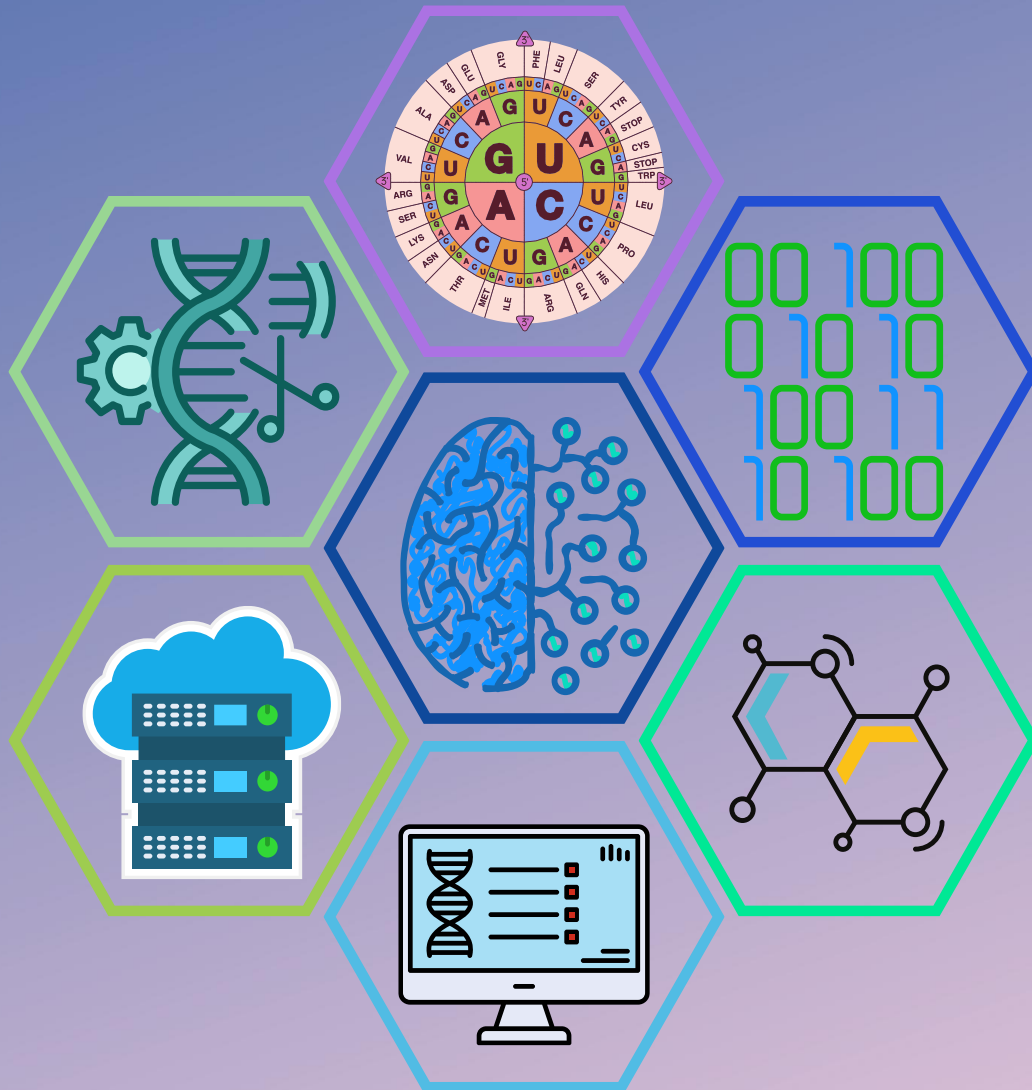


YOUNG MINDS AT WORK: BLENDING HUMAN GENETICS AND BIOINFORMATICS

Abstract Book



On-line workshop
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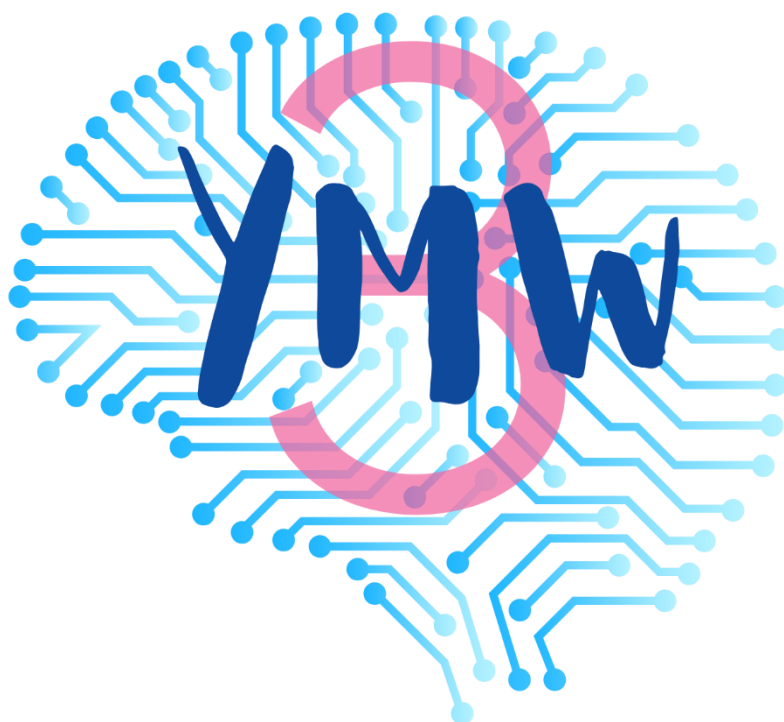


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YMW 2026 Organizing Team

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Invited Speakers

INVITED SPEAKERS

Brunella Franco

Università degli Studi di Napoli "Federico II", Napoli

"The journey from gene identification to basic cell biology"

Alexander Hoischen

Radboudumc University medical center Nijmegen

"Long-read genome sequencing – the next rare disease revolution"

Alexandre Reymond

Center for Integrative Genomics, University of Lausanne

"Copy number variants effects"

Christian Giliissen

Radboudumc University medical center Nijmegen

"Blending Genetics and Bioinformatics to study neurodevelopmental disorders"

Isidro Cortes

EMBL-EBI, United Kingdom

"Computational early cancer genome analysis at a national scale"

AWARDS

Oral communication

Sabrina Busciglio

University of Parma

“Beyond DNA Sequence: DNA methylation as a functional classifier in germline neurodevelopmental disorders and somatic rare tumors”

Poster

Aurora Gronchi

University of Rome “Tor Vergata”

“A Cross-Tissue Meta-Analysis of Transcriptomic Data to Define a Robust Molecular Identity of Human Fibrosis”

Oral Abstracts

A Graph-Based Transcriptomic Framework Unravels Divergent Immune Trajectories in Systemic Lupus Erythematosus

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Background: Transcriptomics is pivotal for uncovering molecular mechanisms in complex diseases, yet substantial patient heterogeneity often masks biological signals. Furthermore, traditional clustering approaches are constrained by rigid geometric assumptions, overlooking the intricate non-linear relationships among clinical samples.

Methods: A graph-based analytical framework was developed to systematically deconvolve disease heterogeneity. The pipeline employs feature selection via median absolute deviation, followed by the construction of a patient similarity network using mutual k-nearest neighbors and weighted cosine similarity. Edge weights are refined using Euclidean distances in low-dimensional space, with long-range connections pruned to enhance local structure. Community structures are identified by integrating multiple detection algorithms (Louvain, Walktrap, and Fast Greedy) to maximize modularity. The resulting subtypes are validated against clinical indices, immune cell infiltration profiles, and functional pathways.

Results: Applied to a peripheral blood cohort of 1,760 patients with systemic lupus erythematosus (SLE), the framework robustly identified eight molecular subtypes. These subtypes demonstrated high intra-group molecular consistency and inter-group heterogeneity in clinical phenotypes and immunological profiles, validating the biological relevance of the network partitioning. Topologically, Subtypes 1 and 5 clustered proximally to healthy controls, representing "clinically quiescent" states; Subtype 1 was characterized by suppressed interferon signaling, while Subtype 5 showed upregulated collagen trimerization. Tracing the disease trajectory toward severity, Subtype 6 emerged as a critical intermediate state bridging quiescence and destruction, clinically presenting with elevated anti-double-stranded DNA (anti-dsDNA) antibodies. Furthermore, occupying the most distal network position, Subtype 7 exhibited the most severe clinical features, including the highest anti-dsDNA positivity and significant hypocomplementemia. Transcriptomically, this subtype was defined by hyper-activated cell cycle, DNA replication, and translation machinery. Immunological deconvolution revealed a specific expansion of circulating monocytes and CD8⁺ T cells, coinciding with relative neutropenia. Conversely, Subtype 8 revealed an alternative severe trajectory dominated by neutrophilic inflammation and suppressed metabolic activity. This distinction highlights a divergence in pathogenic mechanisms—lymphoid proliferation versus neutrophil-driven inflammation—at the severe end of the spectrum.

Conclusions: This network-based framework successfully transcends static clustering to deconvolve SLE heterogeneity into clinically actionable phenotypes—ranging from immunological quiescence to divergent severe states dominated by CD8⁺ T cell proliferation versus neutrophilic inflammation.

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Beyond DNA sequence: DNA methylation as a functional classifier in germline neurodevelopmental disorders and somatic rare tumors

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Background: Accurate variant interpretation and disease classification remain major challenges in both germline neurodevelopmental disorders (NDDs) and rare tumor diagnostics. DNA methylation profiling represents a functional molecular layer capturing the downstream consequences of genetic alterations. In the germline setting, disease-specific episignatures support interpretation of variants in chromatin-related genes such as SETD5. In oncology, methylation-based classifiers refine tumor stratification by integrating histopathological and genomic information. This study aimed to combine human genetics and bioinformatics approaches to evaluate DNA methylation profiling as a unifying functional classifier across germline and somatic contexts.

Methods: For the germline cohort, genome-wide DNA methylation profiles were generated from peripheral blood DNA of patients carrying pathogenic or likely pathogenic SETD5 variants using the Illumina Infinium EPICv2 array. Raw data were processed with the R package MethylCallR. Differentially methylated positions (DMPs) were identified and pathway/GO enrichment analyses performed. To increase statistical power and assess epigenetic overlap, we included 54 healthy controls and patients with other chromatinopathies: Sotos (n=20), CHARGE (n=20), Kabuki (n=20), Coffin–Siris (n=20), and Nicolaides–Baraitser (n=16) syndromes. In the somatic setting, genome-wide methylation profiles were generated from DNA extracted from FFPE rare tumor samples and analyzed using a validated methylation-based classification platform (Epignostix), enabling supervised classification with integrated quality control metrics.

Results: Principal component and clustering analyses clearly separated affected individuals from controls. Two siblings harboring the same SETD5 variant but showing discordant phenotypes exhibited divergent methylation profiles: the mildly affected sibling clustered with controls, whereas the severely affected sibling displayed a markedly altered methylation pattern. These findings support an epigenetic modulatory layer influencing genotype–phenotype correlation, reinforcing an epi-phenotype–genotype relationship. In the somatic cohort, methylation-based classification showed strong concordance with histopathological and clinical assessment, confirming the reliability of the classifier on FFPE material. Integrating genomic, histological, and epigenetic data enabled refined molecular tumor identity assignment, highlighting methylation profiling as a systems-level readout of the cumulative impact of somatic alterations.

Conclusion: Integrating human genetics and computational epigenomics demonstrates that DNA methylation profiling provides a cross-context functional framework bridging genetic variation and clinical phenotype, strengthening precision medicine implementation.

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Ruan Jian Qing Mai Formula Attenuates NINJ1-Mediated Macrophage Ferroptosis to Improve Diabetic Wound Healing

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Background: Impaired diabetic foot ulcer (DFU) healing is driven by unresolved inflammation and cellular dysfunction. While macrophage ferroptosis is implicated in this pathology, its specific mechanisms and intercellular impact remain unclear. This study integrates single-cell transcriptomics and biomedical approaches to investigate macrophage ferroptosis in DFU and evaluate the therapeutic mechanism of the Ruan Jian Qing Mai formula (RJQM) targeting the NINJ1-STAT3 axis.

Methods: We analyzed single-cell (scRNA-seq) and bulk RNA-seq data from human DFU tissues to assess ferroptosis activity, cellular communication networks, and differential gene expression. These bioinformatics findings were validated using a streptozotocin-induced diabetic mouse model and macrophages stimulated with high glucose and lipopolysaccharide. Protein and gene expression, ferroptosis markers, and macrophage-endothelial crosstalk (migration and tube formation) were evaluated in vitro and in vivo.

Results: scRNA-seq profiling revealed pronounced cellular heterogeneity, identifying macrophages as the population with the highest ferroptosis activity scores in the wound microenvironment. Intercellular communication analysis demonstrated profoundly disrupted macrophage-endothelial crosstalk in DFU. Furthermore, intersecting differentially expressed genes with protein-protein interaction networks identified STAT3 as a core hub transcription factor. Experimentally, RJQM accelerated healing in diabetic mice by suppressing macrophage ferroptosis, reducing iron overload, and enhancing GPX4/xCT expression. Validating the bioinformatics predictions, RJQM effectively disrupted a positive feedback loop involving NINJ1, ferroptosis, and downstream STAT3 activation. Finally, conditioned media assays confirmed that RJQM rescued impaired macrophage-endothelial crosstalk, restoring pro-angiogenic endothelial cell functions in a NINJ1-dependent manner.

Conclusion: Blending bioinformatics with experimental validation, we establish that RJQM promotes DFU healing by targeting the NINJ1-STAT3-ferroptosis pathway in macrophages. This suppresses inflammation and restores critical macrophage-endothelial communication, highlighting a novel therapeutic strategy for diabetic wounds.

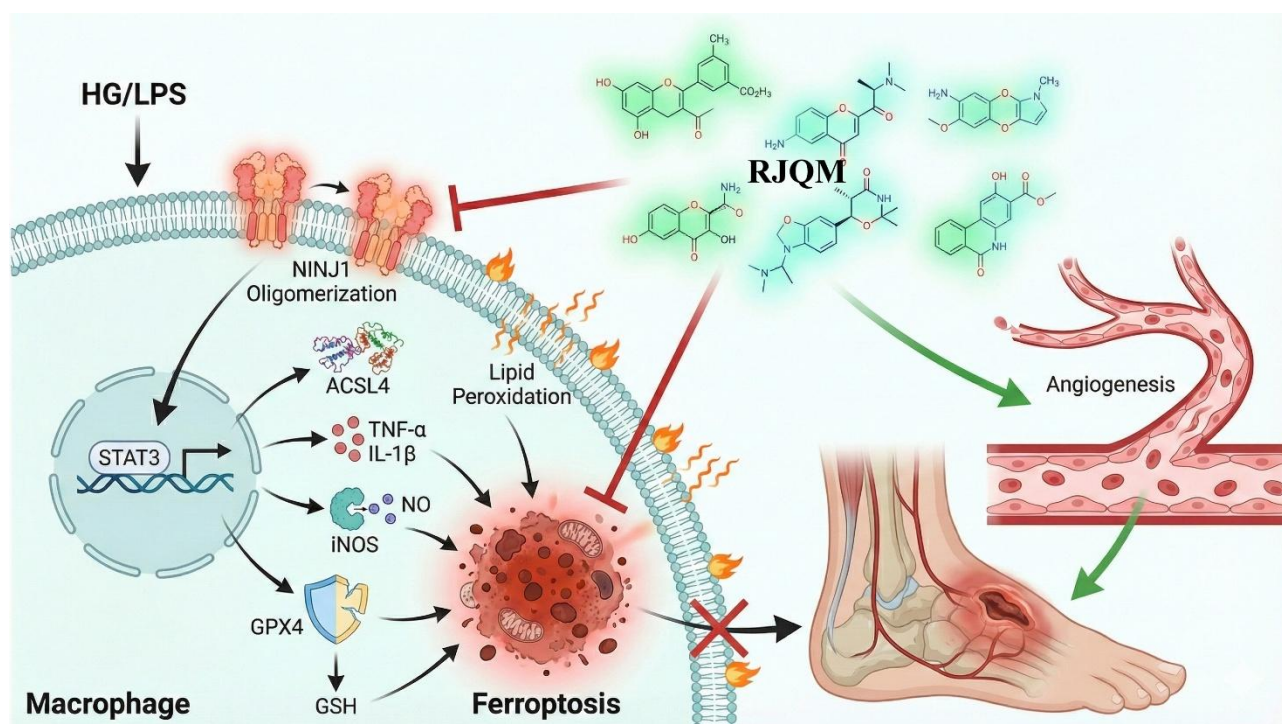


Figure 1. Schematic illustration of the proposed molecular mechanism. In a high glucose and lipopolysaccharide (HG/LPS) environment, macrophage ferroptosis is induced, impairing diabetic wound healing. The process is initiated by the activation of the NINJ1 signaling pathway, which promotes the transcription of STAT3, ACSL4, and pro-inflammatory factors including TNF- α , IL-1 β , and iNOS. The subsequent upregulation of NINJ1 expression and its oligomerization, coupled with a decrease in intracellular glutathione (GSH) and the inactivation of glutathione peroxidase 4 (GPX4), leads to an accumulation of lipid peroxidation. This, along with increased ACSL4, culminates in ferroptosis. The ferroptotic cell death and the associated inflammatory response inhibit angiogenesis and cell migration, thereby delaying the healing of diabetic wounds. The "Ruan Jian Qing Mai Formulation" is shown to intervene in this pathway, mitigating ferroptosis and promoting wound healing.

Integrating RNA-Seq to Improve Molecular Diagnosis in Unresolved Genetic Diseases

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Background: Rare genetic disorders are a heterogeneous group of conditions that remain challenging to diagnose. Despite advances in Whole-Exome (WES) and Whole-Genome Sequencing (WGS) [1,2,3], about 50% of patients lack a molecular diagnosis[4], partly due to the difficult interpretation of non-canonical splicing and regulatory variants[5]. RNA sequencing (RNA-Seq) offers a complementary approach to identify transcriptional alterations, including aberrant gene expression and splicing defects in unresolved cases[6]. This project aims to improve the diagnostic rate in children and adults with infancy-onset suspected rare genetic disorders lacking a molecular diagnosis, using a two-step strategy: WES followed, in negative cases, by RNA-Seq of patient-derived fibroblasts. Although disease-relevant tissues are ideal for transcriptomic analysis, they are often inaccessible. Skin-derived fibroblasts offer a minimally invasive alternative with broad transcriptomic coverage[7,8,9].

