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Microbiome-Linked Metabolic Architecture of Accelerated Biological Aging in Humans

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Supplemental Table 1. Matching balance after age- and sex-matched nearest-neighbor matching

Matching variable	ASD cohort	Matched HC cohort	ASD n	HC n	Standardized mean difference
Age, overall (years)	69.28 ± 12.10	69.22 ± 11.99	120	480	0.005
Age, female subgroup (years)	68.99 ± 11.92	68.93 ± 11.79	107	428	0.005
Age, male subgroup (years)	71.62 ± 13.77	71.60 ± 13.34	13	52	0.001
Female sex, n (%)	107 (89.2%)	428 (89.2%)	120	480	0
Male sex, n (%)	13 (10.8%)	52 (10.8%)	120	480	0

Supplemental Table 2. Floor-proxy observations for proteomic analytes

Analyte	n at floor-proxy	Total n	%	Comment
IL-1β	6	120	5	Possible attenuation by floor effects
IL-6	1	120	0.8	Minimal
TNF-α	1	120	0.8	Minimal
Adiponectin	1	120	0.8	Minimal

Supplemental table 3. Baseline characteristics of healthy controls

Variable	Female, n=2,814	Male, n=2,456	P value
Anthropometrics Measures			
Age at exam (years)	53.81 ± 12.62	51.88 ± 11.88	<0.001**
PhenoAge (years)	44.48 ± 12.46	45.68 ± 12.01	<0.001**
PhenoAgeAccel (years)	−9.93 ± 3.02	−6.83 ± 2.9	<0.001**
BMI (kg/m²)	22.16 ± 3.55	23.98 ± 3.41	<0.001**
Body fat (%)	30.20 ± 7.02	21.90 ± 5.58	<0.001**
Blood Biomarkers			
WBC (×10³/μL)	4.91 ± 1.32	5.23 ± 1.37	<0.001**
Lymphocyte fraction (%)	28.30 ± 8.99	24.66 ± 10.65	<0.001**
MCV (fL)	92.57 ± 4.97	94.96 ± 3.62	<0.001**
RDW-CV (%)	12.6 ± 1.2	13.1 ± 1.1	<0.001**
Albumin (g/dL)	4.40 ± 0.26	4.49 ± 0.26	<0.001**
ALP (U/L)	65.59 ± 19.98	68.19 ± 18.5	<0.001**
Blood glucose (mg/dL)	98.22 ± 12.58	102.5 ± 12.28	<0.001**
CRP (mg/dL)	0.13 ± 0.27	0.14 ± 0.26	0.221
Creatinine (mg/dL)	0.67 ± 0.1	0.90 ± 0.12	<0.001**
Smoking status			
Never	2159 (76.7)	817 (33.3)	<0.001**
Former	469 (16.7)	954 (38.8)	
Current	185 (6.6)	684 (27.9)	
Alcohol use			
None	1620 (57.6)	687 (28)	<0.001**
Occasional	822 (29.2)	939 (38.2)	
Daily	371 (13.2)	828 (33.7)	
Sleep duration			
0–5 h	210 (7.5)	189 (7.7)	0.343
5–7 h	2051 (72.9)	1732 (70.5)	
7–9 h	543 (19.3)	528 (21.5)	
≥9 h	10 (0.4)	7 (0.3)	
Means and standard deviations. Asterisks indicate statistical significance (*p < 0.05, **p < 0.005).			

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Supplemental Table 4. Top 50 metabolites associated with PhenoAge in the ASD cohort

