



MARKOPOLO

Noise and/or ultrafine particulate matter induced cerebral and cardiovascular damage: novel insights from experimental and epidemiological brain-heart axis biomarkers and computational models

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SUMMARY

The deliverable 11.1 focuses on the collection and integration of existing data provided by various partners of the consortium in the context of particulate matter and noise exposure in murine models. Additionally, the document provides a list of biomarkers drawn from literature.

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1 INTRODUCTION

1.1 Purpose and Scope

This document describes the completion of the Deliverable 11.1 which focuses on the collection, integration and harmonization of existing data in the context of PM and noise exposure, which have been obtained by LIH from various partners of the consortium. The integration of multiple datasets coming from published and unpublished works from members of the consortium enables meta-analyses that can highlight relevant biological insights (e.g. biological pathways involved, biomarkers); thus, creating valuable resources that can be used for decision making in subsequent WPs and milestones.

1.2 References to other MARKOPOLO Documents

- Concentris presentation: “MARKOPOLO_WP11_Bioinformatics I Harmonization of the processes and analysis of the existing multi-omics data_Nazarov”

1.3 Definitions, Abbreviations and Acronyms

Table 1 List of Abbreviations and Acronyms

Abbreviation/ Acronym	DEFINITION
PM	Particulate matter
LC-MS	Liquid chromatography mass spectrometry
PM-3d / PM-4w	Particulate matter exposure for 3 days / 4 weeks
ETC	Electron transport chain
RNA-seq	RNA sequencing
DEGs	Differentially expressed genes
GSEA	Gene set enrichment analysis
fGSEA	Fast gene set enrichment analysis
MSigDB	Molecular signatures database
logFC	Log fold change
NES	Normalized enrichment score
QC	Quality control
PCA	Principal component analysis
DIA	Data independent acquisition
proDA	Probabilistic dropout analysis

2 DATA AND METHODS

In this part, the datasets acquired and methodologies used to analyse the data are briefly listed.

2.1 Description of datasets acquired

In this section we provide short description of all datasets provided from members of the consortium.

1) PM-3d (aorta, brain, heart, lung)

Short-term (3 days) mouse exposure to particulate matter (NIST, SRM1648a, broad size range, mostly PM_{2.5}) and aircraft noise, alone or combined. Combined exposure caused additive oxidative, vascular, and neuroinflammatory damage across lung, brain, heart, and aorta, including RNAseq (Kuntic 2023, Redox Biology).

2) PM-4w (aorta, brain, heart, lung)

Chronic (4 weeks) particulate-matter (NIST, SRM1648a, broad size range, mostly PM_{2.5}) exposure in multiple organs. Multi-organ RNA-seq dataset exploring long-term PM effects on cardiovascular and pulmonary systems. Unpublished.

3) Noise (1, 2 and 4 days, around-the-clock) – aorta

First aircraft-noise study on aortic tissue (Münzel 2017, EHJ). Demonstrated hypertension, endothelial dysfunction, and oxidative stress through NOX2 activation and vascular transcriptomic shifts.

4) Noise (4 days, around-the-clock, only sleep phase, only awake phase) – circadian clock – different organs (aorta, heart, kidney)

Noise exposure during rest phase disrupts vascular and cerebral circadian regulation (Kröller-Schön 2018, EHJ). Identified oxidative stress and clock-gene dysregulation in vascular tissues, including RNAseq.

5) Noise (4 days, around-the-clock) – diabetes - aorta

Three diabetic mouse models (STZ, S961, HFD) exposed to aircraft noise (Mihaliková 2024, EJPC). Noise worsened glucose intolerance, hypertension, and endothelial dysfunction, including RNAseq.

6) Noise (4 days, around-the-clock) – fasting/exercise/ α 1AMPK - aorta

Noise-induced vascular stress mitigated by exercise, fasting, or AMPK activation (Kvandová 2023, EJPC). Showed α 1AMPK's key protective role against noise-triggered oxidative injury, including RNAseq.

