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# A thorough examination of GWAS data on dysregulation of miRNA networks and lipid metabolism pathways in metabolic syndrome

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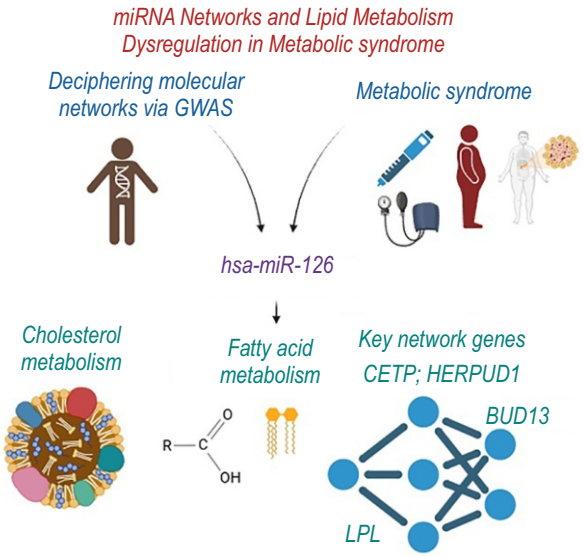
## Abstract

**Aim:** Metabolic syndrome (MetS) is a clustering of risk factors that increases susceptibility to type 2 diabetes and cardiovascular disease. This study aimed to perform a comprehensive bioinformatic analysis of genomic data to elucidate molecular pathways underlying MetS.

**Methodology:** GWAS data from previous MetS studies were analyzed using TargetScan, miRTarBase, Reactome Pathways, KEGG, protein–protein interaction (PPI) networks, and Gene Ontology (GO) mapping. Integration of these datasets identified key miRNAs, metabolic pathways, biological processes, and molecular activities associated with MetS.

**Results:** hsa-miR-126 was markedly enriched and strongly correlated with MetS. Pathway analysis highlighted cholesterol metabolism ($p < 0.05$) and plasma lipoprotein remodeling ($p < 0.05$) as significant contributors. GO analysis revealed triglyceride homeostasis ($p < 0.05$) and very-low-density lipoprotein particle remodeling ($p < 0.05$) as a key biological processes. Metabolomic analysis established strong links between triacylglycerol and glycerol metabolism. Lipid transport and metabolism emerged as central to MetS pathogenesis, with notable enrichments for high-density lipoprotein particles ($p < 0.05$) and phosphatidylcholine-sterol O-acyltransferase activator activity ($p < 0.05$).

**Interpretation:** This comprehensive analysis indicates that dysregulation of lipid metabolism is a major pathway in MetS, with specific miRNAs functioning as critical regulatory molecules. These insights suggest potential therapeutic strategies targeting miRNA-mediated regulation of lipid metabolism.




**Key words:** Cholesterol homeostasis, Lipid metabolism, Lipoprotein remodeling, Metabolic syndrome, miRNA regulation

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## Introduction

Metabolic syndrome (MetS) is a multifaceted cluster of interrelated physiological, biochemical, clinical, and metabolic variables that directly elevates the risk of cardiovascular disease, type 2 diabetes mellitus, and all-cause mortality (Islam *et al.,* 2024). Approximately 20–30% of adults worldwide are affected by MetS, and its prevalence continues to rise at an alarming pace, posing a serious public health challenge (Li *et al.,* 2022). The diagnosis of MetS is made when at least three of the following five factors are present: central obesity, hypertriglyceridemia, impaired glucose tolerance, elevated blood pressure, and reduced high-density lipoprotein (HDL) cholesterol levels (Mohamed *et al.,* 2023). Although these clinical characteristics are well documented, the underlying molecular and genetic mechanisms contributing to MetS remain incompletely understood. Recent advances in genome-wide association studies (GWAS) have enabled the identification of genetic variants associated with complex diseases and traits, including MetS. By investigating numerous loci linked to blood pressure regulation, glucose homeostasis, lipid metabolism, and obesity, GWAS has provided valuable insights into the genetic architecture of MetS components (Timasheva *et al.,* 2024).

Nevertheless, translating these associations into mechanistic understanding is challenging and requires integrative approaches that combine pathway analysis, functional annotation, and multi-omics genomics data. Such strategies are crucial to uncovering how these genetic signals interact and converge on biological processes that drive disease pathogenesis. MicroRNAs (miRNAs), small non-coding RNAs that regulate gene expression post-transcriptionally, have emerged as key players in the molecular landscape of MetS (Ghamlouche *et al.,* 2023). By binding to 3' untranslated regions of target mRNAs, miRNAs mediate mRNA degradation or translational repression, thereby influencing numerous physiological and pathological mechanisms. Mounting evidence highlights the involvement of miRNAs in the development of MetS and its components by modulating lipid metabolism, insulin signaling and adipogenesis (Fodor *et al.,* 2021).