Metabolite	rho	P value	fdr_q
Gluconic acid	0.52	1.6173E-09	3.4287E-07
N2-Phenylacetylglutamine	0.49	1.5337E-08	1.6257E-06
Cystine	0.44	5.026E-07	2.91E-05
Trimethylamine N-oxide	0.44	5.4906E-07	2.91E-05
3-Indoxylsulfuric acid	0.43	9.7494E-07	4.1337E-05
Choline	0.42	2.0608E-06	7.1828E-05
Propionylcarnitine	0.42	2.3717E-06	7.1828E-05
Phe	0.41	3.716E-06	9.8475E-05
SDMA	0.40	4.4998E-06	0.000106
1H-Imidazole-4-propionic acid	0.38	1.5683E-05	0.00033248
Galacturonic acid **Glucuronic acid**	0.35	6.9497E-05	0.00133939
1-Methyladenosine	0.35	9.2683E-05	0.0016374
Malic acid	0.35	0.000108	0.00176879
H-Asp(Gly-OH)-OH	0.34	0.000128	0.001936
N,N-Dimethylglycine	0.34	0.000155	0.00219526
Kynurenine	0.32	0.000458	0.00606737
γ-Glu-Ile **γ-Glu-Leu**	0.31	0.000599	0.00746396
p-Cresol sulfate	0.29	0.001136	0.01337639
N6,N6,N6-Trimethyllysine	0.28	0.001944	0.02169094
Ornithine	0.27	0.002710	0.02872566
Tyr	0.27	0.003062	0.02952586
Isocitric acid	0.27	0.003064	0.02952586
Ile	0.26	0.003650	0.03326013
Leu	0.26	0.003901	0.03326013
Val	0.26	0.003922	0.03326013
N-Acetylgalactosamine 4-sulfate	0.26	0.004440	0.03559333
N-(1-Deoxy-1-fructosyl)valine	0.26	0.004533	0.03559333
Glycocholic acid	0.25	0.005415	0.04099683
N-(1-Deoxy-1-fructosyl)leucine	0.25	0.005719	0.04131644
Creatinine	0.25	0.005940	0.04131644
Urea	0.25	0.006042	0.04131644
Trimethoprim	0.25	0.006469	0.04285573
Sarcosine	0.24	0.007014	0.04498682
Guanidoacetic acid	-0.24	0.007215	0.04498682
γ-Glu-Phe	0.24	0.007430	0.04500492
Cystathionine	0.23	0.010083	0.05937942
1-Methyl-4-imidazoleacetic acid	0.23	0.010884	0.06236224
S-Sulfocysteine	0.23	0.012094	0.06746995
Ethylmalonic acid	0.22	0.014520	0.07793623
Glutamic acid γ-methyl ester **O-Acetylhomoserine** **2-Aminoadipic acid**	0.22	0.014705	0.07793623
β-Ala	0.22	0.015584	0.08057911
Glu	0.22	0.016702	0.08430355
γ-Butyrobetaine	0.22	0.018335	0.09039386
5-Oxoproline	0.21	0.019383	0.09338995
1-Methylhistidine **3-Methylhistidine**	0.21	0.020087	0.09463237
cis-Aconitic acid	0.21	0.023167	0.10615466
Lactic acid	0.21	0.023534	0.10615466
Acetoacetic acid	-0.20	0.026086	0.11521272
Theobromine	-0.20	0.029261	0.12659825
γ-Glu-Lys_divalent	0.20	0.031816	0.13223619

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Supplemental table 5. Top 50 metabolites correlated with PhenoAgeAccel

Metabolite	rho	p	fdr_q
Lactic acid	0.35	6.96E-05	0.01476
Malic acid	0.33	0.000197	0.018334
Glycocholic acid	0.33	0.000259	0.018334
Galacturonic acid **Glucuronic acid**	0.30	0.000853	0.04523
Guanidoacetic acid	-0.29	0.001245	0.049092
S-Carboxymethylcysteine	0.29	0.001389	0.049092
Gluconic acid	0.28	0.002088	0.063249
Pro	0.26	0.003576	0.088833
Thiodiglycolic acid	0.26	0.003887	0.088833
Trimethoprim	0.26	0.00419	0.088833
β-Ala	0.25	0.00505	0.097335
1H-Imidazole-4-propionic acid	0.24	0.008653	0.14273
Pyruvic acid	0.24	0.009075	0.14273
Phe	0.24	0.009567	0.14273
Ile	0.23	0.010099	0.14273
Ethyl glucuronide	0.23	0.011374	0.148765
Lenticin	-0.23	0.011929	0.148765
Met	0.22	0.01526	0.179732
Pipecolic acid	0.22	0.017191	0.18879
γ-Glu-Ser	-0.22	0.01781	0.18879
N1-Methyl-4-pyridone-5-carboxamide	-0.21	0.02041	0.189838
N-(1-Deoxy-1-fructosyl)leucine	0.21	0.020623	0.189838
N-(1-Deoxy-1-fructosyl)valine	0.21	0.020886	0.189838
Taurine	-0.21	0.021491	0.189838
Glu	0.21	0.022746	0.192883
N-Acetylputrescine	0.20	0.024995	0.200365
Trimethylamine N-oxide	0.20	0.025518	0.200365
Fumaric acid	0.20	0.028406	0.215071
Asp	0.20	0.031499	0.22494
Hypoxanthine	0.20	0.031831	0.22494
Theobromine	-0.19	0.0347	0.226959
Tranexamic acid	-0.19	0.035625	0.226959
Leu	0.19	0.036071	0.226959
Maleic acid	0.19	0.03656	0.226959
5-Hydroxylysine	0.19	0.037501	0.226959
γ-Glu-Ile **γ-Glu-Leu**	0.19	0.03854	0.226959
Sarcosine	0.19	0.040153	0.230066
Ala	0.18	0.043314	0.241231
Val	0.18	0.044377	0.241231
Tyr	0.17	0.060803	0.319494
N6-Methyllysine	0.17	0.061789	0.319494
Choline	0.17	0.065877	0.327376
1-Methylnicotinamide	-0.17	0.066402	0.327376
Orotidine	0.17	0.070891	0.341567
Urocanic acid	-0.16	0.074469	0.350832
Phosphorylcholine	-0.16	0.078057	0.359739
SDMA	0.16	0.088196	0.396123
Pantothenic acid	0.15	0.091471	0.396123
ADMA	0.15	0.091557	0.396123
Propionylcarnitine	0.15	0.097921	0.40104