Methods: A dedicated computational pipeline was developed and benchmarked using RNA-Seq data from patients carrying known splicing or expression-altering variants (n=7). The analysis focuses on three transcriptomic signatures: aberrant gene expression[10], splicing abnormalities[11], and extreme allelic imbalance. Public fibroblast RNA-Seq datasets were integrated to increase statistical power[12]. The workflow includes data preprocessing, quantification, and outlier detection using a one-vs-all approach. Candidate genes are prioritized through integration of transcriptomic findings with clinical phenotypes.

Results: Fibroblasts from three healthy controls and eighteen patients were isolated and characterized. Analysis of different culture passages revealed variability in gene expression profiles, leading to a selection of a standardized passage for subsequent analyses. In the validation cohort (n=7), the pipeline identified four out of six known disease-associated genes at the expression level and detected the expected splicing defect. The two genes that were not detected had expression levels that fell below, or were at, the threshold established in the analysis. We are currently sequencing and analyzing the patients (n=11) for whom the underlying genetic cause has not yet been identified.

Conclusion: Integration of RNA-Seq with genomic data is expected to improve diagnostic yield and advance understanding of disease mechanisms, ultimately supporting more precise and personalized care. Previous studies reported an 8-36% increase in diagnostic rates with RNA-Seq[6], and a similar improvement is anticipated in our cohort.

Acknowledgments

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Characterization of Brain Microbiome in Neurodegenerative Disease via Metatranscriptomics

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Background: The human microbiome, comprising bacteria, archaea, viruses, and eukaryotic microbes, inhabits various body sites, including traditionally sterile regions such as the lungs, bloodstream, and brain (Ogunrinola et al., 2020). Microorganisms modulate human physiology through host-microbe interactions, and dysregulation of the gut-brain-microbiota axis has been implicated in neurological disorders, including neurodegenerative diseases (Martin et al., 2018). Standard RNA-Seq analyses of human tissues typically focus on host transcripts, while a substantial fraction of reads remains unmapped and is often discarded. These unmapped reads may contain biologically informative microbial sequences and can be leveraged to profile active microbial communities in human brain samples.

Methods: Five publicly available human brain transcriptomic datasets were collected from the GEO database, each comprising Alzheimer's Disease (AD) patients and controls. Following quality control and read filtering, samples were aligned to the human reference genome using a splice-aware aligner. Unmapped reads were extracted and analyzed by metatranscriptomics, including taxonomic classification and functional profiling. Microbial diversity and abundance were quantified, and differential abundance was assessed within each study and across studies via meta-analysis. In addition, microbial genome-level analyses were performed to compare transcriptional signals across taxa and identify shared expressed genes. The workflow is summarized in Figure 1.

Results: Across 300 AD patients and 168 controls, actively transcribed microbial taxa were detected from unmapped RNA-Seq reads. Differential abundance analyses revealed study-specific signals, and meta-analysis identified 14 taxa consistently associated with AD, including *Salmonella enterica* and *Streptococcus pneumoniae*. Genome-level comparisons further supported shared gene-expression patterns across multiple taxa.

Conclusion: These findings demonstrate that unmapped RNA-Seq reads from human brain transcriptomes can be repurposed for sensitive metatranscriptomic profiling, enabling cross-study integration of microbial features in Alzheimer's disease. Notably, *Streptococcus pneumoniae*, identified by meta-analysis, has been linked to meningitis (Gil et al., 2023). This framework provides a scalable strategy for investigating active microbial communities in neurodegeneration and can be extended to compare microbial and functional patterns across multiple neurodegenerative diseases.

Workflow

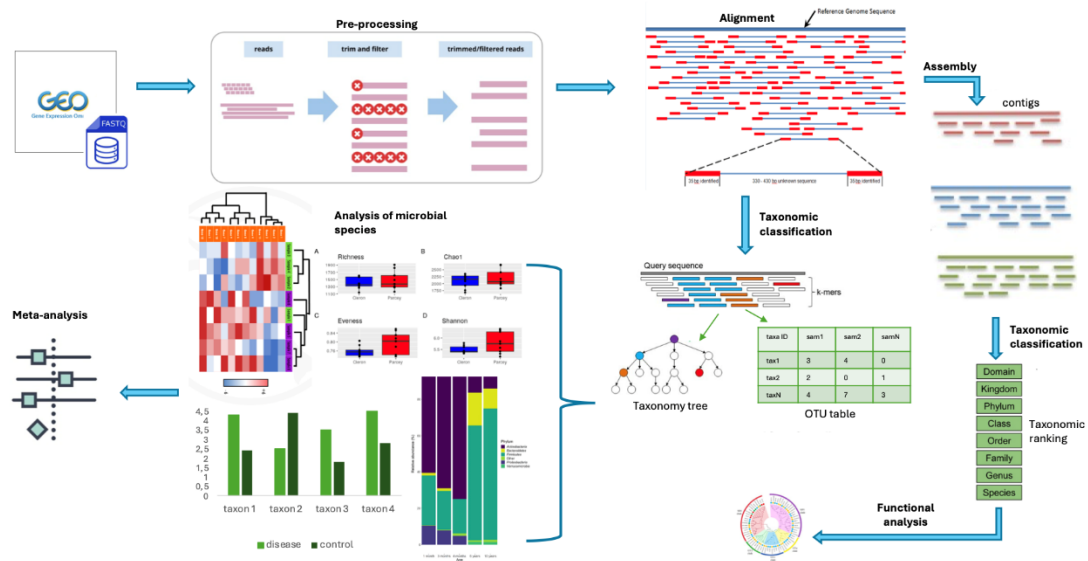


Fig. 1 Overview of the project workflow, including data collection, read filtering, alignment to the human reference genome, and downstream microbial analyses.

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Do phenolic metabotypes reflect genetic risk signatures for cardiometabolic diseases? Exploring genomic complexity using the PGS Catalog

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Background: (Poly)phenols are bioactive compounds abundant in plant-based food, associated with cardiometabolic and anti-inflammatory benefits. However, responses to (poly)phenol intake vary widely among individuals. Metabotyping – clustering individuals by metabolic profiles – allows to capture this variability and partially explains association with disease risk. In parallel, advances in genomics allowed the quantification of genetic predisposition through polygenic scores (PGS), aggregating findings from genome-wide association studies. The integration of phenolic metabotypes and PGS has not yet been explored. In this study, we investigated whether phenolic metabotypes reflect underlying genetic risk for cardiometabolic traits and diseases.

Methods: We analysed data from the Oral Polyphenol Challenge Test (OPCT) study, including genetic and metabolic profiles. The OPCT cohorts comprised 300 individuals, aged 18-74 years, with slightly more female (57%), of Southern European origin, and - in general - good health. Individuals were classified as low (qL) and high (qH) phenolic producers, based on previously identified metabotypes. After genotyping (Illumina GSA) and genotype imputation, scores from the PGS Catalog were computed and compared between metabotypes using non-parametric tests. Principal Component Analysis (PCA) identified the most contributing PGS, and a composite metaPGS was derived. Computational annotation and functional analyses were performed on genes underlying significant PGS.

Results: A total of 4,489 PGS calculated from the PGS Catalog were tested, identifying 124 significant PGS differently associated with metabotypes ($p < 0.05$). The most represented categories were anthropometric traits (32 PGS), cardiometabolic traits (31 PGS) and cancer (21 PGS). Notable association included ovarian cancer ($p = 0.0008$), HDL cholesterol ($p = 0.003$), type 2 diabetes ($p = 0.006$), and coronary artery disease ($p = 0.013$). PCA revealed components enriched for brain morphology (PC1, $R^2 = 0.069$) and cardiometabolic traits (PC2, $R^2 = 0.05$). The metaPGS explained a small portion of phenotypic variability between metabotypes ($R^2 = 0.048$, $p = 0.00551$). Functional analysis highlighted genes involved in (poly)phenol metabolism and bioavailability, suggesting possible pleiotropic effects.

Conclusion: This study explored the possibilities of merging personalized nutrition (metabotypes) and personalized genomics (PGS) approaches. Integrating metabotypes with PGS uncovers genetic stratification relevant to (poly)phenol metabolism and cardiometabolic risk, offering novel opportunities for more accurate precision nutrition strategies.

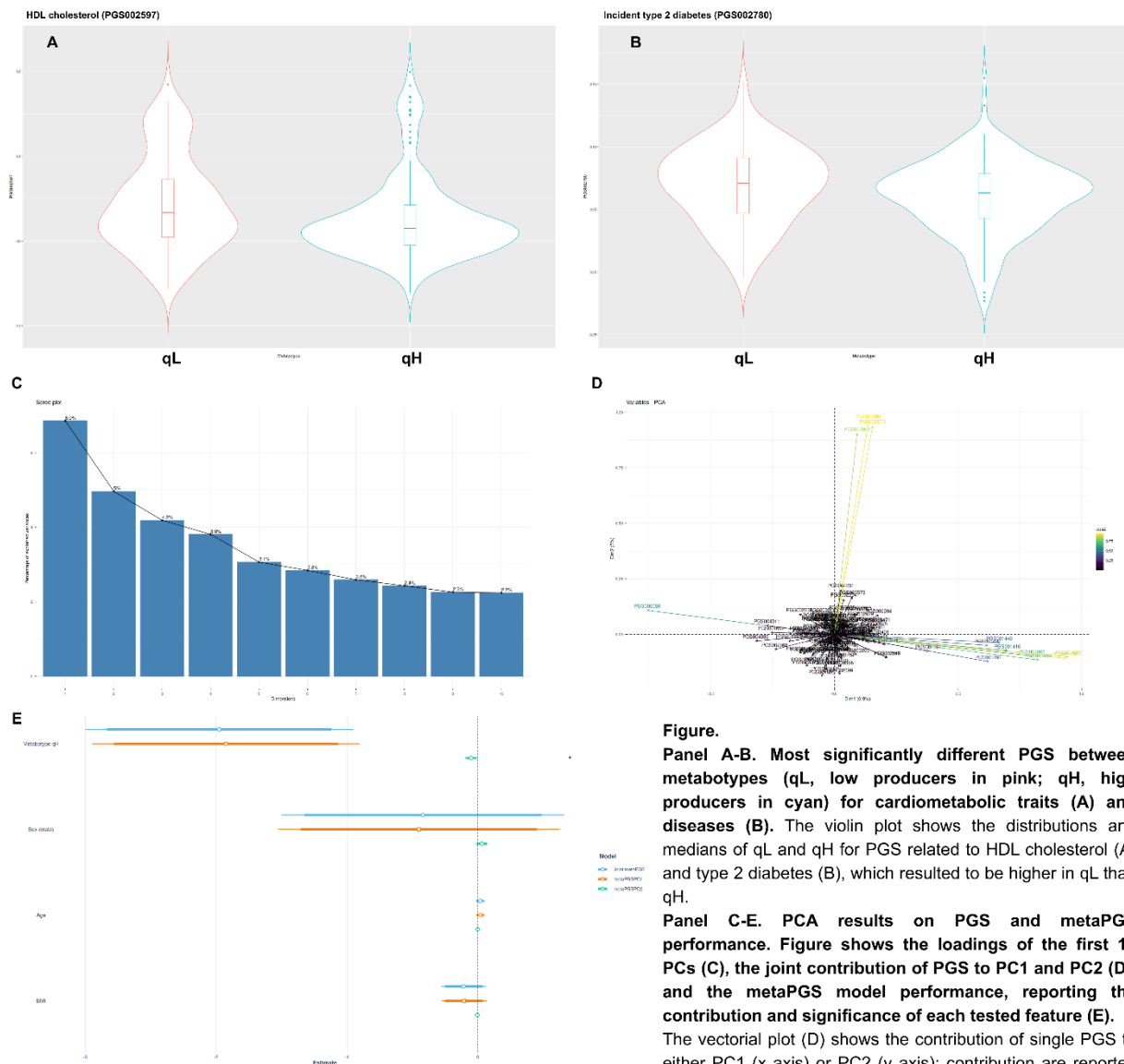


Figure.

Panel A-B. Most significantly different PGS between metabolotypes (qL, low producers in pink; qH, high producers in cyan) for cardiometabolic traits (A) and diseases (B). The violin plot shows the distributions and medians of qL and qH for PGS related to HDL cholesterol (A) and type 2 diabetes (B), which resulted to be higher in qL than qH.

Panel C-E. PCA results on PGS and metaPGS performance. Figure shows the loadings of the first 10 PCs (C), the joint contribution of PGS to PC1 and PC2 (D), and the metaPGS model performance, reporting the contribution and significance of each tested feature (E).

The vectorial plot (D) shows the contribution of single PGS to either PC1 (x axis) or PC2 (y axis); contribution are reported with their cos2 value, colour-coded as follow: black (no significant contribution), dark blue (low contribution), green (medium contribution), yellow (high contribution).

Acknowledgments

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GWAS by subtraction to Disentangle RBD Genetic Background from α -Synucleinopathies

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Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by loss of muscle atonia and abnormal behaviors occurring during REM sleep. Idiopathic RBD (iRBD) is recognized as the strongest prodromal hallmark of α -synucleinopathies, with a conversion rate to a neurodegenerative condition that reaches up to 96.6% at 15 years of follow-up. RBD-converters display a more severe clinical trajectory compared to those that do not present with RBD. However, the extent to which iRBD represents a distinct genetic entity or an early manifestation of neurodegeneration remains unclear.

To address this, we deployed a GWAS-by-subtraction approach via Genomic Structural Equation Modeling (GenomicSEM) on the largest available GWAS data for PD, RBD, DLB, and MSA to design a two-latent factors (hereafter F1 and F2) model to disentangle the genetic architecture of iRBD from the shared genomic liability across α -synucleinopathies.

Our analysis highlighted the SNCA locus as a key genetic regulator of iRBD susceptibility, both at coding and non-coding levels. Colocalization via MetaXCan with eQTL and sQTL signals further emphasized its central role in regulating brain-specific transcriptomic levels, mostly affecting the substantia nigra and the cerebellum.

Using Linkage Disequilibrium Score Regression (LDSC) and Mendelian Randomization (MR), we evaluated genomic correlations and potential causal associations between the two latent variables and a set of highly heritable traits and several neuroanatomical outcomes. LDSC revealed a positive genomic correlation between F1 and daytime napping, telomere length and parental lifespan, while F2 showed an opposite trend.

MR revealed that increased F1 liability is associated with reduced volumes of the mid-anterior corpus callosum and right accumbens, and increased precuneus volume, suggesting early genetically driven structural changes that may serve as biomarkers for RBD progression.

In conclusion, iRBD liability is embedded within a genetic signature providing compelling evidence for its high likelihood of phenoconversion, supporting a continuum model of α -synucleinopathies disease risk, where early neuroanatomical changes may serve as biomarkers to aid in disease stratification and risk prediction, potentially improving early intervention strategies.

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Integrated Genetics and Multi-omics Analysis Identifies Myeloid-derived CHI3L1 as a Core Driver of Neutrophil Storms and Structural Remodeling in Ulcerative Colitis

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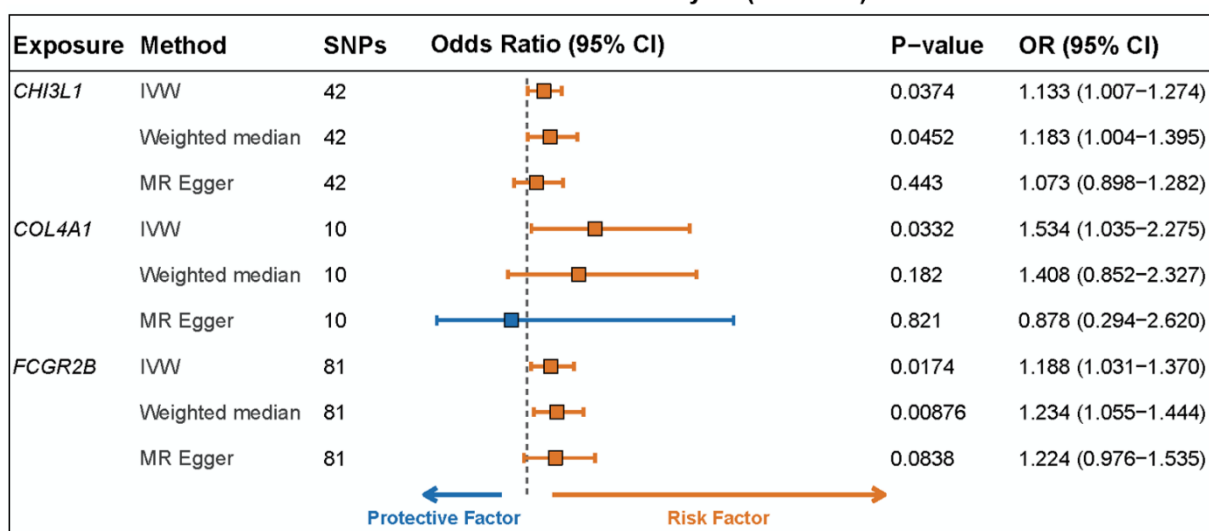
Background and Aims: Ulcerative colitis (UC) is characterized by acute inflammatory flares and the catastrophic loss of basement membrane (BM) integrity. This study aims to identify the core regulatory hub driving the transition from inflammation to pathological BM remodeling and early fibrosis, elucidating the underlying cascade mechanisms.

Methods: We utilized Mendelian Randomization (MR) to establish the causal link between CHI3L1 and UC. Single-cell RNA sequencing (scRNA-seq) integrated with network-based in silico knockout (virtual perturbation) quantified CHI3L1's regulatory weight. Multi-omics integration (microbiome/metabolomics) delineated pathways driving dysbiosis and metabolic toxicity. Findings were validated through in vivo pharmacological blockade, assessing immune infiltration, metabolic disturbances, and BM damage.

Results: In the UC microenvironment, barrier dysfunction-derived PAMPs/DAMPs drive explosive myeloid CHI3L1 expression. MR confirmed a robust causal association between CHI3L1 and UC risk, positively correlating with neutrophil infiltration. ScRNA-seq and virtual perturbation analysis identified CHI3L1 as a central orchestrator of neutrophil destructive potential, exerting a 26.8-fold regulatory drive on the matrix-degrading enzyme MMP9. Crucially, KEGG enrichment of perturbed genes revealed the IL-17 signaling pathway as the primary downstream network regulated by CHI3L1, positioning it as a pivotal amplifier of IL-17-mediated inflammation. CHI3L1 surge induced secondary dysbiosis (Roseburia depletion and Bacteroides expansion) and D-Sphingosine accumulation, exacerbating genomic oxidative stress. Structural dynamic analysis showed MMP9 and COL4A1 exhibit profound "pathological synchrony" ($r=0.90$), unveiling a "degradation-deposition" vicious cycle leading to BM collapse. Finally, pharmacological CHI3L1 inhibition suppressed neutrophil storms, reversed oxidative damage, and restored organized COL4A1 deposition.

