPIN1, PECR, SLC27A2	5.10E-04

Table 4.1 continued from previous page

	Carbon metabolism	ACAT2, ALDH6A1, GPT2, PCCB, PC, SHMT1, SUCLG2	1.90E-06
	Lipid metabolism	ACACA, ECI1, ECI2, GCDH,HADH	6.70E-03
	Glucose metabolism	AKT2, DCXR, PDHA1,PDK2	5.10E-04
Cluster 2	Carbohydrate metabolism	AKT2, CHIT1, DCXR, PHKA2,	9.40E-04
E. score: 2.68		PDHA1, PDK2	
	Glucose metabolic process	AKT2, DCXR, PDHA1, PDK2	1.90E-02
Cluster 3	Fatty acid degradation	ACAT2, ADH1B, ECI1, ECI2,	8.70E-04
E. score: 1.97		GCDH,HADH	
	Lipid metabolism	RAB7A, CHKA, ECI1, ECHDC2,	5.90E-02
Cluster 4 E. score: 1.71		LPIN1, PLA2G5	
Cluster 5 E. score: 1.32	Carbon metabolism	ACO2, PCCA, PDHA1,SDHA	1.00E-01
Cluster 6	Insulin signaling pathway	AKT2, SHC1, EIF4EBP1,PHKA2	1.60E-01
E. score: 1.01	Metabolism of xenobiotic	ADH1B, ALDH1A3, HSD11B1	3.20E-01
Cluster 7	Cytochrome P450		
E. score: 0.95	P13K-AKT Signaling pathway	AKT2, GNG7, FLT4, COL6A6,	2.50E-01

Table 4.1 continued from previous page

Cluster 8 E. score: 0.79		E1F4EBP, VEGFA	
Cluster 9 E. score: 0.75	Cell cycle	DAB2IP, EGFL6, ING1, TXNIP	5.00E-01
	Kinase	ACVR1C, CSK, CKB, CDKN2B,	8.40E-01
Cluster 10 E. score: 0.12		PDK1,SIK2, STK38	
	Domain; protein kinase	ACVR1C, CSK, SIK2, STK38	9.20E-01
	Signaling peptide	CPAMD8, TNFATP6, TNP73-AS1,	5.50E-01
Cluster 11 E. score: 0.11		VSTM4, APOO, AIFM1, ASPN, BPHL, CDHR3, CALU, CHIT1, COL6A6, EVI2B, FGFRL1, FLT4, HSPA13, OXNAD1, PLA2G5, PDK1L2, PTPRC, RET, SERPINI1, SNED1, TLR8, TF, TMED4, TREM2, VEGFA	
Cluster 12 E. score: 0.02	Lysine degradation	ACAT2, AASS, GCDH, HADH	1.00E-01

TABLE 4.2: Top clusters gene ontologies of DEGs of Chronic inflammation

Categories	**GO term**	**Hits**	**P-value**
Cluster 1 E score: 2.64	Nucleotide binding	PFKFB3, ABL2, BUB1B, DDX17, HBS1L, NEK2, PIF1, RAD54L	4.2E-5
		CENPE, CIT, CDK1, IRAK2, KIF14	
Cluster 2 E. score: 5.89	Cell cycle	PFKFB3, ABL2, BUB1B, DDX17 NEK2, PIF1, RAD54L,	1.2E-10
		CENPE, CIT CDK1, KIF14	
Cluster 3 E. score: 3.56	Regulation of cyclin-dependent protein serine/threonine kinase activity	CDC25A, CDC25C, CCNA2 CDKN3, GADD45A, PKMYT1	1.6E-8
	Sonic Hedgehog (SHH) Receptor Ptc1 Regulates cell cycle	CDC25A, CDC25C, CDK1	8.6E-4
Cluster 4 E. score: 3.16	Regulation of cyclin-dependent protein serine/threonine kinase activity	CDC25C, CDKN3, DUSP4, SSH1	5.3E-5
Cluster 5 E. score: 0.35	Inflammatory response	CCL14, TNFRSF21, IRAK2, IL18RAP	4.4E-2
	Immune response	CCL14, TNFRSF21, IL18RAP	2.2E-1

TABLE 4.3: Top clusters gene ontologies of DEGs of Insulin resistance

Categories	**GO term**	**Hits**	**P-value**
Cluster 1 E. score: 11.26	Lipid metabolism	HMGCR, H MGCS1, DHCR7, ELOVL5, MID1IP1, NSDHL, SCAP, AACS, ACACA, ACSL5, EHHADH, FDPS FDFT1, FADS1, FADS2,FASN, GAL3ST1, HSD17B7, INSIG1, IDI1, LSS, LPCAT3, MSMO1, MVK, PLA2G10, PLA2G16, PMVK, PCSK9 SREBF1,THRSP, TM7SF2	1.70E-20
	Sterol metabolism	HMGCR, HMGCS1, DHCR7, NSDHL, SCAP, FDPS, FDFT1, INSIG1, IDI1, MSMO1,MVK, PMVK PCSK9, SREBF1, TM7SF2	7.70E-17

Table 4.3 continued from previous page

	Cholesterol metabolism	HMGCR, HMGCS1, DHCR7, NSDHL, SCAP, FDPS, FDFT1, INSIG1, IDI1, MVK, PMVK, PCSK9, SREBF1, TM7SF2	2.90E-16
	Metabolic pathway	HMGCR, HMGCS1, DHCR7, NSDHL,ACACA, ACSL5, ACSS2 ALDH5A1, ALDOC, EHHADH FDPS, FDFT1, FASN, GAL3ST1 HSD17B7,INPP1, IDI1, LSS, MDH1, MTHFD1L, MMAB, MSMO1, MVK PLA2G10, PLA2G16, PMVK, PKLR, RDH11,TM7SF2	9.10E-05
	Domain, protein kinase	TAOK3, ACVR2B, AURKB CAMK1D, CHEK1, COQ8A, CDK14	3.60E-03
Cluster 2 E. score: 2.73		DCLK1, NTRK2, STK3, ULK1	

Table 4.3 continued from previous page

	Protein kinase like domain	TAOK3, ACVR2B, AURKB CAMK1D, CHEK1, COQ8A, CDK14 DCLK1, EEF2K, NTRK2,STK3, ULK1	4.20E-03
Cluster 3 E. score: 1.24	Protein kinase catalytic domain	CLK3, NEK6, CAMKK2, MAPK11 MYO3B,PRKCA	2.50E-01
	Kinase	CLK3, NEK6, WNK1, CAMKK2 IRAK4, MAPK11, MYO3B,PI4KA PRKCA	3.00E-01
	Lipid catabolic process	IAH1, PLA2G12A, PLCG2	2.40E-01
Cluster 4 E. score: 0.68			
	Lipid metabolism	ACADVL, GALC, IAH1, PLA2G12A PLCG2, PTPMT1	4.20E-01
	Inflammatory mediator regulation of TRP channels	MAPK11, PLCG2, PRKCA, TRPV1	9.40E-02
Cluster 5 E. score: 0.67	Intracellular signal transduction	GNB1L, WNK1, ASB7, MAPK11,	1.60E-01

Table 4.3 continued from previous page

		PLCG2,PLEKHM1, PRKCA, ZBTB33	
	Cell cycle	ERCC6L, AURKB, CHEK1, CDK14	1.20E-01
Cluster 6 E.- score: 0.54	Mitochondrial	ESCO2, MCM5, MCM6, PTTG1	
	inner membrane	TXNIP	
		ABCA9, ACADVL, CHCHD3	8.50E-01
Cluster 7 E. score: 0.48		PTPMT1	

4.2.4.3 GenCLiP 2.0

In order to reveal the DEGs network analysis and GO annotations from literature, GenCLip which is used for human gene function and network analysis had been used. It is a web based text mining human gene function and network analysis server, which identifies biological functions and molecular netwroks in a given gene list [111]. A default cutoff criteria had been chosen to perform its GO clustering i.e p value $<$ 1e-4 and hit $>$ 7. Among three modules in GenCLip [119] two of them have been used, Gene cluster with literature profiles, and literature mining gene networks. In order to get the clusters of DEGs 116 genes of obesity, 161 of insulin resistance and 67 of chronic inflammation (an output of enriched clusters genes, from DAVID) were uploaded to input data box, with the selection of identifier as official gene symbol. As a result, gene information, gene cluster with literature profile with above default cutoff criteria, and literature mining gene network were performed. For obesity, among 116 genes, 17 was given, with 12 clusters having E scores given in form of table as well as a heatmap of that table, and a network of these genes were generated respectively and shown in figure 4.1.