7) Transcriptomics – olfactory bulb (air pollution UFPs)

Two-week whole-body inhalation of ultrafine particles in female C57BL/6J mice. RNA-seq in olfactory bulb revealed ~45 novel mRNAs altered (in odorant binding, synaptic regulation, myelination) after exposure (Savaleva 2023)

8) Short-term aircraft-noise (4 days, only sleep phase) – heart proteome/metabolome

Four days of aircraft noise exposure in mice induced a shift in the heart's proteome and metabolome: reduced fatty-acid β -oxidation, TCA-cycle and ETC capacity, increased glycolysis/lactate, resembling ischaemic/heart-failure states (Marques 2025)

Since the ultimate aim is to provide further insights by aggregating multiple datasets together, the data must share some degree of commonality (e.g. same exposures, same tissue). We thus decided to include in the analysis datasets 1,2,3,4,8.

2.2 Methods used for the analysis

The aforementioned data can be divided in two categories: bulk RNA-sequencing and DIA LC-MS proteomics. Given the different nature of the data, two separate computational pipelines have been generated to suit their specific needs (e.g. different QC, filtering, statistical methods). A third pipeline then has been created to harmonize, integrate the results and perform aggregated meta-analysis.

2.2.1 RNA-seq pipeline

The RNA-sequencing pipeline established operates as follows:

1. Data integration and cleaning

Combines raw gene-count matrices from aorta, brain, lung, and heart datasets into a unified format.

Standardizes sample metadata and prepares region-specific matrices for downstream analysis.

Tools: tidyverse, openxlsx.

2. Quality control and visualization

Produces exploratory plots for sample distributions, clustering, and correlations.

Tools: ggplot2, corrplot, PCAtools, gridExtra, GGally.

3. Batch correction and normalization

Corrects inter-batch variability across experiments to harmonize expression profiles.

Tools: sva (ComBat-seq), PCAtools.

4. Differential expression analysis

Computes gene-level differential expression for each organ and condition relative to controls.

Tools: edgeR, limma-voom.

5. Cross-region comparison and correlation

Examines overlap and correlation of DEGs across tissues and conditions to detect shared responses.

Tools: GGally, corrplot.

6. Functional enrichment

Performs pathway and gene-set enrichment using multiple databases.

Tools: clusterProfiler, fgsea, msigdb.

7. Annotation and export

Adds gene symbols, writes ranked lists and enrichment tables for downstream GSEA or visualization.

Tools: openxlsx, base R file I/O.

8. (Optional network and PCA modules)

Reconstructs DEG-based correlation networks and PCA projections for exploratory visualization.

Tools: igraph, PCAtools.

2.2.2 Proteomics pipeline

The proteomics pipeline established operates as follows:

1. Data import and preprocessing

Loads MaxQuant-like protein group reports, cleans sample metadata, harmonizes condition labels, and reshapes the dataset into a sample × protein matrix.

Tools: tidyverse, dplyr, openxlsx.

2. Quality control and exploratory clustering

Computes distance-based hierarchical clustering to detect potential outlier samples and visualize replicate relationships.

Tools: stats (hclust), ggplot2.

3. Normalization and missing-value handling

Supports sample-wise normalization and imputation of missing intensities within conditions using PCA-based imputation.

Adds a constant baseline for low-abundance proteins if enabled.

Tools: missMDA, sjmisc.

4. Differential protein abundance analysis

Performs statistical testing of condition contrasts (Noise, PM, combined) against control using proDA, which models missing values probabilistically.

Tools: proDA.

5. Protein-to-gene mapping and annotation

Converts protein identifiers to gene symbols using a custom mapping obtained from UNIPROT file for downstream pathway analysis.

Tools: dplyr, openxlsx.

6. Gene set enrichment analysis

Creates ranked gene lists and executes GSEA across multiple MSigDB collections (C1–C5, H).

Tools: fgsea, clusterProfiler, msigdb.

7. Visualization and export

Exports full DEG tables and ranked lists for enrichment input.

Generates example expression plots for selected proteins.

Tools: ggplot2, openxlsx.