Conclusions: CHI3L1 is a central hub bridging IL-17-mediated immune dysregulation with intestinal structural failure. The CHI3L1/IL-17/Neutrophil axis triggers an "oxidation-degradation" cascade that disrupts BM repair kinetics, accelerating pathological remodeling and fibrosis in UC.

Mendelian Randomization Analysis ($P < 5e-8$)



Acknowledgments

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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On the development of neoantigen vaccine for HCC cure: from genomics of Hepatitis B Virus (HBV) integration in liver cells genome to immunological host response toward neoepitopes as novel immunotherapeutic approaches

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Background: Chronic hepatitis B virus (HBV) infection remains a major global health burden and is a leading cause of hepatocellular carcinoma (HCC), for which curative options are limited and systemic therapies provide only modest survival benefit. Beyond chronic inflammation, HBV contributes to hepatocarcinogenesis through integration into the hepatocyte genome, a hallmark detected in most HBV-related HCC (HBV-HCC). Integrated HBV sequences can be transcriptionally active and generate virus–host chimeric transcripts encoding junction-derived neo-peptides, key for developing personalized immunotherapeutic strategies, including neoantigen T cell-based vaccines.

Methods: HBV integration events (virus-host junctions) were identified from RNA-sequencing data of patients-derived tumors using a dual-calling pipeline, consisting of independent alignments against both the HBV and human reference genomes. Integration breakpoints were then used to reconstruct virus–host chimeric coding sequences. For integrations occurring at the 3' end of human genes, the native human coding frame was preserved for in silico translation. In contrast, for 5' integrations all three possible reading frames were systematically evaluated to identify potential candidate translated chimeric proteins. Junction-spanning candidate peptides were generated by using IEDB MHC class I prediction tools for the chimeric sequences. Only patient-specific HLA alleles were considered, and peptide lengths were tailored to allele preferences, prioritizing 9–11-mers. Candidate epitopes were selected on the basis of the NetMHCpan EL rank, based on binding affinity scores, and antigen processing features, and by applying stringent selection thresholds to enrich for highly probable MHC class-I ligands.

Results: We reconstructed multiple patient-specific chimeric junction regions across distinct genomic loci and generated translated protein fragments encompassing integration sites. From these sequences, we identified a subset of junction-spanning peptides with favorable predicted HLA class I presentation in the corresponding patient. Several candidates displayed strong predicted binding and processing scores, supporting their potential immunogenicity. Selected peptides will undergo synthesis and functional in vitro validation to evaluate CD8⁺ T-cell activation in a patient-specific context.

Conclusions: By integrating viral integration mapping with HLA-restricted epitope prediction, we propose a scalable and personalized strategy to uncover actionable neoantigens arising from HBV integration events. This approach provides a translational framework for precision immunotherapy development in HBV-related liver cancer.

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Machine Learning–Based Innovation in Clinical Biochemical Markers for Enhanced Early Detection of Colorectal Cancer

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Background: Early detection of colorectal adenocarcinoma (COAD) remains challenging due to the limited sensitivity of conventional serum markers such as CEA. This study systematically evaluates the diagnostic potential of routine clinical biochemical markers in early-stage COAD and explores a translational strategy for repurposing established markers for early screening.

Methods: High-throughput serum biochemical profiling was conducted in 205 newly diagnosed, treatment-naïve early-stage colorectal cancer patients and 100 healthy controls. Based on literature curation, non-specific proteins and protein complexes were excluded, yielding 15 single-gene-encoded serum proteins suitable for integration with transcriptomic data. Machine learning, Mendelian randomization, and multi-omics analyses were applied to identify optimal biomarkers and assess their clinical relevance, prognostic value, and early diagnostic performance. Mechanistic features and drug sensitivity were further investigated using weighted gene co-expression network analysis (WGCNA), peripheral immune profiling, spatial transcriptomics, single-cell sequencing, and next-generation sequencing (NGS).

Results: Glutathione reductase (GSR) demonstrated exceptional robustness and causal relevance across multidimensional analyses. Reduced GSR transcript levels were significantly associated with disease progression, poor therapeutic response, increased mortality, and inferior overall survival, independent of clinical covariates. Serum GSR activity declined with tumor progression and, when combined with CEA, CA125, and CA199, achieved an AUC of 0.911 for early detection. Functional analyses indicated that GSR is primarily involved in redox regulation, glutathione metabolism, and mitochondrial homeostasis. Low-GSR patients exhibited reduced peripheral NK cell proportions, while tumors with low GSR showed decreased PD-1⁺ exhausted T cells. Low-GSR tumors displayed reduced sensitivity to targeted therapies, whereas high-GSR tumors were enriched for PIK3CA mutations and were more responsive to Afatinib, Gefitinib, AZD2014, and MK2206. Low-GSR tumors may therefore represent candidates for future metabolic intervention strategies.

Conclusions: GSR is a robust serum biomarker for early COAD that significantly enhances the diagnostic performance of CEA and provides independent prognostic and therapeutic guidance.

Acknowledgments

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Integrative Multi-Omics Analysis to Explore the Molecular Impact of Dietary (Poly)phenols on Humans

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Background: Inter-individual variability represents a major challenge in understanding how dietary (poly)phenols (PPs) influence cardiometabolic (CM) health and in translating their biological effects into effective precision nutrition strategies. Such variability arises from the complex interplay between host genetics, molecular regulation, and gut microbiome composition, which together modulate PP metabolism and bioactivity.

Methods: As a starting point, we relied on the stratification into PP metabolic phenotypes (metabotypes) defined within the Oral (Poly)phenol Challenge Test, a study involving 300 Southern European volunteers (18–74 years) recruited in Parma. Participants underwent a standardized PP challenge consisting of tablets rich in multiple classes of dietary PPs and provided blood, urine and faecal samples. Based on urinary PP derived metabolites, individuals were clustered into two metabotypes: metabotype 1 (low PP producers) and metabotype 2 (high PP producers), associated with a higher or a lower CM-risk due to the beneficial function exerted by PP metabolites. We then integrated genomics, metabolomics and metagenomics to investigate molecular determinants underlying these metabotypes, using DIABLO (mixOmics), and advance precision nutrition approaches.

Results: Integrative multi omics analyses revealed coordinated genetic–microbial–metabolic associations (Fig. 1), with clear molecular metabotype-specific signatures. In low producers, the higher relative abundance of taxa (*Enterobacter*, *Streptococcus*, and *Eubacterium*) may point to a microbial community with reduced functional efficiency. In contrast, high producers were strongly associated with sulphated PP metabolites (3-Hydroxy-2-methoxybenzene-1-sulfate, 2,3-Dihydroxybenzene-1-sulfate and 2-Hydroxy-3-methoxybenzene-1-sulfate) reflecting enhanced capability for producing bioactive phenolic conjugates. At the genomic level, both metabotypes were associated with intergenic and intronic variants, including rs10485285, rs9341828 and rs8003061 (on TSHR) for metabotype 1, and rs9283799, rs9359428 and rs36093037 (on TSHR) for metabotype 2. Although the precise mechanisms remain to be defined, these variants may contribute to inter-individual differences in PP metabolism through modulating gene expression and metabolic enzyme activity.

Conclusion: Together, host genetic variants, PP derived metabolites, and microbial taxa form a multi layered signature distinguishing the two metabotypes and serving as promising biomarkers for metabotype profiling and CM-risk investigation. This integrative framework suggests unprecedented molecular axes linking human genetics, gut microbiome composition, and specific PP metabolites, offering novel mechanistic insights into inter-individual variability and CM diseases.

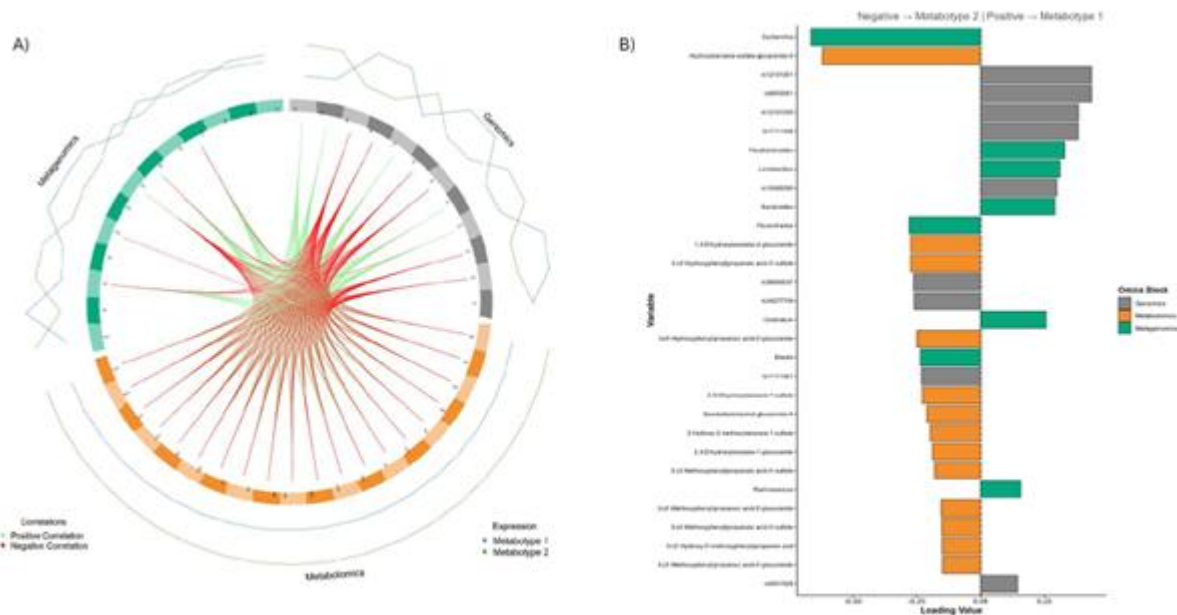


Figure 1 Multi-omics integration. A. Circos plot. Correlations between variables omics layers including SNPs (S, genomics), identified as codes: S for SNPs, B for gut bacterial genera (metagenomics), and PP metabolites (M, metabolomics). External lines indicate the association with one of the two metabolite types: blue, metabolite 1, low PP producers; green: metabolite 2, high PP producers. B. Loadings plot. Top 30 significant omics features discriminating the two metabolite types: positive loadings (right side) show contribution to metabolite 1, while negative loadings (left side) show contribution to metabolite 2. Reported bars are colour-coded considering the referring omics layer: genomics (grey), metabolomics (orange), metagenomics (green).

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Poster Abstracts

A Cross-Tissue Meta-Analysis of Transcriptomic Data to Define a Robust Molecular Identity of Human Fibrosis

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Background: Fibrosis is characterized by excessive deposition of extracellular matrix proteins, leading to tissue scarring and organ dysfunction (Distler et al. 2019). It is a major contributor to global mortality, involved in up to 45% of deaths (Wynn 2008). While triggers and initial sites of injury are often organ-specific, later stages of disease are driven by shared core signaling pathways (Rieder et al. 2025). Despite these commonalities, most studies focus on single organs and the few approved antifibrotics are strictly disease-specific. There is a critical need to define a universal molecular identity of fibrosis that could serve as a pan-fibrotic target across diverse clinical contexts.

Methods: We are developing a computational meta-analysis pipeline, illustrated in Figure 1, to integrate publicly available transcriptomic datasets from the Gene Expression Omnibus (GEO) across four organs: lung, kidney, liver, and skin. We systematically selected all available bulk transcriptomic studies with at least 2 fibrotic and 2 non-fibrotic samples. Following curation, metadata annotation, and normalization, we extract higher-order features including pathway activity, transcription factors activity, and cell-type deconvolution using single-cell datasets as reference. We employ two meta-analytical strategies: (1) a random-effects meta-analysis, adapting a previously established framework (Appierdo et al. 2025), to identify significant features and group them into shared immuno-fibrotic modules; (2) a meta-analysis of regression coefficients to define a common gene signature for identifying fibrosis in unknown samples. These modules and signatures will be validated across independent cohorts to ensure robustness.

Preliminary results: To date, we have curated 92 datasets comprising 4,644 samples across four tissues (32 lung, 29 liver, 20 kidney, and 11 skin). We have established the core analysis pipeline and are currently refining its components to improve the identification of shared immuno-fibrotic modules and the derivation of a pan-fibrotic gene signature.

Conclusion: Through this cross-organ approach, we aim to highlight shared fibrosis mechanisms and identify new targets for pan-fibrotic therapeutic intervention. Future work will involve expanding to additional organs and investigating whether fibrotic signatures correlate with clinical outcomes or therapeutic resistance in oncology.

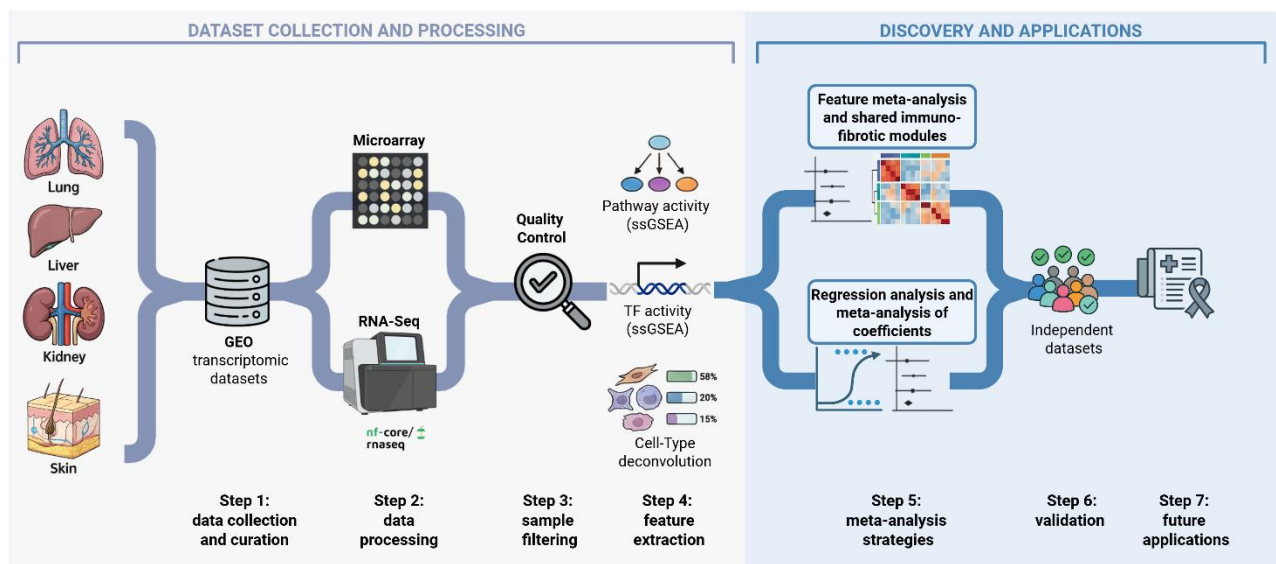


Figure 1: Computational workflow for cross-tissue meta-analysis.

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Huangqi Polysaccharides Attenuates Platelet Activation by Acetate Upregulation and Olfr78 Activation

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Background: Astragalus Polysaccharide (APS), the main active component of the traditional Chinese herb *Astragalus membranaceus*, exerts well-documented cardiovascular protective effects. However, its specific mechanism and role in regulating platelet activation and thrombosis remain to be clarified.

Methods: In this study, spontaneously hypertensive rats (SHR) were orally administered APS to evaluate its effects on platelet activation and thrombosis. The underlying mechanism was investigated using gut microbiota analysis, metabolomic profiling, antibiotic intervention, and acetate supplementation. Platelet function was assessed by aggregation, integrin $\alpha\text{IIb}\beta\text{3}$ activation, P-selectin expression, spreading, and clot retraction assays. The involvement of the cAMP/PKA/VASP pathway and olfactory receptor Olfr78 was explored via qPCR/RT-PCR, Cellular Thermal Shift Assay (CETSA), Drug Affinity Responsive Target Stability (DARTS), and Olfr78-knockout mice.

Results: Oral APS significantly inhibited platelet activation and thrombosis in SHR by remodeling the gut microbiota and increasing plasma acetate levels, rather than by directly acting on platelets. APS elevated gut microbial α -diversity, enriched beneficial bacteria, reduced pathogenic bacteria, and increased acetate production. Antibiotic-mediated microbiota depletion abolished the antiplatelet effect of APS, while acetate supplementation restored it. Acetate directly suppressed platelet aggregation, integrin $\alpha\text{IIb}\beta\text{3}$ activation, P-selectin exposure, spreading, and clot retraction. Mechanistically, acetate activated the platelet cAMP/PKA/VASP signaling pathway and targeted Olfr78, which was verified to be expressed on platelets. In Olfr78-knockout mice, acetate-mediated antiplatelet effects were lost. Moreover, APS alleviated myocardial ischemia-reperfusion (I/R) injury in SHR.

Conclusion: This study reveals that APS inhibits platelet activation and thrombosis via the microbiota-acetate-Olfr78 axis. Olfr78 serves as a novel therapeutic target for thrombotic diseases. These findings provide a theoretical basis for developing APS-based antiplatelet agents and support the "microbiota-metabolite-platelet" axis as a promising integrated strategy for hypertension-associated thrombosis.