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Supplemental Table 6. Overlapping metabolites between PhenoAge and PhenoAgeAccel

Metabolite	Inferred pathway
1H-Imidazole-4-propionic acid	Amino acid metabolism
Choline	Microbiome / TMAO / uremic
Galacturonic acid **Glucuronic acid**	Amino acid metabolism
Glu	Amino acid metabolism
Gluconic acid	Amino acid metabolism
Glycocholic acid	Amino acid metabolism
Guanidoacetic acid	Other
Ile	Amino acid metabolism
Lactic acid	Mitochondria / energy
Leu	Amino acid metabolism
Malic acid	Mitochondria / energy
N-(1-Deoxy-1-fructosyl)leucine	Glycation / AGE
N-(1-Deoxy-1-fructosyl)valine	Glycation / AGE
Phe	Amino acid metabolism
Propionylcarnitine	Microbiome / TMAO / uremic
SDMA	Other
Sarcosine	Redox / one-carbon
Theobromine	Other
Trimethoprim	Other
Trimethylamine N-oxide	Microbiome / TMAO / uremic
Tyr	Amino acid metabolism
Val	Amino acid metabolism
β -Ala	Other
γ -Glu-Ile **γ -Glu-Leu**	Amino acid metabolism

Supplemental table 7. Spearman's rank correlations among PhenoAge, inflammatory cytokines, adiponectin, and TMAO-related metrics.

Variable pair	Spearman's ρ	p value	95% CI
PhenoAge			
IL-1β (pg/ml)	0.167	0.068	-0.011, 0.337
IL-6 (pg/ml)	0.399	<0.001**	0.238, 0.541
TNF-α (pg/ml)	0.5	<0.001**	0.352, 0.623
Adiponectin (μg/ml)	−0.008	0.932	-0.187, 0.171
TMAO Pathway Index			
IL-1β (pg/ml)	0.098	0.289	-0.083, 0.272
IL-6 (pg/ml)	0.053	0.568	-0.128, 0.230
TNF-α (pg/ml)	0.311	<0.001**	0.139, 0.464
Adiponectin (μg/ml)	0.055	0.551	-0.126, 0.232
TMAO			
IL-1β (pg/ml)	0.161	0.078	-0.018, 0.331
IL-6 (pg/ml)	0.137	0.135	-0.043, 0.309
TNF-α (pg/ml)	0.242	0.008*	0.066, 0.404
Adiponectin (μg/ml)	0.038	0.682	-0.142, 0.216
TML			
IL-1β (pg/ml)	0.048	0.599	-0.132, 0.226
IL-6 (pg/ml)	0.126	0.172	-0.055, 0.298
TNF-α (pg/ml)	0.146	0.112	-0.034, 0.317
Adiponectin (μg/ml)	0.039	0.673	-0.141, 0.217
Choline			
IL-1β (pg/ml)	0.088	0.338	-0.093, 0.263
IL-6 (pg/ml)	0.059	0.521	-0.121, 0.236

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TNF-α (pg/ml)	0.312	<0.001**	0.140, 0.465
Adiponectin (μg/ml)	-0.055	0.552	-0.232, 0.126
DMG			
IL-1β (pg/ml)	-0.034	0.713	-0.212, 0.146
IL-6 (pg/ml)	0.057	0.538	-0.124, 0.234
TNF-α (pg/ml)	0.183	0.046*	0.003, 0.350
Adiponectin (μg/ml)	0.141	0.125	-0.039, 0.312
Asterisks indicate statistical significance (*p < 0.05, **p < 0.005).			