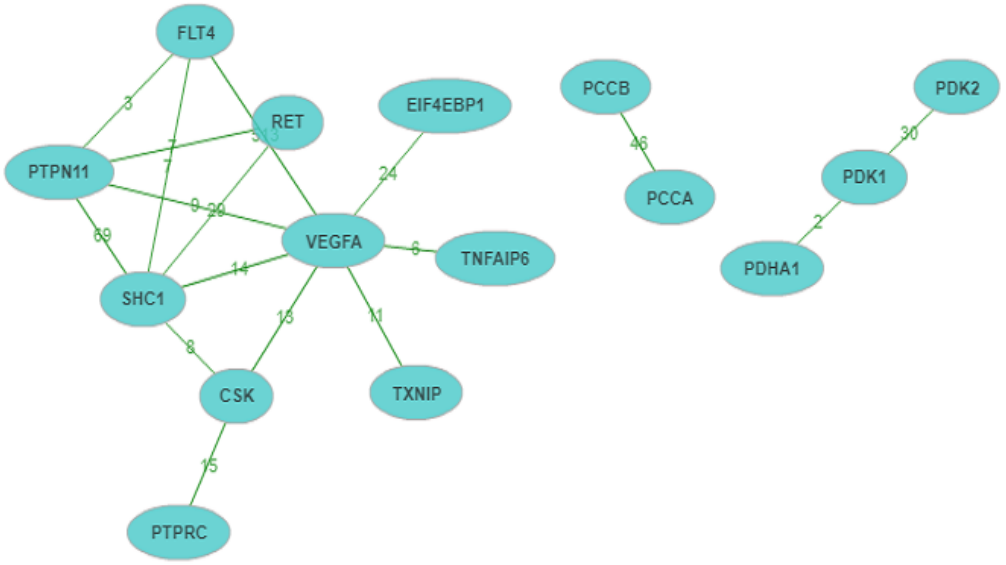

FIGURE 4.1: Total of 15 genes from17 related pairs of DEGs form a network, the number showing the co cites/ overlap from literature mining. The number mentioning of abstracts for a particular gene and its pair genes In case of insulin resistance when 161 genes were uploaded, among these 161 genes 25 were given, with 14 clusters. Network of genes is shown in figure 4.2.

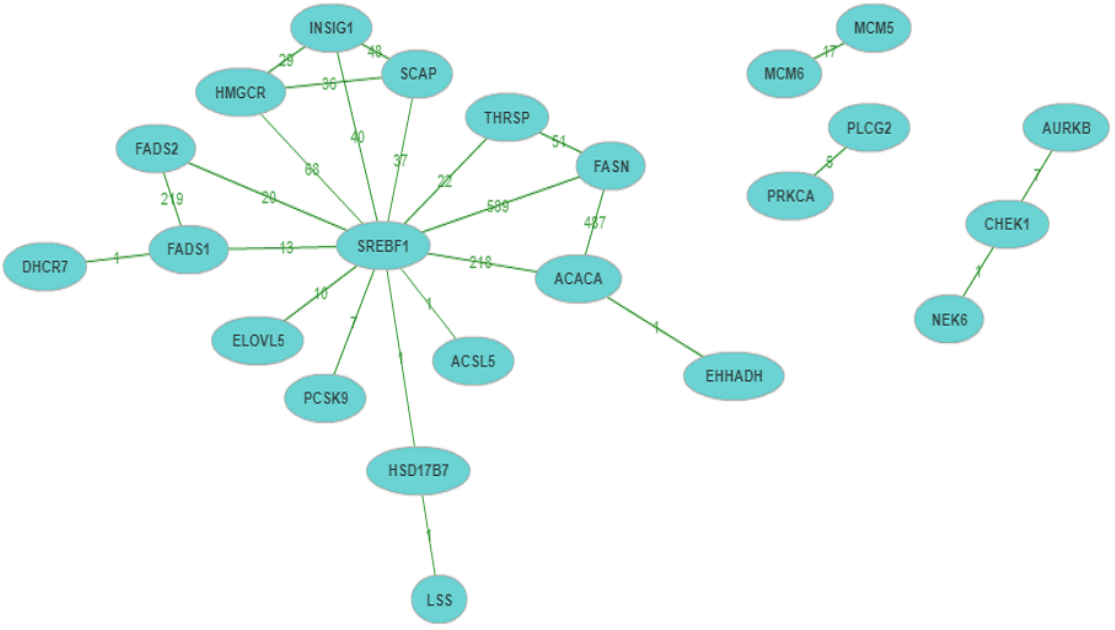

FIGURE 4.2: Total of 23 genes from 25 related pairs of DEGs form a network, the number showing the co cites/ overlap from literature mining. The number mentioning of abstracts for a particular gene and its pair genes

For Chronic inflammation, among 67 genes,18 was given, with 11 clusters having E scores given in form of table and a network of these genes were generated respectively and shown in figure 4.3.

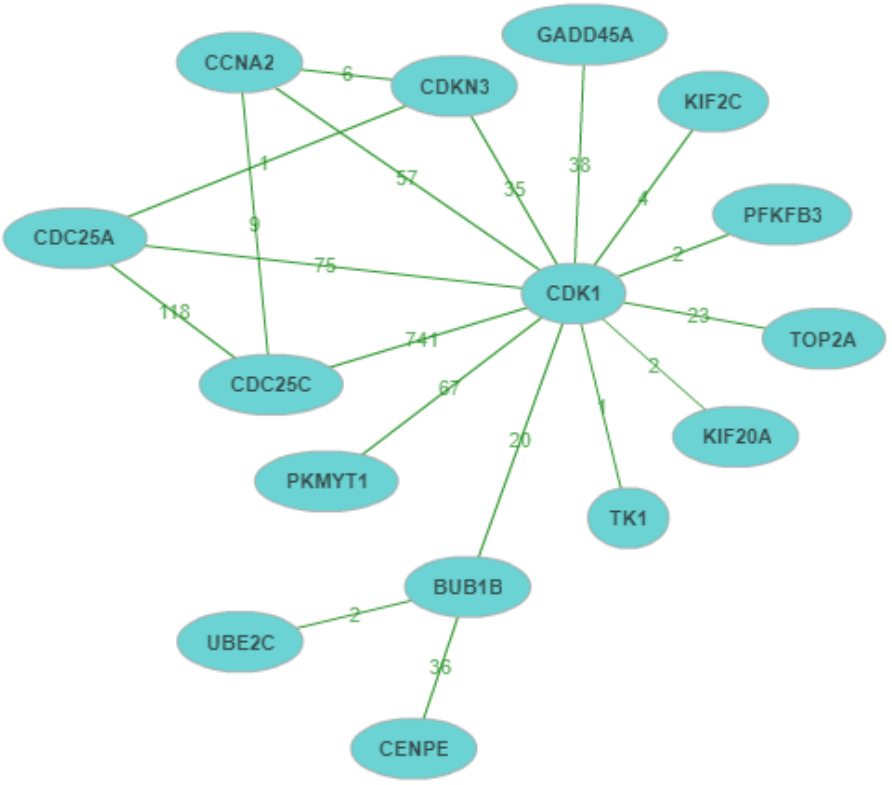

FIGURE 4.3: Total of 15 genes from 18 related pairs of DEGs form a network, the number showing the co cites/ overlap from literature mining.