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Multi-Dimensional Genomic Analysis and Risk Prediction for Antipsychotic-Induced Liver Injury

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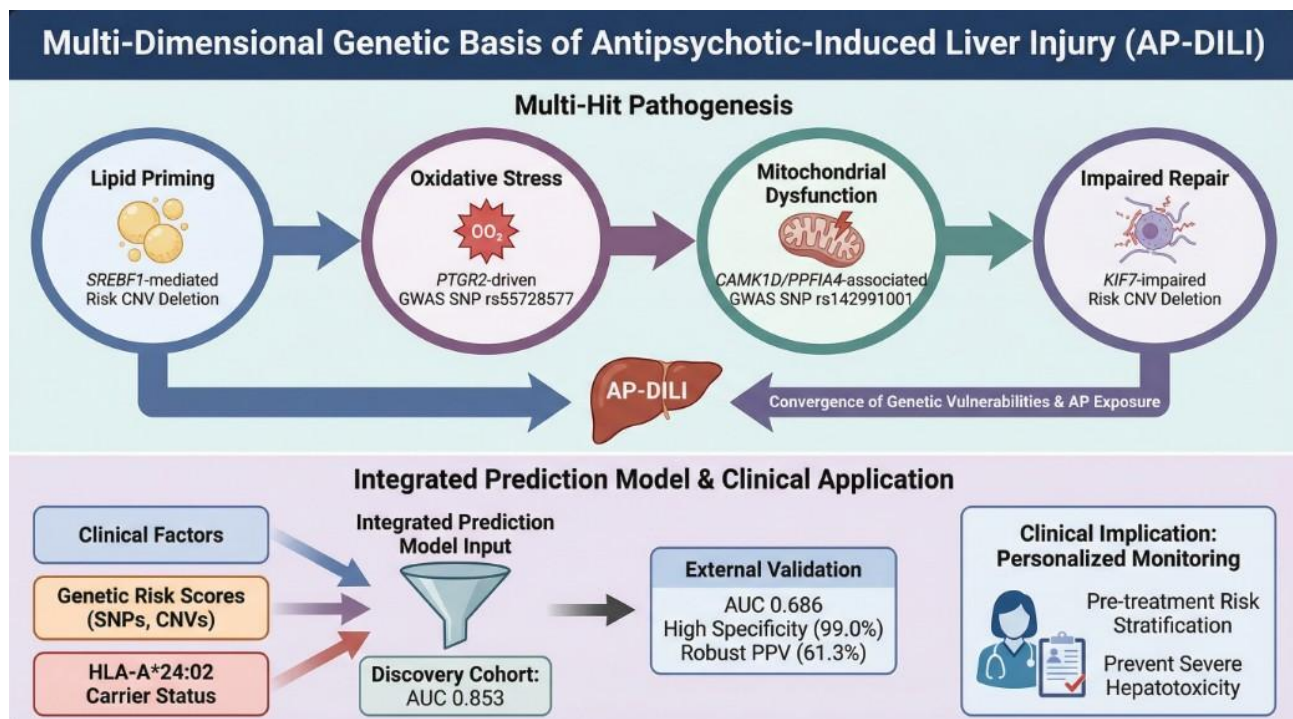
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Background: Antipsychotic-induced liver injury (AP-DILI) is a rare but serious adverse reaction that often necessitates treatment discontinuation and poses significant management challenges in schizophrenia^{1, 2}. However, its genetic architecture remains poorly defined, and no pre-treatment risk stratification tools are currently available³.

Methods: We conducted a multi-dimensional genetic study in a Han Chinese cohort comprising 101 severe AP-DILI cases (defined by Hy's Law) and 975 controls. We integrated genome-wide association study (GWAS), copy number variant (CNV) analysis, and HLA allele imputation to identify susceptibility loci. An integrated risk prediction model was constructed combining clinical factors with genetic risk scores derived from significant variants. Model performance was evaluated in the discovery cohort and externally validated in an independent cohort of 1,210 individuals.

Results: GWAS identified two genome-wide significant loci: PTGR2 (involved in oxidative stress; OR=4.46, $P=2.73 \times 10^{-8}$) and CAMK1D (implicated in mitochondrial function; OR=2.21, $P=3.74 \times 10^{-8}$). CNV analysis revealed eight risk-associated deletions, notably affecting KIF7 (a regulator of tissue repair; OR=7.17) and SREBF1 (a master regulator of lipid metabolism; OR=10.41). The convergence of these signals across distinct pathways is consistent with a "multi-hit" model of AP-DILI susceptibility, involving lipid dysregulation, oxidative stress, mitochondrial dysfunction, and impaired repair. Building on these findings, we developed an integrated risk prediction model incorporating clinical factors, polygenic risk scores, CNV risk scores, and HLA-A*24:02 carrier status. The model achieved an AUC of 0.853 in the discovery cohort. In external validation, despite phenotypic heterogeneity (using a non-strict cohort of clinician-reported abnormalities rather than confirmed severe cases), the model retained 99.0% specificity and a 61.3% positive predictive value. Although sensitivity was modest, the high specificity and PPV suggest the model's potential utility in real-world pharmacovigilance for prioritizing high-risk patients.

Conclusion: This study delineates the multifactorial genetic basis of AP-DILI and provides genetic evidence supporting a "multi-hit" pathogenesis model involving lipid metabolism, oxidative stress, and tissue repair deficits. The resulting high-specificity prediction tool offers a potential strategy for pre-treatment risk stratification, which could enable personalized monitoring to prevent severe hepatotoxicity in clinical practice.



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Role and mechanism of TNFRSF1A⁺ hepatocellular carcinoma stem cells in invasion and metastasis

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Background: The pronounced heterogeneity and unfavorable prognosis of hepatocellular carcinoma (HCC) are closely associated with the presence of cancer stem cells (CSCs). However, the specific CSC subpopulation driving invasion and metastasis, along with its targetable molecular mechanisms, remains to be fully elucidated. This study aimed to identify a novel invasive CSC subset and explore potential therapeutic strategies targeting it.

Methods: Through bioinformatic analysis of public single-cell RNA sequencing (scRNA-seq) datasets, we identified a distinct TNFR1⁺ CSC subpopulation in HCC. Subsequently, immunofluorescence staining of clinical specimens was performed for validation. The functional roles of this subset were assessed both in vitro and in vivo using flow cytometric sorting, Transwell invasion assays, wound healing assays, colony formation assays, and zebrafish xenograft models. Mechanistic investigations were conducted using RNA sequencing and western blotting. Finally, a phenotype-based drug screening approach was employed to identify potential targeting compounds.

Results: A TNFR1⁺ CSC subpopulation was identified within the HCC single-cell atlas. Analysis of clinical samples suggested a potential correlation between the enrichment of this subpopulation and higher grades of microvascular invasion as well as larger tumor size. In vitro and in vivo experiments demonstrated that sorted TNFR1⁺ EpCAM⁺ cells exhibited enhanced invasive and colony-forming capabilities in vitro and displayed increased tumorigenicity and invasive potential in zebrafish models. These phenomena were potentially associated with the activation of the TNF- α /TNFR1/NF- κ B signaling pathway. Furthermore, the natural compound shikonin was found to suppress the invasive capacity of tumor cells. Preliminary mechanistic exploration suggested that shikonin's effects might involve interference with the phosphorylation process of NF- κ B.

Conclusion: Through multidimensional analysis, this study implicates the TNFR1⁺ CSC subpopulation as a functionally relevant subset associated with the invasive phenotype in HCC. The data not only reveal the potential critical role of the TNF- α /TNFR1/NF- κ B pathway but also preliminarily suggest shikonin as a candidate molecule for targeting this subpopulation, providing a foundation for future investigations.

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Combinatorial Barcoding Meets In Vivo CRISPR: Establishing sci-RNA-seq3 as a Phenotypic Readout

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Background: Single-cell RNA sequencing has transformed our understanding of cellular heterogeneity in complex tissues. The DECODE project aims to systematically map context-dependent genetic networks in *Drosophila melanogaster* by combining CRISPR-based perturbations with single-cell transcriptomics. To achieve the high throughput and cost-effectiveness required for large-scale perturbation studies, this work sought to establish sci-RNA-seq3, a combinatorial indexing-based single-nucleus RNA sequencing platform, as a reliable and scalable alternative to commercial droplet-based methods for profiling the *Drosophila* midgut, a well-characterized model system for studying intestinal stem cell biology and tissue homeostasis.

Methods: Nuclei were isolated from whole adult *Drosophila melanogaster* bodies and processed using the three-round sci-RNA-seq3 combinatorial indexing protocol. Sequencing was performed on an Illumina NextSeq 2000. Raw data were processed with the sci-rocket bioinformatics pipeline, adapted for the EMBL high-performance computing cluster. Quality control was performed in ScanPy; ribosomal gene content was used as a proxy for RNA quality, since mitochondrial content is uninformative in nuclear preparations. Doublets were detected and removed using Scrublet. The filtered dataset was then subjected to dimensionality reduction, clustering, and UMAP visualization. Cell type annotation was carried out by semi-supervised label transfer using scANVI, with the Alzheimer's Disease Fly Cell Atlas as reference.

Results: Sequencing generated approximately 369 million read pairs, of which 92.5% mapped to the *Drosophila melanogaster* reference genome. After quality control and doublet removal, 29,514 high-quality nuclei were retained for downstream analysis. The dataset showed features characteristic of single-nucleus sequencing, including substantial intronic content and no mitochondrial reads. Label transfer via scANVI achieved high prediction confidence across 13,842 common genes (mean score 0.983, median 1.000). The observed cellular composition was biologically consistent with known *Drosophila* anatomy.

Conclusion: sci-RNA-seq3 produces biologically meaningful single-nucleus transcriptomic data that is consistent with existing *Drosophila* atlases. The platform captures the cellular diversity of the midgut, including stem and progenitor populations relevant to the study of intestinal homeostasis. Its high throughput, low cost per nucleus, and compatibility with frozen samples make it appropriate for experiments requiring simultaneous profiling of multiple CRISPR perturbation conditions, providing the technical foundation for mapping context-dependent genetic networks in the *Drosophila* intestine.

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For this purpose, we designed a novel, easy-to-use standalone command-line tool able to reliably discriminate long reads originated by either mtDNA or NuMTs and generated by Oxford Nanopore Technologies (ONT) sequencing based on the known lack of CpG methylation in human mtDNA. MitSorter aligns the reads to the mitochondrial genome incorporating base modification calls directly from raw POD5 files. The resulting BAM file is then partitioned into two separate BAM files: one containing unmethylated reads and the other containing methylated reads. You can see the entire workflow in the image attached.

Tool's features, computational framework, validation approach, and its potential applications in other genomic research areas are widely described in a recent publication in *Bioinformatics Advances*, available at <https://doi.org/10.1093/bioadv/vbaf135>. Source code and documentation, are available at <https://github.com/asvarvara/MitSorter>.



Integrating long-read phasing and methylation profiling to establish the causative role of de novo UBE3A variants in Angelman Syndrome

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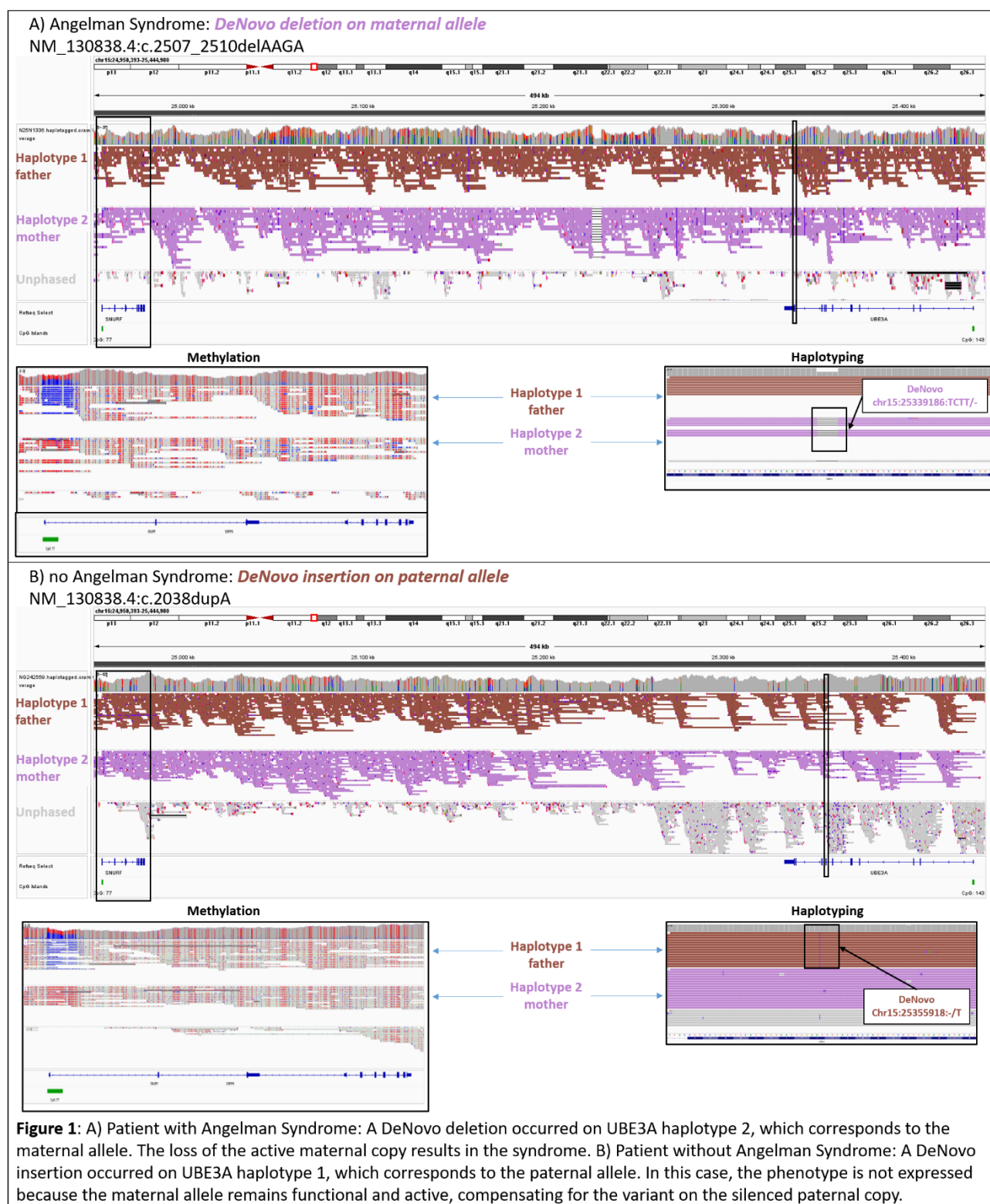
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Background: Angelman syndrome (AS) is a neurodevelopmental disorder that arises from loss of function of the maternally expressed UBE3A gene in neurons. Although some cases involve abnormal methylation of the SNRPN/SNURF imprinting control region on chromosome 15q11–q13, confirming the pathogenicity of de novo UBE3A sequence variants requires determining the parental origin of the affected allele, as only maternal variants disrupt UBE3A expression in the brain [1]. Nanopore sequencing provides a key advantage in this context because it enables the direct detection of 5-methylcytosine at CpG sites. This allows genetic variant analysis, haplotype phasing, and allele-specific methylation profiling to be integrated into a single workflow, without the need for additional assays. In this study, we investigated two unrelated patients in whom trio-exome sequencing had previously revealed distinct de novo variants in the UBE3A gene (NM_130838.4:c.2507_2510delAAGA, NM_130838.4:c.2038dupA). Both variants met ACMG criteria for classification as pathogenic (Class 5).

Methods: To further characterize these findings, we performed whole-genome Oxford Nanopore sequencing. Basecalling and modified-base detection were carried out using Dorado, and a human variation workflow was applied to enable phasing and methylation-aware interpretation. We specifically examined the methylation status of the imprinted SNRPN promoter region to determine the parental origin of each UBE3A variant through haplotype phasing.

Results: AS was confirmed in one patient, as the c.2507_2510delAAGA was on the maternal allele (Fig1,A). In the second patient, AS was not confirmed, as the c.2038dupA was identified on the paternal allele (Fig1,B).

Conclusion: Our results show that Nanopore-based profiling is a robust, single-assay strategy for resolving the clinical significance of de novo UBE3A variants. By combining long-read phasing with methylation analysis, this approach reliably discriminates between maternal and paternal alleles. This integrated workflow streamlines molecular diagnosis of AS by unifying variant detection and imprinting assessment in a single, efficient process.



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Chen Lan Hua Formula alleviates *Candida albicans* infection by inhibiting NF- κ B/NLRP3-mediated macrophage pyroptosis through the activation of SIRT3/FOXO3A-dependent mitophagy

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Background: Invasive candidiasis, primarily caused by *Candida albicans*, exhibits high mortality driven by excessive inflammation and pyroptosis. The traditional Chinese medicine Chen Lan Hua Formula (CLH) demonstrates clinical efficacy against fungal infections, but its pharmacological mechanisms remain unclear.

Methods: A systemic *C. albicans* murine model and *C. albicans*-macrophage (Raw264.7 and J774A.1) co-culture systems were established. Efficacy and mechanisms were evaluated via survival rates, fungal burden, pathology, transcriptomics, and protein expression analysis. Sirt3-knockdown macrophages were generated using shRNA to investigate SIRT3's role. Additionally, CLH constituents were characterized via HPLC, and individual herbs were validated in vivo through formula deconstruction.

Results: In vivo, CLH significantly improved survival, decreased fungal burden, and attenuated kidney inflammation. Transcriptomics linked CLH targets to macrophage pyroptosis and mitophagy. In vitro, CLH suppressed *C. albicans*-induced NLRP3 inflammasome activation and pyroptosis (reducing GSDMD-NT, IL-1 β , and IL-18). Mechanistically, CLH upregulated SIRT3, which activated the FOXO3A–PINK1–Parkin pathway to promote mitophagy. This process cleared damaged mitochondria and reduced mROS, thereby preventing NLRP3 activation. Sirt3 knockdown abolished these protective effects. Formula deconstruction identified *Artemisia capillaris* Thunb. and *Solidago decurrens* Lour. as the primary active anti-candida constituents.

Conclusion: CLH protects against *C. albicans* infection by promoting SIRT3/FOXO3A-dependent mitophagy, which subsequently inhibits NLRP3/NF- κ B-mediated macrophage pyroptosis.

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Label-free identification of macrophage subtypes with different NINJ1 expression levels by single-cell Raman spectroscopy and explainable deep learning

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Background: NINJ1 is a key regulator of plasma membrane integrity and a terminal executor of membrane rupture during pyroptosis. Subtle alterations in NINJ1 expression can modulate inflammatory signaling without inducing overt morphological changes, posing a challenge for label-free identification of immune cell subtypes differing primarily at the gene expression level.

Methods: An analytical framework integrating single-cell Raman spectroscopy (SCRS), deep learning, and explainable artificial intelligence (XAI) was established for non-destructive discrimination of RAW264.7 macrophages with differential NINJ1 expression. Eleven machine learning algorithms were evaluated, among which a Long Short-Term Memory (LSTM) network was selected for optimal performance. SHAP-based interpretability analysis was employed to identify key spectral features contributing to classification. Pharmacological validation was conducted using a DNA-damaging agent to induce endogenous NINJ1 upregulation.

Results: Although average Raman spectra were highly similar and unsupervised analyses failed to separate cell states, supervised models successfully extracted discriminative features from high-dimensional spectral data. The LSTM network achieved the best performance, with overall accuracy exceeding 98% and superior F1-score and AUC values. SHAP analysis identified a combinatorial Raman signature, including peaks at 1373.6, 1604.8, 1003.3, and 1208.6 cm^{-1} , suggesting membrane-associated protein conformational changes and lipid microenvironment remodeling linked to NINJ1 modulation. The trained LSTM model sensitively detected drug-induced NINJ1 upregulation, with prediction distributions consistent with protein-level validation.

Conclusion: An interpretable deep learning-assisted SCRS platform was established for high-sensitivity, label-free phenotyping of genetically and pharmacologically modulated immune cells. The approach enables detection of subtle gene expression differences without labelling, offering broad potential for immune cell analysis.