Supplemental Table 8. Renal function–adjusted sensitivity analyses

Analysis	Outcome	Estimate	95% CI	P value
Unadjusted correlation	TPI – PhenoAge	rho = 0.48	—	3.9×10^{-8}***
Adjusted model (+ age, sex, BMI, smoking, eGFR)	PhenoAge	β = 2.92	0.74 to 5.10	0.009*
Broader covariate model (+ alcohol, sleep, occupation)	PhenoAge	β = 2.92	0.73 to 5.12	0.01*
TMAO alone adjusted model	PhenoAge	β = 0.54	-0.79 to 1.87	0.424
TPI; TMAO Pathway Index. † Adjusted for age, sex, BMI, and estimated glomerular filtration rate. Asterisks indicate statistical significance (*p < 0.05, **p < 0.005).				

Supplemental Table 9. Robustness analyses for the TMAO Pathway Index

Model	Outcome	β	95% CI	P value
Full TPI	PhenoAge	2.92	0.74 to 5.10	0.009*
TMAO alone	PhenoAge	0.54	-0.79 to 1.87	0.424
TPI without TMAO	PhenoAge	2.67	0.74 to 4.61	0.007*
TPI without TML	PhenoAge	2.26	0.47 to 4.05	0.014*
TPI without choline	PhenoAge	2.44	0.25 to 4.62	0.029*
TPI without DMG	PhenoAge	2.46	0.26 to 4.67	0.029*
Asterisks indicate statistical significance (*p < 0.05, **p < 0.005).				

Supplemental Table 10. Exploratory sex interaction analysis

Interaction term	β	95% CI	P value	Interpretation
TPI × male sex	8.19	0.86 to 15.51	0.029	Exploratory only due to small male sample size
Asterisks indicate statistical significance (*p < 0.05).				

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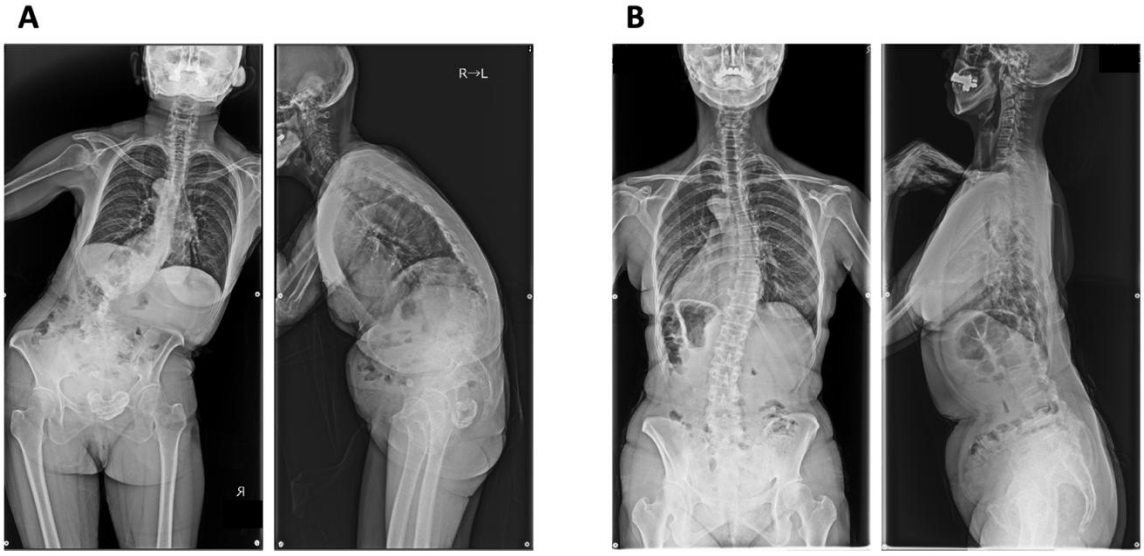
Supplemental Figure

Supplemental figure 1. Representative Patient Demonstrating Clinical, Functional, and Radiographic Characteristics



A. Representative 59-year-old female ASD patient showing severe coronal and sagittal malalignment (Cobb83°, CSVL = 136 mm, C7SVA 221 mm) on standing PA/lateral radiographs. Clinical data demonstrated frailty (mFI-11 = 0.45), reduced muscle mass (SMI 4.8 kg/m²), osteoporosis (T-score –3.1), limited mobility (6MWD 233 m), and elevated biological aging (PhenoAge 59.1-year-old; PhenoAgeAccel +1.10 years). ODI 44.4%, EQ-5D 0.621, SII 602.5.