Notably, miR-126 and miR-29a have been implicated in metabolic disorders and proposed as promising biomarkers and therapeutic targets (Yamada *et al.,* 2021). These regulatory molecules provide an additional layer of complexity to MetS pathophysiology and represent a potential bridge between genetic predisposition and environmental factors. Among the hallmark features of MetS, dysregulation of lipid metabolism plays a central role. Alterations in lipoprotein composition, transport, and metabolic processing are critical to disease's progression (Dakal *et al.,* 2025). The assembly, remodeling, and clearance of lipoproteins depend on a tightly regulated network of enzymes, transfer proteins and receptors (Kumari *et al.,* 2021). Dyslipidemia—characterized by elevated triglycerides, low HDL cholesterol, and an increased concentration of small dense low-density lipoprotein (LDL) particles—is a common clinical manifestation of MetS and is closely tied to perturbations in these pathways (Stoicescu *et al.,* 2025). Parallel to lipid metabolism, endoplasmic reticulum (ER) stress has been implicated in the pathogenesis of MetS (AlBashtawi *et al.,* 2024). Disturbances in ER function activate the unfolded protein response (UPR), which regulates calcium homeostasis, lipid synthesis and protein folding. Chronic ER stress, often observed in obesity, insulin resistance, and systemic inflammation, may exacerbate metabolic imbalances and contribute to disease progression (Ajoolabady *et al.,* 2022). Understanding the interplay between lipid metabolic pathways and ER stress can therefore offer deeper insight into the mechanistic basis of MetS.

Despite substantial progress in delineating individual aspects of MetS, few studies have conducted integrative analyses across multiple molecular datasets. Given the complexity of syndrome, a system biology strategy incorporating genomics, transcriptomics, and metabolomics is essential to fully elucidate its underlying mechanisms (Nafie *et al.,* 2025). Such integrative approaches can identify key regulatory networks and biological pathways, revealing novel therapeutic targets and potential biomarkers. Addressing this need, the present research undertook a comprehensive analysis of GWAS data related to MetS using a suite of bioinformatic resources to detect significant miRNAs, pathways, biological processes, and molecular functions associated with the disease. Databases including TargetScan, miRTarBase, Reactome, KEGG and Gene Ontology were employed to define the molecular landscape of MetS, while metabolomic analysis was performed to identify relevant metabolites and explore their roles in disease pathophysiology (Nafie *et al.,* 2025). Through this integrative methodology, the aim was to uncover critical miRNAs, key lipid metabolism and transport pathways, and the intricate relationships among cellular components, molecular processes, and biological functions that collectively drive MetS, ultimately highlighting novel targets for diagnosis and therapeutic intervention.

## Materials and Methods

**Data acquisition and preprocessing:** Genome-wide association study (GWAS) datasets from previous metabolic syndrome investigations served as a primary data source for this study (Sollis *et al.,* 2023). These datasets included genetic variants significantly linked to metabolic syndrome (MetS) and its individual components.

**miRNA target prediction and validation:** Identification of miRNAs associated with MetS followed a two-step strategy. Initially, potential binding sites in the 3' untranslated regions of genes residing in MetS-linked regions were predicted *in silico* with TargetScan (Min and Yoon, 2010). This algorithm considers seed match quality, sequence conservation, and site context to generate a ranked list of candidate miRNAs. To strengthen the predictions, experimentally validated interactions were retrieved from miRTarBase (Cui *et al.,* 2024). Only miRNAs supported by both TargetScan predictions and miRTarBase evidence were

**Table 1:** Enrichment analysis of the input gene set. Each row corresponds to a specific biological term or pathway. The Overlap column indicates the number of input genes associated with the term, while the Genes column lists the specific overlapping genes. Statistical significance is reported as the P-value, with the Adjusted P-value accounting for multiple testing. The Odds Ratio reflects the strength of association between the input genes and the term, and the Combined Score integrates both significance and enrichment magnitude, with higher values indicating stronger enrichment.

| Term | P-value | Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|
| hsa-miR-126 | p <0.05 | 0.123078 | 65.7053 | 464.1914 | *APOA5;HERPUD1* |
| hsa-miR-4327 | p <0.05 | 0.468071 | 9.012018 | 37.3605 | *CETP;APOA5;HERPUD1* |
| hsa-miR-369-5p | p <0.05 | 0.468071 | 20.51917 | 58.84862 | *LPL* |
| hsa-miR-3678-5p | p <0.05 | 0.468071 | 16.81617 | 45.02107 | *CETP* |
| hsa-miR-4776-5p | p <0.05 | 0.468071 | 6.033987 | 15.91834 | *LPL;APOA5* |
| hsa-miR-487b | p <0.05 | 0.468071 | 15.12107 | 38.95157 | *HERPUD1* |

retained for downstream analysis, ensuring high-confidence target selection.