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Coptisine inhibits macrophage M1 polarization by activating the Nrf2/HO-1/HMGB1 signaling pathway for the treatment of diabetic ulcers

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Background: Diabetic ulcers (DU) are common diabetic complications, where hyperglycemia-induced chronic inflammation impairs healing. Macrophages are crucial for repair but undergo pro-inflammatory M1 polarization in high-glucose conditions, perpetuating inflammation. Given limited clinical therapies, we investigated Coptisine, known for anti-inflammatory properties, to elucidate its mechanism in DU healing, focusing on the Nrf2/HO-1/HMGB1 pathway, mitochondrial homeostasis, and M1 macrophage polarization.

Methods: scRNA-seq profiled microenvironmental differences between DU and normal wounds. We established an STZ-induced diabetic mouse skin defect model and an HG+LPS-induced RAW264.7 macrophage inflammation model. CETSA confirmed Coptisine-Nrf2 binding. Western blot, RT-qPCR, immunofluorescence, ELISA, and flow cytometry assessed inflammatory markers, M1 polarization, pathway components, and ROS. Mitochondrial function was evaluated via JC-1 and ATP assays. The Nrf2 inhibitor ML385 was used for mechanistic validation in vivo and in vitro.

Results: scRNA-seq showed reduced Nrf2 and enriched ROS pathways in DU macrophages. Coptisine directly bound and stabilized Nrf2. It dose-dependently accelerated wound healing, reduced pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6) and iNOS, upregulated Nrf2/HO-1, and suppressed HMGB1 release in vivo. In vitro, it blocked M1 polarization, decreased ROS, and restored mitochondrial membrane potential and ATP. ML385 reversed these effects, increasing HMGB1, impairing mitochondria, and exacerbating inflammation.

Conclusion: Coptisine directly activates Nrf2, downregulating HMGB1 to maintain mitochondrial homeostasis. This inhibits M1 macrophage polarization driven by mitochondrial dysfunction, breaking the chronic inflammation cycle and significantly promoting diabetic ulcer healing.

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Cyanidin-3-O-glucoside promotes the resolution of deep vein thrombosis in mice by suppressing macrophage M1 polarization via the HIF-1 α pathway

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Background: Deep vein thrombosis (DVT) is a vascular disorder characterised by the abnormal formation of thrombi within the deep veins, which obstructs venous blood flow and can lead to severe, potentially life-threatening complications. Macrophages play a pivotal role in thrombus resolution. Cyanidin-3-O-glucoside (C3G), the most common naturally occurring anthocyanin compound, has been demonstrated to possess anti-inflammatory and antioxidant properties. However, its specific function in thrombosis resolution remains elusive. **Purpose:** This study aimed to examine how C3G influences deep vein thrombosis resolution and to clarify the mechanisms behind thrombus resolution.

Methods: A murine model of stasis-induced DVT was created by ligating the inferior vena cava. C3G was dissolved in water and given by oral gavage starting three days before IVC ligation, then once daily until the day prior of tissue collection. Thrombus resolution was evaluated using histological staining, gelatin zymography, western blotting, and immunofluorescence. Additionally, in vitro macrophage polarisation models and a CoCl₂-induced hypoxic model were employed to assess the protective effects of C3G pretreatment on macrophages.

Results: C3G significantly reduced thrombus weight, length, and cross-sectional area in mice 14 days after DVT induction. It promoted thrombus resolution by enhancing macrophage infiltration while suppressing M1 macrophage polarisation and inflammatory responses. C3G also improved both systemic and local hypoxia by downregulating HIF-1 α expression, thereby enhancing antioxidant capacity and alleviating oxidative stress. In in vitro experiments, C3G inhibited the expression of M1 polarisation markers and inflammatory cytokines in LPS and IFN- γ -stimulated BMDMs. Moreover, in the CoCl₂-induced hypoxia model, C3G reduced ROS generation, increased the BCL2/Bax ratio, and inhibited apoptosis. It also downregulated HIF-1 α and its downstream glycolytic enzymes, thereby suppressing hypoxia-induced metabolic reprogramming and improving cellular hypoxic conditions.

Conclusion: C3G facilitates late-stage thrombus resolution by recruiting macrophages to the thrombus, suppressing their M1 polarisation, and modulating the HIF-1 α pathway. These findings suggest that C3G holds promise as a potential prophylactic strategy for DVT.

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Phytochemical Demethoxycurcumin suppresses hepatic stellate cell activation to alleviate fibrosis and stress-related liver injury by inhibiting STAT3 phosphorylation, a Non-canonical SMAD Pathway of TGF- β signaling

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Background: Liver fibrosis is a critical pathological process in the progression of chronic liver diseases, and if left untreated, it progresses to severe outcomes, such as liver cirrhosis, liver failure, and liver cancer, posing a major global health burden with approximately 2 million deaths each year. Huaji Ruangan Decoction (HJRGD), a classic formula rooted in traditional Chinese medicine, has shown therapeutic effects in clinical treatment of liver fibrosis. However, its mechanisms of action and functional ingredients remain largely unknown. Here, we explored the active phytochemicals, derived from HJRGD and able to recapitulate the beneficial effects of HJRGD, and investigated their mechanisms of action.

Methods: We integrated experimental models with computational approaches to elucidate HJRGD's anti-fibrotic mechanisms. A carbon tetrachloride (CCl₄) -induced mouse model was established to evaluate HJRGD's effects through histopathology, Western blot, and biochemical analyses. UHPLC-MS identified compounds in HJRGD. For computational analyses, we employed SwissTargetPrediction for compound-target prediction and GeneCards for fibrosis-related gene mining. Using R studio, we performed KEGG, GO, DisGeNET, and Reactome enrichment analyses to construct compound-target-pathway networks. Molecular docking using AutoDock was conducted to validate key compound-target interactions. Human and mouse hepatic stellate cell lines were used for in vitro validation.

Results: HJRGD significantly reduced liver fibrosis, injury, and inflammation in mice, with marked inhibition of STAT3 phosphorylation among TGF β signaling pathways. UHPLC-MS identified 160 compounds in HJRGD. Bioinformatics tools and multi-database enrichment analyses (KEGG, GO, DisGeNET, Reactome) prioritized demethoxycurcumin as a candidate targeting STAT3, revealing enrichment fibrosis-related pathways. AutoDock molecular docking confirmed that demethoxycurcumin binds to STAT3, thereby stabilizing the protein and preventing the conformational changes in its SH2 domain that are essential for ensuing tyrosine phosphorylation and dimer formation. In vitro studies validated that demethoxycurcumin suppressed STAT3 phosphorylation and inhibited TGF β -SMAD3 transcriptional activity in hepatic stellate cells, recapitulating HJRGD's anti-fibrotic effects.

Conclusion: Demethoxycurcumin is a key active component of HJRGD that directly targets STAT3 to exert anti-fibrotic effects. Integrating experimental models with bioinformatics tools (KEGG, GO, DisGeNET, Reactome enrichment) and molecular docking (AutoDock) revealed this mechanism, positioning demethoxycurcumin as a promising lead for anti-fibrosis drug development.

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Decoding the Therapeutic Mechanisms of Kaempferol in Peripheral Artery Disease: Insights from Single-Cell Transcriptomics and Network Pharmacology

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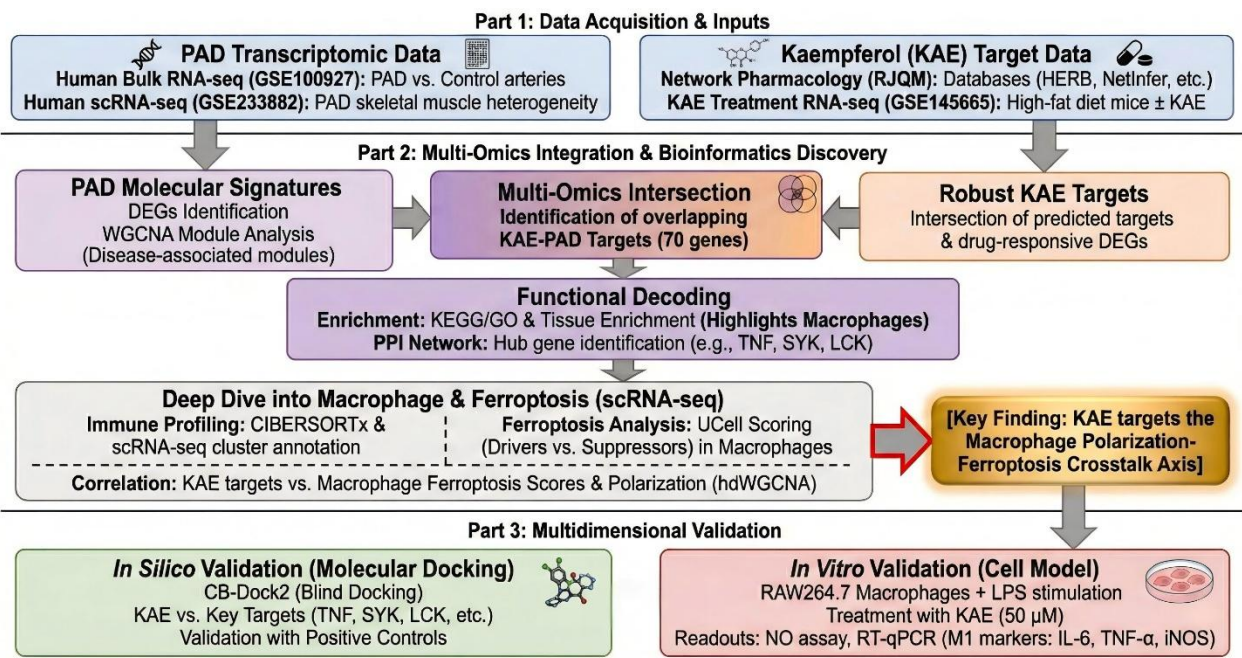
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Background: Peripheral artery disease (PAD) is a chronic ischemic condition driven by vascular occlusion and metabolic-immune dysregulation. Kaempferol, the core active component of the clinically effective Ruan Jian Qing Mai formula, shows therapeutic promise for PAD, yet its precise molecular mechanisms remain elusive.

Methods: We employed an integrative multi-omics framework combining bulk transcriptomics, single-cell RNA sequencing (scRNA-seq), network pharmacology, and in vitro experimental validation to decode kaempferol's therapeutic targets.

Results: Transcriptomic profiling and weighted gene co-expression network analysis (WGCNA) revealed extensive transcriptional remodeling in PAD, highlighting key networks associated with vascular inflammation. Network pharmacology and multi-database integration identified 70 kaempferol-targeted genes linked to macrophage activation and lipid metabolism. Single-cell analysis and CIBERSORTx pinpointed macrophages as the primary cellular mediators. Specific kaempferol targets were enriched in macrophages and associated with $\gamma\delta$ T-cell infiltration and distinct polarization modules. In vitro assays using RAW264.7 cells confirmed that kaempferol significantly suppresses LPS-induced M1 macrophage polarization and inflammatory cytokine expression. Furthermore, ferroptosis scoring and molecular docking indicated that kaempferol modulates heightened ferroptosis susceptibility in PAD macrophages by strongly binding to key regulatory targets.

Conclusion: Kaempferol exerts its therapeutic efficacy in PAD primarily by attenuating M1 macrophage polarization and modulating ferroptosis. These multi-omics insights provide a robust mechanistic foundation and potential molecular targets for precision PAD interventions.



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Causal Relationship between Hormone levels and lung cancer

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Background: Lung cancer is a highly prevalent neoplastic disease in various regions of the world, but the mechanism of its occurrence, development, and metastasis is not clear. Different hormone levels have different potential roles in the occurrence, development, and metastasis of lung cancer, but the association between hormone levels and lung cancer is not clear.

Objective: This study aims to explore the causal relationship between hormone levels and lung cancer using Mendelian randomization. Sensitivity and heterogeneity tests were conducted to ensure the reliability of the results, offering insights into the prevention, diagnosis, and treatment of lung cancer.

Methods: We employed a two-sample Mendelian randomization (MR) analysis using large-scale publicly available genome-wide association studies (GWAS) data to assess the causal relationship between hormone levels and lung cancer. We explored the causal relationship between 17 hormones and three subtypes of lung cancer. The inverse variance weighted (IVW) method was used as the primary analysis, while MR-Egger, Weighted Median, Weighted Mode, and Simple Median were applied as supplementary methods. Sensitivity and heterogeneity tests were conducted to ensure the robustness of the findings.

Results: We identified six hormone levels significantly associated with lung squamous cell carcinoma (LUSC): Total testosterone, Oestradiol, Thyrotropin-releasing hormone, Fasting insulin, Parathyroid hormone, and Glucocorticoid. Among them, Total testosterone, Oestradiol and Thyrotropin-releasing hormone were negatively correlated with morbidity. Insulin, Prolactin levels and Parathyroid hormone were positively correlated with morbidity. Five hormone levels were significantly associated with lung adenocarcinoma (LUAD): Luteinizing hormone, Thyroid hormones, Insulin, Prolactin levels and Parathyroid hormone. Luteinizing hormone and Thyroid hormones were negatively correlated with morbidity, while Insulin, Prolactin levels and Parathyroid hormone were positively correlated with morbidity. Similarly, five hormone levels were linked to small cell lung cancer (SCLC): Total testosterone, Luteinizing hormone, Estradiol, PTHrP, and Insulin. Total testosterone and Luteinizing hormone were negatively correlated with morbidity, while Estradiol, PTHrP, and Insulin were positively correlated with morbidity. Several hormones were associated with different subtypes of lung cancer.

Conclusion: This study demonstrates significant causal relationships between specific hormone levels and various types of lung cancer, providing valuable insights for prevention, diagnosis, and treatment strategies.

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Rapid Identification of Chinese Herbal Pieces Using Raman Spectroscopy Combined with Deep Learning

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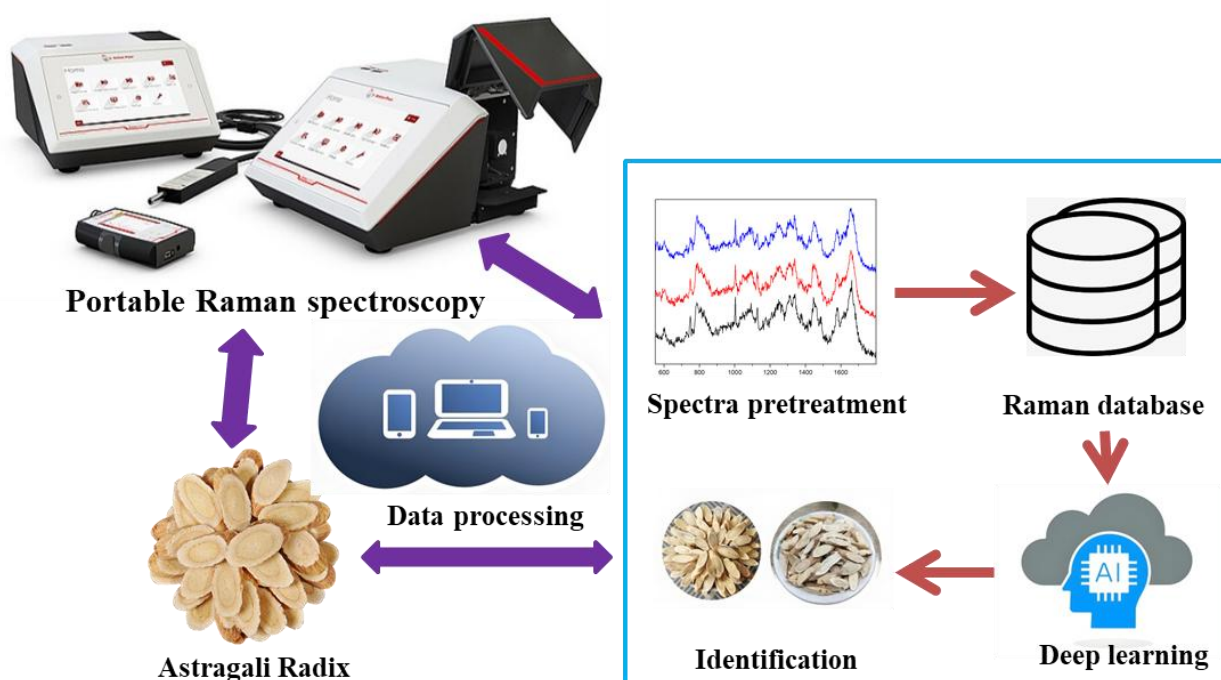
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Background: Ensuring the quality and authenticity of Traditional Chinese Medicine (TCM) decoction pieces is vital for clinical efficacy. Current methods like HPLC are time-consuming and unsuitable for on-site screening. While Raman spectroscopy offers a rapid, non-destructive alternative, it faces challenges from fluorescence interference and complex data analysis. There is an urgent need for accurate, real-time identification techniques.

Methods: This study integrates portable 1064-nm Raman spectroscopy with deep learning to identify easily confused TCMs: Glycyrrhizae Radix et Rhizoma, Astragali Radix, and Scutellariae Radix. Experimental parameters were optimized to minimize fluorescence. Spectral data underwent preprocessing via Air-PLS baseline correction and Savitzky-Golay smoothing, followed by feature extraction using Competitive Adaptive Reweighted Sampling (CARS). A Convolutional Neural Network (CNN) model was developed and trained on this processed data to classify species based on their spectral fingerprints.

Results: The optimized protocol successfully acquired distinct fingerprint spectra for the selected samples. Using the 1064-nm portable system, the study effectively distinguished the three morphologically similar herbs. The constructed CNN-LSTM deep learning model achieved a classification accuracy of 97.44% on validation and test sets. These results confirm that combining Raman spectroscopy with deep learning effectively captures and differentiates subtle chemical features inherent to various TCM decoction pieces, overcoming traditional analytical limitations.

Conclusion: Integrating portable 1064-nm Raman spectroscopy with deep learning provides a powerful, practical solution for the rapid, on-site, and non-destructive identification of TCM pieces. This approach delivers objective, accurate, and real-time quality control, addressing the shortcomings of conventional laboratory methods. The establishment of this technology and its associated spectral database will significantly enhance TCM standardization, safety, and monitoring across clinical, manufacturing, and distribution sectors, promoting modernization in herbal medicine quality assurance.



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San Huang Xiao Yan recipe promoted wound healing in high-fat diet /streptozotocin-induced diabetic ulcer mice by inhibiting Th17 cell differentiation

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Background: Diabetic ulcers are a severe diabetes complication, often resulting in amputations. The San Huang Xiao Yan recipe (SHXY) has been effective in treating these ulcers, but its mechanisms and active components are unclear. This study aims to confirm SHXY's effectiveness and understand its action mechanism.

