Supplementary Material

NMR-based Urinary Biomarkers in Pediatric Primary Mitochondrial Disorders and Chronic Kidney Disease: Shared Mitochondrial Dysfunction, Diverging Biosignatures

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Supplementary Material

Supplementary Methods

Clinical and Laboratory Data Collection

Data extracted from clinical records included family history and detailed clinical presentation, encompassing neuromuscular symptoms, renal involvement, and other organ-specific manifestations. Regular medication use was also documented. Laboratory evaluation comprised blood counts, blood urea nitrogen (BUN), plasma and urinary creatinine, urea, and serum electrolytes (sodium, potassium, chloride, calcium). Urinary analyses included total proteinuria, microalbuminuria, α-1 and β-2 microglobulins, fractional excretion of sodium (FENa), tubular phosphate reabsorption (TmP/GFR), calciuria, glycosuria, urine pH, and specific gravity. Renal ultrasonography was performed in all patients with suspected or confirmed kidney disease.

eGFR calculation

Patients were further classified according to the KDIGO 2024 guidelines, with glomerular filtration rate (GFR) estimated using the creatinine-based CKiD U25 Equation (or the average- creatinine-cystatin based U25 Equation when available), except for patients younger than 12 months, where eGFR was calculated using the Scharwartz formula, and for patients older than 18 years, where eGFR was based on CKD-EPI 2021 guidelines.

Chromatography of urinary organic acids

Same-sample chromatography of urinary organic acids was achieved by gas chromatography-mass spectrometry (GC-MS) with two internal standards (3-phenylbutyrate and 3-tricarballylic acid) in an equivalent volume of 5 mmol/L creatinine, following acidification, salt addition, and extraction with trimethylsilyl (TMS).

Spectral Pre-processing

Spectral region exclusion was applied to eliminate regions corresponding to the water peak and the highly variable urea signal, which could introduce artefacts in multivariate analysis. Spectral alignment and binning were performed prior to normalization.

^{1}H-NMR

Urinary ^{1}H-NMR analysis was performed on samples thawed at room temperature, after centrifugation, buffered with a phosphate solution (KH_2PO_4 in D_2O with 0.1% sodium salt of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid - TSP), and pH adjusted to 7.40 (±0.02). ^{1}H-NMR spectra were obtained using a 5 mm inverse probe at 500 MHz on a Bruker AVANCE III 500 spectrometer.

NMR spectra were recorded on a Bruker AVANCE III 500 spectrometer (Bruker, Rheinstetten, Germany), operating at 500.18 MHz for 1H observation, using a 5 mm inverse TXI probe, at 298 K. For each sample, one unidimensional (1D) 1H NMR spectrum was acquired using the “noesypr1d” pulse sequence (Bruker library), with water presaturation, a spectral width of 7002.8 Hz, 128 scans, 32 k data points, a 2.3 s acquisition time and a 4 s relaxation delay. Each free-induction decay was zero-filled to 64 k points and multiplied by a 0.3 Hz exponential line-broadening function prior to Fourier transform. The spectra were manually phased and baseline-corrected, and chemical shifts were referenced internally to the TSP signal (δ 0.00). Spectra were acquired with a NOESY-1D pulse sequence applying a 0.3 Hz line-broadening factor, followed by Fourier transformation, manual phase, baseline correction, and TSP peak calibration at 0.00 ppm (TOPSPIN© software). Peak alignment was manually adjusted with Amix-Viewer© (version 3.9.14), and the data matrix was processed in MATLAB©. Three samples were randomly selected and independently reprocessed to validate reproducibility in spectrum preparation and alignment steps.

Peak assignment

Peak assignment was manually performed on Chenomx NMR Suite© 8.5 and supported by an internally curated spectral library (published as Supplementary Table S2 in Ferreira et al., 2024), the Human Metabolome Database (HMDB), and literature. Identified metabolites are reported using standard nomenclature and HMDB identifiers where available. Resonances that could not be confidently annotated were retained in the analysis and labeled as unidentified signals according to resonance (U_{ppm}). A complete list of identified metabolites, including chemical shifts, reference sources, and notes on assignment confidence, is provided in Supplementary Tables S2 and S3.

Sample Exclusion Criteria and Outlier Detection

Samples collected during acute decompensations or intercurrent infections were excluded based on clinical metadata and multivariate outlier detection (Figure S1), ensuring that profiles reflected patients' baseline metabolic state.

Identification of Unknown Metabolites

Unknown metabolites were considered for putative annotation only if they met the following criteria: *i*) spectral peaks were consistent and well-resolved across the sample set; and *ii*) the peak area was integrated and included in univariate or multivariate statistical analysis.

Metabolites not fulfilling these criteria were excluded from annotation but retained in the dataset as unidentified features). Candidate chemical classes or structures were assigned based on chemical shift, multiplicity, and visual comparison with reference spectra available in the Human Metabolome Database (HMDB).

Confidence levels for metabolite annotation were classified according to the Metabolomics Standards Initiative (MSI) recommendations (Sumner et al., 2007):

- MSI Level 1 – Identified compound: unambiguous identification based on comparison of spectral data (^{1}H-NMR or MS) and chromatographic retention time with those of an authentic standard analyzed under identical experimental conditions.
- MSI Level 2 – Putatively annotated compound: Putative identification based on spectral similarity (chemical shift and multiplicity in NMR, or m/z fragmentation in MS) with reference databases, without confirmation with an authentic standard.
- MSI Level 3 – Putatively characterized compound class: Putative classification into a chemical class (e.g., aromatic nitrogen-containing compound) based on characteristic spectral features, without specific compound assignment.
- MSI Level 4 – Unknown compound: A reproducible signal detected by NMR or MS with no sufficient information to assign chemical identity or class.

Inclusion and exclusion criteria

Primary mitochondrial disorders (PMD): Inclusion required a confirmed molecular diagnosis consistent with a primary mitochondrial disorder, including mutations in mitochondrial DNA (mtDNA) or nuclear genes involved in mitochondrial function.

Suspected mitochondrial disease (SMD): These cases were selected based on suggestive clinical and biochemical features (e.g., elevated Lactate and/or alanine, neuromuscular symptoms, developmental delay, radiological findings compatible with mitochondrial dysfunction) but without a confirmed genetic diagnosis. Only individuals with persistent suspicion of PMD and no alternative diagnosis after genetic testing were retained.

Chronic kidney disease (CKD): Patients with established CKD of any etiology, stages 1–5 according to KDIGO classification, were included. The subgroup CKD stage 1- 2 included patients with mild-to-moderate renal dysfunction (eGFR ≥60 mL/min/1.73m^2), while CKD stage 3-5 included advanced CKD (eGFR <60 mL/min/1.73m^2). Patients with nephrotic syndrome were included if renal function was chronically affected.

Controls: Children and adolescents followed in the nephrology outpatient clinic for minor non-progressive conditions (e.g., vesicoureteral reflux, transient abnormalities on renal ultrasound) were included as controls. All had normal renal function (eGFR >90 mL/min/1.73m^2) and no abnormal urinalysis at inclusion.