4.2.4.4 KEGG Mapper

KEGG mapper is a database used for KEGG mapping, three databases integrated are pathways, (brite, network and diseases) which contains the experimental data from published literature and represented in terms of KEGG [112]. KEGG is an interactive collection of 18 databases categorized into four categories: systems, genomics, chemicals, and health information. KEGG Mapper is a set of KEGG mapping tools for databases of Pathway, Brite, and Module. In order to search pathways, 16 genes of obesity, 161 genes of insulin resistance and 67 genes of chronic inflammation were used separately with default parameters, and pathways for Homo sapiens were selected. It reveals three types of pathways, simple pathways with genes, network of pathways and diseases pathways related to DEGs. Against 116 DEGs of obesity, 21 brite pathways, 48 networks and 64 disease pathways were retrieved. In case of insulin resistance 161 DEGs were uploaded and 19 brite pathways, 37 networks and 31 disease pathways, for chronic inflammation , 17 brite pathways, 39 networks and 11 disease pathways were retrieved.

4.2.5 Gene interaction network

For cross checking of the gene functional association network, FunCoup (https://funcoup.sbc.su.se) is used. It is a web-based framework having data of 22 model organisms [120]. In search bar, gene list retrieved from KEGG pathways was uploaded. Selected species was Homo sapiens. Network, interactors and interactions were fetched out. Functional coupling network for obesity, insulin resistance and chronic inflammation are shown in figures 4.4, 4.5 and 4.6 respectively.

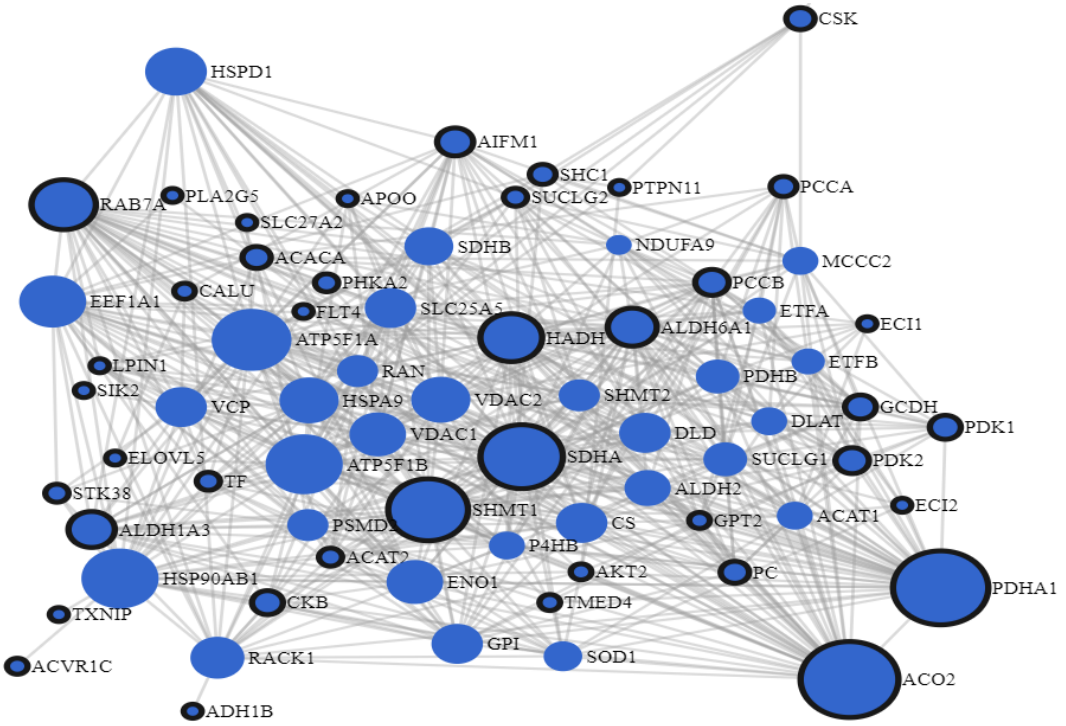

FIGURE 4.4: 59 out of 277 query genes were found. Total 89 genes present with 588 links between them.

4.2.6 Protein-Protein Network Construction and Analysis of Functional Modules in Protein-Protein Interactions

Once the DEGs pathways enrichment analysis had been done, with different tools their protein protein network construction was done to achieve the results that

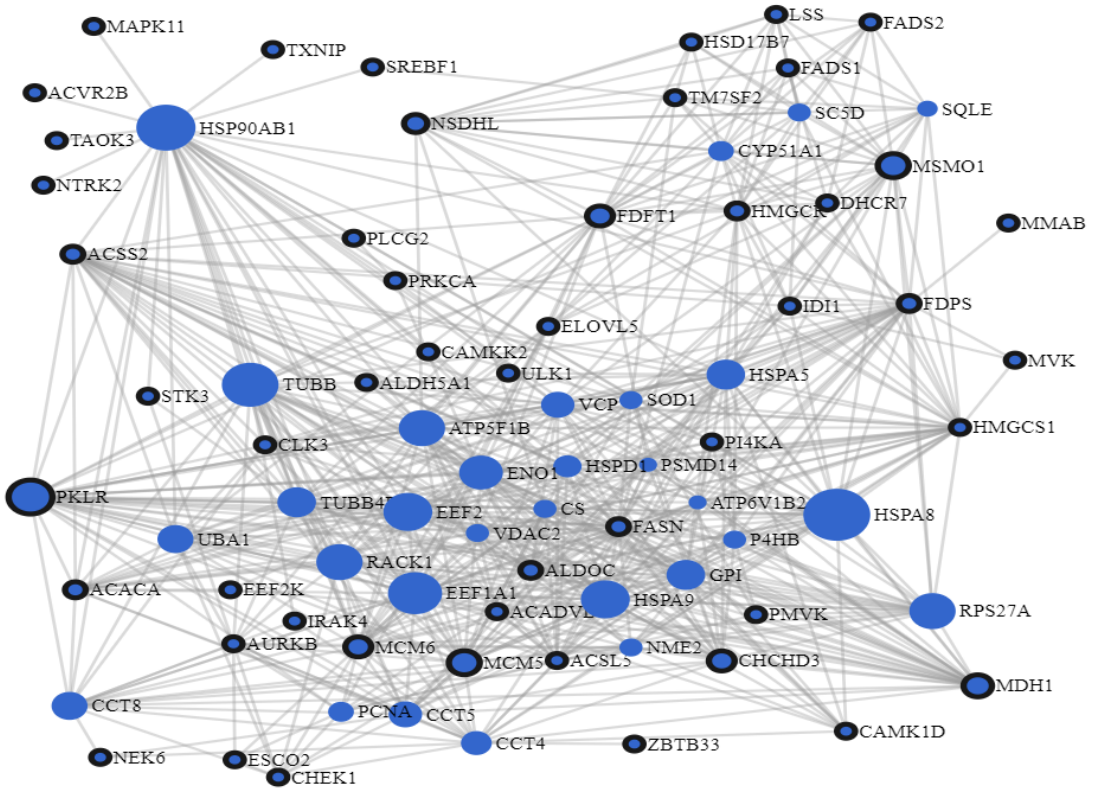

FIGURE 4.5: 63 out of 279 query genes were found. Total 93 genes present having 613 links between them.