B. Representative 56-year-old female ASD patient showing moderate coronal and sagittal malalignment (Cobb33°, CSVL = -18 mm, C7SVA 74 mm) on standing PA/lateral radiographs. Clinical data demonstrated a robust phenotype (mFI-11 = 0.09), preserved muscle mass (SMI 6.0 kg/m²), and near-normal bone density (T-score –0.6). Mobility was well maintained (6MWD 448 m), and biological aging indices indicated substantially younger biological age (PhenoAge 46.2-year-old; PhenoAgeAccel –9.81 years). ODI 11.1%, EQ-5D 0.728, SII 440.0.

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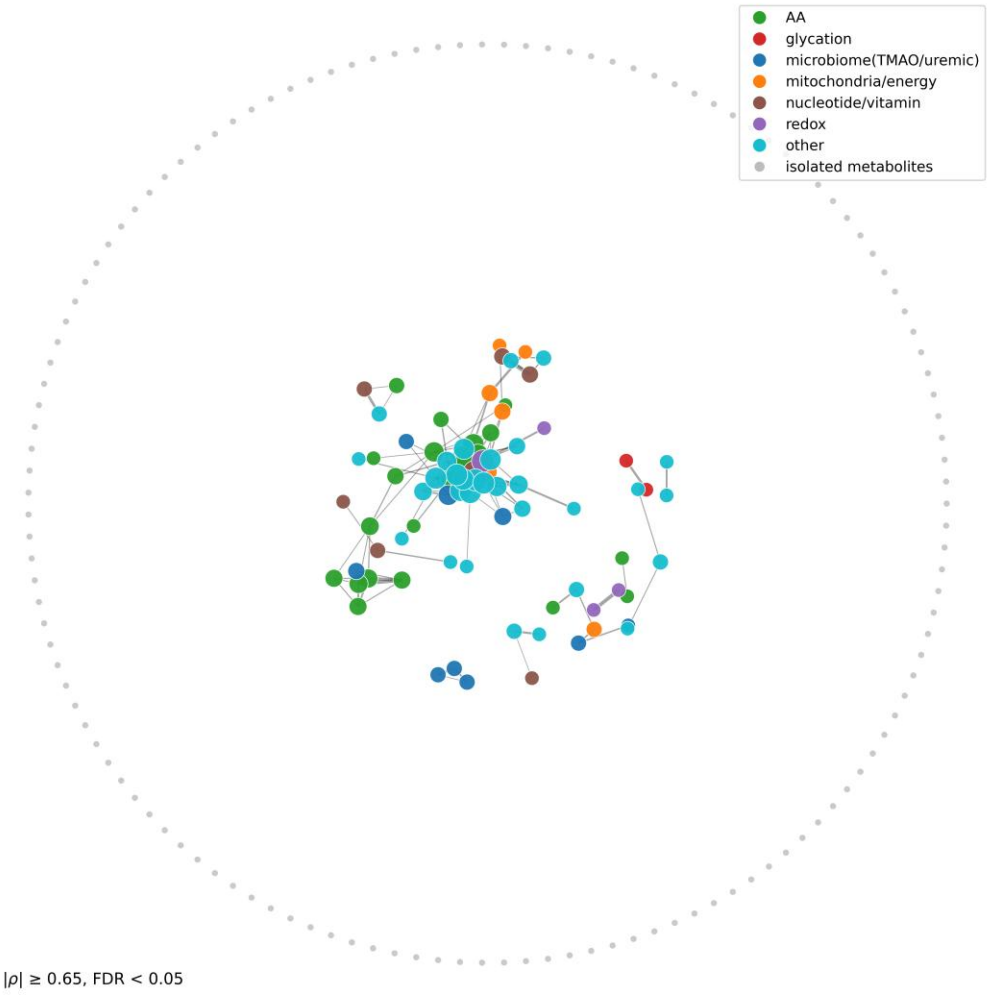
Supplementary figure 2. Global metabolite correlation network in ASD.
Nodes represent plasma metabolites and edges indicate significant pairwise correlations ($|\rho| \geq 0.65$, FDR < 0.05). Nodes are color-coded by major biochemical domains, including glycation, microbiome-derived methylamine metabolism (TMAO axis), amino acid metabolism, mitochondrial/TCA-cycle pathways, nucleotide/vitamin metabolism, and redox regulation. The network reveals a limited number of highly interconnected metabolic hubs within a predominantly sparse structure, indicating that biological aging is characterized by coordinated metabolic reorganization rather than diffuse biochemical alterations.