Methods: We used bioinformatics and mass spectrometry to identify SHXY's key targets for treating diabetic ulcers. C57BL/6J mice, fed a high-fat diet and treated with streptozotocin, underwent skin injury to create a diabetic ulcer model. Mice received varying oral doses of SHXY for a week. We measured blood glucose, body weight, and wound size on days 0, 3, and 7. Inflammatory factors were assessed with ELISA, while HE and Masson staining examined skin tissue changes. We used flow cytometry to study CD4⁺ T cell subpopulations, and qPCR and Western blot for protein and mRNA changes in ulcer tissues. Immunofluorescence checked IL-17A and CD31 levels. In vitro, naive CD4⁺ T cells were isolated and differentiated into Th1, Th2, Th17, and Treg cells to test SHXY's effects.

Results: Diabetic ulcer mice showed slower wound healing, higher spleen and thymus indices compared to the normal group. Their skin wounds had more fibrous tissue, deformed lumens, and increased mucus, with higher levels of IL-17A, ROR γ T, IL-6, IL-1 β , and CXCL1. SHXY and its active components helped heal wounds, improved skin integrity, reduced inflammation, and increased collagen at the wound site. This was achieved by lowering IL-17A and related proteins like HIF-1 α , MAPK, and MMP9 in immune organs and ulcers. SHXY and its active components also boosted the angiogenesis protein CD31 and TGF- β expression, aiding ulcer healing.

Conclusions: SHXY and its active components may help treat diabetic ulcers by inhibiting Th17 cell differentiation, which reduces inflammatory cytokines and calms immune responses, thus providing a scientific basis for the clinical application.

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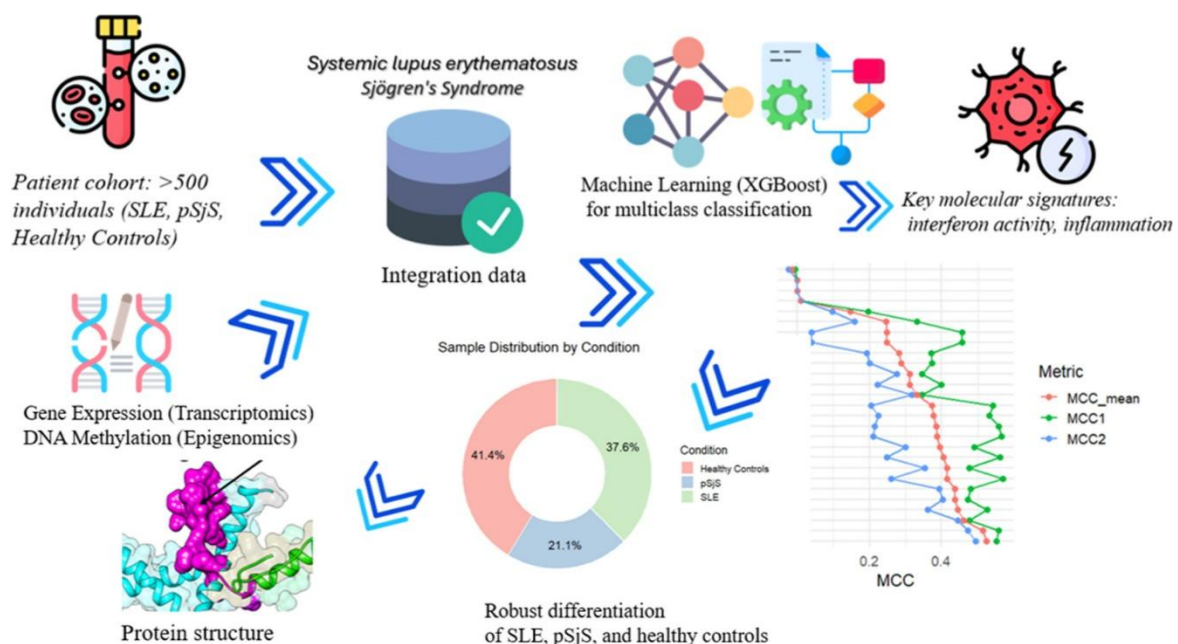
Integrative machine learning of gene expression and DNA methylation for the accurate diagnosis of systemic lupus erythematosus and Sjögren's syndrome

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Systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSjS) are systemic autoimmune diseases with overlapping clinical symptoms, complicating accurate diagnosis. This study leverages integrative machine learning of gene expression and DNA methylation data to improve diagnostic precision and biomarker discovery. Using multi-omics data from 211 SLE patients, 178 pSjS patients, and 220 healthy controls, we implemented an eXtreme Gradient Boosting (XGBoost) multiclass classifier to distinguish between SLE, pSjS, and healthy individuals. Model performance varied across molecular clusters, with the highest predictive accuracy in the interferon cluster (MCC = 0.78). Feature selection identified 85 key genes and 850 CpG sites, which enhanced diagnostic performance and were enriched in interferon and interleukin signaling pathways. External validation using independent datasets confirmed the robustness of these biomarkers. Our findings demonstrate that machine learning-driven integration of transcriptomic and epigenomic data offers a powerful approach for the differential diagnosis of SLE and pSjS, with potential clinical applicability.



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ADAR Enzymes and Circular RNA Generation within the Supraspliceosome

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Adenosine Deaminases Acting on RNA (ADARs) convert adenosines to inosines within double-stranded RNA structures (dsRNAs), which are recognized as guanosines by the splicing and translation machineries. ADAR1 and ADAR2 enzymes have been identified within the supraspliceosome, a huge dynamic complex of four native spliceosomes connected by pre-mRNA. The supraspliceosome, where linear splicing and alternative splicing occur, was recently shown to contain circular RNAs (circRNAs), generated from pre-mRNA through back-splicing. Generation of circRNA is promoted by base-pairing of the circRNA flanking introns. Although circRNAs are of low abundance, they are more stable than their linear counterparts and are prevalent in the brain. ADARs, initially considered circRNA biogenesis repressors due to their editing capability, seem now more complex, requiring further research.

Our study investigates the role of ADAR-mediated RNA editing on circRNA formation. Through minigene transfection and Sanger sequencing analyses, we focus on the formation of circMAN2A1 3→2 and circUPF1 16→13 within the supraspliceosome. The findings indicate that the nucleus and cytoplasm harbor these transfected circRNAs, whereas the cytoplasm harbors endogenous circMAN2A1. Interestingly, ADAR1-p150 increases both circUPF1 and circMAN2A1 RNA expression levels, whereas ADAR1-p110 has a stronger effect on circMAN2A1. Notably, ADAR-p150 enhances the translation of specific transfected circRNAs. Our findings suggest ADARs as potent regulators of circRNA biogenesis and translation.

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Oroxylin A Accelerates Diabetic Wound Healing through Nrf2 Mediated Suppression of Neutrophil Extracellular Traps and Activation of Fibroblasts

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Background: Diabetic wound healing represents a major clinical challenge, characterized by persistent inflammation and impaired tissue regeneration. Oroxylin A is a flavonoid isolated from *Scutellaria baicalensis* Georgi, a key herb in the Sanhuang Xiaoyan Formula. This herb has been widely used in traditional Chinese medicine for anti-inflammatory therapy and wound care; however, the role and underlying molecular mechanism of Oroxylin A in diabetic wound healing remain unclear.

Methods: A diabetic mouse model was established in C57BL/6J mice by high fat diet feeding combined with streptozotocin injection, followed by full thickness skin excision on the back to create a diabetic wound model. Histological analysis, enzyme linked immunosorbent assay (ELISA), quantitative real time polymerase chain reaction (qPCR), immunofluorescence, and Western blot were performed to detect the expression levels of inflammatory markers, collagen deposition, cytokines, neutrophil extracellular traps (NETs), and α smooth muscle actin (α SMA). In vitro, the effects of Oroxylin A on neutrophil extracellular trap formation (NETosis), reactive oxygen species (ROS) production, and nuclear factor erythroid 2 related factor 2 (Nrf2) activation were investigated in neutrophils induced by high glucose and phorbol 12 myristate 13 acetate (PMA). In fibroblasts cultured under high glucose conditions, the regulatory effects of Oroxylin A on cell proliferation, migration, and differentiation were examined.

Results: Oroxylin A treatment significantly accelerated wound closure and increased collagen deposition in diabetic mice, while reducing the levels of inflammatory cytokines and NET formation. In vitro results demonstrated that Oroxylin A decreased high glucose/PMA induced NET production and ROS generation in neutrophils, and these effects were reversed by the Nrf2 inhibitor ML385. In fibroblasts, Oroxylin A upregulated α SMA expression and enhanced cell proliferation, migration, and activation.

Conclusion: Oroxylin A accelerates diabetic wound healing through a dual mechanism: on the one hand, it activates the Nrf2 pathway in neutrophils to suppress oxidative stress and NETosis; on the other hand, it promotes the proliferation, migration, and activation of fibroblasts.

Acknowledgments

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Ethyl caffeate reprograms macrophage immunometabolism via the SIRT3–FOXO3A–AKT axis to enhance host defense against *Candida auris*

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Background: *Candida auris* (*C. auris*) is an emerging multidrug-resistant fungal pathogen. Current antifungals are often insufficient, creating a need for host-directed strategies. Sirtuin 3 (SIRT3) is a mitochondrial deacetylase that regulates redox homeostasis, but its role in antifungal macrophage defense is not well defined. We examined how SIRT3 shapes macrophage responses to *C. auris*. We also evaluated ethyl caffeate (EC) as a host-directed modulator.

Methods: We used murine macrophages with Sirt3 knockdown or overexpression. We quantified phagocytosis, intracellular fungal survival, mitochondrial ROS dynamics, and macrophage cell integrity using imaging, flow cytometry, CFU assays, and LDH release. We profiled infection-induced transcriptional programs by RNA-seq and performed pathway analyses. We tested EC both in vitro and in systemic infection models in *Drosophila* and mice. We assessed pathway markers by immunoblotting and immunofluorescence. We used the SIRT3 inhibitor 3-TYP to test inhibition sensitivity.

Results: SIRT3 deficiency impaired macrophage antifungal function and was accompanied by redox imbalance. mtROS regulation was disrupted in a biphasic pattern, with an early spike followed by late depletion. Transcriptomics linked SIRT3-dependent programs to FOXO and PI3K–AKT signaling. In macrophages, SIRT3 status tracked with FOXO3A acetylation and AKT phosphorylation. EC showed weak direct antifungal activity in vitro but improved outcomes in systemic infection models. EC treatment increased SIRT3 abundance and was associated with reduced FOXO3A acetylation and restrained infection-associated AKT activation. These functional and signaling effects were largely sensitive to SIRT3 inhibition by 3-TYP.

Conclusion: This study connects SIRT3-dependent redox control to FOXO3A–AKT signaling during *C. auris* infection. It also supports EC as a host-directed candidate that improves antifungal defense in vivo while limiting inflammatory injury.

Acknowledgments

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DNA barcoding for the detection of potential adulterants in commercial oregano

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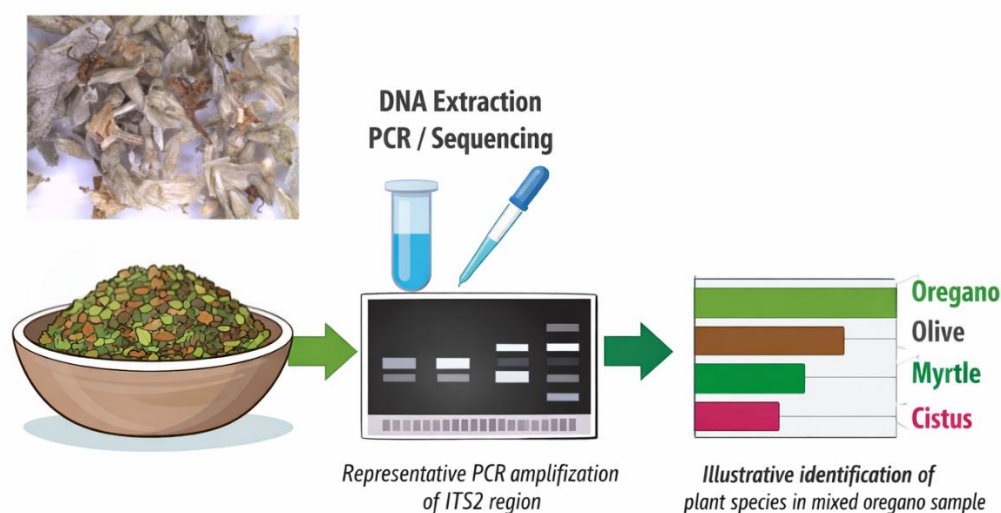
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Background: Oregano (*Origanum vulgare*) is a widely used culinary herb, highly appreciated for its aroma and flavor. However, commercial oregano is prone to adulteration, as dried and ground products are visually indistinguishable from leaves of other plant species, such as olive (*Olea europaea*), myrtle (*Myrtus communis*), and rockrose (*Cistus* spp.). Ensuring the authenticity of oregano is essential for consumer protection and quality control in the spice industry. DNA barcoding offers a molecular approach to identify plant species in processed herbal products, overcoming the limitations of traditional morphological analysis.

Methods: Commercial oregano samples were hypothetically collected and processed for DNA analysis. DNA was extracted from the samples using standard plant extraction protocols. The ITS2 region was amplified via polymerase chain reaction (PCR) using universal plant primers, and products were visualized through agarose gel electrophoresis. Sequencing of the amplified fragments would then allow comparison against reference sequences in databases such as GenBank or BOLD, enabling the identification of all plant species present in each sample. This workflow illustrates how DNA barcoding can be applied for the detection of adulteration in ground spices.

Results: Using DNA barcoding, it is expected that the sequences corresponding to *Origanum vulgare* would be detected as the main component in authentic samples, whereas additional sequences from other plant species could indicate potential adulteration. This approach provides a clear identification of each species present, demonstrating the method's capability to resolve complex mixtures of plant material and to distinguish between closely related or morphologically similar herbs.

Conclusion: DNA barcoding represents a powerful tool for the authentication of commercial oregano, providing reliable molecular evidence for the presence of adulterant species. By integrating this molecular approach, spice producers and regulatory agencies can enhance quality assurance, prevent fraudulent labeling, and maintain consumer confidence in herbal products.



Illustrative schematic of mixed oregano sample and DNA barcoding to detect potential adulterants.

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Functional Architecture of Human–Microbiome Protein–Protein Interactions in Autism Spectrum Disorder: A Two-Tier Network Topology Framework

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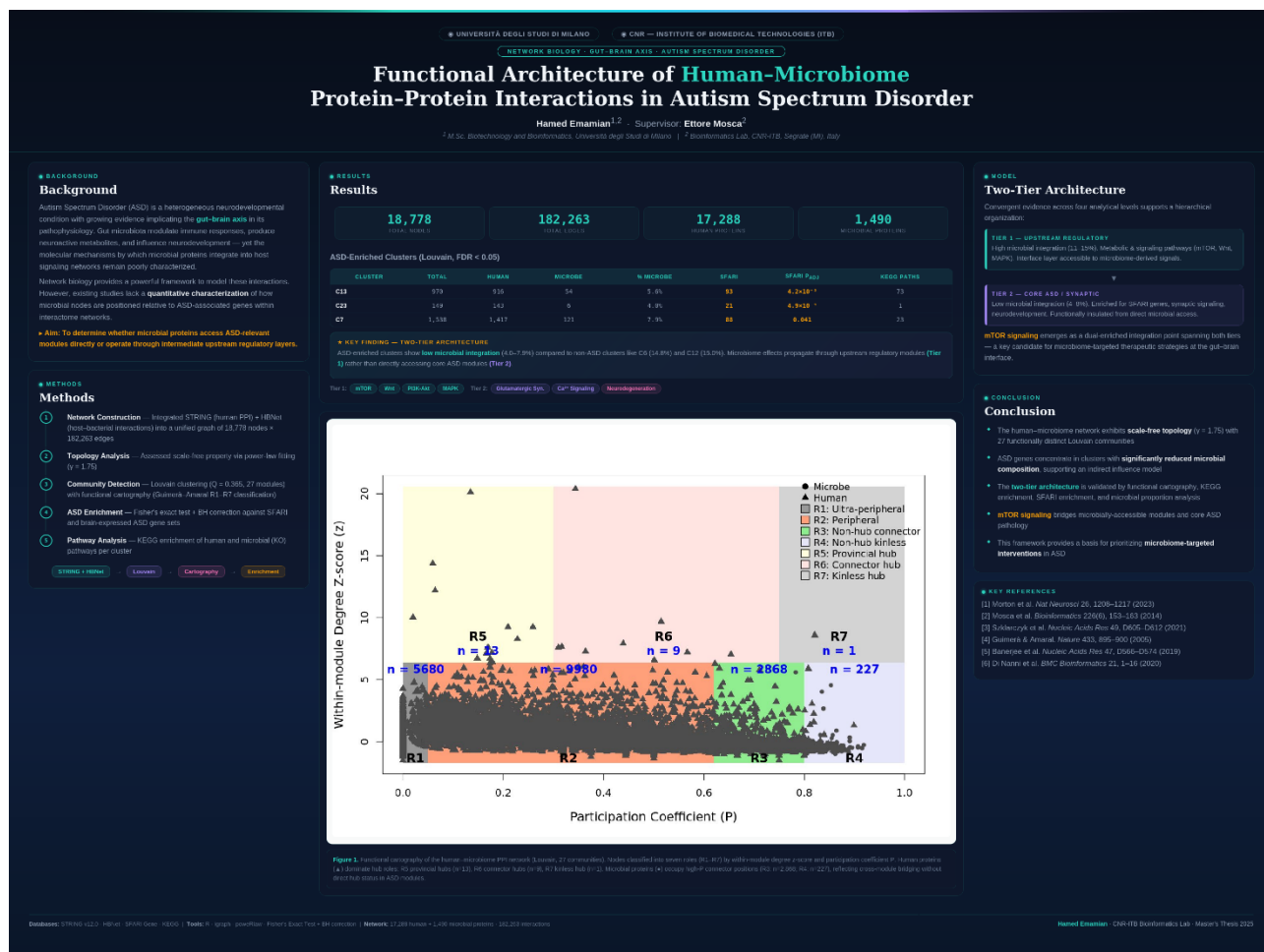
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Background: Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental condition with increasing evidence implicating the gut-brain axis in its pathophysiology. Gut microbiota modulates immune responses, produces neuroactive metabolites, and influence neurodevelopment through the gut-brain axis. However, the molecular mechanisms by which microbial proteins integrate into host protein interaction networks relevant to ASD remain poorly characterized. Network biology offers a quantitative framework to map these complex cross-kingdom interactions, yet no study has systematically assessed the topological positioning of microbial proteins relative to ASD-associated genes within a unified human-microbiome interactome.