**Pathway and functional enrichment:** Significant miRNAs were subjected to pathway enrichment using Reactome (Croft *et al.,* 2011) and KEGG (Kanehisa *et al.,* 2017) to highlight metabolic and lipid-transport pathways disrupted in MetS. Gene Ontology (GO) analysis further clarified the relevant cellular components, molecular functions and biological processes. Integrating miRNA regulatory networks with enriched pathways allowed visualization of miRNA–mRNA interactions, revealing potential therapeutic targets and biomarkers. This combined framework offers a foundation for future precision-medicine approaches to MetS.

## Results and Discussion

Metabolic syndrome is a multifactorial disorder characterized by dyslipidemia, insulin resistance and cardiovascular risk, where microRNAs (miRNAs) and key regulatory genes play critical roles in pathophysiology. Bioinformatic prediction using TargetScan and miRTarBase identified several miRNAs linked to metabolic syndrome. Table 1 highlights the primary miRNAs predicted through TargetScan 2017. Among them, hsa-miR-126 demonstrated the strongest association, targeting APOA5 and HERPUD1, genes involved in lipid metabolism and endoplasmic reticulum (ER) stress, respectively (Li *et al.,* 2023). Other miRNAs, such as hsa-miR-4327 and hsa-miR-369-5p, were linked with CETP and LPL, proteins critical for triglyceride and cholesterol regulation (Fernández-Tussy *et al.,* 2021). The high odds ratio and combined scores suggest a potential role of these miRNAs in regulating lipid homeostasis and cellular stress response, indicating their potential as biomarkers and therapeutic targets for metabolic syndrome.

The TargetScan predictions carry strong mechanistic logic when examined against what is known about MetS biology. hsa-miR-126 is perhaps the best characterised vascular miRNA, primarily known for maintaining endothelial integrity and regulating VEGF-mediated angiogenesis through suppression of SPRED1 and PIK3R2. Its enrichment here targeting APOA5 and HERPUD1 extends its known biology into lipid homeostasis and ER stress regulation — both central features of MetS pathophysiology. APOA5 is a well-established determinant of plasma triglyceride levels, and miR-126-mediated suppression of APOA5 would be expected to impair VLDL catabolism and elevate circulating triglycerides, a hallmark feature of MetS dyslipidaemia (Rasmi *et al.,* 2025). The pairing of hsa-miR-4327 with CETP and APOA5 is also noteworthy — CETP (cholesteryl ester transfer protein) mediates bidirectional transfer of cholesteryl esters and triglycerides between HDL and apoB-containing lipoproteins (Oestereich *et al.,* 2022); its post-transcriptional suppression by miR-4327 could theoretically raise HDL-C and reduce atherogenic lipoprotein conversion, suggesting a potentially protective miRNA-mediated mechanism that may be disrupted in MetS. hsa-miR-369-5p targeting LPL connects miRNA regulation directly to lipoprotein lipase activity — the principal enzyme responsible for triglyceride hydrolysis in peripheral tissues, whose deficiency or dysfunction is a recognised contributor to the hypertriglyceridaemia of MetS.

miRTarBase analysis, identified hsa-miR-3162-3p with a remarkably high odds ratio of 86.73, strongly associated with APOA5, highlighting its pivotal role in lipid metabolism. Additionally, hsa-let-7a-5p targets *BUD13* and *HERPUD1*, genes implicated in lipid transport and ER stress. Low p-values suggest these miRNAs contribute to the molecular pathophysiology of metabolic syndrome and could be exploited as novel diagnostic or therapeutic targets. The miRTarBase results reinforce and extend the TargetScan findings with the added confidence of experimental validation. The remarkably high odds ratio for hsa-miR-3162-3p targeting APOA5 (OR = 86.73) places this miRNA among the strongest computational associations in the entire analysis, and its experimental validation means this is not a prediction artefact — this miRNA-gene pair has been confirmed in at least one biological system. Given APOA5's established role as a co-factor that activates LPL-mediated triglyceride hydrolysis on vascular endothelial surfaces, suppression of APOA5 by miR-3162-3p would reduce LPL activation, and thereby contribute to postprandial hypertriglyceridaemia—a phenotype clinically characteristic of MetS and strongly predictive of cardiovascular events. hsa-let-7a-5p targeting BUD13 and HERPUD1 adds another dimension: let-7 family miRNAs are among the most