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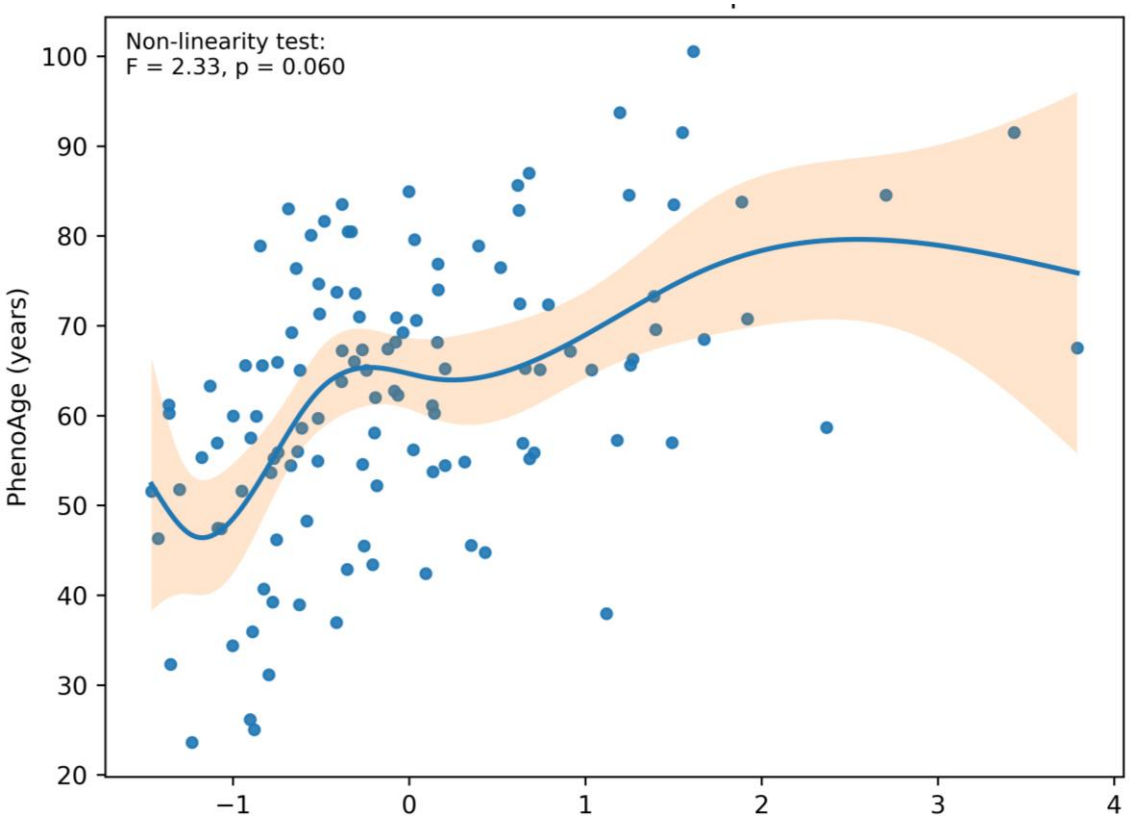
Supplementary figure 3. Non-linear association between the TMAO Pathway Index and PhenoAge assessed by restricted cubic spline analysis.
The association between the TMAO Pathway Index and PhenoAge was evaluated using restricted cubic spline regression with four knots placed at standard quantiles. Solid lines represent spline-estimated mean PhenoAge values, and shaded areas indicate 95% confidence intervals. Individual dots represent ASD patients. The test for non-linearity showed no statistically significant deviation from linearity (F = 2.33, p = 0.060), supporting the appropriateness of linear modeling in the main analyses.