General exclusion criteria: Patients were excluded if they met any of the following:

- Age <1 month at the time of sample collection, due to known immaturity of renal function and metabolic pathways;
- Post-renal transplant status, due to profound metabolic changes related to immunosuppression and renal graft function;
- Presence of acute intercurrent illness or metabolic decompensation at the time of sampling, based on clinical information;
- Known or suspected secondary causes of mitochondrial dysfunction, such as intoxications or acquired neurometabolic conditions.

In addition to the predefined screening criteria (Supplementary Table S1), a number of additional exclusions were applied during the analytical phase, based on diagnostic clarification, data quality, or unexpected findings:

- **P01** (PMD group): excluded due to inconclusive diagnosis, based on an oligosymptomatic presentation and a mitochondrial DNA variant of uncertain significance (VUS) with heteroplasmy <20% in blood, which was considered insufficient to confirm primary mitochondrial disease;
- **P23** (SMD group): excluded following multivariate outlier detection in principal component analysis (PCA);
- **P27** (Control group): excluded due to unexplained metabolic acidosis revealed during screening;
- **Additional urine samples** from PMD patients collected during acute clinical decompensation were excluded from the main analysis to avoid confounding effects of transient metabolic alterations.;
- **Patientes with PMD and predominant CKD** (P15 and P25).

Table S1 – Detailed inclusion and exclusion criteria

Group	Inclusion Criteria	Exclusion Criteria
Applicable to all	Age >1M and <20 y	Active UTI
Primary mitochondrial disorders (PMD)	Genetically confirmed mitochondrial disease (nDNA or mtDNA)	
Suspected mitochondrial disease (SMD)	Suspected PMD based on clinical/biochemical presentation without genetic confirmation	Other diagnoses confirmed by genetic studies
Chronic Kidney disease (CKD)	eGFRcr 90 mL/min/1.73m²*	Transplant recipient CKD due to PMD
Control	Otherwise healthy patients with any mild condition (eg; transitory creatinine elevation, CAKUT, or post-UTI follow-up) without evidence of CKD (eGFR; normal urinalysis, normal acid-base balance)	Other known organ/system involvement or development issues Other suspected or confirmed (eg. genetically) conditions

*** Calculated by creatinine-based Chronic Kidney Disease in Children (CKiD) U25 Equation except for patients younger than 12 months, where eGFR is based on the Schwartz formula, and for patients older than 18 years, where eGFR is based on CKD-EPI 2021 guidelines.;**
Abbreviations: M: months; Y: years; CAKUT: Congenital Anomalies of the Kidney and Urinary Tract; eGFRcr: creatinine based estimated glomerular filtration rate; UTI: urinary tract infection.

Figure S1. Recruitment Flowchart

Figure S1. Flowchart of participant selection and exclusions during the recruitment process. Final groups used in the statistical analysis are shown at the bottom, with the number of patients and urine samples per group.

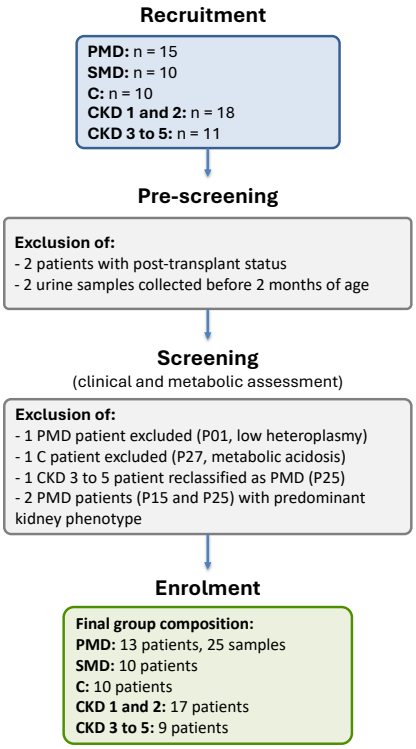

Figure S1. **Flowchart of participant selection and exclusions during the recruitment process. Final groups used in the statistical analysis are shown at the bottom, with the number of patients and urine samples per group. PMD: primary mitochondrial disorder; SMD: suspected mitochondrial disorder; C: control; CKD: chronic kidney disease.**

Table S2 – List of identified metabolites

Metabolite name, chemical shift (ppm), multiplicity. All metabolites in this table were assigned with a confidence of Putative identified metabolite (MSI Level 2) according to Metabolomics Standards Initiative - MSI), reference database (HMDB). Indicate which metabolites were quantified and included in statistical analyses. Abbreviations: AA: amino acid; HMDB: Human Metabolome Database; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Main pathway	Compound Name	HMDB ID	KEGG	(δ ppm (multiplicity)	Quantified? (yes/no)
Aromatic AA metabolism	Phenylalanine	HMDB0000159	C00079	3.15 (dd/m), 4.00 (m), 7.32 (m)	yes
Alanine metabolism	L-Alanine	HMDB0000161	C00041	1.48 (d), 3.78 (q)	yes
Amine metabolism	Dimethylamine	HMDB0000087	C00543	2.71 ppm (s)	yes
Aromatic AA metabolism	Phenylalanine	HMDB0000159	C00079	3.15 (dd/m), 4.00 (m), 7.32 (m)	yes
Aromatic compound metabolism	p-Cresol	HMDB0001858	C01468	2.28 (s); 6.80–7.20 (d)	no
Beta-alanine metabolism	beta-Alanine	HMDB0000056	C00099	2.57 (t); 3.38 (t)	no
β -oxidation / Short-chain fatty acid metabolism	α-Hydroxyisobutyrate	HMDB0000729	NA	1.26 (s), 1.31 (s)	yes
β -oxidation / Short-chain fatty acid metabolism	L-Carnitine	HMDB0000062	C00487	3.22 (s); 3.64 (s)	no
Beta-oxidation/ketone metabolism	4-Hydroxybutyrate	HMDB0000710	C00989	2.45 (t); 4.15 (t)	no
Beta-oxidation/ketone metabolism	Acetate	HMDB0000042	C00033	1.92 (s)	yes
Beta-oxidation/ketone metabolism	Acetoacetate	HMDB0000060	C00164	2.27 (s); 3.42 (s)	no
Beta-oxidation/ketone metabolism	Acetone	HMDB0001659	C00207	2.23 (s)	no
Branched-chain AA metabolism	2-Methylacetoacetate	HMDB0003771	NA	1.20 (d); 2.25 (s); 3.45 (s)	no
Branched-chain AA metabolism	3-Methyl-2-oxovalerate	HMDB0000491	C00671	0.92 (d), 1.10 (d), 2.20 (m), 2.55 (m)	yes
Branched-chain AA metabolism	Isoleucine	HMDB0000172	C00407	0.94 (d); 1.49 (m); 1.71 (m)	no
Branched-chain AA metabolism	Isoleucine	HMDB0000172	C00407	0.94 (t), 1.02 (d), 1.28 (m), 1.46 (m), 3.67 (d)	yes
Branched-chain AA metabolism	L-Valine	HMDB0000883	C00183	0.99 (d), 1.04 (d), 2.27 (m), 3.62 (d)	yes
Branched-chain AA metabolism	Leucine	HMDB0000687	C00123	0.96 (d), 0.97 (d), 1.69 (m), 1.70 (m), 3.74 (t)	yes
Carbohydrate metabolism	D-Glucose	HMDB0000122	C00031	3.20–5.40 (m)	no
Carbohydrate metabolism	Glucose 1-phosphate	HMDB0001586	C00103	3.30–5.50 (m)	no
Carbohydrate metabolism	Glucose 6-phosphate	HMDB0001401	C00092	3.30–5.50 (m)	no
Carbohydrate metabolism	Levoglucosan	HMDB0000640	NA	3.30–5.40 (m)	no
Carbohydrate metabolism	myo-Inositol	HMDB0000211	C00137	3.26 (m); 3.52 (m); 3.61 (m); 3.64 (m)	no
Carbohydrate metabolism	Uridine diphosphate glucose	HMDB0000286	C00029	3.20–5.60 (m); 7.90 (d)	no
Creatine metabolism	Creatine	HMDB0000064	C00300	3.04 (s), 3.93 (s)	yes
Creatine metabolism	Creatinine	HMDB0000562	C00791	3.05 (s), 4.06 (s)	yes
Creatine metabolism	GuanidoAcetate	HMDB0000128	C00581	3.77 (s); 3.82 (s)	no
Creatine metabolism	GuanidoAcetate	HMDB0000128	C00581	3.77 (s); 3.82 (s)	no
Dicarboxylic acid metabolism	Methylsuccinate	HMDB0001844	NA	1,09 (d), 2.12 (dd), 2.51 (dd), 2.61 (dd)	yes

