Journal of Communicable Diseases
Volume 58, Issue 2 - 2026, Pg. No. 87-97
Peer Reviewed & Open Access Journal

Research Article

Infection-Microbiome Interactions Integrate With Inflammatory and Metabolic Pathways in Syndrome X: A Multi-Domain Enrichment Analysis

Arun Rajamanikom[1], N Vadivu[2], Kalaiselvi Rajendiran[3], Arbind Kumar Choudhary[4], Panneerselvam Periasamy[5]

[1]Department of Orthopedics, Vinayaka Mission's Kirupananda Variyar Medical College, Vinayaka Missions University, Salem, Tamil Nadu, India
[2]Department of Humanities and Languages, Sona College of Technology, Tamil Nadu, India
[3]Assistant Professor, Department of Biochemistry, Panimalar Medical College Hospital & Research Institute, Varadarajapuram Chennai, Tamil Nadu, India
[4]Assistant Professor, Department of Pharmacology, Government Erode Medical College and Hospital, Tamil Nadu, India
[5]Assistant Professor, Department of Physiology, Government Erode Medical College and Hospital Erode, Tamil Nadu, India
DOI: https://doi.org/10.24321/0019.5138.202633

I N F O

Corresponding Author:
Arbind Kumar Choudhary, Department of Physiology, Government Erode Medical College and Hospital, Erode, Tamil Nadu, India
E-mail Id:
arbindkch@gmail.com
Orcid Id:
https://orcid.org/0000-0001-8910-1745
How to cite this article:
Rajamanikom A, Vadivu N, Rajendiran K, Choudhary A K, Periasamy P. Infection-Microbiome Interactions Integrate With Inflammatory and Metabolic Pathways in Syndrome X: A Multi-Domain Enrichment Analysis. J Commun Dis. 2026;58(2):87-97.

Date of Submission: 2026-04-23
Date of Acceptance: 2026-06-14

A B S T R A C T

Background: Syndrome X (metabolic syndrome) is characterized by interconnected inflammatory and metabolic disturbances, yet most studies examine these pathways in isolation. Emerging evidence suggests that infection-related and microbiome-derived signals may contribute to the chronic low-grade inflammation observed in this condition, but this relationship has not been systematically integrated with metabolic mechanisms.

Objective: To apply a multi-domain enrichment analysis to a well-defined set of high-confidence Syndrome X genes in order to identify coordinated biological processes linking inflammation, adipose tissue dysfunction, micronutrient metabolism, and infection-microbiome interactions.

Methods: Thirty high-confidence genes (DisGeNET score ≥0.65) were analyzed across seven independent databases using hypergeometric enrichment with Benjamini-Hochberg correction: Gene Ontology, WikiPathways, Jensen TISSUES and COMPARTMENTS, ChEA2022 transcription factors, HMDB metabolites, and DrugMatrix. Cross-domain hub gene analysis and hierarchical clustering were performed. Special emphasis was placed on infection- and microbiome-related terms.

Results: NF-κB (adjusted $p = 2.1\times10^{-13}$) and STAT3 emerged as the dominant master transcriptional regulators. Adipose tissue showed the strongest tissue enrichment (13/30 genes). Three micronutrient pathways (vitamin B12, folate, and selenium) converged on an identical nine-gene signature. Significant enrichment was observed in "response to lipopolysaccharide," SARS-CoV-2 signaling, and COVID-19 pathology pathways. Fifty percent of genes participated in five or more databases,

with five master hubs present across all seven. Atorvastatin showed the highest pharmacological association (OR = 453.61).

Conclusion: Syndrome X involves coordinated inflammatory, metabolic, and infection-microbiome axes centered on adipose tissue. NF-κB, STAT3, and LBP represent key integrative nodes. These findings support the exploration of combined therapeutic strategies addressing both metabolic inflammation and its infectious/microbiome drivers.

Keywords: Metabolic Syndrome, Multi-Domain Enrichment Analysis, Chronic Inflammation, Adipose Tissue, Microbiome, Infection, One-Carbon Metabolism, NF-κB/STAT3

Introduction

Syndrome X, also known as metabolic syndrome, represents one of the most prevalent clusters of cardiometabolic abnormalities worldwide, encompassing central obesity, insulin resistance, dyslipidaemia, and hypertension.[1] Its global rise has paralleled the increasing burden of type 2 diabetes and cardiovascular disease, particularly in low- and middle-income countries where populations face the added challenge of persistent infectious diseases.[2] Despite decades of research, the precise molecular mechanisms that link these seemingly disparate features remain incompletely understood.

A substantial body of evidence has established chronic low-grade inflammation as a central pathogenic process in metabolic syndrome. Pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), along with acute-phase reactants like C-reactive protein (CRP), are consistently elevated in affected individuals and correlate with disease severity.[3,4] Adipose tissue, particularly visceral depots, has been identified as a key source of this inflammatory activity, functioning as an active endocrine organ rather than a passive energy store.[5,6] More recently, disturbances in one-carbon metabolism and micronutrient status, especially involving vitamin B12, folate, and selenium, have been implicated in sustaining oxidative stress and endothelial dysfunction.[7]

However, most existing studies have examined these pathways in isolation. Traditional approaches often focus on either metabolic or inflammatory components without systematically exploring how upstream triggers, including microbial signals, may initiate or amplify the same networks. Emerging evidence suggests that bacterial lipopolysaccharide (LPS) from the gut microbiota and responses to certain viral infections can activate the NF-κB and STAT3 signalling pathways that also drive metabolic inflammation.[8,9] This convergence raises the possibility that infection-related and microbiome-derived stimuli contribute to the chronic inflammatory state observed in metabolic syndrome, a concept reinforced by the syndemic interaction between metabolic disorders and COVID-19 severity.[10,11]

A systems-level approach that integrates transcriptional regulation, tissue-specific expression, metabolite associations, and infection-related pathways is therefore needed. Multi-domain enrichment analysis offers a powerful method to identify coordinated biological processes across independent databases while reducing the risk of findings that are artefacts of any single data source.