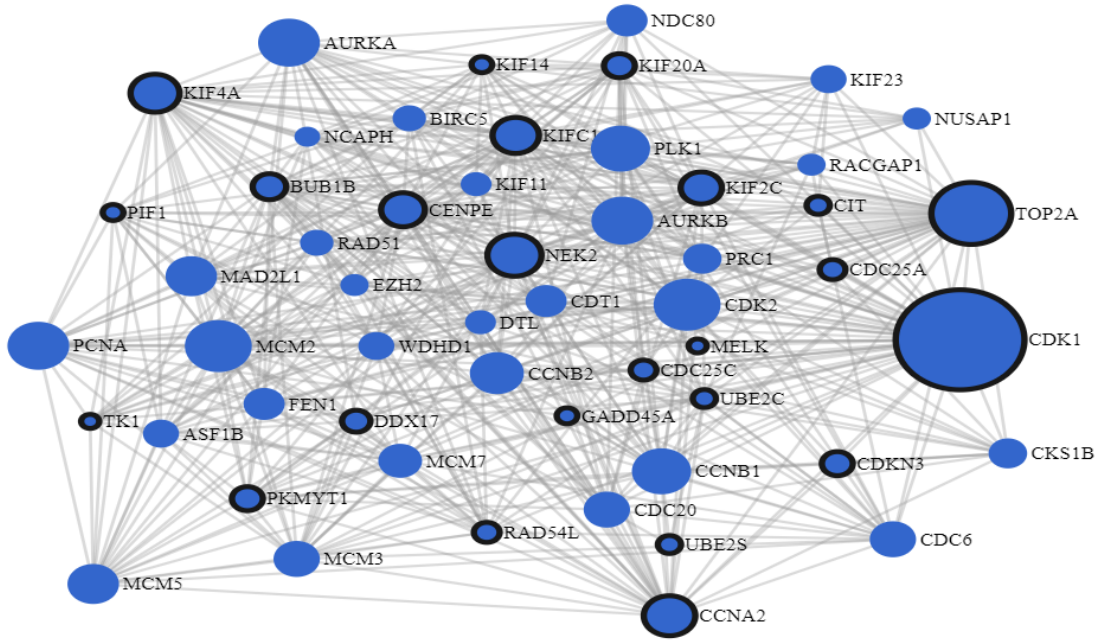

FIGURE 4.6: 35 out of 184 query genes were found. Total 65 genes present having 533 links between them.

how proteins interact with each other in differentially expressed genes. Network Analyst is a web based tool which helps in meta-analysis of data by showing

protein-protein interactions (PPIs) of gene expression data. It calculates the values based on centrality. It is used to identify hub genes. It is an important point because it shows the nodes which holds main central position in a network. Betweenness centrality is to measure mediation of a particular node in network. If nodes, like contract, link, transaction etc. go through a single node present in a hub, then this one node is more essential having high centrality of betweenness [113].

In this research PPI of two modules were selected, generic PPI and tissue specific PPI. On main homepage gene list input had been selected. Selecting Homo sapiens as specified organism and Official gene symbol in Id type, gene list retrieved from KEGG pathway mapper were uploaded. Selecting PPI followed by selecting generic PPI and tissue specific PPI separately. By choosing STRING interactome with default cut-off score 900, results were shown in the form of visual networks along with seeds, nodes and edges and genelists.

Total genes retrieved as a result of KEGG pathway mapping of DEGs, were uploaded in search box. In these modules, PPI were visible along with data in tabular form. Those genes which betweenness centrality occurs above 0 for sub networks were selected. Hence final 41 DEGs of obesity, 46 DEGs of insulin resistance and 36 DEGs of inflammation were retrieved from both Genric and tissue-specific PPIs in network analyst.

4.2.6.1 Generic Protein Protein Interactions

To get protein protein interations with generic from STRING interactome with none of parameters and experimentally validated forum was used. For obesity 277 genes (KEGG pathway result) were uploaded, total 9 subnetworks were retrieved, about 409 nodes, 548 edges and 32 seeds in network were given in form of major subnetwork. Major subnetwork is given in figure 4.7. For Insulin resistance 279 genes were uploaded in gene query box, total 7 subnetworks were retrieved, about 527 nodes, 662 edges and 38 seeds in network were given in form of major subnetwork. Major subnetwork is given in figure 4.8

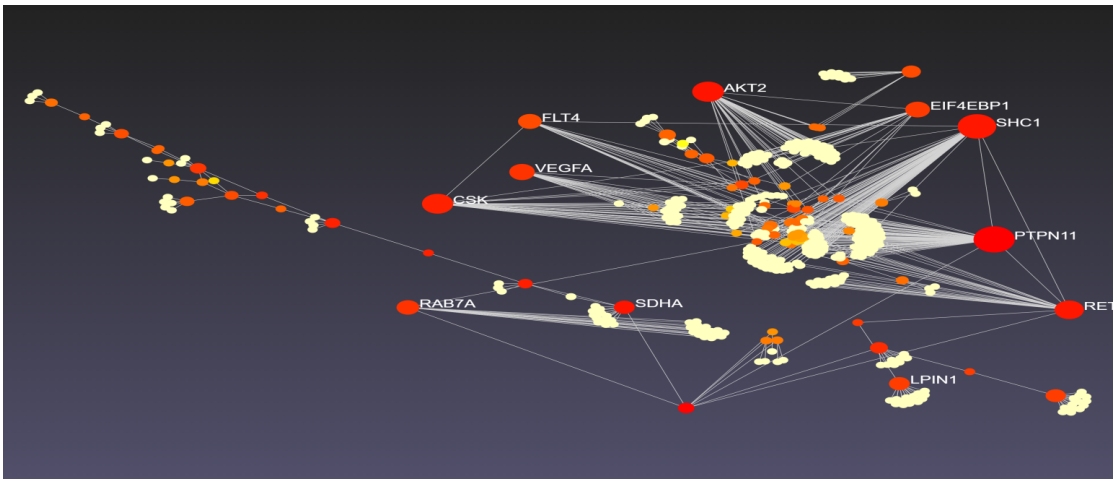

FIGURE 4.7: This pictorial presentation shows a typical view of protein-protein interaction network analysis and visualization. There are 409 nodes, 548 edges to be built in the network. Nodes represent seed (significant) proteins shown in red color while edges establish relationships among proteins, white color showing the proteins interactions in the network.

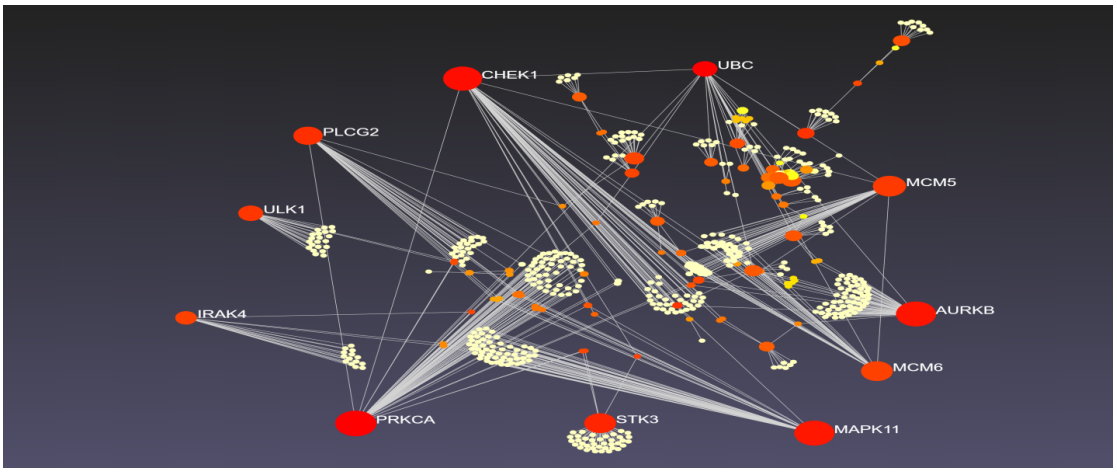

FIGURE 4.8: This pictorial presentation shows a typical view of protein-protein interaction network analysis and visualization. There are 527 nodes, 662 edges to be built in the network. Nodes represent seed (significant) proteins shown in red color while edges establish relationships among proteins, white color showing the proteins interactions in the network

Chronic inflammation KEGG mapping result in 184 genes, which were uploaded in gene query box, total 5 subnetworks were retrieved, and about 446 nodes, 669 edges and 28 seeds in network were given in form of major subnetwork. Major subnetwork is given in figure 4.9.pictorial presentation shows a typical view of protein-protein interaction network analysis and visualization. There are 446 nodes, 669 edges to be built in the network. Nodes represent seed (significant) proteins shown in red color while edges establish relationships among proteins,

white color showing the proteins interactions in the network.