Dopamine metabolism	Homovanillic acid	HMDB0000118	C05582	3.43 (s), 6.76 (d), 6.79 (dd), 7.10 (d)	yes
Ethanol metabolism	Ethanol	HMDB0000108	C00469	1.17 (t), 3,65 (q)	yes
Fatty acid metabolism	3-Methyladipate	HMDB0000555	NA	0.90–2.30 (m)	no
Fatty acid metabolism	Adipate	HMDB0000448	C06104	1.60 (m); 2.30 (t)	no
Glucoronate pathway	D-Glucuronate	HMDB0000127	C00191	5.26 (d), 3.20–4.00 (m)	yes
Glycine and serine metabolism	Glycine	HMDB0000123	C00037	3.56 (s)	yes
Glycolysis/Krebs cycle	cis-Aconitate	HMDB0000072	C00417	3,44 (d), 6,58 (s)	yes
Glycolysis/Krebs cycle	Citrate	HMDB0000094	C00158	2.52 (dd), 2.67 (dd)	yes
Glycolysis/Krebs cycle	Ethylmalonate	HMDB0000622	NA	1.25 (t); 2.40 (q)	no
Glycolysis/Krebs cycle	Fumarate	HMDB0000134	C00122	6.51 (s)	yes
Glycolysis/Krebs cycle	Isocitrate	HMDB0000193	C00311	2.60–2.85 (dd); 4.10 (m)	no
Glycolysis/Krebs cycle	Malonate	HMDB0000691	C00383	2.65 (s)	no
Glycolysis/Krebs cycle	Oxoglutarate	HMDB0000208	C00026	2.45 (t); 3.00 (s)	no
Glycolysis/Krebs cycle	Pyruvate	HMDB0000243	C00022	2.37 (s)	yes
Glycolysis/Krebs cycle	Succinate	HMDB0000254	C00042	2.41 (s)	yes
Glycolysis/Krebs cycle	trans-Aconitate	HMDB0000958	C02341	6.34 (s)	yes
Glyoxylate and dicarboxylate metabolism	Glycolate	HMDB0000115	C03547	3.98 (s)	yes
Heme biosynthesis	5-Aminolevulinic acid	HMDB0001149	C00430	2.42 (dt), 2.51 dt), 2.74 (t), 3.81 (s)	yes
Histidine metabolism	1-Methylhistidine	HMDB0000001	C01152	3.34 (s), 7.01 (s), 8.15 (s)	yes
Histidine metabolism	Histidine	HMDB0000177	C00135	3.15 (dd), 3.24 (dd), 3.98 (t), 7.07 (s), 7.82 (s)	yes
Ketone metabolism	3-Hydroxybutyrate	HMDB0000011	C01089	1.19 (d), 2.24 (dd), 2.39 (dd), 4.15 (m)	yes
Leucine degradation	Isovalerate	HMDB0000718	C08262	0.90 (d); 2.05 (m)	no
Leucine metabolism	3-Hydroxyisovalerate	HMDB0000754	C20827	1.25 (s), 2.35 (s)	yes
Lysine degradation	2-Aminoadipate	HMDB0302754	C00956	1.63 (m), 1.81 (m), 2.07 (m), 2.23 (t), 2.33 (m), 3.86 (m)	yes
Lysine degradation	Lysine	HMDB0000182	C00047	1.47 (m); 1.75 (m); 3.03 (m)	no
Lysine degradation	Pipecolic acid	HMDB0000070	C00408	1.50–2.10 (m); 3.35 (m)	no
Methylamine metabolism	Trimethylamine N-oxide	HMDB0000925	C01104	3.27 (s)	no
Methylation metabolism	Betaine	HMDB0000043	C00719	3.27 (s), 3.90 (s)	yes
Nicotinate metabolism	Trigonelline	HMDB0000875	C01004	4.43 (s), 7.11 (d), 8.07 (d), 8.85 (s)	yes
One carbon pool by folate	Formate	HMDB0000142	C00058	8.44 (s)	yes
Phenylalanine / Tyrosine metabolism	Phenylacetate	HMDB0000209	C07086	3.60 (s); 7.35 (m)	no
Phenylalanine / Tyrosine metabolism	Succinylacetone	HMDB0000635	NA	2.60 (s); 2.70 (s)	no
Phenylalanine metabolism	Phenylacetylglycine	HMDB0000821	C05598	3.64 (s), 3.82 (s), 7.20–7.40 (m)	yes
Propionate metabolism / TCA-related	Methylmalonate	HMDB0000202	C02170	1.23 (d)	yes
Purine metabolism	Hypoxanthine	HMDB0000157	C00262	8,18 (s), 8.20 (s)	yes
Purine metabolism	Inosine	HMDB0000195	C00294	3.86 (dd), 3.93 (dd), 4.34 dd),4.38 (td), 4.78 (dd), 6.09 (d), 8.49 (s)	yes
Purine metabolism	Xanthine	HMDB0000292	C00385	7.85 (s); 8.12 (s)	no
Pyrimidine metabolism	Thymine	HMDB0000262	C00178	1.90 (s); 7.55 (s); 11.2 (br s)	no
Pyrimidine metabolism	Uracil	HMDB0000300	C00106	5.80 (d); 7.50 (d)	no