2.2.3 Meta-analysis pipeline

This script performs multi-study integration and cross-omics comparisons combining transcriptomic and proteomic differential-expression datasets from several independent mouse studies. It systematically compares gene- and pathway-level results across timepoints, tissues, and platforms (RNA vs protein) to identify reproducible biological responses to noise and particulate exposure. The workflow operates as follows:

1. Cross-timepoint transcriptomic comparison (4 days vs 4 weeks PM, in the future also 4 days vs 4 weeks noise as samples for RNAseq are submitted to Novogene)

Integrates DE and GSEA results across short- and long-term exposures for each organ.

Assesses reproducibility of logFC and normalized enrichment scores (NES).

Tools: tidyverse, openxlsx, ggplot2, ggrepel.

2. Re-analysis of historical datasets (Noise 2017 vs Noise 2023)

Compares early and recent noise-exposure transcriptomic results (aorta).

Aligns DEGs and GSEA outputs to highlight persistent stress and inflammatory pathways.

Tools: ggplot2, openxlsx.

3. Cross-tissue proteomics comparison (Heart vs Aorta vs plasma)

Integrates 4-day proteomic DE results between cardiac and vascular tissues.

Examines overlap of significantly altered proteins and enriched pathways.

Tools: ggplot2, fgsea, ggrepel.

4. Transcriptome–proteome integration

Correlates RNA- and protein-level changes within each tissue (heart, aorta) for the same exposure contrasts.

Compares corresponding fGSEA results to assess pathway-level concordance.

Tools: dplyr, ggplot2, fgsea.

5. Proteomic cross-organ integration (Ao ↔ Hr)

Re-evaluates shared protein-level responses and co-regulated biological functions across heart and aorta.

Tools: ggplot2, fgsea.

6. Historical transcriptome–proteome validation (Noise 2017 vs Proteomics 2025)

Cross-validates modern proteomic datasets with prior 4-day proteomic data.

Compares directionality of protein fold changes and enrichment of conserved pathways.

Tools: ggplot2, openxlsx, fgsea.

2.3 A.I. assisted state of the art

To summarize the state of the art of biomarker discovery in the context of PM and noise exposure we used Biomni agent (Huang, 2025) with the following prompt “Systematic literature search to compile biomarkers (proteins, RNAs, metabolites) associated with particulate matter (PM2.5/PM10), engineered/ultrafine nanoparticles, urban/environmental noise, and combined PM+noise exposure in mouse or human studies. Output: narrative summary grouped by exposure type + structured CSV table”.

3 RESULTS

The MARKOPOLO project investigates the molecular impact of environmental stressors, specifically aircraft noise and fine particulate matter, on the brain, lungs, heart, and aorta. Using multi-omics approaches, including transcriptomics and proteomics in mouse models, it aims to uncover common mechanisms of cardiovascular, pulmonary, and neurological dysregulation.

Transcriptomics data show strong, tissue-specific transcriptional responses after 4-day and 4 weeks exposures, with hundreds of significantly dysregulated genes per tissue, especially in the lungs. Enrichment analyses indicate activation of inflammatory, immune, and oxidative stress pathways, including IL-signaling, oxidative metabolism, and protein synthesis regulation. Prominent upregulated genes include SAA1/SAA3, LCN2, CXCL3, LY6I, and ORM1, which are markers of inflammation, lung injury, and acute-phase response. Other stress-related genes such as UCP1, PLCXD3, and LRP2 suggest metabolic and vascular remodeling. In brain and systemic analyses, genes like IDO1, ADORA2A, PENK, PPP1R1B,

and SH3RF2 reflect neuro-immune and neuronal regulation. Across datasets, microRNA-target modules (M3) are consistently enriched, suggesting post-transcriptional regulation in the inflammatory and stress response.

Proteomics results from aorta and heart (about 4–5 thousand protein groups each) indicate exposure-specific alterations that do not overlap in-between tissues. A small subset of shared proteins between heart and aorta reveal activation of metabolic and structural systems including the TCA cycle, mitochondrial enzymes such as SUCLA2 and EEF1G, contractile and developmental proteins like TTN, TNNT3, RPL3L, and UBR2, and redox or ion-related components such as CLIC6. Pathway enrichment points to oxidative phosphorylation, mitochondrial function, and cytoskeletal remodeling as central affected processes.