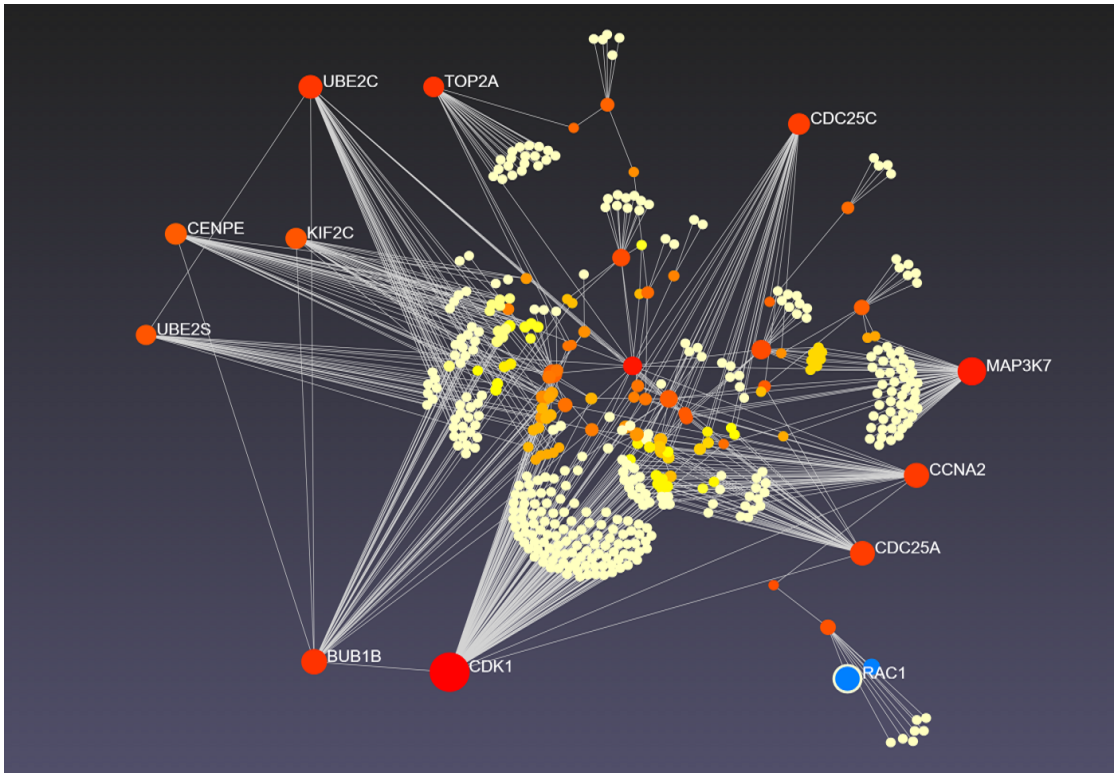

FIGURE 4.9: This pictorial presentation shows a typical view of protein-protein interaction network analysis and visualization. There are 446 nodes, 669 edges to be built in the network. Nodes represent seed (significant) proteins shown in red color while edges establish relationships among proteins, white color showing the proteins interactions in the network

4.2.6.2 Tissue-Specific Protein Protein Interaction

Similarly for Tissue-specific interactions analysis was done. Selection of specific tissues for each disease was done. For Obesity, adipose tissue was selected as a result 6 subnetworks were retrieved. In main subnetwork 590 nodes, 43 seeds and 714 edges were given in form of major network given in figure 4.10. A typical view of tissue-specific PPIs shows network analysis and visualization. There are 590 nodes, 43 seeds and 714 edges. red color is highlighting the nodes which are domains interacting with human genes, while the seed nodes in orange and yellow representing the interactions of domains with those of human genes.

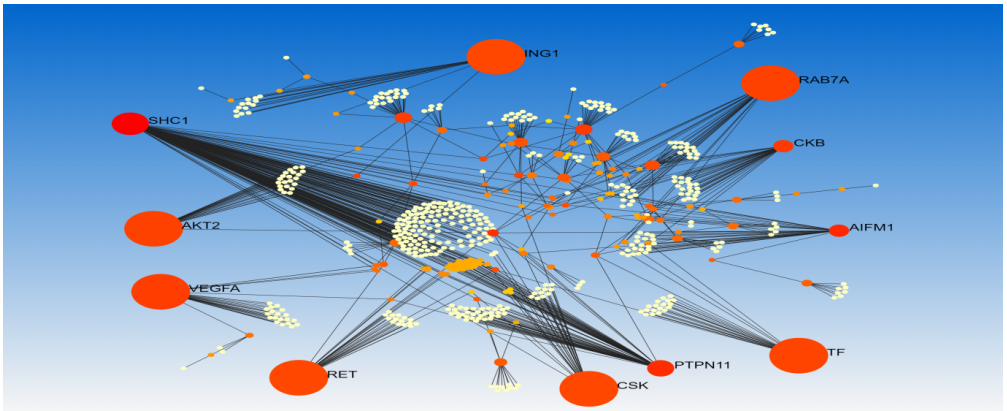

FIGURE 4.10: A typical view of tissue-specific PPIs shows network analysis and visualization. There are 590 nodes, 43 seeds and 714 edges. red color is highlighting the nodes which are domains interacting with human genes, while the seed nodes in orange and yellow representing the interactions of domains with those of human genes.

For insulin resistance, liver tissue was selected as result 6 subnetworks were retrieved. In main subnetwork 709 nodes, 46 seeds and 847 edges are present and shown in figure 4.11.

A typical view of tissue-specific PPIs shows network analysis and visualization. There are 709 nodes, 46 seeds and 847 edges. red color is highlighting the nodes which are domains interacting with human genes, while the seed nodes in orange and yellow representing the interactions of domains with those of human genes.

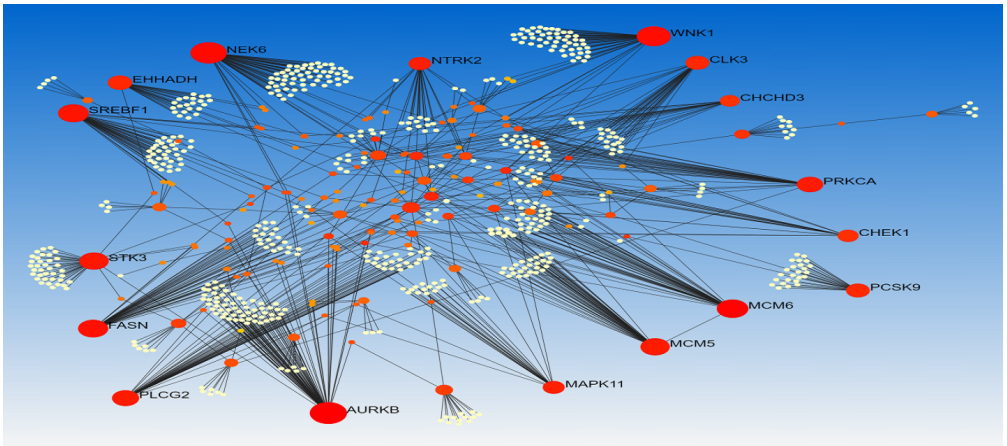

FIGURE 4.11: A typical view of tissue-specific PPIs shows network analysis and visualization. There are709 nodes, 46 seeds and 847 edges. red color is highlighting the nodes which are domains interacting with human genes, while the seed nodes in orange and yellow representing the interactions of domains with those of human genes.