Pyruvate metabolism	Lactate	HMDB0000190	C00186	1.33 (d), 4.11 (q)	yes
Redox / Cofactor metabolism	NAD	HMDB0000902	C00003	6.10 (d); 8.60 (s); 9.30 (s)	no
Redox / Cofactor metabolism	NADPH	HMDB0000221	C00005	6.05 (d); 8.55 (s); 9.25 (s)	no
Short-chain fatty acid metabolism	2-Hydroxyisobutyrate	HMDB0242161	NA	1.26 (s); 1,31 (s)	no
Short-chain fatty acid metabolism	Butyrate	HMDB0000039	C00246	0.88 (t), 1.54 (m), 2.14 (t)	yes
Sulfur-containing AA metabolism	Methionine	HMDB0000696	C00073	2.10 (s); 2.65 (t); 3.88 (t)	no
Sulfur-containing AA metabolism	Taurine	HMDB0000251	C00245	3.27 (t); 3.43 (t)	no
Threonine metabolism	L-Threonine	HMDB0000167	C00188	1.32 (d); 4.22 (m)	no
Tryptophan metabolism	Kynurenic acid	HMDB0000715	C01717	6.10 (s); 7.60 (s)	no
Tryptophan metabolism	Kynurenine	HMDB0000684	C00328	6.90 (d); 7.40 (d); 7.60 (s)	no
Tyrosine / Phenylalanine metabolism	4-Hydroxyphenyl Acetate	HMDB0060390	C13636	3.54 (s), 6.70 (d), 7.05 (d)	yes
Tyrosine metabolism	L-Tyrosine	HMDB0000158	C00082	6.88 (d); 7.20 (d); 3.93 (dd)	no
Urea cycle	Guanidinosuccinate	HMDB0003157	C03139	2.15–2.60 (m); 3.60 (s); 6.60 (s)	no
Urea cycle	Urea	HMDB0000294	C00086	5.83 (br s)	yes
Valine metabolism	(S)-3-Hydroxyisobutyrate	HMDB0000023	C06001	1,11 (d), 2,65 (m), 3,68 (m)	yes
Vitamin (B2) / Cofactor	Riboflavin	HMDB0000244	C00255	2.38 (2), 3.50 (dd), 3.70 (td), 3.81 (ddd), 384 (t), 4.02 (ddd), 3.24 (dd), 7.29 (s)	yes
Vitamin / Cofactor	Biotin	HMDB0000030	C00120	1.30–4.50 (m)	no
Xenobiotic metabolism	4-Aminohippurate	HMDB0001867	D06890	3.85 (s), 6.60 (d), 6.74 (d), 7.75 (d)	yes
Xenobiotic metabolism	Acetaminophen	HMDB0001859	C06804	2.15 (s); 6.50 (d); 7.25 (d)	no
Xenobiotic metabolism	Aspirin	HMDB0001879	C01405	2.30 (s); 7.25–8.00 (m)	no
Xenobiotic metabolism	Hippurate	HMDB0000714	C01586	3.96 (d), 7.54 (m), 7,63 (tt) 7.83 (dd)	yes
Xenobiotic metabolism	N,N-Dimethylformamide	HMDB0001888	C03134	2.91 (s), 2.99 (s), 8.03 (s)	yes
Xenobiotic metabolism	Propylene glycol	HMDB0001881	C00583	1.13 (d), 3,43 (dd), 3,53 (dd), 3,86 m)	yes

[1] Multiplicty: s: singlet; d: doublet; dd: doublet of doublets; ddt: doublet of doublets of triplets; t: triplet; q: quartet; m: multiplet; br: broad

[2] Common name as described in HMDB

Table S3 – Possible identification of unknown metabolites

Putatively annotated unknown urinary metabolites included in univariate statistical analysis. Putative identities or chemical classes were suggested based on spectral characteristics (chemical shift and multiplicity) and comparison with spectral databases (CHEMOMX, HMDB). MSI levels reflect the confidence of annotation according to the Metabolomics Standards Initiative.

Peak ID	δ (ppm)	Multiplicity	Possible Assignments	MSI Level	Notes
U1.47	1.47	m	Isocaproic acid	MSI 3	SCFA/BCAA region
U1.81	1.81	d	2-Aminoadipate or 2-Hydroxyglutarate	MSI 3	
U2.21	2,2	s	Adipate	MSI 3	
U6.50	6.5	s	Aromatic xenobiotic	MSI 4	Unidentified exogenous compound
U6.68	6.68	s	Phenolic compound	MSI 3	Related to phenolic compounds or hippurate
U7.69	7.69	s	Purine derivative	MSI 3	Derivative of adenine or xanthine
U8.01	8.01	s	NAD^+/$NADP^+$ derivative	MSI 2	Nicotinamide fragment
U8.39	8.39	s	NAD^+/$NADP^+$ derivative	MSI 2	Nicotinamide fragment
U8.53	8.53	s	ATP/ADP	MSI 2	Adenine base
U8.55	8.55	s	ATP/ADP	MSI 2	Adenine base
U8.67	8.67	s	NADPH/NADH	MSI 2	Reduced form of nicotinamide

Table S4 – Clinical and Genetic Characterization of Groups

eGFR was calculated using age-appropriate creatinine-based equations: Schwartz formula (k = 0.45 if <1 year; k = 0.413 if 1–18 years) and CKD-EPI if >18 years.

Group	ID	Age (years)	Sex	Phenotype	Genetic basis	Usual medication	eGFR for age	U25-Cr	U25-CisC	U25 Mean eGFR	MA	CKD_stage_KDIGO24	Proteinuria
PMD	P02	8.7	F	Hypotonia; GDD; basal ganglia lesions	EARS2	Q10, thiamine, riboflavine	194	165.1	x	x	x	G1A1	0.187
PMD	P03	18.8	M	Primary Q10 deficiency	COQ8A	Clonazepam, ibedenona, Q10	105	82.42	x	x	não	G1A1	0.036
PMD	P04	14	F	LS	m.10191T>C	Q10, thiamine, riboflavine	124.8	116.6	x	x	não		0.09
PMD	P05	10.5	M	Cardiomiopathy, myopathy, NSHL, FFT	m.3271T>C	Q10, thiamine, riboflavine	159.6	148.9	x	x	não	G1A1	0.147
PMD	P06	10.4	M	LS	m.8993T>G	Q10, thiamine, riboflavine, arginine	158.9	148.2	x	x	12.8	G1A1	0.124
PMD	P07	4.0	F	FFT, Low stature, miopathy, muscle weakness, ptosis	SLSMD	Levotiroxine,thiamine, riboflavine, Q10, L-carnitine	156.2	103.1	x	x	x	G1A1	x
PMD	P08	15.5	F	LS	m.3946G>A	Thiamine, riboflavine, Q10	91.36	86.47	x	x	x	G1A1	0,072
PMD	P09	4.8	M	Cardiomiopathy intermittent 3-MGA-uria	TAZ	Captopril, spironolactone, carvedilol	129.1	115	x	x	x	G1A1	0.172
PMD	P10	1.0	M	Neonatal severe metabolic acidosis, high Lactate, cardiomyopathy, 3-MGA-uria	TMEM70	Q10, thiamine, riboflavine, arginine, bicarbonate	91.63	79.27	x	x	19.2	G1A1	0.392
PMD	P11	16.9	F	LS	m.8993T>G	Q10, thiamine, riboflavine	135.5	132.4	x	x	x	G1A1	intermittent
PMD	P12	1.1	F	Alpers syndrome	POLG	Clonazepam, phenobarbital, lacosamide, Q10, thiamine, riboflavin	334.5	268.1	x	x	26.1	G1A1	0.522
PMD	P13	8.8	M	Epilepsy and ASD	m.8993T>C	Risperidone, atomexetine, valproate	128.2	118	x	x	x	G1A1	0.118
PMD	P14	9.4	F	Cardiorrespiratory arrests, 3-MGA-uria	LYRM4	None	185.9	159.1	x	x	7.1	G1A1	??
PMD	P15	10.5	F	GRACILE	BCSL1	Q10, thiamine, bicarbonate, phosphate supplementation (Joulie solution), enalapril, esomeprazol, vitamine D	88.8	68.07	87.76	77.92	278.9	G2A3	
PMD	P25	9.8	M	NS, NSHL and myopathy	RR2MB	Q10, thiamine, steroids, micophenate mofetil, pravastatine	83.25	77.37	41.7	59.53	9175	G2A3	11,582
SMD	P16	0.3	M	ALTE, metabolic acidosis, ketosis, high alanine, low arginine, undiagnosed. MDC = 3	-	Arginine	75.4	x	x	x	x	G1A1	x
SMD	P17	16.8	F	Recurrent myositis, easy fatigability; CV 11%; MDC =3	-	Q10	122		x	x	x	G1A1	x
SMD	P18	0.9	F	Neonatal distress with hypoglycemia; early onset hypotonia; spasticity, assimetric ptosis, sensory disturbance; GDD; MDC=3	-	None	109		x	x	x	G1A1	x
SMD	P19	0.2	F	Aregenerative anemia, neutropenia, ethylmalonic aciduria and 3-	-	phenobarbital	82.8		x	x	x	G1A1	x