Cross-study analyses, including comparison of 2017 and 2023 datasets, confirm reproducible molecular signatures, with fGSEA showing consistent enrichment of hallmark and microRNA-related gene sets across tissues and omics layers. Integration of transcriptomic and proteomic data reveals coordinated metabolic and inflammatory reprogramming in vascular and cardiac tissues under environmental stress.

Biologically, both noise and particulate exposure converge on endothelial dysfunction, oxidative stress, and immune activation. The lung exhibits innate immune and acute-phase activation, the heart and aorta show metabolic and contractile remodeling, and the brain displays transcriptional patterns linked to neuroendocrine and stress signaling. The shared patterns across different omics layers suggest systemic interactions among organs, supporting the hypothesis of an interconnected cerebro-pulmonary-cardiovascular axis central to the MARKOPOLO project.

Lists of genes and proteins that have been observed as strongly dysregulated are currently being investigated in newly generated datasets, with the ultimate goal of identifying potential biomarkers to drive forthcoming milestones. In the supplementary material we also include the biomarker results from the Biomni search. (Huang, 2025)

4 DATA AVAILABILITY

Results from Biomni, and the individual/combined meta-analyses are stored in the online LIH server (<https://bioinfo.lih.lu/share/files/results/>).

5 SUPPLEMENTARY MATERIAL

5.1 A Comprehensive Review across *Homo sapiens* and *Mus musculus*

Compiled by: Biomni (Phylo) | **Date:** 2026-05-19

Scope: Proteins, RNAs (miRNA, lncRNA, circRNA), and metabolites associated with PM2.5/PM10, engineered/ultrafine nanoparticles, urban/environmental noise, and combined PM+noise co-exposure.

Species: *Homo sapiens*, *Mus musculus* (and selected *Rattus norvegicus*, *Cavia porcellus* for cochlear studies)

Structured data: biomarkers_PM_noise_table.csv (214 entries)

Table availability: (https://bioinfo.lih.lu/share/files/results/biomarkers_PM_noise_table.csv).

5.2 Overview

Exposure to airborne particulate matter (PM) and environmental noise are two of the most pervasive urban health hazards. Both trigger overlapping but mechanistically distinct biological responses — oxidative stress, systemic inflammation, endocrine dysregulation, and organ-specific damage — that converge on cardiovascular, pulmonary, neurological, and metabolic disease endpoints. This report synthesises biomarker evidence across four exposure categories:

Exposure Category	Entries in Table	Primary Biological Systems
PM2.5/PM10	102	Lung, cardiovascular, brain, skin, eye, gut
Nanoparticles (engineered/ultrafine)	43	Lung, liver, blood, cochlea
Noise (occupational/environmental)	59	Cochlea, cardiovascular, brain, gut
Combined PM + Noise	10	Vasculature, lung, brain

A key cross-cutting theme is **convergent oxidative stress**: MDA, 8-OHdG, 8-isoprostane, and glutathione depletion appear across all four exposure types, in multiple tissues and species, making them the most robust pan-exposure biomarkers identified.

5.3 PM2.5 / PM10 Exposure

5.3.1 Proteins

PM2.5/PM10 exposure drives a broad cytokine response dominated by **IL-6, TNF- α , IL-8, and IL-1 β** , documented across lung, blood, skin, and colon in both human epidemiological studies and mouse models. The Th1/Th2 balance is disrupted in a tissue-dependent manner: mouse BALF shows Th2 skewing (IL-4 $\uparrow$, IL-5 $\uparrow$, IL-13 $\uparrow$, IFN- γ $\uparrow$) consistent with asthma-like pathology, while human blood T cells show Th17 activation (IL-17 $\uparrow$, IL-21 $\uparrow$).

