Integrative bioinformatics analysis revealing genetic and molecular mechanisms underlying obesity

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Abstract
Obesity is a complex disorder with significant genetic and molecular underpinnings. This study employed a multi-step bioinformatics approach, integrating genome-wide association study (GWAS) data, functional enrichment analyses, pathway mapping and network-based investigations to elucidate the genetic and molecular mechanisms underlying obesity. TargetScan, miRTarBase, Reactome Pathways, KEGG, Protein-protein interaction (PPI) analysis and Gene Ontology (GO) mapping were the bioinformatic tools used to perform enrichment analysis on GWAS data identified key obesity-associated genes.

GWAS data identified key obesity-associated genes, were subjected to gene ontology (GO) analysis, revealing their involvement in biological processes, Reactome and KEGG pathway databases enriched analysis highlighted the dysregulation of lipid metabolism, inflammatory pathways and neuronal signalling. Notably, LDL clearance, plasma lipoprotein clearance and cholesterol metabolism pathways were significantly enriched. Protein-protein interaction network analysis identified central hub proteins including PRKACA and UBC, implicating dysregulated protein degradation and inflammatory signaling in obesity pathogenesis. miRNA enrichment analysis revealed the regulatory roles of hsa-miR-33a-5p and hsa-miR-877-5p in lipid metabolism and inflammation. Cell-type enrichment analysis highlighted neuroinflammation and immunological components. These findings underscore the intricate interplay between genetic, metabolic and regulatory factors in obesity, providing valuable insights into potential therapeutic targets and biomarkers. Future research should focus on validating these findings and exploring their applications in obesity prevention and management.

Keywords: GWAS, Gene Ontology, Pathway enrichment, Protein-protein interaction, miRNA, Metabolomics.

Introduction

Obesity is a complex and multifactorial disorder that has emerged as a major public health challenge worldwide[4]. Characterized by excessive fat accumulation, obesity is associated with a range of metabolic complications including insulin resistance, type 2 diabetes, cardiovascular diseases and dyslipidemia[15]. Despite the increasing prevalence of obesity, its underlying genetic and molecular mechanisms remain incompletely understood. While lifestyle factors such as diet and physical activity play crucial roles, genetic predisposition and epigenetic modifications significantly contribute to an individual's susceptibility to obesity. Over the past decade, genome-wide association studies (GWAS) have provided valuable insights into the genetic basis of obesity by identifying several loci associated with body mass index (BMI) and related traits[16].

However, a deeper understanding of how these genes function within cellular pathways and regulatory networks, is essential for developing targeted interventions. Functional genomics analyses including gene ontology (GO) classification, pathway enrichment studies and protein-protein interaction (PPI) networks, offer comprehensive insights into the biological processes underlying obesity. These analyses enable researchers to classify genes based on their molecular functions, cellular components and biological pathways[7], thereby elucidating the mechanisms through which genetic variations influence obesity-related phenotypes.

Furthermore, post-transcriptional regulation by microRNAs (miRNAs) has emerged as a key mechanism in obesity, influencing gene expression and metabolic homeostasis. Investigating miRNA-mediated regulation can help to identify novel biomarkers and therapeutic targets for obesity management[5].

Pathway-based analysis, utilizing databases such as KEGG and reactome, allows for a systematic examination of metabolic processes influenced by obesity-associated genes[11,13]. Given that obesity is closely linked to metabolic disorders, understanding its molecular pathways can reveal potential intervention strategies. Additionally, metabolomics approaches, such as those enabled by MetaboAnalyst, provide insights into how genetic factors influence metabolic pathways, bridging the gap between genomics and metabolism[1]. Identifying metabolite alterations associated with obesity can further refine our understanding of its pathophysiology and aid in precision medicine strategies.

The integration of multiple bioinformatics approaches including GWAS, functional enrichment analysis, miRNA-target interactions and metabolomics, provides a holistic view of obesity's genetic landscape. This study employs these methodologies to identify key obesity-associated genes, to elucidate their functional roles and to explore their

regulatory mechanisms. By linking GWAS findings to metabolic alterations, this research aims to uncover novel insights into obesity's molecular underpinnings, paving the way for improved diagnostic and therapeutic strategies.