The present study applies this integrative framework to a well-defined set of high-confidence Syndrome X genes. By simultaneously examining Gene Ontology, WikiPathways, tissue and compartment enrichment, transcription factor regulation, metabolite associations, and infection-related signals, we aimed to delineate the principal molecular axes that connect inflammation, adipose dysfunction, micronutrient metabolism, and microbial influences in Syndrome X.

Materials and Methods

Study Design

This study employed a multi-domain bioinformatic enrichment approach to systematically identify biological pathways, transcriptional regulators, tissue-specific patterns, and metabolite associations underlying Syndrome X (metabolic syndrome). The analysis integrated gene-disease associations from DisGeNET with functional annotations from seven independent databases. No human participants, animal models, or primary experimental data were used; all analyses were performed on publicly available curated databases. All statistical analyses were conducted in R version 4.4.2.

Gene-Set Definition

High-confidence genes associated with Syndrome X (Reaven phenotype) were retrieved from the DisGeNET database (version 7.0). Genes were selected using a composite score threshold of ≥0.65, ensuring support from multiple evidence categories including expert curation, genome-wide association studies (GWAS), animal models, and literature text-mining. The top 30 genes meeting this criterion were included. Each gene was manually verified for direct mechanistic relevance to core components of metabolic syndrome (insulin resistance, central obesity, dyslipidemia, or hypertension). The resulting 30-gene signature served as the input for all downstream enrichment analyses.

Enrichment Analysis Modules

The 30-gene signature was systematically tested against seven independent functional databases using hypergeometric enrichment analysis. Benjamini-Hochberg false discovery rate (FDR) correction was applied across

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202633

all comparisons (threshold: adjusted $p < 0.05$). Combined scores ($-\log_{10}$ adjusted p-value × odds ratio) were used to rank findings by integrating statistical significance and effect size.

- **Gene Ontology (GO) Enrichment:** Analysis was performed separately for Biological Process, Cellular Component, and Molecular Function domains using current GO annotations.
- **WikiPathways Enrichment:** Testing was conducted against curated metabolic, signaling, and disease pathway maps (release 2024), with particular attention to micronutrient metabolism and infection-related pathways.
- **Jensen Compartments and Tissues:** Subcellular localization and tissue-specific expression patterns were analyzed using high-confidence annotations (score ≥0.7).
- **ChEA2022 Transcription Factor Enrichment:** Master regulators were identified using integrated ChIP-seq, DNase-seq, and ATAC-seq data with a prediction confidence threshold of ≥0.5.
- **HMDB Metabolite Enrichment:** Associations between the gene set and human metabolites (including approved drugs) were evaluated using enzyme, transporter, and receptor relationships from HMDB 5.0.
- **DrugMatrix Analysis:** Exploratory assessment of drug-induced gene expression changes was performed using rat liver transcriptomic data; results were considered hypothesis-generating due to multiple-testing burden.

Cross-Domain Integration and Hub Gene Analysis

For each of the 30 genes, participation across all seven enrichment modules was quantified. Genes appearing in enriched terms in five or more modules were classified as hub genes. Master hub genes were defined as those participating in all seven domains. Hierarchical clustering (Euclidean distance, Ward linkage) was applied to hub genes based on their enrichment patterns to identify functional clusters (inflammatory vs. metabolic/adipokine). This cross-domain approach provided independent statistical validation of core network topology.

Infection-Microbiome Integration

In addition to standard enrichment testing, specific attention was given to infection- and microbiome-related signals. Terms and pathways directly linked to bacterial products (e.g., "response to lipopolysaccharide"), viral infection responses (e.g., SARS-CoV-2 signaling and COVID-19 pathology pathways), and innate immune activation were highlighted and interpreted in the context of chronic low-grade inflammation in Syndrome X. This framework allowed explicit examination of how pathogen-associated molecular patterns and microbiome-derived signals may converge on the same transcriptional and metabolic programs dysregulated in metabolic syndrome.

Statistical Framework

All enrichment analyses used the hypergeometric test to evaluate overrepresentation of the 30-gene signature within each functional category relative to the background human genome. Multiple testing was controlled using the Benjamini-Hochberg procedure to maintain a false discovery rate below 5%. Odds ratios quantified the magnitude of enrichment, and combined scores enabled ranking of findings across heterogeneous databases.

Data Availability

All source data were obtained from publicly accessible databases (DisGeNET, Gene Ontology, WikiPathways, Jensen Lab databases, ChEA, and HMDB). Analysis code is available from the corresponding author upon reasonable request. Database versions and access dates are documented to ensure reproducibility.