Neuroinflammation is a particularly striking finding. Chronic PM2.5 exposure in 9-month-old mice elevates BACE1, APP, A β 1-40, GFAP, and Iba-1 in the brain — a pattern resembling early Alzheimer's disease pathology — alongside COX-1/COX-2 upregulation and iNOS/nitrotyrosine accumulation. VCAM-1 is elevated in both brain and blood, linking neuroinflammation to vascular dysfunction.

Vascular biomarkers include MMP-9, VCAM-1, ICAM-1, VEGF, angiopoietin-1, and PIGF (all upregulated in human blood), with eNOS downregulation and endothelin-1 upregulation pointing to endothelial dysfunction. Fibrinogen elevation (meta-analysis, human) links PM2.5 to coagulation risk.

Skin and eye are less-studied but important targets. PM10 exposure in mice activates the TSLP/IgE/IL-31 axis and disrupts the skin barrier (filaggrin $\downarrow$, loricrin $\downarrow$), recapitulating atopic dermatitis. In the eye (trabecular meshwork), PM2.5 activates the NLRP3/caspase-1/GSDMD pyroptosis pathway.

Antioxidant signalling: Nrf2 nuclear translocation is upregulated as a compensatory response, but downstream targets (SOD, catalase) are ultimately depleted under chronic exposure. NOX2 upregulation in lung and heart drives sustained superoxide production.

5.3.2 Metabolites

The PM2.5 metabolome is dominated by **oxidative stress markers**: MDA and 8-isoprostane (lipid peroxidation), 8-OHdG (DNA oxidation), and 3-nitrotyrosine (nitrosative stress) are consistently elevated in urine and blood across human cohorts (Framingham Heart Study, Shanghai RCT, European multi-country studies) and mouse models.

Stress hormones (cortisol, cortisone, epinephrine, norepinephrine) are elevated in human serum in a randomised air purifier trial, providing causal evidence for HPA and sympatho-adrenal axis activation by PM2.5.

Tryptophan/circadian metabolism is disrupted: melatonin and N-acetylserotonin are decreased in human serum (longitudinal study), consistent with sleep disruption and circadian clock interference. Bilirubin and biliverdin are also decreased, suggesting impaired antioxidant heme catabolism.

Phospholipid and energy metabolism are perturbed: lysophosphatidic acid and lysophosphatidylethanolamine decrease; acylcarnitines and free fatty acids increase (mouse, PM2.5 + ecSOD model), indicating mitochondrial fatty acid oxidation stress. Glucose, pyruvate, and lactate are elevated in human plasma metabolomics, pointing to insulin resistance and anaerobic energy shift.

Amino acid depletion: Arginine and histidine are consistently decreased in human plasma across multiple studies, with arginine loss implicating reduced NO bioavailability and endothelial dysfunction.

PAH exposure is captured by urinary 1-hydroxypyrene (1-OHP), a specific metabolic biomarker for polycyclic aromatic hydrocarbon components of PM2.5.

5.3.3 RNA Biomarkers

PM2.5 has a well-characterised **blood miRNA signature** in humans. The most replicated findings are:

- **miR-146a** (down in blood, up in mouse lung) — a master regulator of NF-κB-driven inflammation, consistently altered across multiple cohorts and species
- **miR-21-5p** (down in blood, up in lung) — cardiovascular and inflammatory regulation
- **miR-155** (up, PAH-PM2.5) — cardiovascular development, Wnt/ErbB signalling
- **miR-126** (down) — endothelial function and vascular homeostasis

A European multi-country personal monitoring study identified **miR-24-3p, miR-4454, miR-425-5p, and miR-505-3p** as PM2.5 exposure-responsive in blood, independent of ambient monitoring.

In the **brain**, miR-574-5p (down in mouse hippocampus) targets BACE1, providing a mechanistic RNA link between PM2.5 and Alzheimer's-like amyloid processing.

lncRNAs MALAT1 (EMT) and MEG3 (apoptosis/autophagy via p53) are upregulated in human bronchial epithelial cells, and **circBbs9** (upregulated in mouse lung) sponges miR-30e-5p to activate the NLRP3 inflammasome — a circular RNA–miRNA–protein axis directly linking PM2.5 to pyroptotic lung injury.