Objectives

1. Gene Ontology (GO) analysis to classify genes based on biological processes, molecular functions and cellular components relevant to weight gain
2. Pathway Analysis to identify disease mechanisms by using KEGG and reactome pathway databases to determine whether mapped genes are enriched in obesity-related pathways
3. Investigating post-transcriptional and epigenetic regulation by performing miRNA enrichment analysis (miRTarBase, TargetScan) to identify regulatory miRNAs targeting key obesity-related genes.
4. Protein-protein interaction (PPI) network analysis to explore interactions between Obesity-related genes and their PPI hubs (using string).
5. Linking GWAS findings to metabolomic changes by utilize MetaboAnalyst to investigate whether mapped genes influence metabolic pathways related to Obesity.

Material and Methods

A multi-step bioinformatics approach utilizing genome-wide association study (GWAS) data, functional enrichment analyses, pathway mapping and network-based investigations was employed to investigate the genetic and molecular mechanisms underlying obesity. GWAS data was obtained from publicly available repositories to identify genes significantly associated with obesity[11]. Further downstream analyses of these mapped genes were done. To ensure the reliability of the selected genetic variants, statistical thresholds and quality control measures were applied, focusing on those with established disease relevance.

Further, GO analysis was performed to classify the mapped genes based on their biological roles. This was done by categorizing genes according to biological processes, molecular functions and cellular components, providing insight into how these genes contribute to obesity. Enrichment analysis was conducted using established tools to highlight key functional groups and molecular mechanisms linked to pathogenesis of obesity[17].

Pathway analysis was conducted using the KEGG and reactome databases[8] to understand the mechanisms involved in weight gain. This step helped to determine whether the identified genes were enriched in obesity-related pathways. The results offered a perspective on how genetic variations influence disease progression.

miRNA enrichment analysis was performed to explore post-transcriptional and epigenetic regulatory mechanisms[6]. Using miRTarBase and target scan databases, miRNAs that target key obesity related genes were identified. This analysis provided insight into the regulatory networks that modulate gene expression which might contribute to disease onset and progression. Understanding these interactions is crucial for identifying potential therapeutic targets.

To explore the functional connectivity between obesity related genes, a protein-protein interaction (PPI) network analysis was conducted. Using the STRING database[12], interaction network was constructed to identify central hub proteins and key signalling nodes involved in disease mechanisms, to pinpoint genes with strong functional associations, which serve critical players in obesity.

Lastly by utilizing MetaboAnalyst[2], GWAS findings were linked to metabolomic changes to investigate whether mapped genes influence metabolic pathways relevant to obesity. This analysis helped to identify potential metabolic biomarkers associated with obesity.

Results

TargetScan and miRTarBase Analysis: hsa-miR-33a-5p (OR = 12.37, p = 0.08600) is a well-known regulator of cholesterol metabolism and fatty acid homeostasis, influencing the expression of genes involved in lipid storage and mobilization. Down regulation of miR-33a-5p has been linked to increased lipid accumulation in adipocytes. hsa-miR-877-5p (OR = 9.38, p = 0.1115) has been associated with insulin resistance and inflammation, suggesting a role in obesity-induced metabolic dysfunction. hsa-miR-4738-5p (OR = 79.21, p = 0.01441) and hsa-miR-4641 (OR = 44.31, p = 0.02521) show strong enrichment displayed in table 1.

Reactome Pathways (2024): Several significantly enriched pathways directly relate to obesity: LDL Clearance (p = 0.00946, OR = 123.28) and Plasma Lipoprotein Clearance (p = 0.01835, OR = 61.59) are critical for maintaining cholesterol homeostasis. Plasma Lipoprotein Assembly, Remodelling and Clearance (p = 0.03688, OR = 29.90) suggest that obesity-associated dyslipidemia is linked to impaired lipoprotein metabolism. DNA Damage Reversal Pathways (p < 0.004, OR > 300) highlight the role of oxidative stress and chronic inflammation as displayed in table 2.

KEGG pathways analysis showed that cholesterol metabolism (p = 0.02473, OR = 45.22) is highly enriched, reinforcing the connection between obesity and lipid dysregulation. Lysosome function (p = 0.06220, OR = 17.38) is also significantly enriched. Neuroactive ligand-receptor interaction (p = 0.158, OR = 6.42) indicates potential links between obesity, appetite regulation and neuroinflammation as depicted in table 3.