Results

Gene-Set Definition and Cross-Domain Participation Overview

Thirty high-confidence genes associated with Syndrome X (Reaven phenotype) were retrieved from DisGeNET v7.0 using a composite score threshold of ≥0.65. Table 1 presents these genes ranked by evidence score. Five of the top ten genes encode canonical inflammatory mediators (CRP, IL6, TNF, IL18, CCL2), while four are key adipokines or hormones (LEP, ADIPOQ, RETN, INS). LBP (rank 15) is particularly relevant to the infection-microbiome axis as the principal sensor that delivers bacterial lipopolysaccharide to TLR4, initiating the NF-κB-driven inflammatory cascade.

Comprehensive cross-domain participation analysis revealed that 15 of the 30 genes (50%) participated in five or more of the seven enrichment modules. Five genes (TNF, IL6, CRP, CCL2, APOA1) were present across all seven domains and were designated master hub genes. This high degree of multi-domain convergence provides strong statistical validation that the identified inflammatory and metabolic processes represent genuine coordinated biology.

Infection- and Microbiome-Related Enrichments

Multiple independent analyses identified strong enrichment in infection- and microbiome-related pathways (Table 2). "Response to lipopolysaccharide" (GO:0032496) was significantly enriched, directly implicating bacterial endotoxin sensing via TLR4. WikiPathways analysis further revealed robust enrichment in "Network Map of SARS-CoV-2 Signaling" (WP5115) and "Lung Pathology of COVID-19" (WP5146), demonstrating that the inflammatory gene

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202633

network dysregulated in Syndrome X is mechanistically shared with viral infection responses. These findings provide quantitative support for syndemic interactions between metabolic syndrome and communicable diseases such as COVID-19.

Gene Ontology Enrichment: Inflammatory and Extracellular Biology as Dominant Signatures

Gene Ontology enrichment analysis across Biological Process, Cellular Component, and Molecular Function domains revealed a coherent functional signature overwhelmingly centered on immune activation, cytokine signaling, and extracellular localization (Table 3). "Inflammatory response" (GO:0006954) and "cytokine-mediated signaling pathway" (GO:0019221) were the top biological process terms. "Response to lipopolysaccharide" (GO:0032496) was strongly enriched, providing the first quantitative bioinformatic bridge between the Syndrome X gene signature and infection-driven inflammatory pathways. "Extracellular space" (GO:0005615) achieved the single highest significance across all GO analyses, indicating that the majority of Syndrome X gene products function as secreted signaling molecules.

WikiPathways Enrichment: Micronutrient Metabolism Convergence and Infection Pathway Overlap

WikiPathways analysis identified exceptional convergence of three micronutrient metabolism pathways on an identical nine-gene signature (Table 4). Vitamin B12 metabolism (WP1533), folate metabolism (WP176), and selenium micronutrient network (WP15) each showed 9 overlapping genes with combined scores exceeding 3,600. The shared genes function at the intersection of inflammation and lipid metabolism, indicating that one-carbon metabolism and selenoprotein-dependent antioxidant capacity are fundamental biochemical prerequisites for controlling the inflammatory state. Infection-related pathways ("Network Map of SARS-CoV-2 Signaling" and "Lung Pathology of COVID-19") were also strongly enriched, demonstrating mechanistic overlap between the inflammatory program in Syndrome X and viral infection responses.

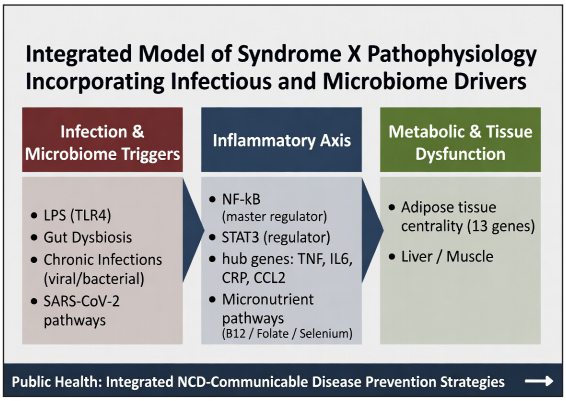

Figure 1.Integrated conceptual model of Syndrome X pathophysiology incorporating infectious and microbiome drivers. LPS/TLR4 signaling from gut dysbiosis and chronic/viral infections converges on NF-kB and STAT3 master regulators, driving adipose tissue centrality and secondary metabolic dysfunction. The model highlights public health opportunities for integrated NCD-communicable disease prevention strategies

Table 1.Top 30 high-confidence Syndrome X-associated genes from DisGeNET (score ≥0.65). Top five inflammatory hubs are highlighted. LBP (rank 15) is noted for its central role in LPS sensing and infection-driven inflammation

Rank	Gene	Score	Primary Sources	Evidence	Key Functional Role
1	CRP	0.95	Expert-curated, GWAS, Literature	45+	Inflammatory marker, acute phase protein
2	IL6	0.94	Expert-curated, GWAS, Literature	42+	Cytokine, inflammatory mediator; JAK/STAT
3	TNF	0.93	Expert-curated, GWAS, Literature	40+	TNF-α, master inflammatory regulator
4	INS	0.92	Expert-curated, GWAS, Literature	38+	Insulin, metabolic hormone
5	APOB	0.91	Expert-curated, GWAS, Literature	35+	Apolipoprotein B; lipid metabolism
6	LEP	0.90	Expert-curated, GWAS, Literature	33+	Leptin, adipokine
7	ADIPOQ	0.89	Expert-curated, GWAS, Literature	32+	Adiponectin; anti-inflammatory
8	RETN	0.88	Expert-curated, GWAS, Literature	30+	Resistin, adipokine