Chronic inflammation includes only 2 subnetwork, Where one is main subnetwork having 599 nodes, 29 seeds and 696 edges were given in form of major subnetwork given in figure 4.12.

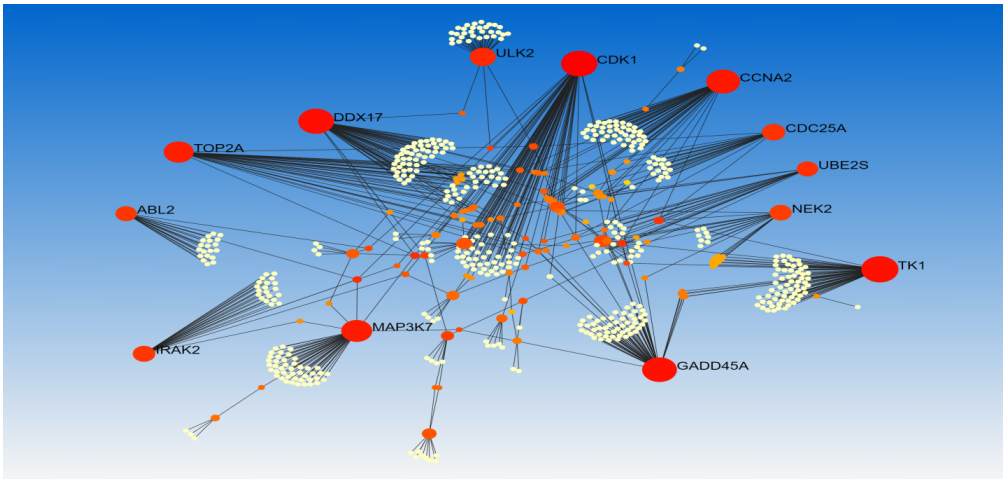

FIGURE 4.12: A typical view of tissue-specific PPIs shows network analysis and visualization. There are599 nodes, 29 seeds and 696 edges. red color is highlighting the nodes which are domains interacting with human genes, while the seed nodes in orange and yellow representing the interactions of domains with those of human genes.

4.2.7 Retrieval of KEGG Data for Functional Modules

EnrichNet is a tool used for network based enrichment analysis for assessment of functional association of DEGs. It manifests novel graphical based statistical values, which explore the molecular networks connecting two genes set with new visualization of sub networks structure [114]. In order to get the functional modules of DEGs of obesity, insulin resistance and chronic inflammation, list of DEGs which were retrieved by network analyst were given as an input in enrichnet tool, using its defualt settings of molecular network of string and identifier format as ensembl id and started analysis. For each of three diseases, search of generic PPI and tissue-specific PPI were done individually and 18 lists of pathways were saved. Furthermore, annotation databases (KEGG, Reactome and NCI pathways interaction database) were used for analysis of functional module. As a result similarity ranking of gene set vs pathways were displayed with pathways, significance of network distance distribution (XD score), significance of overlap (Fisher

test, Q test) and pathways gene set. For retrieval of functional modules, three different annotated databases results, were analysed and compare three databases results with KEGG pathways. Total those pathways were selected as functional which were present in all three databases of obesity, insulin resistance and chronic inflammation and showed cross talks among the genes. Hence, 34 pathways were validated from all three generic PPIs databases, 41 pathways were validated from all three tissue specific PPIs databases of obesity, insulin resistance and chronic inflammation. List of pathways is shown in table 4.4 and 4.5

TABLE 4.4: Validated pathways of generic PPI from KEGG, NCI and Reactome using EnrichNet

Sr.no	**Pathway involved in Generic PPIs of Obesity, Insulin Resistance and Chronic Inflammation**
1	mTOR signaling pathway
2	Progesterone-mediated oocyte maturation
3	Regulation of autophagy
4	Cell cycle
5	Leishmaniasis
6	p53 signaling pathway
7	PPAR signaling pathway
8	RIG-I-like receptor signaling pathway
9	NOD-like receptor signaling pathway
10	ErbB signaling pathway
11	Apoptosis
12	MAPK signaling pathway
13	Pyruvate metabolism
14	VEGF signaling pathway
15	T cell receptor signaling pathway
16	Neurotrophin signaling pathway
17	Wnt signaling pathway
18	Toll-like receptor signaling pathway
19	Oocyte meiosis (inflammation,

Table 4.4 continued from previous page

20	Fc epsilon RI signaling pathway
21	Non-small cell lung cancer
22	Citrate cycle (TCA cycle)
23	Epithelial cell signaling in Helicobacter pylori infection
24	Fc gamma R-mediated phagocytosis
25	Chag as disease
26	Shigellosis
27	Natural killer cell mediated cytotoxicity
28	B cell receptor signaling pathway
29	TGF-beta signaling pathway
30	Tight junction
31	Insulin signaling pathway
32	Pathways in cancer
33	Metabolic pathways
34	Cytokine-cytokine receptor interaction

TABLE 4.5: Validated pathways of tissue specificPPI from KEGG, NCI and Reactome using EnrichNet,

Sr.no	**Pathway involved in tissue-specific PPIs of Obesity, Insulin Resistance and Chronic Inflammation**
1	Cell cycle
2	Progesterone-mediated oocyte maturation
3	Regulation of autophagy
4	mTOR signaling pathway
5	NOD-like receptor signaling pathway
6	p53 signaling pathway
7	RIG-I-like receptor signaling pathway
8	Leishmaniasis
9	ErbB signaling pathway

Table 4.5 continued from previous page

10	Neurotrophin signaling pathway
11	MAPK signaling pathway
12	Wnt signaling pathway
13	Metabolic pathways
14	Lysine degradation
15	Chagas disease
16	Shigellosis
17	Terpenoid backbone biosynthesis
18	VEGF signaling pathway
19	Fc epsilon RI signaling pathway
20	Non-small cell lung cancer
21	Glioma
22	Epithelial cell signaling in Helicobacter pylori infection
23	Leukocyte transendothelial migration
24	Tryptophan metabolism
25	Fatty acid metabolism
26	Valine, leucine and isoleucine degradation
27	Fc gamma R-mediated phagocytosis
28	Toll-like receptor signaling pathway
29	Oocyte meiosis
30	T cell receptor signaling pathway
31	Cytokine-cytokine receptor interaction
32	Amoebiasis
33	Insulin signaling pathway
34	Glycolysis / Gluconeogenesis
35	B cell receptor signaling pathway
36	Apoptosis
37	Natural killer cell mediated cytotoxicity
38	Tight junction

Table 4.5 continued from previous page

39	Pathways in cancer
40	Focal adhesion
41	Adipocytokine signaling pathway

4.2.7.1 KEGG Database

Annotation of pathways with their XD-score had been given in table which shows their network distance distribution, and significance of overlap by fisher test and Q value had been given with the genes set overlaping in a given pathway.

The fisher test, q value and XD score are given in graphical form. The overall genes absolute pearson correlation between XD score and fisher q value is 0.86 and XD score significance threshold is calculated as 0.33 for DEGs of obesity. XD score and fisher q value is 0.80 and XD score significance threshold is calculated as 0.47 for DEGs of Insulin resistance. For chronic inflammation XD score and fisher q value is 0.66 and XD score significance threshold is calculated as 0.37.