**Table 2:** Enrichment analysis of the input gene set. Each row corresponds to a specific biological term or pathway. The Overlap column indicates the number of input genes associated with the term, while the Genes column lists the specific overlapping genes. Statistical significance is reported as the P-value, with the Adjusted P-value accounting for multiple testing. The Odds Ratio reflects the strength of association between the input genes and the term, and the Combined Score integrates both significance and enrichment magnitude, with higher values indicating stronger enrichment

| Term | P-value | Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|
| Plasma lipoprotein remodeling | p <0.05 | p <0.05 | 643.9677 | 10451.33 | *CETP;LPL;APOA5* |
| Plasma lipoprotein assembly, remodeling, and clearance | p <0.05 | p <0.05 | 276.6944 | 3821.363 | *CETP;LPL;APOA5* |
| Chylomicron remodeling | p <0.05 | p <0.05 | 1249.125 | 15739.23 | *LPL;APOA5* |
| Assembly of active LPL and LIPC lipase complexes | p <0.05 | p <0.05 | 587.5588 | 6619.668 | *LPL;APOA5* |
| Transport of small molecules | p <0.05 | p <0.05 | 26.73093 | 188.3587 | *CETP;LPL;APOA5* |

phylogenetically conserved miRNAs and have well-established roles in metabolic regulation, including suppression of insulin secretion and adipogenesis. BUD13 is a component of the retention and splicing (RES) complex involved in pre-mRNA splicing, and its dysregulation may affect splicing of metabolic genes, while HERPUD1 connects this miRNA to ER protein quality control — a pathway increasingly recognised as central to insulin resistance and lipotoxicity in MetS.

Functional pathway analyses further elucidate the involvement of these genes in metabolic regulation. Table 2 presents Reactome pathways associated with metabolic syndrome. Genes such as *CETP, LPL* and *APOA5* are enriched in plasma lipoprotein and chylomicron remodeling pathways, with odds ratio exceeding 600 and 1,200, respectively, indicating their central role in lipid processing. LDL and HDL remodeling pathways are also significantly enriched, linking dyslipidemia to disease development. ER stress-related pathways, including *ATF4* activation and PERK signaling, involve *HERPUD1,* suggesting a connection between cellular stress and metabolic dysfunction. These pathways provide insights into potential therapeutic interventions targeting lipid metabolism and ER homeostasis.

The Reactome pathway enrichment data represents one of the strongest signals in this entire study, and the biological interpretations deserve careful attention. The extraordinary odds ratio for Plasma Lipoprotein Remodelling (OR = 643.97) and Chylomicron Remodelling (OR = 1249.13) driven by CETP, LPL, and APOA5 reflects the tight clustering of MetS genetic variants around the post-prandial lipoprotein processing machinery. CETP facilitates the exchange of triglycerides from VLDL and chylomicrons for cholesteryl esters from HDL, generating triglyceride-enriched HDL that is rapidly cleared and contributing to the characteristically low HDL-C of MetS. LPL and APOA5 together control the rate of triglyceride hydrolysis from circulating lipoproteins at vascular surfaces — when this system is genetically perturbed, postprandial lipid clearance slows, plasma triglycerides accumulate, and atherogenic remnant lipoprotein particles pile up (Chen *et al.,* 2021). The ER stress-related pathway enrichment (ATF4 activation, PERK signalling via HERPUD1) is also mechanistically meaningful — chronic lipid overload in adipocytes and hepatocytes triggers ER stress, activating the unfolded protein response which in turn upregulates lipogenic gene expression through SREBP-1c, creating a feed-forward loop where dyslipidaemia and ER stress mutually amplify each other in MetS (Karin and Kim, 2025). KEGG pathway analysis identifies cholesterol metabolism and PPAR signaling as critical processes, emphasizing lipid regulation and metabolic balance. Genes APOA5, CETP and LPL contribute significantly, while glycerolipid metabolism is enriched, supporting its role in lipid storage and transport. HERPUD1's involvement in ER protein processing links ER stress to metabolic syndrome. Additionally, LPL's role in neurodegenerative conditions, such as Alzheimer's disease, suggests broader implications of metabolic regulation in systemic health (Bruce *et al.,* 2020) (Table 3).