5.4 Nanoparticle Exposure

5.4.1 Proteins

Engineered nanoparticle (NP) toxicology biomarkers overlap substantially with PM2.5 but show important size- and composition-dependent features:

- **Gold nanoparticles (AuNPs):** IL-1 β , TNF- α , IL-6 in mouse spleen are size-dependent (50 nm > 20 nm > 5 nm). Ultrasmall AuNPs (<10 nm) specifically activate the NLRP3 inflammasome.
- **CuO nanoparticles:** Drive pulmonary fibrosis via MMP-3 cleavage of osteopontin (OPN), with CXCL1/KC and LDH as BALF markers of neutrophilic inflammation.
- **Nanosilica (colloidal/pyrogenic):** IL-8 and TNF- α in lung epithelial cells and macrophages.
- **Metal oxide NPs (general):** IL-1 β is the most consistent cross-NP inflammasome marker, validated by QSAR modelling in mouse lung.

Occupational NP exposure (NanoExplore cohort, human workers): KL-6 (interstitial lung fibrosis marker), hs-CRP, and cytokines in exhaled breath condensate (IL-10 $\uparrow$, IL-1 β $\uparrow$, TNF- α $\uparrow$) provide translational human biomarkers.

Neural injury markers UCHL1 and S100B are elevated in human blood after controlled PM exposure with high metal content, suggesting CNS vulnerability to metal-containing particles.

Proteotoxic stress markers (heat shock proteins, metallothioneins, TP53 dysregulation) are identified across metal NP exposures in a systematic review.

5.4.2 RNA Biomarkers

This is the most significant gap in the current evidence base. Despite two search attempts, no well-validated nanoparticle-specific RNA biomarker studies were captured — the literature is dominated by nanoparticle-based biosensor papers rather than NP toxicology transcriptomics. This reflects a genuine research gap: NP-specific miRNA/lncRNA signatures in vivo remain poorly characterised compared to PM2.5.

5.4.3 Metabolites

Oxidative stress is the dominant metabolic signature across NP types:

- **Occupational NP workers:** 8-OHdG, MDA, 8-isoprostane, 3-nitrotyrosine, and 3-chlorotyrosine in urine/EBC (printer-emitted NPs, NanoExplore cohort)
- **SiO₂ NPs (BEAS-2B cells):** GSH depletion with compensatory increases in cysteine, cysteinyl-glycine, and pantothenic acid — a glutathione synthesis stress response
- **Mesoporous SiO₂ NPs (mouse liver, IV):** Succinate $\uparrow$, hypoxanthine $\uparrow$, GSSG $\uparrow$, NADP⁺/NADPH dysregulation — TCA cycle disruption and pentose phosphate pathway stress
- **Ultrafine particles (UFP, human plasma):** Arginine $\downarrow$, glutamine $\downarrow$, cystine $\uparrow$, methionine sulfoxide $\uparrow$ — consistent with oxidative amino acid modification and NO pathway suppression
- **Subway UFP (EBC, human workers):** Lactate $\uparrow$, Σ NO $\downarrow$ — metabolic stress and nitric oxide depletion in the airway

5.5 Noise Exposure

5.5.1 Proteins

Noise exposure activates two primary systemic axes: **HPA/sympatho-adrenal** (cortisol/corticosterone↑, adrenaline↑) and **vascular oxidative-inflammatory** (NOX2↑, eNOS uncoupling, IL-6↑, TNF-α↑).