				methylglutaconic aciduria (3MGA); neonatal distress without sentinel event									
SMD	P20	18.7	F	FTT; LV hypertrophy cardiomiopathy without heart failure; muscle biopsy with multiple RC deficiencies; MDC =3	-	Q10, thiamine, riboflavine	136	118.5	x	x	x	G1A1	x
SMD	P21	3.7	M	Progressive spasticity (++ lower limb), GDD; MDC=3	-	Q10, thiamine, riboflavine	125.3		x	x	13.8	G1A1	0.13
SMD	P22	4.5	F	Episodic ataxia and sonnomlence; low stature; intermittent high Lactate and alanine; hyperintense cerebellar lesions. Krebs metabolites in OAs; MDC=5	-	Q10, thiamine, riboflavine	123.3	101.8	x	x	0	G1A1	0
SMD	P23	19.5	F	Epileptic encephalopathy, absent language, GDD/ID; MDC=4	-	Vigabatrine, zonisamide, lacosamide, ketogenic diet	132	108.3	x	x	0	G1A1	0
SMD	P24	2.2	F	FFT, low stature.muscle weakness, paradoxal ketosis, persistent high Lactate and alanine, distal tubular acidosis, Krebs metabolites in OAs, MDC=6	-	Q10, thiamine, riboflavine	118.4		x	x	<9	G1A1	0.239
C	P26	2.7	M	CAKUTRenal duplication with bilateral pyelectasis	-	None	122	108.1	96.6	102.4	8.3	G1A1	0.194
C	P28	3.1	F	Previous UTI	-	None	162.7	132.4	92.9	112.7	13.8	G1A1	0.23
C	P29	9.8	M	Crossed renal ectopia	-	None	129.1	111.9	106.1	109	9.9	G1A1	0.1
C	P30	13.3	M	Ureterohydronephrosis	-	None	102.9	106.1	91.8	98.95	6.3	G1A1	0.114
C	P31	12.4	F	Renal duplication with bilateral pyelectasis	-	None	109.7	100.4			<3,4	G1A1	0.063
C	P32	8.1	F	Vesicoureteral reflux	-	None	122.8	104.8	80.6	92.7	<3,4	G1A1	0.125
C	P33	13.5	M	Vesicoureteral reflux with spontaneous regressions	-	None	110.8	119.4	89.9	104.7	26.3	G1A1	0.146
C	P34	13.9	M	Transient creatinine elevation	-	None	100.1	107.9	90.8	99.35	7.4	G1A1	0.06
C	P35	18.2	M	Posterior urethral valve (corrected)	-	None	130	107.4	90.5	98.9	9.5	G1A1	0.05
C	P36	16.8	M	Solitary kidney	-	None	85.66	105.4	89.7	97.55	11.9	G1A1	0.06
CKD 1 - 2	P37	6.6	M	Ataxia Telangiectasia	ATM	None	112.5	102.9			16.9	G1A1	0.15
CKD 1 - 2	P38	15.8	M	Polymalformative syndrome with CAKUT	-	None	70.45	82.9	73.1	78	17.3	G2A1	0.054
CKD 1 - 2	P39	17.9	F	CAKUT with multicystic kidneys	-	None	113	83.3	84.1	83.7	3.1	G2A1	0.053
CKD 1 - 2	P40	14.3	M	Renal Agenesia and ASD	-	None	79.85	86	89.9	87.95	2.3	G2A1	0.044
CKD 1 - 2	P41	8.1	F	PKD	-	None	129.1	112.8	90.7	101.8	7.2	G1A1	0.158
CKD 1 - 2	P42	17.8	M	Steroid-dependent NS	-	Steroids, Mycophenolate mofetil	138	127	96.4	111.7	4.5	G1A1	0.043
CKD 1 - 2	P43	11.1	M	X-L Hypophosphatemic Rickets (PHEX)	PHEX	Burosumab	134	117.1	70.7	93.9	16.5	G1A1	0.13
CKD 1 - 2	P44	7.2	M	X-L Hypophosphatemic Rickets	PHEX	Burosumab	163	149.1	120.6	134.9	5.4	G1A1	0.144
CKD 1 - 2	P45	17.1	M	Alport Syndrome	COL4A3	None	88.87	113.2	87.6	100.4	14.4	G1A1	0.064
CKD 1 - 2	P46	4.8	M	Steroid-sensitive NS; ASD	-	Steroids, Mycophenolate mofetil	259.9	234			516	G1A3	0.864