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Detailed Methods

1. Healthy control selection and matching

Selection

- Population source: 10,163 adults aged ≥30 years from an institutional health-check program (2020–2023)
- Exclusion criteria: malignancy, inflammatory disease, diabetes mellitus, cardiovascular disease, pregnancy
- Eligible healthy control pool: 5,270 adults (2,814 females; 2,456 males)

Patient population

- Adult spinal deformity (ASD), ≥30 years, radiographic criteria:
  C7 sagittal vertical axis ≥5 cm, pelvic tilt ≥25°, coronal Cobb ≥20°
- Exclusions: active infection, malignancy, severe hepatic dysfunction

Matching

- Algorithm: nearest-neighbor matching (1:4)
- Matching variables: exact matching on sex; nearest-neighbor matching on age
- Matching without replacement
- Balance assessment: visual inspection and standardized mean difference (SMD <0.1)

Outcome

- Primary outcome: PhenoAge (continuous)

Primary statistical inference

- Paired comparison between ASD patients and the mean of four matched controls
- Paired t-test with sex-stratified secondary analyses
- Each ASD case was paired to the mean value of matched controls
- Analyses performed in Python (NumPy, SciPy, statsmodels)
- Two-sided significance threshold: $\alpha = 0.05$

2. Calculation of PhenoAge and PhenoAgeAccel

PhenoAge was calculated according to the method originally proposed by Levine et al., using a composite mortality score derived from nine clinical biomarkers and chronological age. Specifically, PhenoAge was computed as:

$$PhenoAge = 141.50225 + \ln\{-0.00553 \times \ln(1 - Mortality\ Score)\} / 0.09165.$$

The mortality score was calculated using the following equation:

$$Mortality\ Score = -19.9067 - 0.0336 \times albumin\ (g/L) + 0.0095 \times creatinine\ (\mu mol/L) + 0.1953 \times serum\ glucose\ (mmol/L) + 0.0954 \times In(C\text{-}reactive\ protein\ (mg/dL)) - 0.0120 \times lymphocyte\ percentage\ (\%) + 0.0268 \times mean\ corpuscular\ volume\ (fL) + 0.3306 \times red\ cell\ distribution\ width\ (\%) + 0.0019 \times alkaline\ phosphatase\ (U/L) + 0.0554 \times white\ blood\ cell\ count\ (10^3\ cells/\mu L) + 0.0804 \times chronological\ age\ (years).$$

PhenoAge acceleration (PhenoAgeAccel) was defined as the difference between PhenoAge and chronological age, calculated as:

$$PhenoAgeAccel = PhenoAge - chronological\ age.$$

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For participants in the healthy control cohort, when individual biomarker values required for PhenoAge calculation were missing, age-stratified reference values derived from previously reported data on healthy Japanese populations were used as substitutes, categorized by 10-year age intervals. Individuals with missing data for two or more required parameters were excluded from the analysis. In the ASD cohort, complete data for all variables were required for inclusion in PhenoAge analyses.

3. Metabolomics – Sample processing & QC

Fasting venous blood samples were collected in EDTA tubes in the morning and centrifuged at 1,500 × g for 10 min at 4 °C within 2 h of collection. Plasma was aliquoted and stored at −80 °C and subjected to a single freeze–thaw cycle prior to analysis.

Metabolomic profiling was performed using capillary electrophoresis–mass spectrometry (CE–MS; Human Metabolome Technologies, Japan), enabling quantification of approximately 1,100 hydrophilic metabolites.

Quality control (QC) samples were inserted every 10 analytical runs to monitor analytical stability. Metabolites with coefficients of variation exceeding 20% in pooled QC samples were excluded. Retention time drift was maintained below 1%, and principal component analysis (PCA) was used to confirm clustering and analytical consistency of QC samples.

Raw metabolomic data were $\log_{10}$-transformed and Pareto-scaled prior to statistical analyses. Metabolites with missing values in more than 30% of samples were removed using a uniform threshold across all analyses.

Multiple testing in metabolome-wide association analyses was controlled using the Benjamini–Hochberg false discovery rate (FDR). Metabolite rankings are presented for descriptive prioritization, whereas statistical inference was based on FDR-adjusted significance.

Correlation analyses

- Spearman correlation matrices
- Variables tested: PhenoAge, PhenoAgeAccel, TMAO, TML, choline, DMG, IDO activity, SII, SMI, handgrip, 6MWD

IDO activity was defined as the kynurenine-to-tryptophan ratio.