The KEGG pathway results complement the Reactome findings and add several important dimensions. Cholesterol metabolism pathway enrichment (OR = 207.77, CETP and LPL) directly implicates the reverse cholesterol transport pathway — the process by which excess cholesterol is retrieved from peripheral tissues and returned to the liver for excretion (Zhang *et al.,* 2021). CETP's central role in this pathway means that its genetic variation or miRNA-mediated suppression has direct consequences for cholesterol homeostasis, which may explain part of the cardiovascular risk associated with the MetS gene set (Ağagündüz *et al.,* 2025). PPAR signalling pathway enrichment (LPL and APOA5) is particularly relevant to MetS therapy — PPARα agonists (fibrates) and PPARγ agonists (thiazolidinediones) are established pharmacological treatments for dyslipidaemia and insulin resistance of MetS, and their targets LPL and APOA5 emerging here computationally confirms biological plausibility (Cheng *et al.,* 2019). The appearance of Alzheimer's disease pathway through LPL is not coincidental — LPL is expressed in the brain and modulates neuronal lipid homeostasis; the epidemiological overlap between MetS and dementia risk has been long observed, and this shared LPL-mediated pathway provides a molecular basis for that clinical association. Protein processing in ER (HERPUD1) further

**Table 3:** Enrichment analysis of the input gene set. Each row corresponds to a specific biological term or pathway. The Overlap column indicates the number of input genes associated with the term, while the Genes column lists the specific overlapping genes. Statistical significance is reported as the P-value, with the Adjusted P-value accounting for multiple testing. The Odds Ratio reflects the strength of association between the input genes and the term, and the Combined Score integrates both significance and enrichment magnitude, with higher values indicating stronger enrichment

| Term | P-value | Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|
| Cholesterol metabolism | p <0.05 | p <0.05 | 207.7708 | 1932.576 | *CETP;LPL* |
| PPAR signaling pathway | p <0.05 | p <0.05 | 138.3472 | 1177.887 | *LPL;APOA5* |
| Glycerolipid metabolism | p <0.05 | p <0.05 | 66.44667 | 266.3422 | *LPL* |
| Protein processing in endoplasmic reticulum | p <0.05 | p <0.05 | 23.32235 | 69.7643 | *HERPUD1* |
| Alzheimer disease | p <0.05 | p <0.05 | 10.6663 | 23.96575 | *LPL* |

**Table 4:** PPI analysis of the input gene set. Each row corresponds to a specific biological term or pathway. The Overlap column indicates the number of input genes associated with the term, while the Genes column lists the specific overlapping genes. Statistical significance is reported as P-value, with the Adjusted P-value accounting for multiple testing. The Odds Ratio reflects the strength of association between the input genes and the term, and the Combined Score integrates both significance and enrichment magnitude, with higher values indicating stronger enrichment

| Term | P-value | Adjusted P-value | Odds Ratio | Combined Score | Genes |
|---|---|---|---|---|---|
| *EWSR1* | 0.001677 | 0.031863 | 46.43427 | 296.7497 | *CETP;HERPUD1* |
| *HNRNPA1* | 0.038376 | 0.111479 | 30.79845 | 100.4127 | *BUD13* |
| *TOP1* | 0.040117 | 0.111479 | 29.42074 | 94.61551 | *ZPR1* |
| *COPS6* | 0.043303 | 0.111479 | 27.18904 | 85.36112 | *LPL* |
| *RNR2* | 0.046479 | 0.111479 | 25.27006 | 77.54756 | *ZPR1* |
| *RPA2* | 0.047632 | 0.111479 | 24.63727 | 75.002 | *HERPUD1* |
| *PLK1* | 0.048784 | 0.111479 | 24.03515 | 72.59476 | *LPL* |
| *UBQLN4* | 0.050222 | 0.111479 | 23.32235 | 69.7643 | *HERPUD1* |
| *TAF2* | 0.052806 | 0.111479 | 22.13966 | 65.11577 | *ZPR1* |
| *SSC1* | 0.065638 | 0.124711 | 17.65179 | 48.07654 | *BUD13* |

reinforces the ER stress-lipid metabolism nexus already seen in Reactome results, with HERPUD1 acting as a key mediator of the HRD1 ubiquitin ligase complex that degrades misfolded ER proteins accumulating under lipotoxic stress.