CKD 1 - 2	P47	2.8	F	VUR and UTIs ; T1D	-	Insuline	207.4	101.3			2742	G1A3	5.326
CKD 1 - 2	P48	16.9	M	Nutcracker Syndrome	-	0	82.65	101.7			158.7	G1A2	0.255
CKD 1 - 2	P49	11.4	M	Steroid-dependent NS	-	Steroids, cyclosporine, ramipril	150.4	148.4			2020	G1A3	2.419
CKD 1 - 2	P50	14.8	F	PKD	-	None	105.2	100.8	73	86.9	79.3	G1A2	0.161
CKD 1 - 2	P51	16.2	M	Probable IgA Nephropathy	-	Ramipril	97.62	114.9	101.7	108.3	782.3	G1A3	1.075
CKD 1 - 2	P52	7.8	M	Glomerulonephritis - IgA Nephropathy	-	Finerenone	146.8	135.4			3174	G1A3	4.11
CKD 1 - 2	P53	17.3	M	Urethral stricture	-	None	87.58	107.7	87.6	97.65	148	G1A2	0.386
CKD 3 - 5	P54	12.9	F	Cystinosis	CTNS	Cysteamine, phosphate, bicarbonate, sodium citrate, potassium citrate, potassium chloride, paricalcitol, calcium carbonate, vitamin D, iron	26.12	23.9	43.8	33.85	3983	G4A3	6.242
CKD 3 - 5	P55	4.7	M	CAKUT with dysplastic and cystic kidneys	-	Bicarbonate, epoetin α, paricalcitol, vitamin D, folic acid	13.99	12.6	18.3	15.45	2650	G5A3	
CKD 3 - 5	P56	15.7	M	Ochoa syndrome with CAKUT	HNF1B	Calcium carbonate, amlodipine, vitamin D, silodosin, resin, alfacalcidol, iron	43.27	50.9			13.4	G3aA1	
CKD 3 - 5	P57	9.5	M	Joubert syndrome w/ nephronophthisis	TCTN2	Bicarbonate, iron, calcitriol, allopurinol, epoetin alfa, amlodipine, esomeprazole	10.42	9.8	17.6	13.7	194.6	G5A2	
CKD 3 - 5	P58	15.8	M	CAKUT	-	Bicarbonate, calcitriol, enalapril, oxybutynin, tamsulosin, resin	18.95	22.3	22.1	22.2	46.3	G4A2	
CKD 3 - 5	P59	14.6	M	AR-PKD and liver fibrosis	PKHD1	Bicarbonate, epoetin alfa, vitamin D, paricalcitol, iron	23.53	26.5	26.4	26.45	15.7	G4A1	
CKD 3 - 5	P60	8.2	M	Scarring nephropathy; VUR	-	Bicarbonate, epoetin alfa, vitamin D, paricalcitol, enalapril, oxybutynin, somatotropin	16.24	15.2	26.1	20.65	76.4	G4A2	
CKD 3 - 5	P61	15.3	F	MMC; VUR; neurogenic bladder	-	Bicarbonate, epoetin alfa, vitamin D, paricalcitol, iron, calcium carbonate	11.42	10.9	17.9	14.4	3063	G5A3	
CKD 3 - 5	P62	10.8	F	AR-PKD	PKHD1	Bicarbonate, iron, alfacalcidol, sevelamer, epoetin alfa, propranolol, calcium carbonate, vitamin D	14.74	12.8	19	15.9	75.9	G5A1	

***For autosomal diseases, the implicated gene is indicated, whereas for mtDNA, the associated variant is presented; ** CKD stage based on eGFR and albumin-to-creatinine ratio as defined by the KDIGO 2024 Guidelines; for patients younger than 12 months, eGFR is based on Schwartz formula and for patients older than 18 years, eGFR is based on KDIGOEPI 2021 guidelines. Abbreviations: 3-MGA-uria: 3-methylgluthaconic aciduria; ACR**: Albumin-to-creatinine ratio; **AD**: Autosomal Dominant; **AR**: Autosomal Recessive; **ASD**: Autism Spectrum Disorder; **CAKUT**: Congenital Anomalies of the Kidney and Urinary Tract; **DM**: Diabetes Mellitus; **FS**: Fanconi Syndrome; **FTT**: Failure to Thrive; **GRACILE**: Growth retardation, Renal (kidney) dysfunction, Aminoaciduria (presence of amino acids in the urine), Cholestasis, Iron overload, Lactateosis, Early death; **GDD**: Global Developmental Delay; **LS**: Leigh Syndrome; **MDC:** mitochondrial disease criteria; **MMC**: Myelomeningocele; **M**: Male; **NS**: Nephrotic Syndrome; **NSHL**: Neurosensory Hearing Loss; **PKD**: Polycystic Kidney Disease; SLSMD: single large-scale mtDNA deletions; **T1D**: Type 1 Diabetes; **UTI**: Urinary Tract Infection; **VUR**: Vesicoureteral Reflux; **X-L**: X-linked; **Y**: Years; **F**: Female.

Figure S2 – Group validation of CKD and control groups through multivariate analysis

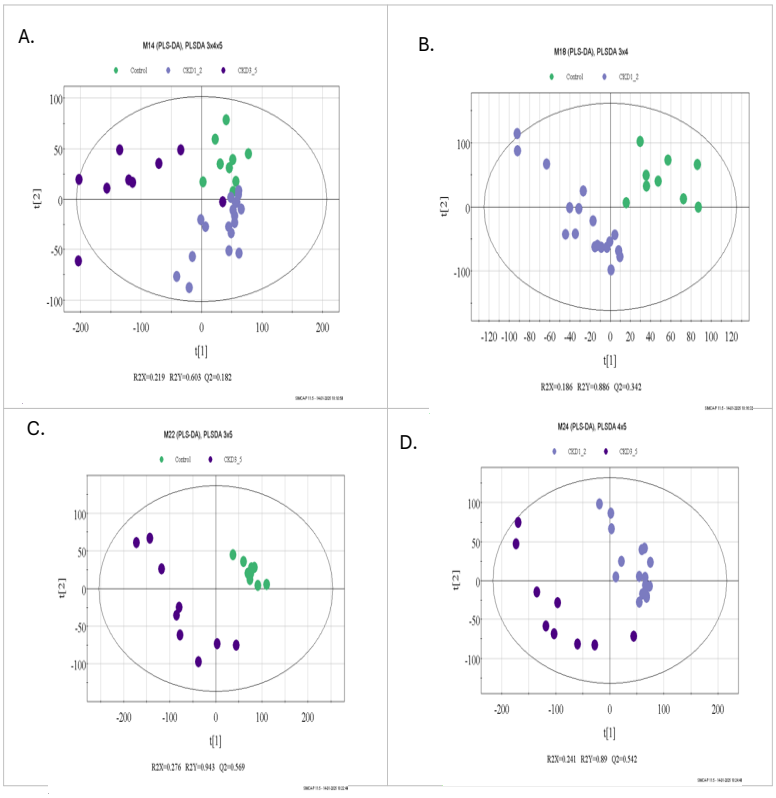

Figure S3. Group validation of CKD and control groups through multivariate analysis using PLS-DA. *Q^2 values indicate the predictive performance of each PLS-DA model*

A. Overview of CKD stages 1-2, CKD stages 3-5, and control groups

B. Control vs CKD stages 1-2 (Q^2 = 0.342)

C. Control vs CKD stages 3-5 (Q^2 = 0.569)

D. CKD stages 1-2 vs CKD stages 3-5 (Q^2 = 0.542)

Table S6 – Quality Parameters of PLS-DA Models used to explore urinary metabolic profiles.