Protein-Protein Interaction (PPI): On string database analysis, number of nodes were 8, edges 25 (versus expected number of edges 5), average node degree 6.25, average local clustering coefficient 0.912 and protein -protein interaction enrichment p value was 2.84e-10 (Figure 1).

Table 1
miRTarBase 2017 Analysis

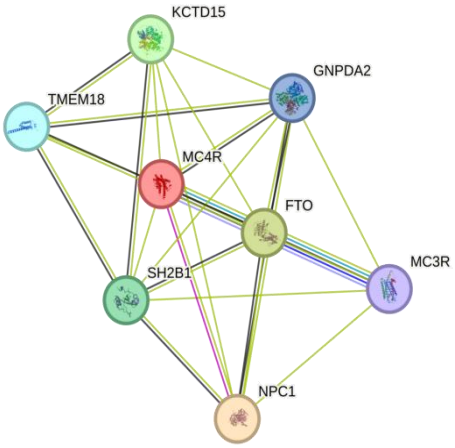
Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	hsa-miR-4738-5p	0.01441	0.2955	79.21	335.86
2	hsa-miR-4641	0.02521	0.2955	44.31	163.08
3	hsa-miR-8060	0.03785	0.2955	29.11	95.32
4	hsa-miR-4266	0.04603	0.2955	23.77	73.18
5	hsa-miR-1233-3p	0.06362	0.2955	16.97	46.76
6	hsa-miR-1225-3p	0.07300	0.2955	14.70	38.46
7	hsa-miR-33a-5p	0.08600	0.2955	12.37	30.34
8	hsa-miR-877-5p	0.1115	0.2955	9.38	20.58
9	hsa-miR-6878-5p	0.1182	0.2955	8.81	18.81
10	mmu-miR-136-5p	0.1337	0.2955	7.71	15.51

Table 2
Reactome Pathways 2024

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	Reversal of Alkylation Damage by DNA Dioxygenases	0.003495	0.03395	370.07	2093.27
2	DNA Damage Reversal	0.003994	0.03395	317.19	1751.86
3	LDL Clearance	0.009462	0.05362	123.28	574.57
4	Plasma Lipoprotein Clearance	0.01835	0.07799	61.59	246.23
5	Transcriptional and Post-Translational Regulation of MITF-M Expression and Activity	0.02521	0.08573	44.31	163.08
6	Plasma Lipoprotein Assembly, Remodelling and Clearance	0.03688	0.1045	29.90	98.68
7	MITF-M-regulated Melanocyte Development	0.06597	0.1602	16.34	44.43
8	G Alpha (S) Signalling Events	0.07627	0.1621	14.04	36.12
9	Peptide Ligand-Binding Receptors	0.09472	0.1789	11.16	26.31
10	DNA Repair	0.1455	0.2389	7.03	13.55

Table 3
KEGG 2021 Human

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	Cholesterol metabolism	0.02473	0.07418	45.22	167.30
2	Lysosome	0.06220	0.09330	17.38	48.27
3	Neuroactive ligand-receptor interaction	0.1580	0.1580	6.42	11.85



Light blue line: curated databases

Purple line: experimentally determined

Blue line: gene co-occurrence

Yellow line: text mining

Black line: co-expression

Violet line: protein homology

Figure 1: Protein-protein interaction by string database

Proteins are the nodes of the network. The hypothesised functional relationships are represented by the edges. An edge was drawn in evidence mode with seven lines of varying colours; lines indicate the presence of the seven various categories of evidence that were considered in anticipating the relationships as follows (Figure 1):

Gene Ontology (GO) Analysis

GO Biological Process (2023): Intestinal cholesterol absorption (p = 0.00449, OR = 277.53) directly relates to obesity-associated hypercholesterolemia. Positive regulation of bone resorption (p = 0.00499, OR = 246.68) is relevant to obesity-induced osteoporosis and regulation of feeding behavior (p = 0.00697, OR = 170.74) suggests a connection between obesity, appetite dysregulation as shown in table 4.