4.2.7.2 Reactome Database

XD score and fisher q value and XD score significance threshold for Obesity, Insulin resistance and chronic inflammation is 0.76 and 1.2, 0.83 and 0.4, 0.97 and 1.4 respectively

4.2.7.3 NCI Database

The overall genes absolute pearson correlation between XD score and fisher q value is 0.91 and XD score significance threshold is calculated as 0.56 for Obesity. XD score and fisher q value is 0.67 and XD score significance threshold is calculated as 2.32 for DEGs of Insulin resistance. For chronic inflammation XD score and fisher q value is 0.7 and XD score significance threshold is calculated as 0.92.

4.3 Elucidation of Therapeutic Targets Based on Pathway Crosstalk

The third objective of the study is to elucidate the therapeutic targets based on pathways crosstalks. For this purpose following steps were performed:

4.3.1 Pathway Cross Talk Generation

Gephi is an open source network visualization, analysis and exploration tool used for pathway cross talk. The pathways which showed the cross talks were analyse and visualize with the gephi.

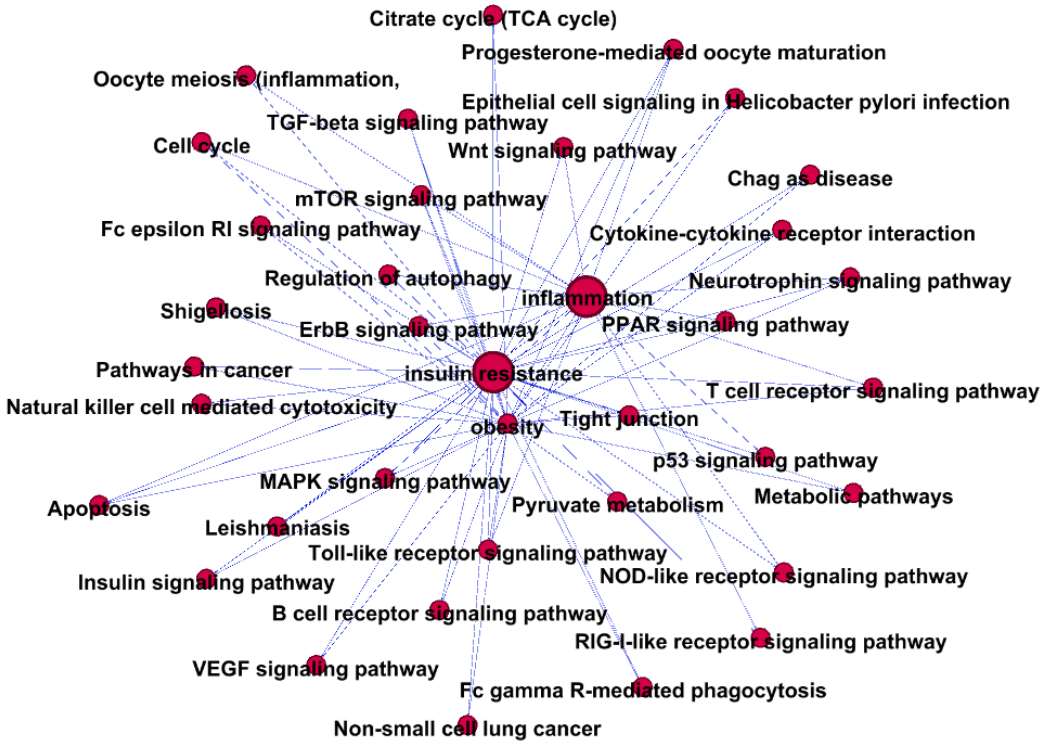

FIGURE 4.13: The bigger nodes in the middle are the root node. represents host diseases pathways, rose red nodes are pathways interacting with the host pathways and blue color represent edges.

The directed pathway cross talks had been generated for DEGs of obesity, insulin resistance and chronic inflammation weighted 1. In module of generic PPI

crosstalk, there were 37 nodes and 79 edges, average density of which were 0.059. The 34 pathways cross talks had been generated, with average degree of 2.135. In module of tissue-specific PPI crosstalk, there were 44 nodes and 92 edges, average density of which were 0.059. The 34 pathways cross talks had been generated, with average degree of 2.091. In module, there were 37 nodes and 79 edges indicating

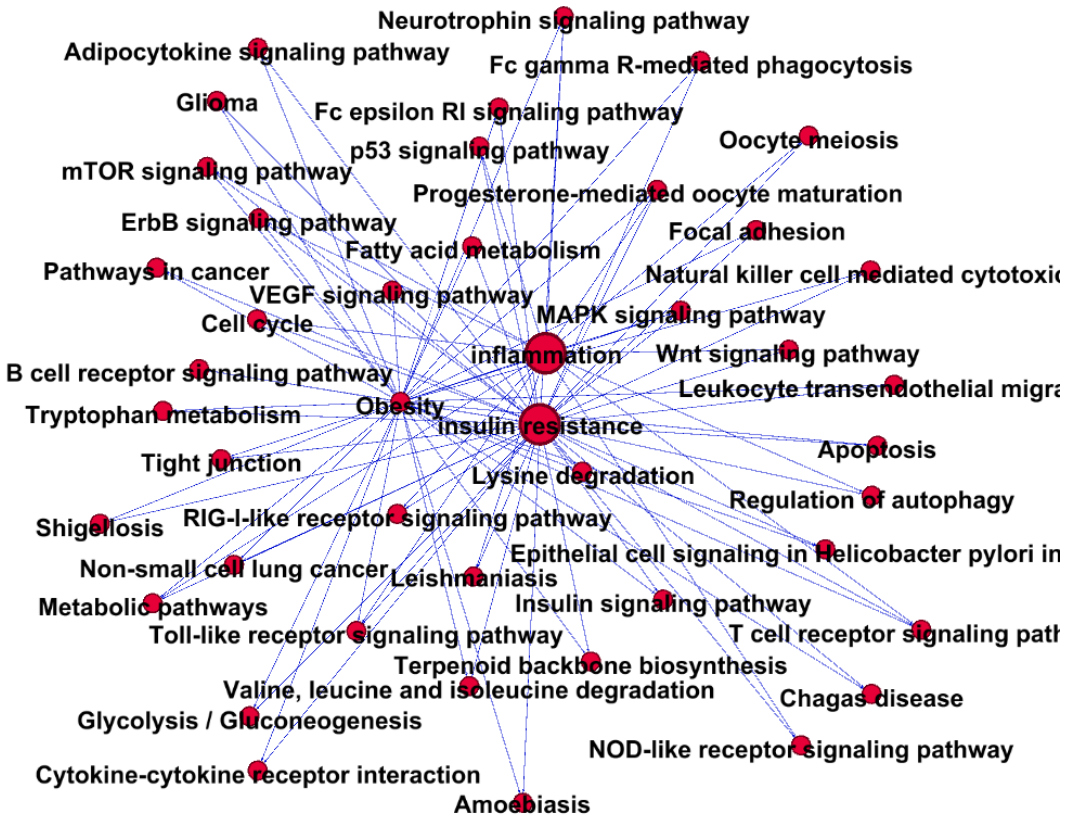

FIGURE 4.14: The bigger nodes in the middle are the root node represents host diseases pathways, rose red nodes are pathways interacting with the host pathways

the crosstalk of 34 pathways of generic PPI. These pathways include mTOR, p53, apoptosis, Wnt, nucleotide excision repair, notch, cell cycle, insulin, toll like receptor etc. In pathway crosstalk network, various new pathways were identified besides already present pathway crosstalk; these remaining pathways generating crosstalk are identified as new crosstalk of pathways. These new crosstalk would help us to identify treatments easily. The DEGs for each disease from NCBI were identified from these novel pathways are considered as target nodes for better and prospective therapeutic targets. In future, by exploring the new pathways crosstalk, novel therapeutic targets would be achieved possibly. These targets

might be able to cure the core symptoms of obesity, insulin resistance and chronic inflammation.