Protein-protein interaction (PPI) analysis identified hub proteins correlated with metabolic syndrome. EWSR1 exhibited the strongest association (odds ratio: 46.43), linking HERPUD1 and CETP in lipid metabolism and ER stress pathways. Proteins HNRNPA1, TOP1 and COPS6 connect RNA processing with cellular stress, while RPA2, UBQLN4 and PLK1 indicate involvement of LPL and HERPUD1 in proteostasis and lipid regulation. Proteins ZPR1 and BUD13 represent potential drug targets, highlighting the regulatory networks underlying metabolic syndrome (Table 4). The PPI network analysis reveals the connective tissue linking MetS molecular players through protein interaction hubs. EWSR1 emerging as the strongest hub (OR = 46.43, connecting CETP and HERPUD1) is a somewhat unexpected finding — EWSR1 is an RNA-binding protein traditionally known from oncology, but recent studies have identified roles in adipocyte differentiation and metabolic gene regulation through its interactions with chromatin remodelling complexes. Its bridging of CETP (lipid metabolism) and HERPUD1 (ER stress) at a protein interaction level suggests that transcriptional and post-transcriptional regulatory machinery may coordinate the lipid-ER stress axis in MetS beyond what pathway analysis alone reveals. HNRNPA1 and SSC1 connecting through BUD13 reinforce the RNA processing dimension in MetS — heterogeneous nuclear ribonucleoproteins regulate splicing of metabolic transcripts including those encoding lipid-handling enzymes (Feng *et al.,* 2022). ZPR1, appearing as an interaction partner of TOP1 and TAF2 and as a potential drug target, is known to mediate signalling between the cell surface receptor for insulin-like growth factors and nuclear gene expression programmes, positioning it at an interesting crossroads between insulin signalling and transcriptional control of metabolic genes. PLK1 and UBQLN4 connecting through LPL and HERPUD1, respectively, point towards cell cycle and protein degradation pathways being entangled with lipid metabolism regulation under MetS conditions.

Metabolomic analysis identifies glycerol and triacylglycerol as significantly enriched metabolites associated with LPL, supporting its role in triglyceride hydrolysis, lipid storage and energy homeostasis. This emphasizes LPL as a potential therapeutic target for dyslipidemia and cardiovascular risk mitigation (Gugliucci, 2023). Gene ontology (GO) analyses further revealed the functional significance of these genes. Biological processes showed enrichment in acylglycerol and