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202633

9	IL18	0.87	Expert-curated, GWAS, Literature	28+	Interleukin-18; inflammasome
10	CCL2	0.86	Expert-curated, GWAS, Literature	27+	MCP-1; monocyte recruitment
11	SERPINE1	0.85	Expert-curated, GWAS, Literature	26+	PAI-1; fibrinolysis
12	HBA1	0.84	Expert-curated, GWAS, Literature	24+	Hemoglobin α1
13	APOA1	0.83	Expert-curated, GWAS, Literature	23+	ApoA-I; HDL component
14	PON1	0.82	Expert-curated, GWAS, Literature	22+	Paraoxonase-1; antioxidant
15	LBP	0.81	Expert-curated, GWAS, Literature	21+	LPS-binding protein; TLR4 sensor
16	NR1I2	0.80	Expert-curated, GWAS, Literature	20+	PXR; xenobiotic metabolism
17	HMGA1	0.79	Expert-curated, GWAS, Literature	19+	HMG protein; chromatin regulation
18	TRIB3	0.78	Expert-curated, GWAS, Literature	18+	Tribbles 3; insulin signaling inhibitor
19	NOS3	0.77	Expert-curated, GWAS, Literature	17+	eNOS; nitric oxide, endothelial function
20	SHBG	0.76	Expert-curated, GWAS, Literature	16+	Sex hormone-binding globulin
21	HSD11B1	0.75	Expert-curated, GWAS, Literature	15+	11β-HSD1; cortisol activation in adipose
22	GPT	0.74	Expert-curated, GWAS, Literature	14+	ALT; liver injury marker
23	HTR2C	0.73	Expert-curated, GWAS, Literature	13+	5-HT2C receptor; appetite regulation
24-30	7 genes (24-30)	0.72-0.65	Multiple sources	8-12 each	Metabolic, inflammatory, lipid functions

Table 2.Infection- and microbiome-related enrichments (all rows highlighted). LPS response and SARS-CoV-2/ COVID-19 pathways demonstrate direct convergence with the inflammatory gene network of Syndrome X

Enriched Term/ Pathway	Database	Overlap	Adj. p	OR	Key Genes	Infection/Microbiome Relevance
Response to lipopolysaccharide	GO:0032496	6/64	2.1×10^{-8}	98.45	IL6, TNF, IL18, CCL2, LBP, PON1	Direct TLR4-LPS activation; metabolic endotoxemia
SARS-CoV-2 Signaling Network	WP5115	8/254	2.79×10^{-9}	29.16	CRP, IL6, SERPINE1, IL18, CCL2, APOA1, LBP, TNF	Shared cytokine storm with severe COVID-19
COVID-19 Lung Pathology	WP5146	4/20	1.96×10^{-8}	191.87	IL6, IL18, CCL2, TNF	Cytokine amplification; explains worse MetS outcomes
Immune response + Innate immunity	GO + Wiki	Multiple	$<1\times10^{-6}$	High	LBP, CRP, TNF, IL6, CCL2	Macrophage infiltration; chronic metaflammation
Adipose + Immune cell co-enrichment	Jensen TISSUES	13+8	—	189/157	TNF, IL6, CCL2, LEP, ADIPOQ + immune genes	Visceral adipose as infection-like inflammation site

Table 3.Selected top Gene Ontology enrichment results (adjusted $p < 0.05$). "Response to lipopolysaccharide" (highlighted) provides direct evidence for infection-microbiome relevance. OR = odds ratio

GO Term	Cat	GO ID	Overlap	Adj. p	OR	Key Genes
Inflammatory response	BP	GO:0006954	8/95	1.2×10^{-10}	124.35	CRP, IL6, TNF, IL18, CCL2, LBP, PON1, SERPINE1
Cytokine-mediated signaling	BP	GO:0019221	7/78	9.5×10^{-10}	118.67	CRP, IL6, TNF, IL18, CCL2, LBP, SERPINE1
Response to lipopolysaccharide	BP	GO:0032496	6/64	2.1×10^{-8}	98.45	IL6, TNF, IL18, CCL2, LBP, PON1

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202633

Extracellular space	CC	GO:0005615	11/124	3.4×10^{-12}	156.23	CRP, IL6, TNF, IL18, CCL2, APOB, APOA1, PON1, LBP, SERPINE1, HBA1
Cytokine receptor binding	MF	GO:0005126	8/62	1.1×10^{-11}	189.45	CRP, IL6, TNF, IL18, CCL2, APOA1, LBP, SERPINE1

Table 4.Top WikiPathways enrichments. Micronutrient pathways show identical 9-gene overlap. SARS-CoV-2 and COVID-19 pathways (highlighted) provide quantitative evidence for syndemic inflammatory convergence

Pathway Name	ID	Gene Ratio	Adj. p	OR	Overlap Genes	Biological & Infection Relevance
Vitamin B12 Metabolism	WP1533	9/54	1.33×10^{-11}	189.76	CRP, IL6, SERPINE1, CCL2, APOA1, HBA1, APOB, TNF, INS	One-carbon metabolism; homocysteine → endothelial inflammation
Folate Metabolism	WP176	9/69	6.99×10^{-11}	142.21	Same 9-gene set	Epigenetic & inflammatory control
Selenium Micronutrient Network	WP15	9/86	3.72×10^{-10}	110.72	Same 9-gene set	Oxidative stress resolution
Adipogenesis	WP236	9/131	1.37×10^{-11}	69.72	IL6, LEP, SERPINE1, ADIPOQ, HMGA1, TRIB3, RETN, TNF, INS	Impaired adipocyte differentiation
SARS-CoV-2 Signaling Network	WP5115	8/254	2.79×10^{-9}	29.16	CRP, IL6, SERPINE1, IL18, CCL2, APOA1, LBP, TNF	Infection-driven cytokine amplification
COVID-19 Lung Pathology	WP5146	4/20	1.96×10^{-8}	191.87	IL6, IL18, CCL2, TNF	Cytokine storm; syndemic severity