4.4 Summary Chart

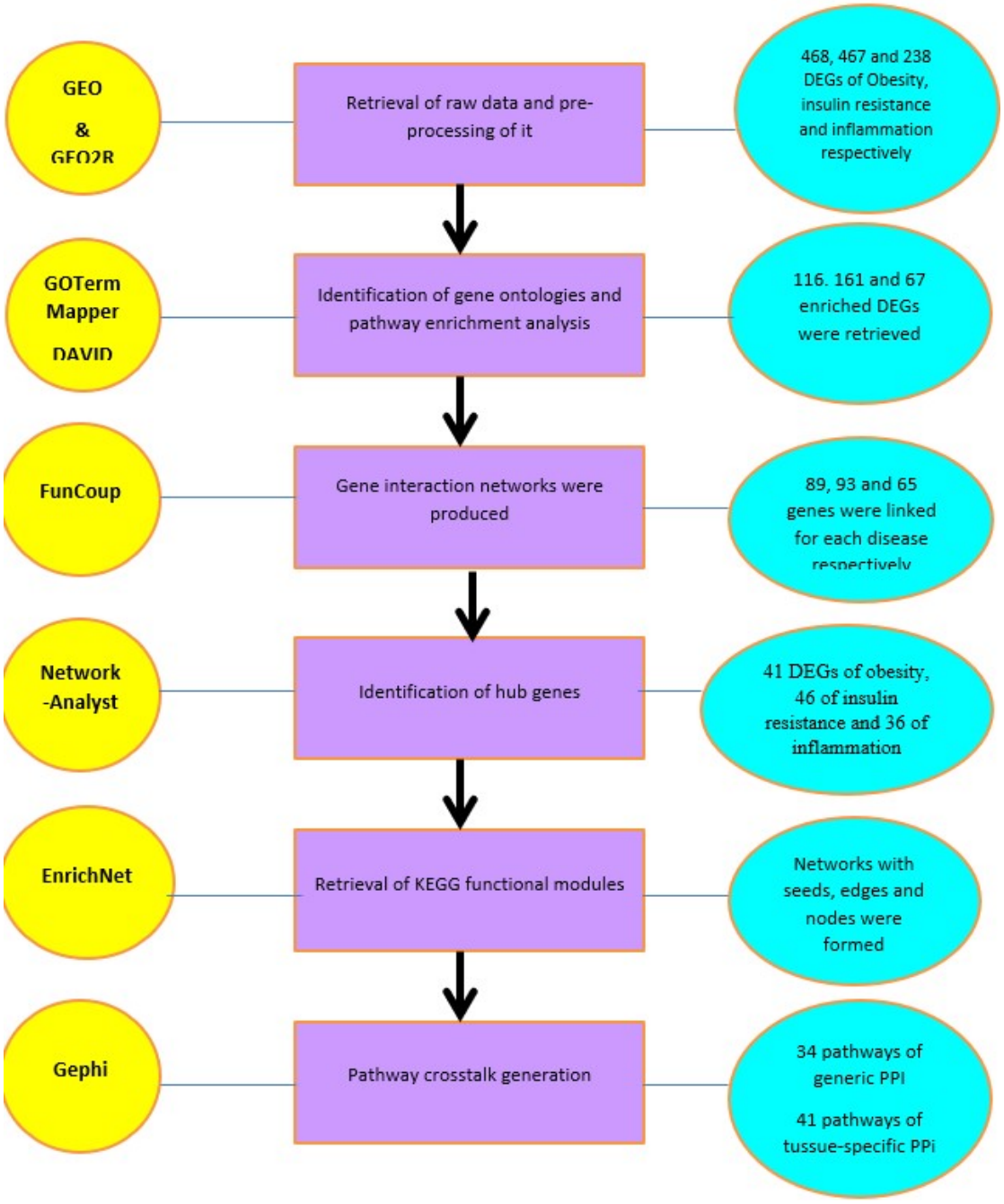

FIGURE 4.15: Summary chart

Chapter 5

Conclusions and Recommendations

Prevalence of diabetes, cardiovascular diseases and other inflammatory diseases is quite common and is increasing exponentially. Both prevalence and occurrence of these comorbidities is increasing and together they add a lot in disease burden. As they usually occur together, chances are quite high that they share a common risk, trigger or molecular mechanism. This research is an effort to explore the common trigger or to check out the overlapping gene involved in the pathways of Obesity, Insulin resistance and chronic inflammation. Therefore, Crosstalk between the pathways is generated to figure out the missing links. First of all pathways involved in Obesity, Insulin resistance and chronic inflammation were retrieved through manual literature review. Pathways mainly involved were metabolic, inflammatory, insulin signaling, cytokines and immune pathways. Most important pathways involved are metabolic (MAPK/ERK, mTOR and P13k/Akt), pro inflammatory (TGF-β, JAK/STAT and NF-κb) signaling pathways. Insulin signaling and adipocytokine signaling pathways are also crucial in development of obesity. After retrieval of key pathways, identification of key gene was performed, for this purpose, key genes which are involved in obesity, insulin resistance and chronic inflammation were retrieved using pathway cross talks. Retrieval of microarray data was performed to identify differentially expressed genes and the total

468 DEGs of obesity, 467 of insulin resistance and 238 for chronic inflammation of microarray data were retrieved from database NCBI. After DEGs identification, gene ontologies were performed using GO term mapper for generic annotations of DEGs. Following the annotations of DEGs pathway enrichment was performed by using DAVID tool and malacard database, from there 6 functional clusters of obesity, 7 of insulin resistance and 7 of chronic inflammation with their respective enrichment score were retrieved (enrichment score with positive values indicates the genes at the top of the list). In this study DEGs were mostly enriched in GO terms of, MAPk, NF-κB, P13-k/AKT/mTOR, cell adhesion molecules, metabolic pathways, immune, inflammatory and insulin signaling pathways. Obesity is usually considered as an outcome of dysregulation in metabolic pathways and for chronic inflammation mainly pro inflammatory pathways and for insulin resistance, insulin signaling pathways were involved. Furthermore, the pathways already reported and have been retrieved from malacard database and matched, only those genes were retrieved whose pathways were matched with enriched pathways. Pathways those are enriched in a gene list are enriched pathways. The validated pathways result in 116 genes of obesity, 161 genes of insulin resistance and 67 genes for chronic inflammation for further analysis. However, for further pathway enrichment GenCLiP was used to identify the networks and pathways based on literature survey. A network of DEGs was presented which gives detail about the genes and literature co cites that how these genes were previously linked with other genes in the network. For the validation and crosstalk among the pathways, KEGG database with other three databases, BioCarta, NCI and reactome databases were used. These three databases validate 34 generic pathways and 41 tissue specific pathways, and shows crosstalk among pathways. In order to achieve third objective of the study, i.e. elucidation of therapeutic targets based on pathway cross talks. Gephi tool were used to generate the cross talks among the pathways. The directed pathways crosstalk had been generated for obesity, insulin resistance and chronic inflammation weighted 1. In module, there were 37 nodes and 79 edges indicating the crosstalk of 34 pathways of generic PPI. These pathways include mTOR, p53, apoptosis, Wnt, nucleotide excision repair, notch, cell cycle, insulin, toll like receptor etc. In pathway crosstalk network, various

new pathways were identified besides already present pathway crosstalk; these remaining pathways generating crosstalk are identified as new crosstalk of pathways. These new crosstalk would help us to identify treatments easily. The DEGs for each disease from NCBI were identified from these novel pathways are considered as target nodes for better and prospective therapeutic targets.. In future, by exploring the new pathways crosstalk, novel therapeutic targets would be achieved possibly. These targets might be able to cure the core symptoms of obesity, insulin resistance and chronic inflammation.

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