Each row corresponds to a PLS-DA model comparing specific diagnostic or phenotypic subgroups based on pareto-scaled urinary ^{1}H-NMR spectra. R^2X and R^2Y represent the fraction of variance explained by the model in the predictors (X) and response (Y) matrices, respectively. Q^2 corresponds to the predictive ability of each model as estimated by 7-fold cross-validation. Models with Q^2 values above 0.5 were considered to have moderate to good predictive performance. Notably, comparisons between PMD and CKD subgroups (CKD stage 1- 2, CKD stage 3-5, and CKD stage 1-2 without microalbuminuria) yielded the highest predictive values, while comparisons involving SMD or subtypes within CKD showed weaker performance, reflecting overlapping metabolic profiles.

Comparison	R2X	R2Y	Q2
PMD x SMD	0.172	0.937	0.317
PMD x C	0.167	0.961	0.52
PMD x CKD stage 1-2	0.184	0.932	0.686
PMD x CKD 1- 2 without microalbuminuria	0.215	0.953	0.691
PMD x CKD 3 TO 5	0.255	0.961	0.688
PMD-3MGA x PMD+3MGA	0.197	0.853	0.0985

Abbreviations: PMD – Primary Mitochondrial Disease; SMD – Secondary Mitochondrial Dysfunction; CKD – Chronic Kidney Disease; MA – Microalbuminuria; 3-MGA – 3-Methylglutaconic Aciduria.

Table S7 – Pearson correlation for total area and creatinine normalizations per metabolite.

Metabolite	Pearson r	Correlation*	p_value	Sign.**
α-Hydroxyisobutyrate	-0.3248	weak (negative)	0.005	**
Glycolate	-0.2827	weak (negative)	0.015	*
Creatinine	-0.0711	none	0.547	ns
3-Hydroxyisobutyrate	-0.0564	none	0.633	ns
Methylmalonate	-0.0138	none	0.907	ns
$U_{7.69}$	0.0997	none	0.398	ns
4-Aminohippurate	0.1084	none	0.358	ns
Phenylalanine	0.1256	none	0.286	ns
Isoleucine	0.1343	none	0.254	ns
Histidine	0.1370	none	0.244	ns
3-Hydroxyisovalerate	0.2323	weak	0.046	*
1-Methylhistidine	0.2650	weak	0.023	*
Urea	0.2709	weak	0.020	*
Methylsuccinate	0.3204	weak	0.005	**
2-Aminoadipate	0.3255	weak	0.005	**
N-Phenylacetylglycine	0.3615	weak	0.002	**
trans-Acotinate	0.3640	weak	0.001	**
Dimethylamine	0.3730	weak	0.001	**
cis-Acotinate	0.3772	weak	0.001	***
$U_{8.39}$	0.3924	weak	0.001	***
U8.55	0.4001	moderate	0.000	***
Methylguanidine	0.4200	moderate	0.000	***
Succinate	0.4476	moderate	0.000	***
U6.68	0.4613	moderate	0.000	***
Inosine	0.4708	moderate	0.000	***
U8.53	0.4714	moderate	0.000	***
Creatine	0.4873	moderate	0.000	***
5-Aminolevulinic acid	0.4902	moderate	0.000	***
Acetate	0.4992	moderate	0.000	***
$U_{1.82}$	0.5039	moderate	0.000	***
Lactate	0.5159	moderate	0.000	***
Glycine	0.5345	moderate	0.000	***
Riboflavin	0.5477	moderate	0.000	***
$U_{8.67}$	0.5578	moderate	0.000	***
Hypoxanthine	0.5855	moderate	0.000	***
Citrate	0.5885	moderate	0.000	***
$U_{2.21}$	0.6046	strong	0.000	***
Betaine	0.6468	strong	0.000	***
Propylene glycol	0.6580	strong	0.000	***
Pyruvate	0.6623	strong	0.000	***
Hippurate	0.7308	strong	0.000	***
$U_{6.50}$	0.7323	strong	0.000	***
Homovanillic acid	0.7762	strong	0.000	***
$U_{1.81}$	0.7965	strong	0.000	***
3-Hydroxybutyrate	0.7981	strong	0.000	***
Trigonelline	0.7988	strong	0.000	***
$U_{8.01}$	0.8251	very strong	0.000	***
1-Methylnicotinamide	0.8540	very strong	0.000	***
Fumarate	0.8554	very strong	0.000	***
N,N-Dimethylformamide	0.8693	very strong	0.000	***
4-Hydroxyphenyl acetate	0.8741	very strong	0.000	***
Formate	0.8778	very strong	0.000	***
Leucine	0.8879	very strong	0.000	***
Valine	0.8965	very strong	0.000	***
Alanine	0.9060	very strong	0.000	***
Butyrate	0.9081	very strong	0.000	***

D-glucoronate	0.9128	very strong	0.000	***
3-Methyl-2-oxovalerate	0.9759	very strong	0.000	***
$U_{1.47}$	0.9847	very strong	0.000	***

*Correlation: none 0.00-0.19; weak 0.20-0.39; moderate 0.40-0.59; strong 0.60-0-79; very strong: 0.8-1.0; **Significance: ns: not significant; * p<0,05; **: p<0,01; ***: p<0,001

Table S8 – List of significant metabolites by group and comparison of normalization methods.