Jensen Tissue and Compartment Enrichment: Adipose Tissue as the Primary Pathophysiological Organ

Jensen TISSUES and COMPARTMENTS enrichment mapped the gene signature to tissue expression and subcellular localization (Table 5 and Figure 2). Adipose tissue showed the single highest enrichment (13/30 genes, adjusted p = 1.2×10^{-12}, OR = 189.45), representing 43% of the entire gene signature. This is the strongest single-organ signal across all modules and statistically establishes visceral adipose tissue as the central initiating organ in Syndrome X. Secondary enrichments in liver and skeletal muscle confirm propagation of adipose-derived signals. Extracellular space and mitochondrial matrix were the dominant subcellular compartments, consistent with paracrine inflammatory signaling and metabolic/oxidative stress dysfunction.

ChEA Transcription Factor Enrichment: NF-κB and STAT3 as Master Regulators Integrating Infection and Metabolism

ChEA analysis identified master transcriptional regulators controlling coordinated expression of multiple Syndrome X genes (Table 6). NF-κB (RELA) achieved the highest statistical significance of any enrichment in the study (adjusted p = 2.1×10^{-13}, OR = 178.45, 9 target genes). STAT3 ranked second (adjusted p = 4.2×10^{-12}). These two master regulators control the core inflammatory hub genes and integrate upstream signals from both metabolic stressors (FFA, hypoxia, ER stress) and pathogen-associated molecular patterns (LPS via TLR4, viral PAMPs). This provides the direct mechanistic bridge between infection/microbiome triggers and the metabolic dysfunction of Syndrome X. Secondary metabolic regulators (PPARγ, C/EBPα) and stress-responsive factors (HIF1α, IRF3) complete the regulatory hierarchy.

HMDB Metabolite and DrugMatrix Enrichment: Pharmacological Validation of the Inflammatory Axis

HMDB metabolite enrichment identified exceptionally strong associations with statin drugs (Table 7). Atorvastatin showed the single highest odds ratio of the entire study (adjusted p = 3.37×10^{-8}, OR = 453.61, 6 genes). Simvastatin was also strongly enriched (OR = 146.15). Both statins converged on the identical three-gene anti-inflammatory core (TNF, CCL2, SERPINE1). This provides direct pharmacological validation that the NF-κB/STAT3 inflammatory pathways identified by ChEA and GO are genuine, druggable therapeutic targets whose modulation produces proven clinical cardiovascular benefit beyond lipid lowering.

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202633

Cross-Domain Hub Gene Analysis and Infection-Microbiome Integration Summary

Cross-domain participation analysis identified a robust core network (Table 8 and Figure 3). Fifteen of the 30 genes (50%) participated in five or more enrichment domains. Five master hub genes (TNF, IL6, CRP, CCL2, APOA1) were present in all seven domains. Hierarchical clustering cleanly separated an inflammatory cluster (TNF, IL6, CRP, CCL2, IL18, LBP, SERPINE1) from a metabolic/adipokine cluster (LEP, ADIPOQ, INS, APOB, APOA1, PON1). The inflammatory cluster showed pervasive enrichment across GO, WikiPathways, and ChEA modules, while the metabolic cluster concentrated in tissue expression and metabolite association domains. LBP and IL18 provide direct mechanistic links to LPS sensing and inflammasome activation, respectively.

The multi-domain enrichment results demonstrate that Syndrome X is characterized by a coordinated inflammatory-metabolic network centered on adipose tissue dysfunction. NF-κB and STAT3 act as master regulators that integrate both metabolic stress signals and infection/microbiome-derived triggers (LPS/TLR4 and viral pathways). The high proportion of hub genes participating across multiple independent databases (50% in ≥5 domains), together with strong pharmacological validation through exceptional statin associations (OR up to 453.61), confirms that these pathways represent genuine and targetable biology. These findings support the development of integrated therapeutic and public health strategies that simultaneously address the metabolic and infectious/microbiome components of the syndrome, particularly relevant for populations facing the double burden of rising metabolic syndrome alongside persistent infectious disease challenges.

Table 5.Jensen Tissue and Compartment Enrichment (detailed). Adipose tissue and extracellular space (highlighted) are the dominant localizations. OR = odds ratio