Methods: The integrated human-microbiome gene-gene interaction network was constructed by combining the STRING database for human PPI and the HBNNet database for host-bacterial interactions. Scale-free topology was assessed via power-law fitting. Community structure was resolved using Louvain clustering (27 modules, $Q=0.365$), and functional cartography based on the Guimera-Amaral framework classified each node into one of seven topological roles (R1-R7). ASD gene enrichment was evaluated by means of Fisher's exact test with Benjamini-Hochberg correction againsts ASD SFARI database (genetic predisposition) and a list of ASD brain expressed genes from the literature. KEGG pathway enrichment was performed for both human and microbial ortholog pathways per cluster.

Results: We defined a human-microbiota interactome that is composed of 18,778 genes (17,288 human, 1,490 microbial) and 182,263 edges exhibited scale-free architecture. Functional cartography of the interactome revealed key microbial hubs. Cluster analysis revealed a modular network composed of 27 communities. Three communities showed significant enrichment in ASD genes (SFARI, $FDR<0.05$): community C13 has 93 ASD genes; community C23 has 21 ASD genes, and community C7 contains 88 ASD genes. Interestingly, these ASD-enriched community contain microbial genes (4.0-7.9%). KEGG analysis identified mTOR, Wnt, PI3K-Akt, and MAPK signaling pathways in microbially-accessible upstream modules, while glutamatergic synapse and calcium signaling pathways characterized the core ASD-enriched clusters.

Conclusion: Our findings reveal a two-tier network architecture in which microbial proteins access upstream regulatory and metabolic modules (Tier 1) but are structurally excluded from core synaptic ASD modules (Tier 2). mTOR signaling emerges as a dual-enriched integration point bridging both tiers, representing a promising candidate target for microbiome-based therapeutic strategies in ASD.



Databases: STRING, HINet, SFARI, KEGG | Tools: R,igraph, powerlaw, Fisher's Exact Test + BH correction | Network: 17,288 human + 1,490 microbial proteins, 182,263 interactions

Hamed Emamiyan · CNR-ITB Biomaterials Lab · Master's Thesis 2023

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HyperCov, a fast and memory-efficient solution for coverage calculation

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Background: Coverage analysis is a fundamental step in quality control and variant calling for Next-Generation Sequencing (NGS) data. However, most tools do not provide comprehensive statistics and do not have real performance improvements from multithreading, leading to performance bottlenecks in analysis pipelines. Here we present HyperCov, a high-performance coverage calculator for NGS data.

Methods: Hypercov leverages AVX2 SIMD vectorization, lock-free atomic task scheduling, and a custom "fast-path" alignment parser to achieve fast and memory-efficient coverage calculation. We tested HyperCov's performance against seven other established coverage calculation tools, samtools, bamtocov, bedtools, megadePTH, mosdepth, pandePTH, sambamba, on the HG001 Illumina short-read and PacBio long-read whole genome sequencing datasets available at <https://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/data/NA12878>. To test multithreading-derived performance improvements, we performed coverage calculation with an increasing number of threads (1, 2, 4, 8, 16, 32) where allowed by the software.

Results: HyperCov supports sorted and unsorted BAM/CRAM formats, and allows custom window size calculations accepting GTF, GFF, and BED interval files and enables GC content calculation if a reference file is provided. Among the tested approaches, HyperCov performed competitively across varying thread counts. Regarding the Illumina dataset, HyperCov provided the best performance at 32 threads, with a peak memory consumption of 1,82GB and 57,1 seconds for the 148GB BAM file. The second best-performing tool was pandePTH with a peak memory consumption of 3,17GB and 73,5 seconds for the same number of threads. Concerning the PacBio dataset, HyperCov peaked again at 32 threads, with a memory consumption of 2,46GB and 7,8 seconds for a 15GB BAM file. Again, the runner-up was pandePTH with a memory consumption of 3,48GB and 9,8 seconds at 32 threads.

Conclusions: HyperCov delivers fast, memory-efficient genomic coverage calculations highly optimized for automated, cloud-based pipelines. By minimizing resource overhead, it provides a scalable, cost-effective solution that overcomes traditional computational bottlenecks in high-throughput sequencing workflows. HyperCov is an open-source MIT-licensed software available at <https://github.com/lisosome/hypercov>.

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bedtools: PMID: 20110278
megadePTH: PMID: 33693500
mosdepth: PMID: 29096012
pandePTH: PMID: 38701418
sambamba: PMID: 25697820

Integrating MS/MS Spectral and Protein Embeddings for Structure-Independent Bioactivity Prediction

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Background: Current machine learning methods for compound-target interaction prediction typically rely on molecular descriptors derived from fully annotated chemical structures. However, molecules analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) experiments consist of fragmentation spectra that require subsequent manual or computational annotation. As a result, existing prediction approaches cannot be directly applied to unannotated or novel compounds. To address this limitation, we propose a pipeline for predicting molecular bioactivities using Spec2Vec [1] embeddings derived from mass spectrometry data as substitutes for conventional molecular fingerprints, facilitating hypothesis generation of potential compound-target associations.

Methods: Based on bioactivity information stored in the Papyrus dataset [2], we propose a proteochemometric model that integrates mass spectra and protein sequence representations. We retrieved a mass spectrometry dataset from the GNPS library [3] and selected spectra acquired in negative ionization mode. Spectra were processed to train a Spec2Vec model and generate spectral embeddings. We obtained bioactivity data from Papyrus by retaining interactions involving natural products and plant metabolites present in the COCONUT [4] database that matched previously processed GNPS spectra, and keeping bioactivities associated with human proteins. We extracted corresponding molecular embeddings from Spec2Vec and target sequence embeddings from the pre-trained UniRep model [5]. Active and inactive classes were defined using a pChEMBL threshold of 5.5. We trained an XGBoost classifier on concatenated compound and protein features, further refined using PCA, and assessed model performance using group-wise 5-fold cross-validation based on protein identifiers. The final dataset comprised 10,092 compound-target pairs, including 714 molecules and 1142 human proteins.

Results: We found that our best-performing model, trained on 500-dimensional spectral embeddings, 5700-dimensional protein embeddings, and 128-dimensional PCA features, achieved an MCC of 0.49, a precision of 0.72, a recall of 0.61, an accuracy of 0.77, and an F1-score of 0.66. This result is close to state-of-the-art performance obtained using known molecular structures, which are more informative but unavailable for unknown compounds.

Conclusion: Early results suggest that spectral embeddings can be integrated into proteochemometric models to predict compound-target interaction without relying on structural annotation, enabling bioactivity modeling directly from mass spectra and the identification of candidate interactions for experimental validation.

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Integrative RNA-seq analysis reveals pathway-level transcriptional signatures in PLP1-associated leukodystrophies

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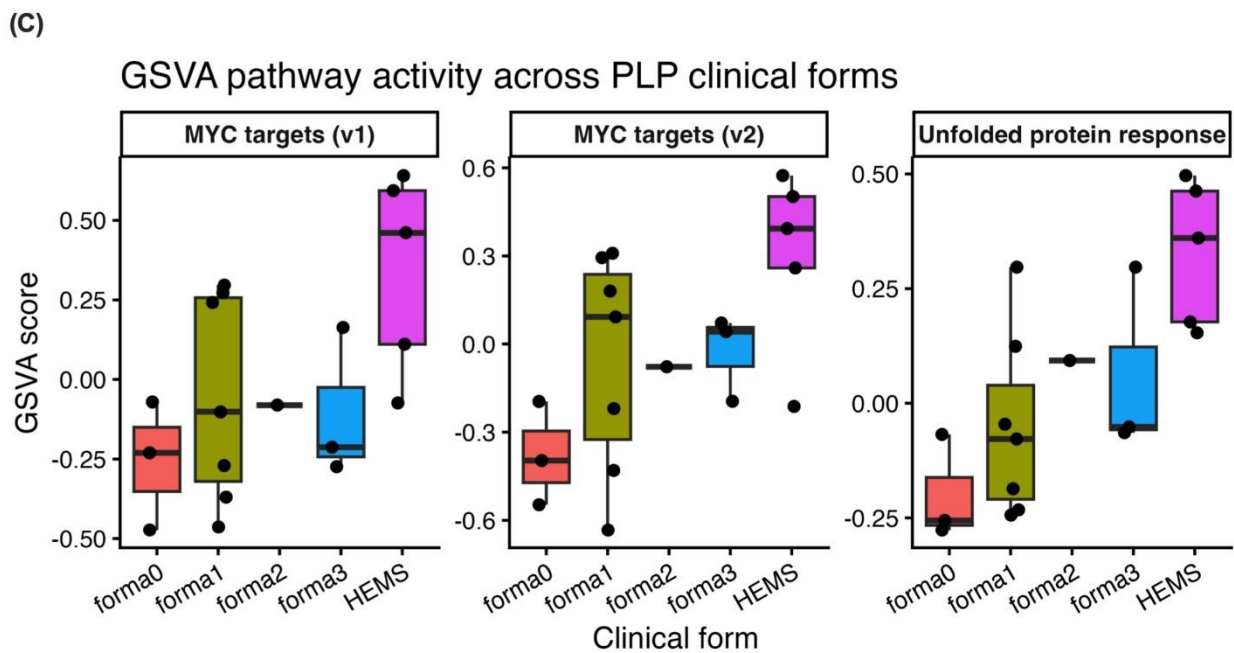
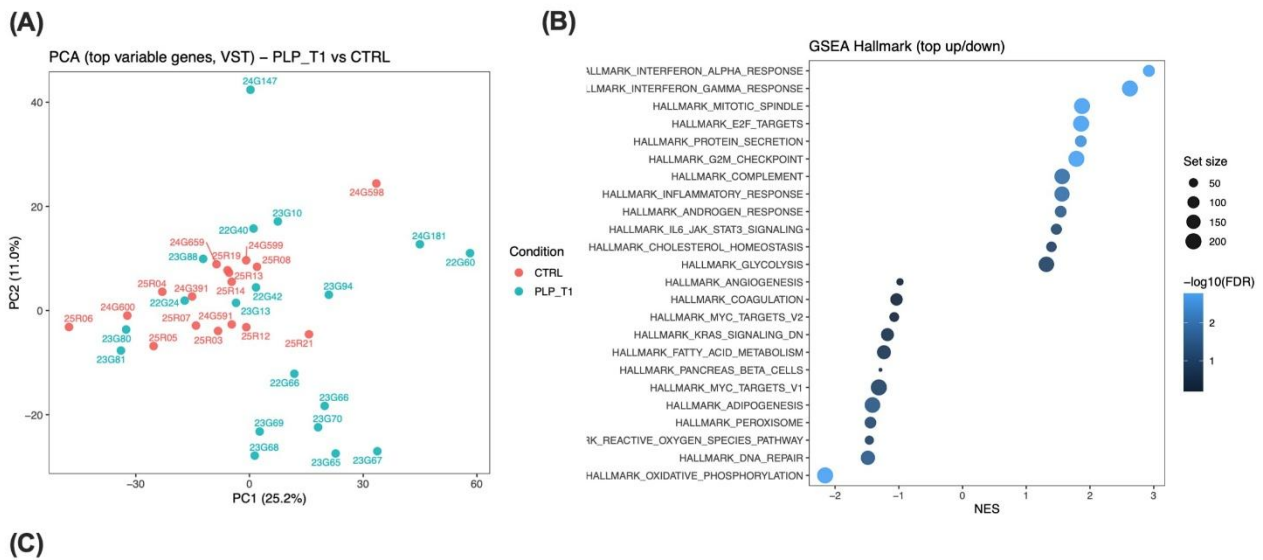
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Background. PLP1-associated disorders are rare X-linked conditions caused by pathogenic variants in the PLP1 gene that includes a wide spectrum of clinical presentations ranging from hypomyelinating leukodystrophy (Pelizeus Merzbacher Disease from form 0 to form 4 and HEMS) to pure a form of spastic paraplegia with normal MRI (SPG2). Although the genetic basis of these disorders is well established, the downstream transcriptional consequences and their relationship with clinical heterogeneity remain poorly understood. Transcriptomic profiling offers an opportunity to investigate disease-related molecular signatures and to explore pathway-level alterations associated with phenotypic variability.

Methods. Bulk RNA-seq data from blood samples of 19 patients with PLP1-related leukodystrophy and 17 healthy controls were analyzed. Raw counts were processed using a DESeq2-based workflow including sequencing run and age as covariates. Differential expression analysis was performed to identify genes deregulated between patients and controls. To investigate pathway-level alterations, preranked Gene Set Enrichment Analysis (GSEA) was conducted using MSigDB collections (Hallmark, Reactome and GO Biological Processes). Gene Set Variation Analysis (GSVA) was additionally applied to estimate pathway activity across samples and to evaluate associations with disease status, clinical forms and mutation types.

Results. Principal component analysis showed no clear separation between PLP1 patients and controls (Fig.1A), suggesting limited global transcriptional differences in blood. Consistently, differential expression analysis identified 13 deregulated genes between PLP1 patients and controls, indicating modest transcriptional changes at the single-gene level. In contrast, pathway-level analyses revealed coordinated transcriptional alterations. GSEA identified 12 significantly enriched Hallmark pathways, including interferon α and interferon γ response, as well as pathways related to cell-cycle regulation (E2F targets, G2M checkpoint) and DNA repair (Fig.1B). GSVA analysis showed limited pathway differences between PLP1 patients and controls. However, when focusing on PLP patients, pathway activity differed across clinical forms. MYC target pathways and the unfolded protein response (UPR) showed significant variation among clinical subgroups (Fig.1C).

Conclusions. Blood RNA-seq shows limited gene-level differences but consistent pathway-level alterations in PLP1-associated disorders. Immune signaling and cellular stress pathways vary across clinical forms, supporting pathway-oriented transcriptomic approaches to investigate phenotypic variability in PLP1-related leukodystrophies.



References and Acknowledgments

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Machine Learning Approaches Outperform Polygenic Risk Scores for Alzheimer's Disease Risk Prediction

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Background. Estimating an individual's risk of developing Alzheimer's disease (AD) is crucial for identifying high-risk individuals that may benefit from targeted preventive strategies aimed at delaying disease onset [1]. Given the well-established role of genetic predisposition in AD, genomic information represents a valuable resource for risk stratification [2]. Polygenic risk scores (PRS) have shown considerable potential for identifying individuals at increased risk based on genomics data [3]. However, PRS models typically rely on the assumption of additive, linear effects of genetic variants on disease risk [4]. In contrast, machine learning (ML) approaches can capture complex and potentially nonlinear relationships among genetic variants, potentially improving risk prediction by integrating information across multiple loci [5].

Objective. This study aims to systematically compare Machine Learning (ML) and Polygenic Risk Score (PRS) approaches by applying both methods to the same set of 150,000 single nucleotide polymorphisms and the same cohort of individuals (N = 989), thereby enabling a direct and unbiased methodological comparison.

Results. Among the evaluated models gradient boosting approaches achieved the highest discriminative performance. CatBoost showed the best mean performance (AUC = 0.69), followed by XGBoost and LightGBM (AUC = 0.67). In contrast, traditional linear and probabilistic classifiers demonstrated lower predictive ability, with Logistic Regression (AUC = 0.59) and Naïve Bayes (AUC = 0.57) yielding modest discrimination. The Bayesian PRS approach implemented via PRS-CS showed limited performance (AUC = 0.55), remaining substantially below boosting-based models in this same-cohort setting.

Conclusions. These results indicate that, in a same-cohort setting, gradient boosting algorithms are more effective at leveraging high-dimensional genotype data for Alzheimer's disease risk prediction than traditional linear or probabilistic classifiers, as well as PRS-based approaches. The higher performance of CatBoost, XGBoost, and LightGBM highlights the importance of modeling complex, non-additive interactions among genetic variants. In contrast, simpler linear or additive methods capture only a limited portion of the genetic signal, resulting in lower predictive accuracy. However, this study is limited by moderate sample size, and further validation in independent cohorts is needed.

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Exploring lamins role in the interplay between chromatin architecture and DNA damage response

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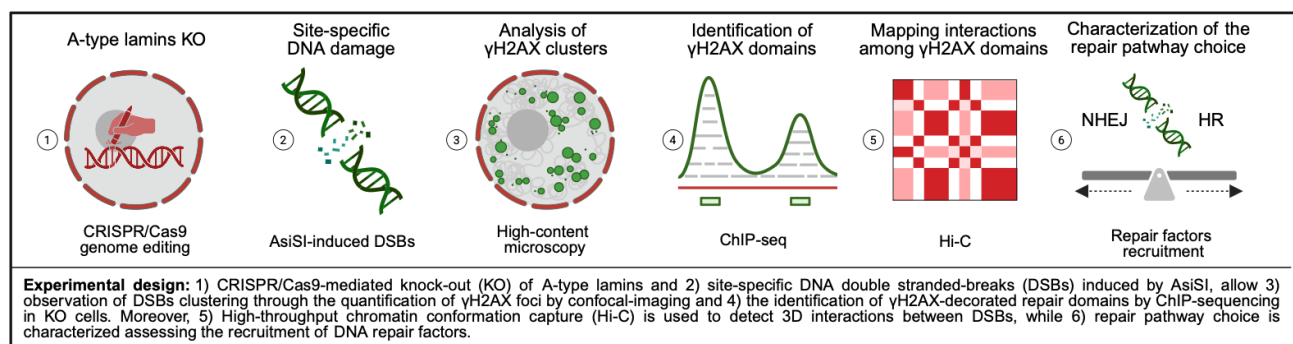
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In eukaryotes, interplay between timely DNA damage response (DDR) and a dynamic chromatin landscape is essential for genome stability [1]. The involvement of nuclear architecture and spatial compartmentalization have become apparent [2], but their role is yet to be fully uncovered. Specifically, in G1-phase, transcriptionally active genes damaged by DNA double-stranded breaks (DSBs), segregate into clusters of DSBs to avoid unfaithful repair. Since this behaviour also depends on nuclear landmarks [3], we focused on nuclear lamina, a meshwork of intermediate filaments composed of A- and B-type lamins, that maintains nuclear shape while serving as a chromatin-binding site.

To explore the role of lamins in the clustering of DSBs, we deleted A-type lamins (LMNA KO) via CRISPR/Cas9 genome editing and generated an immortalized, non-tumorigenic, human cell line expressing the ER-AsiSI system, to inducing site-specific DSBs. Clustering of DSBs can be observed through the aggregation of γ H2AX foci; therefore, we developed a quantitative imaging tool to measure clustering given γ H2AX foci dimensions. ChIP-sequencing was then used to identify γ H2AX-positive AsiSI sites and high-throughput chromosome conformation capture (Hi-C) is currently being employed to detect DSBs interactions. Finally, characterization of repair factors recruitment will highlight any functional imbalance in the repair pathway choice.