- SII was calculated as neutrophils × platelets / lymphocytes.
- SMI was defined as skeletal muscle index derived from body composition analysis.

4. Construction of the TMAO Pathway Index (TPI)

Rationale

Quantifies gut-microbiome → hepatic methylamine → oxidation flux

Components

- TMAO (terminal oxidation product)
- TML (microbial precursor)
- Choline (dietary substrate)
- DMG (oxidation intermediate)

Calculation

Each metabolite was standardized:

$$Z_i = (value_i - mean_i) / SD_i,$$

where mean and SD were calculated from the ASD patients cohort.

The TPI was calculated as:

TPI = (Z_TMAO + Z_TML + Z_choline + Z_DMG) / 4

Statistical modeling

- Primary multivariable model: PhenoAge = β0 + β1(TPI) + β2(age) + β3(sex) + β4(BMI) + β5(smoking) + β6(eGFR) + ε
  Sensitivity model: PhenoAge = β0 + β1(TPI) + β2(age) + β3(sex) + β4(BMI) + β5(smoking) + β6(eGFR) + β7(alcohol) + β8(sleep duration) + β9(occupation) + ε
- Restricted cubic spline: 4 knots (5th, 35th, 65th, 95th percentile)

Missing data

- <5% → multiple imputation (10 iterations; Rubin's rules)

5. Renal function–adjusted sensitivity analyses

To evaluate the potential influence of renal clearance on circulating TMAO–related metabolites, we performed a series of sensitivity analyses accounting for kidney function.

Estimation of renal function

Estimated glomerular filtration rate (eGFR) was calculated using the Japanese Society of Nephrology equation based on serum creatinine, age, and sex:

$$eGFR = 194 \times Cr^{-1.094} \times Age^{-0.287} \times (0.739\ for\ women)$$

where serum creatinine (Cr) was expressed in mg/dL. eGFR values are reported in mL/min/1.73 m².

Exclusion and stratified analyses

To assess whether impaired renal function influenced associations between the TPI and biological aging or inflammatory markers, participants with reduced renal function (eGFR <60 mL/min/1.73 m²) were excluded, and correlation analyses were repeated in the remaining cohort. In addition, analyses were conducted across eGFR strata to evaluate consistency of associations across levels of renal function.

Multivariable adjustment

Multivariable linear regression models were constructed to examine associations between TPI and outcome variables after accounting for renal function. Models were adjusted for age, sex, body mass index, and eGFR.

eGFR-residualized analyses

To further isolate TMAO-related metabolic signals independent of renal clearance, TPI was residualized with respect to eGFR using linear regression. Residuals representing the eGFR-independent component of TPI were then used in correlation analyses with PhenoAge and inflammatory markers.

Statistical analysis

Correlations were assessed using Spearman's rank correlation coefficients. Regression coefficients are presented with 95% confidence intervals. All sensitivity analyses were performed using the same statistical thresholds as in the primary analyses.

6. Proteomic assays

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Plasma samples were analyzed using MSD V-PLEX and U-PLEX platforms.

Analytes

- IL-1β, IL-6, TNF-α (MSD V-PLEX)
- Adiponectin (MSD U-PLEX)

QC

- Internal controls per plate
- CV threshold <10%
- Plasma samples were analyzed using the MSD V-PLEX and U-PLEX platforms on the MESO QuickPlex SQ 120 system. IL-1β, IL-6, and TNF-α were measured using the V-PLEX Proinflammatory Panel 1 Human Kit, and adiponectin was measured using the U-PLEX Human Adiponectin Assay. Samples were analyzed in singlicate, and calibrators were analyzed in duplicate using 8 concentrations. Standard curves were generated using a 4-parameter logistic model with $1/Y^2$ weighting. Reported detection limits for IL-1β, IL-6, and TNF-α were 0.017 pg/mL, 0.082 pg/mL, and 0.044 pg/mL, respectively. In the exported analytic dataset, low-end floor-proxy observations were summarized descriptively for each analyte.

Data processing

- Log transformation
- Mann–Whitney U tests (upper vs. lower tertiles of PhenoAge)
- Spearman correlation ($\alpha = 0.05$)

7. Radiographic assessment

Standing whole-spine radiographs were obtained.

Parameters included: C7SVA, Thoracic kyphosis (TK), Pelvic incidence (PI), Pelvic tilt (PT), Lumbar lordosis (LL), PI–LL mismatch, Coronal Cobb angle, and Central sacral vertical line (CSVL)