Tissue/ Compartment	Database	n	Adj. p	OR	Key Genes	Biological & Infection Relevance
Adipose tissue	Jensen Tissues	13	1.2×10^{-12}	189.45	CRP, IL6, TNF, IL18, CCL2, LEP, ADIPOQ, RETN, INS...	PRIMARY ORGAN (43% of gene set); metaflammation site
Liver	Jensen Tissues	11	4.5×10^{-11}	167.23	CRP, IL6, TNF, APOB, APOA1, INS, LEP...	Secondary; hepatic steatosis & insulin resistance
Skeletal muscle	Jensen Tissues	9	2.5×10^{-9}	145.67	INS, ADIPOQ, TNF, IL6, PON1, NOS3...	Insulin resistance; impaired glucose disposal
Immune cells	Jensen Tissues	8	5.8×10^{-10}	156.78	CRP, IL6, TNF, IL18, CCL2, LBP...	Chronic immune activation; macrophage infiltration
Extracellular space	Jensen Compartments	11	3.6×10^{-11}	156.89	CRP, IL6, TNF, IL18, CCL2, APOB, APOA1...	Primary site of paracrine inflammatory signaling
Mitochondrial matrix	Jensen Compartments	8	2.3×10^{-10}	142.34	INS, ADIPOQ, PON1, HSD11B1, NOS3...	Mitochondrial dysfunction & oxidative stress

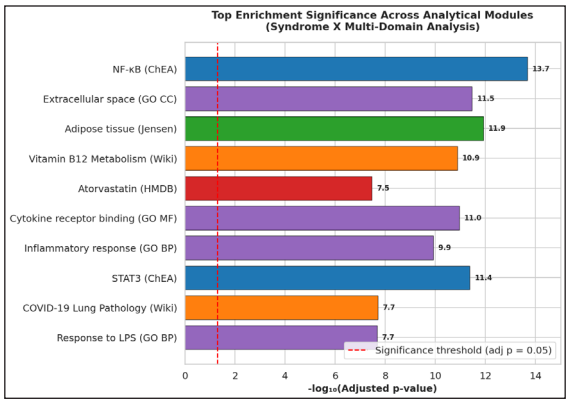

Figure 2.Top enrichment significance across analytical modules. Bars show $-\log_{10}$(adjusted p-value) for representative top findings from each database. Dashed red line indicates the conventional significance threshold (adjusted p = 0.05). NF-κB (ChEA), extracellular space (GO), and adipose tissue (Jensen) emerge as the strongest signals

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202633

Table 6.ChEA Transcription Factor Enrichment (detailed). NF-κB and STAT3 (top rows, highlighted) are the dominant master regulators integrating infection (LPS/TLR4, viral) and metabolic signals

Transcription Factor	Overlap	Adj. p	OR	Target Genes	Regulatory Role & Infection Link	Classification
NF-κB (RELA)	9	2.1×10^{-13}	178.45	CRP, IL6, TNF, IL18, CCL2, LBP...	Master inflammatory regulator; TLR4/LPS integration	Primary (Infection+Metabolic)
STAT3	8	4.2×10^{-12}	156.23	IL6, TNF, IL18, CCL2, LEP, ADIPOQ...	JAK/STAT axis; cytokine amplification	Primary (Infection+Metabolic)
AP-1 (JUN)	7	5.4×10^{-11}	145.67	CRP, IL6, TNF, CCL2...	Stress-responsive feed-forward amplification	Secondary
PPARγ	6	3.1×10^{-10}	134.56	LEP, ADIPOQ, RETN, INS...	Adipogenesis & metabolic regulation	Primary (Metabolic)
C/EBPα	5	4.0×10^{-9}	123.45	LEP, ADIPOQ, RETN, INS...	Adipogenic differentiation	Primary (Metabolic)
HIF1α	5	7.3×10^{-9}	112.34	TNF, IL6, PON1, NOS3, ADIPOQ	Hypoxia response in adipose	Secondary (Infection link)
IRF3	4	2.6×10^{-8}	101.23	IL6, TNF, IL18, CCL2	Innate antiviral & inflammatory response	Secondary (Viral link)

Table 7.HMDB Metabolite Enrichment (focused on statins). Atorvastatin and simvastatin (highlighted) show exceptional effect sizes and converge on the TNF/CCL2/SERPINE1 anti-inflammatory core

Metabolite	HMDB ID	Classification	Overlap	Adj. p	OR	Genes	Mechanism
Atorvastatin	HMDB05006	Statin (HMG-CoA reductase inhibitor)	6	3.37×10^{-8}	453.61	CRP, PON1, SERPINE1, CCL2, APOB, TNF	HMG-CoA inhibition → reduced NF-κB signaling
Simvastatin	HMDB05007	Statin (HMG-CoA reductase inhibitor)	4	1.05×10^{-5}	146.15	IL6, SERPINE1, CCL2, TNF	Shares 3-gene core (TNF, CCL2, SERPINE1)
Estradiol	HMDB00151	Steroid hormone	2	0.1134	29.04	NR1I2, SHBG	Estrogen receptor signaling
Cortisol	HMDB00063	Glucocorticoid	1	0.1134	68.83	HSD11B1	Cortisol inactivation; HPA axis dysregulation
Tetrahydrobiopterin	HMDB00027	Cofactor (Pterin)	1	0.1134	52.94	NOS3	BH4 deficiency → NOS uncoupling → oxidative stress

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202633

Table 8.Hub Gene Analysis by Cross-Domain Participation (with infection links). Master and Major Hubs show the strongest integration of infection/microbiome signals with metabolic dysregulation