GO Molecular Function (2023): Melanocortin receptor activity (p = 0.00249, OR = 555.17) is highly relevant and cholesterol binding (p = 0.02473, OR = 45.22) and sterol binding (p = 0.02912, OR = 38.18) reinforce the involvement of lipid transport and metabolism in obesity as per the analysis shown in table 5.

Table 4
GO Biological Process 2023

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	DNA Dealkylation (GO:0035510)	0.003495	0.03406	370.07	2093.27
2	DNA Dealkylation Involved In DNA Repair (GO:0006307)	0.003495	0.03406	370.07	2093.27
3	Intestinal Cholesterol Absorption (GO:0030299)	0.004492	0.03406	277.53	1500.17
4	Kinetochore Assembly (GO:0051382)	0.004492	0.03406	277.53	1500.17
5	Kinetochore Organization (GO:0051383)	0.004990	0.03406	246.68	1307.49
6	Positive Regulation Of Bone Resorption (GO:0045780)	0.004990	0.03406	246.68	1307.49
7	Intestinal Lipid Absorption (GO:0098856)	0.005488	0.03406	222.00	1155.57
8	Regulation Of Feeding Behavior (GO:0060259)	0.006979	0.03406	170.74	847.70
9	Membrane Raft Organization (GO:0031579)	0.006979	0.03406	170.74	847.70
10	Centromere Complex Assembly (GO:0034508)	0.006979	0.03406	170.74	847.70

Table 5
GO Molecular Function 2023

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	Melanocortin Receptor Activity (GO:0004977)	0.002498	0.02747	555.17	3326.77
2	Ferrous Iron Binding (GO:0008198)	0.01243	0.04004	92.44	405.55
3	Neuropeptide Binding (GO:0042923)	0.01392	0.04004	82.15	351.18
4	Catalytic Activity, Acting On A tRNA (GO:0140101)	0.01933	0.04004	58.34	230.20
5	2-Oxoglutarate-Dependent Dioxygenase Activity (GO:0016706)	0.02032	0.04004	55.42	215.92
6	Cholesterol Binding (GO:0015485)	0.02473	0.04004	45.22	167.30
7	Iron Ion Binding (GO:0005506)	0.02766	0.04004	40.27	144.50
8	Sterol Binding (GO:0032934)	0.02912	0.04004	38.18	135.03

Table 6
Cell Marker 2024

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	Rheaume Et al.Nat Commun.3 Retina Mouse	0.003495	0.02097	370.07	2093.27
2	Neuron Inferior Colliculus Mouse	0.007973	0.02392	147.96	714.91
3	Monocyte Spleen Mouse	0.07019	0.1404	15.31	40.68
4	Plasmacytoid Dendritic Cell Blood Human	0.1567	0.2351	6.48	12.01
5	Type II Spiral Ganglion Neuron Brain Mouse	0.2407	0.2687	3.99	5.68
6	Neuron Brain Mouse	0.2687	0.2687	3.50	4.60

Cell-Type Enrichment: The cell marker 2024 analysis highlighted neuronal and immune cells. Neuron Inferior Colliculus (p = 0.00797, OR = 147.96) and Brain Neurons (p = 0.2687, OR = 3.50) suggest that obesity-induced neuroinflammation and hypothalamic dysfunction may contribute to appetite dysregulation. Plasmacytoid dendritic cells (p = 0.1567, OR = 6.48) suggest that obesity is associated with chronic low-grade inflammation and monocyte spleen (p = 0.07019, OR = 15.31) suggests an overactive immune response as displayed in table 6.

Discussion

The identified microRNAs (miRNAs) play crucial regulatory roles in metabolic pathways, particularly in lipid metabolism and inflammation, two hallmarks of obesity. hsa-miR-33a-5p is a well-known regulator of cholesterol metabolism and fatty acid homeostasis, influencing the expression of genes involved in lipid storage and mobilization. Down regulation of miR-33a-5p has been linked to increased lipid accumulation in adipocytes, a hallmark of obesity. hsa-miR-877-5p has been associated with insulin resistance and inflammation, suggesting a role in obesity-induced metabolic dysfunction. hsa-miR-4738-5p and hsa-miR-4641 show strong enrichment and may serve as novel biomarkers for obesity-related metabolic dysregulation.