Upon inducing site-specific DSBs in LMNA KO cells, analysis of confocal images revealed potential defects in the aggregation of γ H2AX foci and the absence of a cluster-like behavior in G1. ChIP-seq analysis showed the establishment of a comparable number of γ H2AX-positive domains, relative to control cells, although affected by a reduction of γ H2AX deposition surrounding AsiSI sites. Preliminary Hi-C data also suggest the influence of LMNA KO on spatial compartmentalization in the context of DNA damage, with a depletion of long-range genomic contacts and altered dynamics in 3D chromatin rearrangements adjacent to DSBs.

Considering nuclear architecture and chromatin dynamics as an integral part of the response to DNA damage, could unravel how they influence disease mechanisms for the benefit of patients. With these results, we are also encouraged to explore the potential of targeting chromatin organization and its dynamics in the context of genome instability and cancer therapy.



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Benchmarking of GPU-accelerated variant calling pipelines for scalable whole-genome analysis in Lewy Body Dementia

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Background: Neurodegenerative diseases (NDDs) are characterized by progressive neuronal loss driven by biological hallmarks such as pathological protein aggregation, neuroinflammation, and lysosomal dysfunction [1]. Lewy Body Dementia (LBD) is a complex synucleinopathy where the identification of rare and common genetic variants is crucial for understanding disease mechanisms.

Whole genome sequencing (WGS) provides a powerful approach to investigate these variants, although it requires high computational efforts. Since the computational burden of WGS often limits large-scale analysis, we aim to develop a robust and scalable WGS analysis pipeline to support genetic studies in LBD.

Methods: We implemented a WGS analysis framework using NVIDIA Parabricks (v4.4.0-1) on high performance computing environment, leveraging GPU acceleration. Variant calling performance was compared using GATK HaplotypeCaller and Google DeepVariant, two widely used algorithms. The workflow was applied to a WGS dataset including LBD-affected individuals from the DEMENTIA-SEQ study (phs001963.v3.p1) and healthy controls from the NABEC study (phs001300.v6.p1) from dbGAP repository. Detected variants were functionally annotated using Ensembl Variant Effect Predictor (VEP) and BCFtools. Pathogenicity was assessed using in silico predictors, such as CADD, useful to prioritize rare variants (MAF < 1%) potentially linked to neurodegenerative hallmarks.

Results: Preliminary benchmarking indicates that GPU-accelerated frameworks significantly reduces processing times compared to conventional CPU-based pipelines, facilitating the analysis of extensive WGS cohorts. Comparative analysis of the variant calling tools revealed differences in sensitivity and precision for SNVs and InDels detection, highlighting the importance of tool selection for rare variant discovery [2], [3]. The integration of downstream annotation provided a detailed landscape of variants in candidate genes, allowing for the prioritization of those potentially involved in key molecular pathways of neurodegeneration.

Conclusions: By integrating GPU-accelerated variant calling with multidimensional functional annotation, we provide a scalable methodology to handle the complexity of large WGS datasets. This approach not only enhances the discovery of rare genetic risk factors but also bridges the gap between raw genomic data and the biological hallmarks of LBD. This optimized pipeline improves computational efficiency facilitating the interpretation of genomic variants in neurodegenerative studies.

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Network pharmacology and experiments reveal that the Modified Bu-Shen-Yi-Qi formula and its active ingredient baicalein ameliorate BLM-induced lung fibrosis in mice via PI3K/Akt signaling pathway

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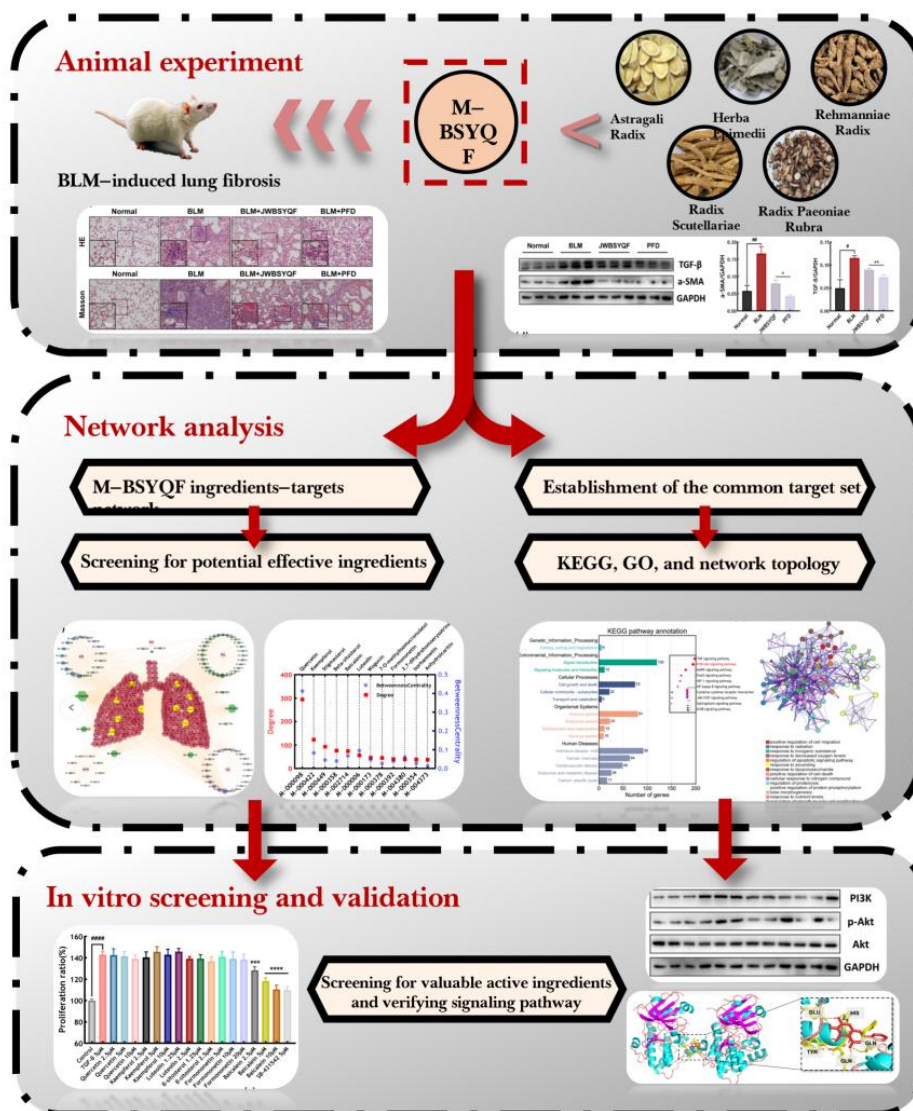
Background: Idiopathic pulmonary fibrosis (IPF) is a progressive, refractory lung disease often closely linked to inflammation. The Modified Bu-Shen-Yi-Qi Formula (M-BSYQF) is a classic Chinese patent formula composed of five herbal ingredients. It is clinically used in China to treat inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease (COPD), but its efficacy for IPF remains unclear. This study aims to evaluate the therapeutic efficacy of M-BSYQF against pulmonary fibrosis and further identify potential active components and molecular pathways.

Methods: In this study, we used a bleomycin-induced mouse model to investigate the therapeutic effect of M-BSYQF on pulmonary fibrosis. To further explore the potential effective ingredients and molecular pathways, we used the network pharmacology approach to construct a drug-ingredient-target network of M-BSYQF. Then, the common target set was established for M-BSYQF, fibroblast, and lung fibrosis. Analyses of the KEGG pathway, GO enrichment, and network topology were performed to identify key biological processes and molecular pathways for the common targets. Finally, a TGF- β 1-induced NIH/3T3 proliferation and activation model was used to validate the possible active ingredients and signaling pathways.

Results: M-BSYQF reversed BLM-induced balf leukocyte levels, pulmonary inflammatory lesions and fibrotic collagen deposition in mice and reduced the levels of α -SMA, Col1a1 and TGF- β . A total of 86 active ingredients were identified, 12 of which were considered as potential effective ingredients, while only baicalein effectively improved TGF- β -induced proliferation and activation of NIH/3T3. KEGG results showed that PI3K/Akt signaling pathway may be the potential action mechanism, and Western Blot demonstrated that both M-BSYQF and baicalein downregulated the protein levels of p-PI3K and p-Akt. The molecular docking results suggested that baicalein may directly act on the catalytic and regulatory subunits of PI3K, and this effect is stronger than direct binding to Akt1.

Conclusion: Our study revealed that baicalein may be the material basis for M-BSYQF in the treatment of pulmonary fibrosis, and the PI3K/Akt signaling pathway may be a common pathway of action for M-BSYQF and baicalein.

M-BSYQF significantly ameliorates bleomycin (BLM)-induced pulmonary fibrosis in mice, and network pharmacology reveals baicalein and the PI3K-AKT pathway as its primary active ingredient and key signaling pathway.



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Optical Genome Mapping and Long-Read Nanopore Sequencing for CSTB molecular diagnosis in Unverricht-Lundborg Disease

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Background: Unverricht-Lundborg disease (ULD), or progressive myoclonic epilepsy type 1A (EPM1A), is an autosomal recessive progressive disorder with childhood onset, characterized by action- and stimulus-induced myoclonus, generalized tonic-clonic seizures (GTCs), ataxia, and cognitive decline. ULD is caused by pathogenic CSTB gene variants or a 5'-UTR dodecamer repeat expansion (30-75 repeats in affected individuals vs 2-3 in the general population), showing inter- and intra-allelic heterogeneity. We aimed to perform molecular diagnosis in two unrelated patients with a suspected ULD by using Optical Genome Mapping (OGM) and Long Read Sequencing (LRS) to detect both CSTB variants and expansions.

Methods: OGM was performed on high-molecular-weight gDNA, followed by nanopore genomic LRS using Oxford Nanopore Technologies.

Results: Patient 1 presented at 11 years with sleep-related GTCs and photosensitivity. After initial seizure control with valproate, seizures recurred at 15 years alongside giant somatosensory evoked potentials (SEPs). Progressive tremor and myoclonus led to loss of ambulation and borderline cognition; at 22 years, brivaracetam improved symptoms. Patient 2 presented at 11 years with myoclonus and GTCs. Neurophysiology evolved to giant SEPs and C-reflex by 18 years. Despite seizure control, tremor and motor decline occurred, partially improved with piracetam, with neuropsychological slowed processing speed. OGM detected potential CSTB 5'-UTR insertions as compound heterozygous in Patient 1 (596bp, 810bp) and homozygous in Patient 2 (659bp). LRS excluded point variants and defined repeats as 50/71 and 50/65, respectively.

Conclusion: This study presents an approach based on OGM and LRS for the molecular diagnosis of CSTB repeat expansion in Unverricht-Lundborg disease. Combined OGM and LRS enable detection, sizing, and haplotyping of CSTB repeat expansions, supporting comprehensive molecular diagnosis of ULD and other repeat expansion disorders.

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Psoriasis modulates inflammatory bowel disease risk and intestinal epithelium lipid metabolism via IL-1 β -producing Macrophages

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Patients with psoriasis have an increased risk of developing inflammatory bowel disease, yet the mechanisms underlying this comorbidity remain unclear. In a clinical cohort, we observed an inverse correlation between psoriasis severity and postprandial plasma apolipoprotein B48 levels, a finding recapitulated in experimental psoriasis mouse models, indicating impaired intestinal lipid handling. To directly interrogate this process, we developed a recombinant photoconvertible apolipoprotein B reporter that enables real-time quantification of endogenous chylomicron production in intestinal organoids and in vivo. Using this system, we demonstrate that psoriasis promotes the expansion of IL-1 β -producing intestinal macrophages, which accelerate apolipoprotein B degradation, impair chylomicron secretion, drive epithelial lipid accumulation, and exacerbate mucosal inflammation. Integrating human and experimental data, our findings implicate macrophage-driven metabolic dysregulation of the intestinal epithelium as a mechanistic link between psoriasis and gut inflammation and highlight intestinal IL-1 β as a potential therapeutic target.

References and Acknowledgments

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Bioinformatics and experimental approaches identify TTN as a diagnostic marker for rheumatoid arthritis with T-cell–enriched expression

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Background: Rheumatoid arthritis is a chronic autoimmune disease with heterogeneous clinical manifestations, and its molecular mechanisms remain incompletely understood. **Objective:** To identify core genes associated with rheumatoid arthritis and evaluate their diagnostic utility, with emphasis on immune relevance and causality.

Methods: Differential expression analysis and weighted gene coexpression network analysis were used to prioritize hub genes. Functional enrichment and protein–protein interaction analyses supported biological interpretation. Diagnostic performance was assessed using a nomogram and receiver operating characteristic analysis. Immune cell infiltration analysis was conducted to identify immune-associated core genes. Two-sample Mendelian randomization using genome-wide association study data was performed to estimate the causal effect of titin on rheumatoid arthritis risk. Validation was conducted in independent public transcriptomic datasets, single-cell RNA sequencing of synovial tissue, and peripheral blood CD4-positive T cells from patients with active rheumatoid arthritis and healthy controls using quantitative real-time polymerase chain reaction and Western blot.

Results: Titin was prioritized as a rheumatoid arthritis–associated core gene and demonstrated favorable diagnostic performance. Titin expression was consistently higher in rheumatoid arthritis than in controls across validation datasets. Single-cell RNA sequencing indicated enriched titin expression in synovial T cells. In peripheral blood CD4-positive T cells, titin was significantly upregulated at both RNA and protein levels in patients with rheumatoid arthritis. Mendelian randomization supported a causal association between genetically predicted titin and rheumatoid arthritis risk.

Conclusion: Integrative analyses identify and validate titin as a rheumatoid arthritis–associated gene with immune relevance, supporting its potential as a diagnostic biomarker.

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The application of proteomics technology in the treatment of deep vein thrombosis

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Background: Deep vein thrombosis (DVT) is a serious vascular condition with complex pathophysiology, and current diagnostic and therapeutic approaches remain suboptimal. Omics technologies offer powerful tools to unravel molecular mechanisms and identify novel biomarkers and therapeutic targets.

Methods: A range of multi-omics approaches were employed, including plasma metabolomics and proteomics integrated with clinical parameters in patient cohorts, whole-genome sequencing within trans-omics programs, and the combination of bulk RNA-seq with single-cell RNA-seq (scRNA-seq) to profile transcriptomic signatures in DVT.

Results: These analyses revealed significant metabolic dysregulation in pathways such as glycolysis/gluconeogenesis and the TCA cycle, and identified a nine-metabolite panel as potential diagnostic biomarkers for post-traumatic DVT. Transcriptomic profiling pinpointed CXCR4 as a potential genetic marker, with scRNA-seq uncovering active roles for B cells, T cells, and monocytes in DVT pathogenesis. Integrative proteogenomic analysis further highlighted the complement and coagulation cascades as significantly altered pathways.

Conclusion: Multi-omics technologies are instrumental in elucidating DVT pathophysiology, identifying novel diagnostic biomarkers, and uncovering potential therapeutic targets, offering new insights for precision management of this condition.

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Gut Microbiota Modulation by Inulin Improves Metabolism and Ovarian Function in Polycystic Ovary Syndrome

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Background. Polycystic ovary syndrome (PCOS) is closely associated with gut microbiota, and managing metabolic disorders related to PCOS has been proposed as an effective intervention to improve the disease. However, the underlying mechanism remains unclear.

Methods. This study explored the alleviative effects of inulin (a gut microbiota regulator) on PCOS. The impacts of inulin on hyperandrogenism and glucolipid metabolism were evaluated in human PCOS cohort and mouse models. Gut microbiota composition, short-chain fatty acids (SCFAs) production, lipopolysaccharide-binding protein (LBP) level and ovarian inflammation were detected. Lipopolysaccharide (LPS) administration and fecal microbiota transplantation (FMT) were further performed to verify the regulatory mechanism.

Results. Inulin treatment observably ameliorated hyperandrogenism and glucolipid metabolism disorders in both PCOS patients and mice. It increased the abundance of microbial co-abundance group (CAG) 12 (including Bifidobacterium and other SCFAs-producing bacteria), enhanced SCFAs biosynthesis capacity and fecal SCFAs levels. Inulin also reduced LBP level and alleviated ovarian inflammation in PCOS mice, while LPS injection reversed the protective effects of inulin. FMT from inulin-treated PCOS patients improved insulin sensitivity, lipid accumulation and thermogenesis, and reduced hyperandrogenism and ovarian inflammation in antibiotic-treated mice.

Conclusion. Gut microbiota mediates the beneficial effects of inulin on metabolic disorders and ovarian dysfunction in PCOS. Modulating gut microbiota is a promising therapeutic strategy for PCOS.

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Association Study of ABCG2 and SLC2A9 Gene Polymorphisms with Gout Susceptibility in Mongolian and Han Populations in Inner Mongolia

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A genome-wide association study (GWAS) in China indicated that the ABCG2 gene is a primary genetic factor regulating gout, while SLC2A9 is the most significant gene influencing blood uric acid levels[1]. Although the Mongolian population in Inner Mongolia has higher rates of alcohol consumption, meat intake, and metabolic syndrome than the Han population[2], their prevalence of hyperuricemia (HUA) is lower[3], suggesting possible genetic differences. Objective: To investigate the influence of ABCG2 and SLC2A9 gene polymorphisms on gout susceptibility in Mongolian and Han populations in Inner Mongolia, aiming to provide insights into the genetic mechanisms underlying gout and to inform individualized treatment strategies for different ethnic groups. Methods: Gout patients and healthy controls from the Mongolian and Han populations in Inner Mongolia were included. Genotyping of ABCG2 (rs13120400, rs1481012, rs2231142, rs3114018) and SLC2A9 (rs1014290, rs16891234, rs3733590, rs3733591, rs3775948, rs4697701, rs9999470) was conducted using mass spectrometry. Various genetic models were employed to analyze the association between ABCG2 and SLC2A9 single nucleotide polymorphisms (SNPs) and gout. Haplotype construction and evaluation of SNP combinations were performed to assess their impact on gout, and genotype distribution differences between the two ethnic groups were analyzed. Results: ABCG2 rs1481012, rs2231142, and SLC2A9 rs3733591 variants increased the risk of gout ($P < 0.05$), while variants in SLC2A9 (rs3775948, rs4697701, rs1014290, rs3733590, rs16891234, rs9999470) and ABCG2 rs3114018 were associated with a reduced gout risk ($P < 0.05$). Specifically, rs1481012 and rs2231142 variants significantly increased gout risk in the Han population but showed no significant association in the Mongolian population. Certain SNPs, such as rs16891234 and rs9999470, demonstrated protective effects in the Mongolian group, which were not observed in the Han group. In the Han population, the ABCG2 CC haplotype might act as a protective factor against gout. Conclusion: ABCG2 and SLC2A9 gene polymorphisms influence gout susceptibility in the Inner Mongolia region. The distribution of ABCG2 gene polymorphisms differs between the Han and Mongolian populations, whereas no significant differences were found in the distribution of SLC2A9 gene polymorphisms.

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