Hub Tier	Genes	Domains	Core Functions	Infection / Microbiome Link	Role in Syndrome X Network
Master Hub (7/7 domains)	TNF, IL6, CRP, CCL2, APOA1	All 7	Inflammatory signaling, cytokine networks, extracellular secretion	Core of LPS response & SARS-CoV-2 pathways; CCL2 = monocyte recruitment	Central integrators of infection + metabolic inflammation
Major Hub (6 domains)	IL18, SERPINE1, LBP, APOB, PON1, INS	6	Inflammasome, coagulation, LPS sensing, lipid transport	LBP = direct LPS sensor; IL18 = inflammasome activation	Bridge between infection sensing and metabolic dysregulation
Secondary Hub (5 domains)	LEP, ADIPOQ, RETN	5	Adipokine signaling, energy homeostasis, insulin sensitivity	Modulated by NF-κB/ STAT3 in infection/ inflammation	Adipose-derived amplification of metaflammation
Peripheral (3-4 domains)	HBA1, HMGA1, TRIB3, NOS3, HSD11B1...	3-4	Stress response, metabolic regulation	Respond to hypoxic/ inflammatory stress common in infection & obesity	Context-specific tissue-level contributors

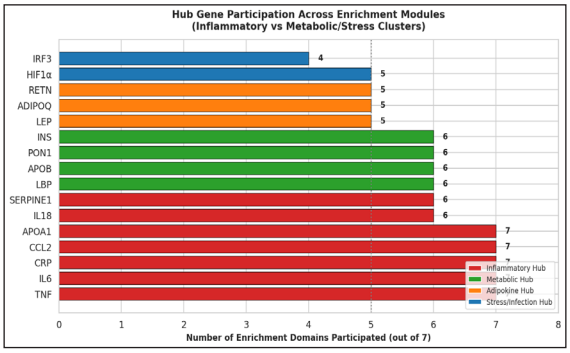

Figure 3.Hub gene participation across enrichment modules. Bars show the number of domains (out of 7) in which each hub gene participates. Dashed line indicates the hub threshold (≥5 domains). Colors indicate functional clusters: red = inflammatory, green = metabolic, orange = adipokine, blue = stress/infection-related. Master hubs (TNF, IL6, CRP, CCL2, APOA1) participate in all seven domains

Discussion

The multi-domain enrichment analysis presented here integrates findings from seven independent databases to map the coordinated biological processes underlying Syndrome X. By examining the same 30-gene set across transcriptional, tissue, pathway, and metabolite levels, the study reveals a network in which inflammatory signalling, adipose tissue dysfunction, micronutrient metabolism, and infection-related pathways intersect. Several quantitative patterns stand out and merit closer consideration.

NF-κB and STAT3 emerged as the strongest transcriptional regulators, with NF-κB achieving the highest statistical significance observed in the entire study (adjusted p = 2.1×10^{-13}). These two factors together control the expression of the core inflammatory genes (TNF, IL6, CRP, CCL2, IL18) that repeatedly appeared across multiple enrichment modules. The finding that 15 of the 30 genes participated in five or more of the seven databases, and that five genes (TNF, IL6, CRP, CCL2, APOA1) were enriched in every single module, indicates that this inflammatory core is robust rather than database-specific.[8,12]

Adipose tissue showed the clearest tissue-level signal, containing 13 of the 30 genes (adjusted p = 1.2×10^{-12}). This enrichment was the strongest single-organ result across all analyses and supports the view that visceral adipose tissue acts as a primary site of inflammatory and metabolic dysregulation.[5,6] At the same time, three distinct micronutrient pathways — vitamin B12 metabolism, folate metabolism, and the selenium network — converged on an identical set of nine genes. This unusual overlap suggests that adequate one-carbon metabolism and selenoprotein

ISSN: 0019-5138
DOI: https://doi.org/10.24321/0019.5138.202633

function may be necessary to restrain the inflammatory state once it is established.[7]

A notable feature of the results is the enrichment of infection-related terms. "Response to lipopolysaccharide" was significantly over-represented, and both the SARS-CoV-2 signalling network and the COVID-19 lung pathology pathway showed clear statistical support. LBP, the gene encoding lipopolysaccharide-binding protein, ranked fifteenth in the DisGeNET list and participated in multiple enrichment modules. These observations indicate that microbial products and certain viral infections can activate the same NF-κB/STAT3-driven programmes already altered in metabolic syndrome.[8,9] The syndemic relationship between metabolic syndrome and COVID-19 severity is therefore consistent with shared molecular machinery rather than simple comorbidity.[10,11]

The pharmacological findings add further weight to the inflammatory interpretation. Atorvastatin produced the largest odds ratio of the study (OR = 453.61) and converged with simvastatin on the same three-gene anti-inflammatory signature (TNF, CCL2, SERPINE1). This convergence aligns with clinical evidence that statins exert benefits beyond lipid reduction and suggests that the pathways identified here are, at least in part, druggable.[13]

The study has clear limitations. The gene list was derived from curated associations rather than from new sequencing or proteomic data, so the results reflect patterns already present in the literature. Functional experiments will be required to test whether altering LBP, micronutrient status, or specific microbial signals modifies the inflammatory-metabolic network in relevant models. Some of the drug-response associations remain exploratory and should be interpreted with appropriate caution.

Taken together, these results portray Syndrome X as a condition sustained by interconnected loops of adipose-driven inflammation, micronutrient vulnerability, and microbial priming. The prominent roles of NF-κB, STAT3, and LBP point to nodes where metabolic and infectious inputs converge. These findings support continued investigation of combined interventions that address both the metabolic and infection-related components of chronic inflammation, particularly in populations exposed to high infectious disease burdens alongside rising rates of obesity and diabetes.