These miRNAs likely influence key obesity-related pathways including adipocyte differentiation, lipid storage and inflammatory signalling, underscoring their potential as therapeutic targets in obesity and metabolic disorders. Results suggest that miR-33a-5P plays important roles in inflammatory responses and cholesterol efflux and that anti-miR-33a-5P may help to prevent inflammatory cytokine-associated macrophage foam cell formation[3].

Several significantly enriched pathways directly relate to obesity and metabolic dysfunction, LDL clearance and plasma lipoprotein clearance are critical for maintaining cholesterol homeostasis. Dysregulation of these pathways is common in obesity, metabolic syndrome and cardiovascular diseases, where impaired LDL clearance leads to hyperlipidemia and atherosclerosis. Plasma lipoprotein assembly, remodelling and clearance suggest that obesity-associated dyslipidemia is linked to impaired lipoprotein metabolism. DNA damage reversal pathways highlight the role of oxidative stress and chronic inflammation in obesity, as increased oxidative damage is a hallmark of adipose tissue dysfunction.

Cholesterol metabolism is highly enriched (p = 0.02473, OR = 45.22), reinforcing the connection between obesity and lipid dysregulation. Lysosome function is also significantly enriched (p = 0.06220, OR = 17.38), suggesting potential impairments in autophagy and intracellular lipid processing in obesity. Neuroactive ligand-receptor interaction (p = 0.158, OR = 6.42) indicates potential links between obesity, appetite regulation and neuroinflammation, which are critical in hypothalamic control of energy balance[10].

Intestinal cholesterol absorption (p = 0.00449, OR = 277.53) directly relates to obesity-associated hypercholesterolemia, as excessive dietary fat intake leads to increased cholesterol absorption. Positive regulation of bone resorption (p = 0.00499, OR = 246.68) is relevant to obesity-induced osteoporosis, as excessive adipose tissue leads to bone mineral density loss. Regulation of feeding behavior (p = 0.00697, OR = 170.74) suggests a connection between obesity, appetite dysregulation and reward-based eating behavior. Cholesterol binding (p = 0.02473, OR = 45.22) and sterol binding (p = 0.02912, OR = 38.18) reinforce the involvement of lipid transport and metabolism in obesity.

PRKACA, catalytic subunit of protein kinase A, regulates lipolysis in adipose tissue. Dysregulation of PRKACA activity (p = 0.1995, OR = 4.95) has been linked to obesity-induced insulin resistance. UBC (Ubiquitin C, p = 0.2395, OR = 4.01) is involved in protein degradation and inflammatory responses. Increased activity of the ubiquitin-proteasome system has been observed in obesity-related inflammation. GABARAPL2 (p = 0.2391, OR = 4.02) is associated with autophagy, a process essential for maintaining lipid metabolism and cellular homeostasis in adipose tissue. Jang et al[9] found that *PRKACA* L206R mutation in CPAs causes high hormonal activity with a limited proliferative capacity, as supported by transcriptome profiling.

The enrichment of these proteins suggests that dysregulated protein degradation, lipid metabolism and inflammatory signaling contribute to the pathophysiology of obesity. Melanocortin receptor activity (p = 0.00249, OR = 555.17) is highly relevant, as melanocortin signaling in the hypothalamus is essential for energy homeostasis and appetite control. Defective melanocortin signaling is a major contributor to genetic and acquired obesity. The cell marker 2024 analysis highlighted neuronal and immune cells, both of which are crucial in obesity-related pathophysiology. Neuron Inferior Colliculus (p = 0.00797, OR = 147.96) and Brain neurons (p = 0.2687, OR = 3.50) suggest that obesity-induced neuroinflammation and hypothalamic dysfunction may contribute to appetite dysregulation.

Plasmacytoid dendritic cells (p = 0.1567, OR = 6.48) suggest that obesity is associated with chronic low-grade inflammation, a key driver of metabolic syndrome and insulin resistance. Monocyte spleen (p = 0.07019, OR = 15.31) suggests an overactive immune response, which is commonly seen in obesity-related inflammation and cardiovascular diseases. Study done by Sridhar et al[14] suggested that impaired activity of MC4R led to rare monogenic forms of obesity whereas gene polymorphisms were related to weight gain and metabolic syndrome. Some MC4R gene polymorphisms were protective against obesity.