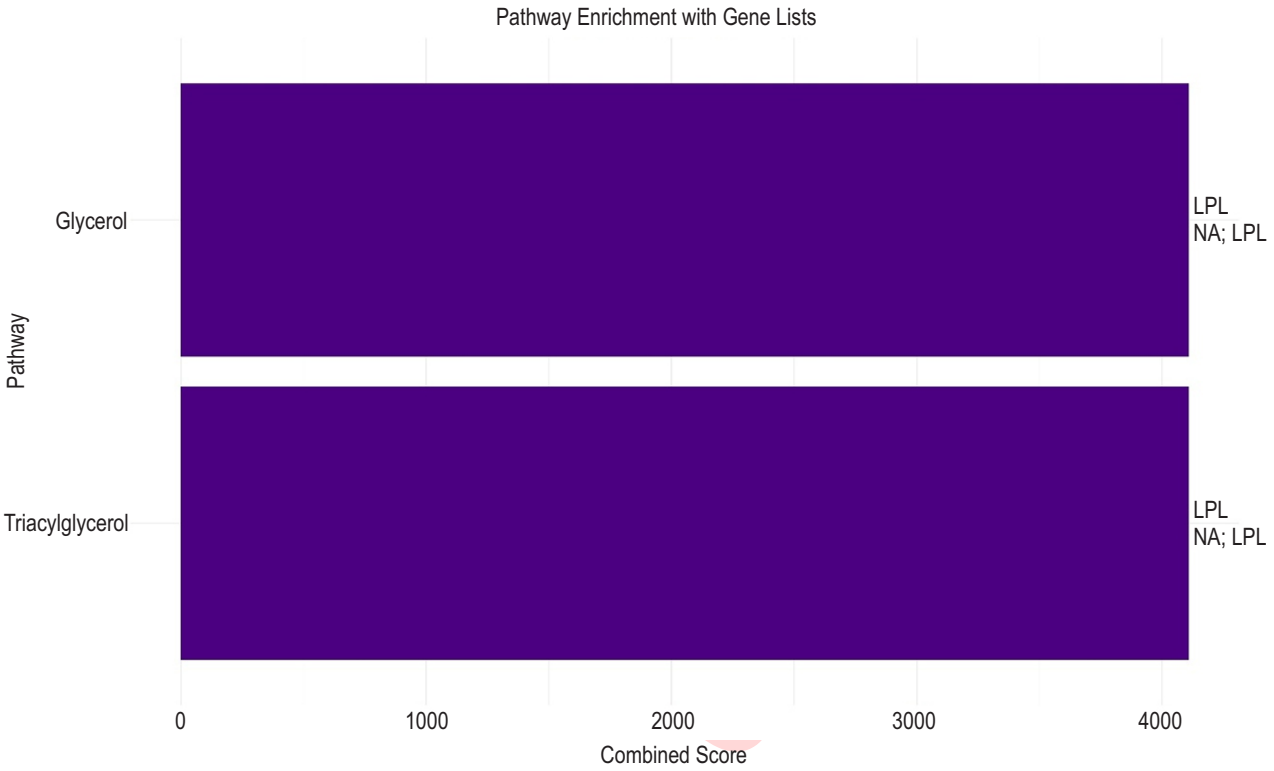


**Fig. 1:** Enriched metabolites and associated genes plotted against the combined score.

triglyceride homeostasis, lipoprotein remodeling and cholesterol regulation, emphasizing CETP, LPL, and APOA5 in lipid transport and cardiovascular function. Cellular components highlighted the presence of these proteins in HDL, LDL, VLDL and chylomicrons, with HERPUD1 associated with the Hrd1p ubiquitin ligase complex, linking protein degradation and lipid transport. Molecular function analysis emphasized cholesterol and sterol transfer, lipase activity, phospholipase functions, and lipoprotein particle binding, reinforcing their role in metabolic regulation.

The GO analysis results provide a coherent functional portrait of the MetS gene set that maps well onto known disease biology. The enrichment of biological processes centred on acylglycerol and triglyceride homeostasis, lipoprotein remodelling, and cholesterol regulation converges on the same lipid handling machinery identified across miRNA, pathway, and PPI analyses—strongly supporting CETP, LPL, and APOA5 as the molecular axis driving MetS-associated lipid dysregulation. The cellular component profile—HDL, LDL, VLDL and chylomicron compartments—reflects the circulating lipoproteins as the primary biological arena in which MetS gene products operate, while HERPUD1's association with the Hrd1p ubiquitin ligase complex in the ER links proteostasis and lipid metabolism within the secretory pathway. This is biologically significant because the ER is not only the site of lipoprotein assembly (apoB-containing lipoproteins are assembled in the ER), but also the organelle most directly stressed by lipid overload. The molecular function enrichment — cholesterol and sterol transfer, lipase activity, phospholipase functions, and lipoprotein particle binding — directly maps to the enzymatic activities of CETP (sterol transfer), LPL (lipase), and APOA5 (lipoprotein binding and LPL activation). This internal consistency across GO categories confirms that the gene set is not capturing random metabolic genes, but a functionally coherent module of lipid-handling proteins whose coordinated dysregulation produces the MetS lipid phenotype. HMDB metabolite analysis (Fig. 1) identified specific triacylglycerol species, such as TG(16:0/16:0/18:0), TG(16:0/16:0/20:0), and TG(16:0/18:0/18:1), strongly associated with LPL activity, confirming its direct involvement in triglyceride metabolism and lipid homeostasis.

The HMDB metabolomics results provide the direct biochemical link between the genetic and functional analyses and the actual metabolic phenotype of MetS. The identification of specific triacylglycerol species—TG(16:0/16:0/18:0), TG(16:0/16:0/20:0), and TG(16:0/18:0/18:1)—as the metabolites most strongly associated with LPL activity is mechanistically precise (Han and Ye, 2021). These are all saturated and monounsaturated long-chain triglycerides, which are the primary substrates for LPL-mediated hydrolysis at vascular endothelial surfaces. Their enrichment in association with LPL confirms that LPL functional capacity is a rate-limiting step for the clearance of these circulating triglycerides, and that genetic variation or miRNA-mediated suppression of LPL expression would

predictably result in elevated circulating concentrations of these specific TG species. This is clinically relevant because not all triglyceride species carry equal cardiovascular risk — long-chain saturated TG species are particularly associated with postprandial lipaemia, hepatic fat deposition, and insulin resistance, and their selective enrichment here connects the MetS gene set directly to the most pathogenic form of dyslipidaemia (Verwer *et al.,* 2020). The additional association with glycerol metabolism is consistent — glycerol is the backbone released upon LPL-mediated TG hydrolysis, and its downstream metabolism through glycerol kinase connects triglyceride catabolism to gluconeogenesis, providing a metabolic link between hypertriglyceridaemia and the glucose dysregulation component of MetS.