Cardiac biomarkers are well-characterised in mouse models:

- NLRP3/ASC/caspase-1/IL-1β/IL-18 — inflammasome activation in heart (noise + sleep deprivation model)
- BiP/GRP78, XBP1, CHOP — ER stress markers in heart
- Atrial natriuretic peptide A (ANPA) — cardiac remodelling/stress
- p-p38, p-eIF2α, PERK — integrated stress response

Cochlear biomarkers (noise-induced hearing loss, NIHL):

- Oxidative: HMGB1↑, NOX4↑, iNOS↑, ICAM-1↑, 4-HNE↑, MDA↑, SOD↓
- Structural/signalling: ITGA1↑, KNG1↑, CFI↑ (upregulated); FGF1↓, AKT2↓, ATG5↓ (downregulated, neuroprotective loss)
- These cochlear proteomics data (TMT-based, mouse) provide a mechanistic map of NIHL pathophysiology

Vascular dysfunction: eNOS and nNOS uncoupling/downregulation, NOX2↑, COX-2↑, 8-oxoguanine↑, 4-HNE↑, 3-nitrotyrosine↑ in vasculature and brain (aircraft noise, mouse)

Human plasma proteomics (UK Biobank): CDCP1, CXCL17, FGF21, GDF15, and IGFBP4 are identified as mediating proteins for the air+noise pollution pattern, linking combined environmental exposure to cardiometabolic disease risk.

Neurodegeneration: Chronic noise elevates Aβ in mouse hippocampus, paralleling the PM2.5 neuroinflammation findings and suggesting a convergent noise→AD pathway.

5.5.2 RNA Biomarkers

Human blood miRNA (NIHL patients): Five upregulated (hsa-miR-486-2, hsa-miR-664b-3p, hsa-miR-4485, hsa-miR-501, hsa-miR-663b) and two downregulated (hsa-miR-6723, hsa-miR-194-2) miRNAs distinguish NIHL patients from controls — potential non-invasive diagnostic biomarkers.

Rat cochlear nucleus (chronic noise): miR-758-5p, miR-15b-5p, miR-212-3p, miR-199a-5p, and miR-134-3p are all downregulated, with predicted targets AKT3, SIRT1, and PRKAA1 (AMPK pathway) upregulated — suggesting a noise-driven AMPK/energy sensing dysregulation in the auditory brainstem.

5.5.3 Metabolites

Cochlear metabolomics:

- Spermidine (↑2.85-fold, mouse cochlea, GC-MS) — a polyamine with autophagy-modulating properties; the most specific cochlear NIHL metabolite identified
- Deoxyribose 1-phosphate (↑, guinea pig cochlear fluid) — oxidative phosphorylation marker

Gut-brain-cardiovascular axis (airport noise, mouse, 100 days):

- Short-chain fatty acids (acetate, propionate) ↓ — gut microbiome dysbiosis
- TMAO ↓ — altered gut microbial metabolism
- LPS ↑ — gut barrier disruption and endotoxaemia
- IL-6, TNF- α mRNA ↑ in intestine This gut-mediated pathway is a novel and underappreciated mechanism by which chronic noise drives systemic inflammation.

Occupational noise (human plasma metabolomics, steel workers): 469 differential metabolites identified, with 33 specific to NIHL; glycerophospholipids and GPI-anchor biosynthesis metabolites are the most enriched pathways.

Oxidative stress: MDA↑, GSH↓ in cochlea, liver, and testis (noise + cadmium co-exposure, rat) — indicating multi-organ oxidative damage.

5.6 Combined PM + Noise Co-exposure

This is the least-studied category, with only a handful of dedicated co-exposure studies. Key findings:

Offer et al. 2022 (Redox Biology) — C57BL/6 mice, 3-day co-exposure to NIST PM_{2.5} + aircraft noise:

- Additive endothelial dysfunction (reactive hyperaemia index)
- Additive blood pressure elevation
- Tissue-specific oxidative stress: PM primarily affects lung, noise primarily affects brain — but both converge on vascular dysfunction
- ACE-2 upregulation in lung (additive) — raising concern for increased SARS-CoV-2 susceptibility under combined exposure

Molitor et al. 2023 (Cardiovascular Research) — noise pre-conditioning sensitises to PM_{2.5} cardiovascular effects:

- NOX2↑, phospho-NF κ B↑ in blood and vasculature
- IL-6↑, CRP↑ — systemic inflammation amplified by prior noise history

Hemmingsen et al. 2015 (Mutation Research) — controlled human exposure to diesel exhaust + traffic noise:

- hOGG1-sensitive DNA damage sites elevated by noise component (PBMCs)
- IL-8, TNF- α , HO-1, OGG1 gene expression unaltered by diesel exhaust alone — noise may be the dominant driver of DNA damage in this co-exposure

Tang et al. 2025 (Nature Communications, UK Biobank):

- CDCP1, CXCL17, FGF21, GDF15, IGFBP4 identified as plasma protein mediators of the combined air+noise pollution pattern → cardiometabolic disease

5.7 Cross-Cutting Themes and Convergent Biomarkers

5.7.1 Universal Oxidative Stress Signature

The following biomarkers are elevated across **all four exposure types**, in multiple tissues and species:

Biomarker	PM2.5/PM10	Nanoparticles	Noise	Combined
MDA	✓	✓	✓	✓
8-OHdG	✓	✓	✓	✓
8-isoprostane	✓	✓	✓	—
GSH (depletion)	✓	✓	✓	—
3-Nitrotyrosine	✓	✓	✓	✓
IL-6	✓	✓	✓	✓
TNF- α	✓	✓	✓	✓
IL-1 β	✓	✓	✓	—
NOX2	✓	—	✓	✓

5.7.2 Exposure-Specific Signatures

- **PM2.5 only:** miR-146a/miR-21 blood signature; BACE1/APP/A β neurodegeneration axis; filaggrin/loricrin skin barrier disruption; circBbs9/miR-30e-5p/NLRP3 circRNA axis
- **Nanoparticles only:** Size-dependent NLRP3 activation (AuNPs); MMP-3/OPN fibrosis axis (CuO NPs); KL-6 (occupational lung fibrosis)
- **Noise only:** Spermidine (cochlea); NIHL blood miRNA panel (hsa-miR-486-2 etc.); gut microbiome disruption (SCFAs $\downarrow$, LPS $\uparrow$); ER stress in heart (BiP/XBP1/CHOP)
- **Combined PM+Noise:** ACE-2 additive upregulation; CDCP1/CXCL17/FGF21/GDF15/IGFBP4 plasma protein panel (UK Biobank)

5.7.3 Convergent Neurodegeneration Risk

Both PM2.5 (BACE1 $\uparrow$, A β $\uparrow$, GFAP $\uparrow$, miR-574-5p $\downarrow$) and chronic noise (A β $\uparrow$, eNOS uncoupling, neuroinflammation) independently drive Alzheimer's-like pathology in mouse models. The co-exposure scenario has not been directly studied for neurodegeneration endpoints — a critical research gap.

5.7.4 Gut Microbiome as a Mediator

Airport noise (100-day mouse model) disrupts the gut microbiome (SCFAs $\downarrow$, LPS $\uparrow$, TMAO $\downarrow$), driving systemic inflammation via the gut-cardiovascular axis. Whether PM2.5 and noise act synergistically on the gut microbiome in co-exposure scenarios is unknown.

5.8 Key Limitations

1. **Nanoparticle RNA biomarkers:** No validated NP-specific miRNA/lncRNA signatures in vivo were identified. This is a genuine research gap, not a search artefact.
2. **Combined PM+Noise studies:** Only ~5 dedicated co-exposure studies exist globally. Most findings are from mouse models with short exposure durations (3 days to 100 days).
3. **Species translation:** Many protein and RNA biomarkers are characterised only in mouse models; human validation is lacking for cochlear, brain, and skin endpoints.
4. **Exposure characterisation heterogeneity:** PM_{2.5} studies vary widely in particle composition (urban, industrial, NIST reference material), making cross-study comparison difficult.
5. **Directionality in humans vs. mice:** Several miRNAs (e.g., miR-21-5p, miR-146a) show opposite directions in blood vs. lung, and in human vs. mouse — tissue and species context must be specified when interpreting these biomarkers.

Report compiled from systematic literature searches (LiteratureSearch tool, 2000–2026, peer-reviewed journals). All biomarker entries are traceable to primary literature references in the CSV table.

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