MC4R could also have anti-inflammatory and neuroprotective effect[14].

The findings of this study highlight the intricate interplay between genetic, metabolic and regulatory factors contributing to obesity. The results underscore the pivotal role of lipid metabolism, inflammatory pathways and neuronal regulation in obesity. One of the most significant findings from the miRNA enrichment analysis was the identification of hsa-miR-33a-5p as a critical regulator of cholesterol metabolism and fatty acid homeostasis. This miRNA has been previously implicated in lipid storage and mobilization and its down regulation has been linked to increased lipid accumulation in adipocytes. Additionally, hsa-miR-877-5p was found to be associated with insulin resistance and inflammation, key hallmarks of obesity-induced metabolic dysfunction. These results align with previous studies emphasizing the role of miRNAs in metabolic regulation and suggest potential therapeutic targets for obesity management.

Pathway enrichment analysis using reactome and KEGG databases provided further insights into the metabolic dysregulation associated with obesity. The enrichment of LDL clearance and plasma lipoprotein clearance pathways underscores the significance of lipid metabolism in obesity pathophysiology. Impaired LDL clearance leads to hyperlipidemia and increases the risk of cardiovascular diseases, a well-documented comorbidity in obese individuals. Similarly, the observed enrichment in DNA damage reversal pathways suggests that oxidative stress and chronic inflammation play a role in obesity-associated complications. Given that excessive adiposity is linked to increased oxidative stress, these findings highlight potential avenues for therapeutic interventions targeting oxidative damage.

The PPI network analysis revealed significant interactions among obesity-associated proteins, with central hub nodes representing key metabolic regulators. PRKACA, a catalytic subunit of protein kinase A, emerged as a significant node within the network. This protein is known to regulate lipolysis and has been implicated in insulin resistance. Additionally, UBC, involved in protein degradation and inflammatory responses, was identified as a crucial player in obesity-related inflammation. These findings support the hypothesis that dysregulated protein degradation and inflammatory signaling contribute to obesity pathogenesis.

Gene ontology (GO) analysis further reinforced these observations by linking obesity-associated genes to crucial biological processes such as cholesterol absorption, feeding behavior regulation and bone resorption. The enrichment of genes involved in intestinal cholesterol absorption suggests a direct connection between excessive dietary fat intake and obesity-associated hypercholesterolemia. Additionally, the regulation of feeding behavior pathway highlights the involvement of neural mechanisms in obesity, reinforcing the role of hypothalamic dysfunction in energy balance dysregulation.

Cell-type enrichment analysis provided additional layers of complexity in our understanding of obesity. The presence of obesity-associated genes in neuronal and immune cell populations indicates that obesity is not merely a metabolic disorder but also involves neuroinflammatory and immunological components. The enrichment of genes in brain neurons and dendritic cells suggests potential links between neuroinflammation, appetite dysregulation and immune activation in obesity. These findings support emerging evidence that obesity is characterized by chronic low-grade inflammation which contributes to metabolic dysfunction and insulin resistance.

Overall, the integration of multiple bioinformatics approaches has enabled the identification of key regulatory pathways, potential biomarkers and therapeutic targets. Future research should focus on validating these findings through experimental studies and exploring their clinical applications in obesity prevention and treatment. Understanding the complex genetic architecture of obesity will be crucial in developing personalized interventions that target specific molecular pathways, ultimately improving obesity management and reducing associated health risks.

Conclusion

The results indicate a strong link between lipid metabolism, inflammation and neuronal dysfunction in obesity. Enriched pathways in cholesterol metabolism, LDL clearance and lipid storage suggest that obesity alters lipid homeostasis, predisposing individuals to dyslipidemia and cardiovascular diseases. The neuroactive ligand-receptor interaction pathway and melanocortin receptor activity highlight hypothalamic dysfunction, which is central to appetite dysregulation and energy balance impairment in obesity.

The enrichment of immune-related cells, DNA repair pathways and protein degradation mechanisms suggests that obesity promotes oxidative stress and inflammatory signaling, increasing the risk of insulin resistance and metabolic disorders. The involvement of lysosomal pathways, PRKACA and UBC indicates that obesity-related autophagy impairment could lead to lipotoxicity and mitochondrial dysfunction.

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