RNA-seq analyses highlighted consistent downregulation of *HERPUD1, LPL, ZPR1* and *BUD13,* and upregulation of *CETP, APOA5, LPL, HERPUD1* and *ZPR1* across multiple datasets, reinforcing their relevance in lipid metabolism, cellular stress and immunomodulation.The RNA-seq GEO signature analysis results are particularly informative because they confirm the directional dysregulation of the MetS gene set in actual gene expression datasets. The consistent downregulation of HERPUD1, LPL, ZPR1 and BUD13 across multiple datasets is biologically coherent—reduced LPL expression in adipose tissue and skeletal muscle is a well-documented feature of insulin-resistant and obese states, and its downregulation in MetS-related expression datasets here confirms that the computational predictions are capturing real transcriptomic biology rather than statistical artefacts. HERPUD1 downregulation is also physiologically meaningful: when ER protein quality control is overwhelmed by chronic lipid and nutrient excess, HERPUD1 expression is initially upregulated but may subsequently collapse as prolonged ER stress induces cell death signalling, and its downregulation in late-stage or chronic MetS-associated datasets is consistent with this progression. The parallel upregulation of CETP, APOA5, LPL, HERPUD1 and ZPR1 in other datasets likely reflects compensatory responses or early-stage lipid overload contexts, suggesting that these genes are bidirectionally regulated depending on the metabolic state and stage of MetS progression — a nuance that underscores the importance of considering disease trajectory when interpreting cross-sectional transcriptomic associations.

Collectively, these findings elucidate the molecular and functional landscape of metabolic syndrome, emphasizing miRNA regulation, lipid metabolism, ER stress response and potential therapeutic targets. *APOA5, CETP, LPL* and *HERPUD1* emerged as central nodes integrating lipid homeostasis and cellular stress pathways, providing a robust framework for understanding metabolic syndrome pathophysiology and guiding biomarker development and therapeutic strategies. Viewing the entire dataset together, a highly consistent molecular model of MetS emerges from this multi-platform bioinformatics analysis. At the centre of this model sit four genes — APOA5, CETP, LPL and HERPUD1—that appear as key nodes across virtually every analytical layer: miRNA targeting, Reactome and KEGG pathways, PPI networks, GO functional categories, metabolomics, and RNA-seq signatures. APOA5 and LPL jointly control the rate of postprandial triglyceride clearance, and their combined suppression through multiple miRNAs (miR-126, miR-3162-3p, miR-4776-5p, let-7a-5p) provides a post-transcriptional mechanism for the hypertriglyceridaemia of MetS. CETP mediates the transfer of cholesteryl esters from HDL to apoB-lipoproteins, and its enrichment across pathways and PPI hubs connects the low HDL-C and elevated atherogenic lipoprotein burden that characterise MetS dyslipidaemia. HERPUD1 bridges lipid metabolism and ER stress — it is the molecular link between the chronic lipid overload seen in MetS and the cellular stress response that amplifies insulin resistance and inflammatory signalling. What makes this analysis particularly noteworthy is the identification of miR-126 as the most significantly enriched miRNA with the highest combined score (OR = 65.71).

This is clinically meaningful because circulating miR-126 levels have been reported as reduced in patients with type 2 diabetes, obesity, and early cardiovascular disease — conditions that are tightly clustered within MetS. The hypothesis emerging from this analysis is that miR-126 downregulation in MetS represents a causal event rather than merely a biomarker, driving APOA5 suppression and thereby creating the triglyceride-rich, HDL-low lipoprotein phenotype at the post-transcriptional level. Restoring miR-126 expression or mimicking its activity therapeutically could, therefore, represent a conceptually novel and multi-target approach to correcting MetS dyslipidaemia. For the Indian context, where MetS prevalence is rising steeply and tends to manifest at lower BMI thresholds than in Western populations, the identification of miRNA-based and LPL/CETP-centred molecular targets is particularly relevant, as pharmacogenomic variation in LPL and CETP is known to differ across South Asian populations and may contribute to the unique lipid phenotype of Indian MetS patients (Mohan *et al.,* 2025).

Metabolic syndrome (MetS) is a multifactorial disorder influenced by genetic, molecular, and environmental factors, increasing cardiovascular and metabolic risk (Jha *et al.,* 2023). Our bioinformatic analysis of GWAS data identified key miRNAs, including downregulation of miR-126 and miR-132, which regulate angiogenesis, endothelial function, inflammation, and insulin resistance (Yu *et al.,* 2020; Zhang *et al.,* 2014). Gene Ontology enrichment highlighted lipid droplets, mitochondria, glucose metabolism, and lipid transport as critical components, with ER stress linking metabolic dysregulation to systemic inflammation (Jha *et al.,* 2017; Yuan *et al.,* 2022). Integration of miRNA regulatory networks with metabolic pathways revealed potential therapeutic targets and biomarkers, supporting precision medicine strategies for MetS management (Ağagündüz *et al.,* 2025). This study relies on computational approaches, which may introduce ancestry-specific biases and limit generalizability. The lack of longitudinal data and absence of validation in independent clinical cohorts further constrain the robustness of the findings. Additionally, translating identified biomarkers into clinical diagnostic or therapeutic applications remains challenging. These

limitations can be addressed in future work by leveraging larger, more diverse datasets and integrating in silico, in vivo, and clinical experiments to validate and extend the findings.

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