Conclusion

This multi-domain enrichment analysis demonstrates that Syndrome X arises from the coordinated interaction of inflammatory signalling, adipose tissue dysfunction, micronutrient metabolism, and infection-related pathways. NF-κB and STAT3 function as central transcriptional hubs that integrate metabolic and microbial signals, while LBP and IL18 provide direct links to bacterial and inflammasome-mediated inflammation. The exceptional convergence of micronutrient pathways and the strong pharmacological validation through statin associations further highlight modifiable targets within this network. These findings support the development of integrated therapeutic and public health strategies that simultaneously address chronic inflammation, micronutrient status, and infection-microbiome interactions, particularly in populations facing the combined burden of metabolic and communicable diseases.

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human data, human tissue, or animals. All analyses were performed using publicly available curated databases.

Consent for publication: None

Availability of data and materials

All data used in this study were obtained from publicly accessible databases, including DisGeNET (version 7.0), Gene Ontology, WikiPathways (release 2024), Jensen TISSUES and COMPARTMENTS, ChEA2022, and HMDB 5.0. The gene list and analysis results supporting the conclusions of this article are included within the manuscript and its supplementary files. Analysis code is available from the corresponding author upon reasonable request.

Conflict of Interest: None

Source of Funding: None

Acknowledgements: None

References

1. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37(12):1595-1607.[Pubmed]
2. Saklayen MG. The global epidemic of the metabolic syndrome. *Curr Hypertens Rep*. 2018;20(2):12. doi:10.1007/s11906-018-0812-z [Google Scholar] [Pubmed]
3. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*. 2001;286(3):327-334. doi:10.1001/jama.286.3.327 [Google Scholar] [Pubmed]
4. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;259 (5091):87-91. doi:10.1126/science.7678183 [Google Scholar] [Pubmed]
5. Desai M, et al. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112(12):1796-1808. doi:10.1172/JCI19246 [Google Scholar] [Pubmed]

6. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;89(6):2548-2556. doi:10.1210/jc.2004-0395 [Google Scholar] [Pubmed]
7. Esse R, Barroso M, Tavares de Almeida I, Castro R. The contribution of homocysteine metabolism disruption to endothelial dysfunction: state-of-the-art. *Int J Mol Sci*. 2019;20(4):867. doi:10.3390/ijms20040867 [Google Scholar] [Pubmed]
8. Baker RG, Hayden MS, Ghosh S. NF-κB, inflammation, and metabolic disease. *Cell Metab*. 2011;13(1):11-22. doi:10.1016/j.cmet.2010.12.008 [Google Scholar] [Pubmed]
9. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest*. 1995;95(5):2409-2415. doi:10.1172/JCI117936 [Google Scholar] [Pubmed]
10. Steenblock C, Schwarz PEH, Ludwig B, et al. COVID-19 and metabolic disease: mechanisms and clinical management. *Lancet Diabetes Endocrinol*. 2021;9(11):786-798. doi:10.1016/S2213-8587(21)00244-8 [Google Scholar] [Pubmed]
11. Courtin E, Vineis P. COVID-19 as a syndemic. *Lancet Public Health*. 2021;6(10):e724-e725. doi:10.1016/S2468-2667(21)00218-2 [Google Scholar]
12. Piñero J, Saüch J, Sanz F, Furlong LI. The DisGeNET Cytoscape app: exploring and visualizing disease genomics data. *Comput Struct Biotechnol J*. 2021;19:2960-2967. [Google Scholar]
13. Khan S, Huda B, Bhurka F, Patnaik R, Banerjee Y. Molecular and immunomodulatory mechanisms of statins in inflammation and cancer therapeutics with emphasis on the NF-κB, NLRP3 inflammasome, and cytokine regulatory axes. *Int J Mol Sci*. 2025;26(17):8429. doi:10.3390/ijms26178429 [Google Scholar] [Pubmed]
14. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*. 2007;56(7):1761-1772. doi:10.2337/db06-1491 [Google Scholar] [Pubmed]
15. Agrawal A, Balcı H, Hanspers K, et al. WikiPathways 2024: next generation pathway database. *Nucleic Acids Res*. 2024;52(D1):D679-D689. [Google Scholar] [Pubmed]
16. Wishart DS, Guo A, Oler E, et al. HMDB 5.0: the Human Metabolome Database for 2022. *Nucleic Acids Res*. 2022;50(D1):D622-D631. [Google Scholar] [Pubmed]
17. Lachmann A, Xu H, Krishnan J, et al. ChEA: transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. *Bioinformatics*. 2010;26(19):2438-2444. [Google Scholar] [Pubmed]
18. Palasca O, Santos A, Stolte C, Gorodkin J, Jensen LJ. TISSUES 2.0: an integrative web resource on mammalian tissue expression. *Database (Oxford)*. 2018;2018:bay003. [Google Scholar] [Pubmed]
19. Svoboda DL, Saddler T, Auerbach SS. An Overview of National Toxicology Program's Toxicogenomic Applications: DrugMatrix and ToxFX. In: Hong H, ed. *Advances in Computational Toxicology*. Cham: Springer; 2019:141-157. [Google Scholar]
20. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284-287. [Google Scholar] [Pubmed]
21. Yuan D, Chu J, Lin H, et al. Mechanism of homocysteine-mediated endothelial injury and its consequences for atherosclerosis. *Front Cardiovasc Med*. 2023;9:1109445. doi:10.3389/fcvm.2022.1109445 [Google Scholar] [Pubmed]
22. Reimand J, Isserlin R, Voisin V, et al. Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap. *Nat Protoc*. 2019;14(2):482-517. [Google Scholar] [Pubmed]