	Compound name	ppm	Total area normalization			Creatinine normalization			Significance[1]
			Fold change	Hedge's Effect size	p-value	Fold change	Hedge's Effect size	p-value	
PMD vs CKD stages 1-2	1-Methylnicotinamide	8.98	1.19	0.24	0.003	1.46	-0.26	0.845	** / ns
	2-Aminoadipate	2.29	0.74	-0.74	0.003	1.13	-0.20	0.869	** / ns
	3-Hydroxyisobutyrate	1.08	1.07	0.22	0.457	1.41	-0.55	0.043	ns / *
	3-Hydroxyisovalerate	1.27	1.17	0.44	0.170	1.49	-0.56	0.007	ns / **
	3-Methyl-2-oxovalerate	0.89	2.06	0.41	0.650	8.03	-0.42	0.031	ns / *
	4-Aminohippurate	7.75	1.43	0.79	0.028	2.11	-0.87	0.015	* / *
	4-HydroxyphenylAcetate	3.45	1.17	0.40	0.028	1.84	-0.49	0.014	* / *
	Alanine	1.48	1.19	0.60	0.039	1.62	-0.82	0.014	* / *
	Butyrate	0.90	1.69	0.52	0.031	4.96	-0.45	0.009	* / **
	cis-Acotinate	3.11	1.11	0.17	0.006	1.61	-0.73	0.043	** / *
	Creatine	3.94	1.40	0.59	0.123	1.88	-0.75	0.028	ns / *
	Dimethylamine	2.72	0.96	-0.14	0.934	1.54	-0.65	0.048	ns / *
	Formate	8.47	2.01	0.53	0.103	3.32	-0.68	0.035	ns / *
	Fumarate	6.53	1.98	0.95	0.005	3.67	-0.77	0.012	** / *
	Histidine	7.10	0.43	-1.20	0.001	0.79	0.40	0.320	** / ns
	Homovanillic acid	6.94	1.38	0.86	0.031	2.45	-0.86	0.009	* / **
	Hypoxanthine	8.20	1.12	0.23	0.773	1.57	-0.57	0.020	ns / *
	Isoleucine	0.93	1.10	0.45	0.015	1.81	-0.62	0.028	* / *
	Lactate	1.34	2.59	0.54	0.005	2.83	-0.70	0.001	** / **
	Methylmalonate	1.23	1.02	0.04	0.837	1.35	-0.46	0.017	ns / *
	Propylene glycol	1.14	1.57	0.78	0.035	2.37	-0.69	0.007	* / **
	Pyruvate	2.38	1.48	0.74	0.408	2.18	-0.97	0.008	ns / **
	Succinate	2.41	1.28	0.68	0.086	2.09	-0.76	0.048	ns / *
	$U_{1.81}$	1.81	0.46	-0.40	0.017	0.89	0.08	0.805	* / ns
	$U_{6.50}$	6.50	1.57	0.56	0.008	4.40	-0.59	0.017	** / *
	$U_{8.01}$	8.01	0.60	-0.86	0.005	0.85	0.29	0.621	** / ns
	$U_{8.67}$	8.67	0.48	-0.99	0.020	0.67	0.42	0.229	* / ns
	Valine	1.05	0.78	-0.91	0.025	1.01	-0.02	0.263	* / ns
	1-Methylhistidine	7.03	0.77	-0.70	0.035	1.29	-0.42	0.483	* / ns
PMD vs CKD stages 3-5	3-Hydroxyisovalerate	1.27	1.99	1.66	0.000	1.98	-1.08	0.009	*** / **
	4-Aminohippurate	7.75	1.59	0.91	0.036	1.80	-0.69	0.164	* / ns
	cis-Acotinate	3.11	1.60	0.68	0.006	1.92	-0.93	0.017	** / *
	Formate	8.47	2.57	0.55	0.009	3.49	-0.60	0.164	** / ns
	D-glucoronate	5.27	0.09	-1.41	0.071	0.08	0.89	0.014	ns / *
	Glycine	3.57	1.30	0.68	0.001	1.39	-0.24	0.431	** / ns
	Hippurate	7.57	1.40	0.37	0.0111	2.08	-0.51	0.164	* / ns
	Homovanillic acid	6.94	2.28	1.63	0.000	3.08	-0.84	0.003	*** / **
	Isoleucine	0.93	0.85	-0.65	0.021	1.00	0.00	0.695	* / ns
	Leucine	0.96	0.60	-0.86	0.021	0.52	0.44	0.471	* / ns
	Methylmalonate	1.23	1.51	0.92	0.025	1.71	-1.06	0.003	* / **
	Phenylalanine	7.43	1.32	0.43	0.0026	1.47	-0.43	0.845	** / ns
	Propylene glycol	1.14	2.08	1.13	0.014	2.25	-0.57	0.030	* / *
	Riboflavin	7.96	1.96	0.35	0.0014	2.29	-0.44	0.431	** / ns
	$U_{8.01}$	8.01	1.55	0.49	0.017	2.17	-1.12	0.009	* / **
	$U_{8.53}$	8.53	1.66	1.15	0.009	1.88	-0.85	0.096	** / ns
PMD vs Control	4-Aminohippurate	7.75	1.83	1.18	0.000	2.88	1.07	0.005	*** / **
	cis-Acotinate	3.11	1.43	0.64	0.018	2.01	1.01	0.015	* / *
	Creatine	3.94	1.60	0.76	0.058	2.16	0.89	0.036	ns / *
	Creatinine	4.06	0.77	-0.62	0.036	1.06	0.25	0.605	* / ns
	Dimethylamine	2.72	0.74	-1.03	0.049	1.08	0.11	0.927	* / ns
	Fumarate	6.53	2.04	0.88	0.004	3.96	0.69	0.004	** / **
	Histidine	7.10	0.41	-1.15	0.006	0.66	-0.55	0.376	** / ns
	Homovanillic acid	6.94	1.56	1.02	0.012	2.68	0.79	0.012	* / *
	Hypoxanthine	8.20	1.30	0.64	0.648	2.04	0.90	0.006	ns / **
	Methylguanidine	2.83	1.14	0.47	0.058	1.68	0.91	0.026	ns / *
	N-Phenylacetylglycine	3.70	1.12	0.41	0.605	1.82	0.75	0.049	ns / *
	trans-Acotinate	6.60	1.00	0.01	0.693	1.46	0.70	0.049	ns / *
	$U_{6.50}$	6.50	1.49	0.48	0.030	3.86	0.57	0.077	* / ns
PMD vs SMD	Creatinine	4.06	1.10	0.19	0.262	0.91	-0.35	0.017	ns / *
	Histidine	7.10	0.48	-1.41	0.017	0.48	-1.48	0.002	* / **
	Hypoxanthine	8.20	0.36	-0.95	0.096	0.49	-0.70	0.043	ns / *
	Isoleucine	0.93	0.84	-0.60	0.030	0.74	-0.35	0.235	* / ns
	Methylmalonate	1.23	1.20	0.26	0.021	0.80	-0.27	1.000	* / ns

	Phenylalanine	7.43	1.13	0.19	0.0089	0.94	-0.07	0.647	** / ns
	Propylene glycol	1.14	1.59	0.63	0.030	1.97	0.51	0.357	* / ns
	$U_{1.81}$	1.81	0.82	-0.25	0.036	0.70	-0.33	0.357	* / ns
	$U_{2.21}$	2.21	0.83	-0.25	0.004	0.97	-0.03	0.647	** / ns
	$U_{8.55}$	8.55	1.45	0.86	0.036	1.23	0.27	0.845	* / ns
SMD vs Control	3-Hydroxybutyrate	1.21	1.03	0.04	0.095	3.29	0.61	0.001	ns / ***
	3-Hydroxyisobutyrate	1.08	1.19	0.22	0.604	1.47	0.23	0.022	ns / *
	3-Hydroxyisovalerate	1.27	0.83	-0.19	0.356	1.85	0.61	0.044	ns / *
	3-Methyl-2-oxovalerate	0.89	1.09	0.12	0.604	1.72	0.27	0.004	ns / **
	4-Aminohippurate	7.75	0.92	-0.06	0.243	3.64	1.71	0.000	ns / ***
	5-Aminolevulinic acid	2.79	1.73	0.75	0.968	1.78	0.49	0.022	ns / *
	Acetate	1.93	1.04	0.04	0.079	7.65	2.50	0.035	ns / *
	alpha-Hydroxyisobutyrate	1.36	1.04	0.05	0.356	1.54	0.35	0.035	ns / *
	Butyrate	0.90	1.06	0.07	0.156	1.52	0.14	0.013	ns / *
	cis-Acotinate	3.11	1.87	1.04	0.133	3.24	0.91	0.002	ns / **
	Creatinine	4.06	0.67	-0.49	0.400	1.80	0.97	0.010	ns / *
	Ethanol	1.19	0.99	-0.01	0.0076	3.52	0.42	0.0021	** / **
	Fumarate	6.53	2.09	0.83	0.028	2.02	0.20	0.008	* / **
	D-glucoronate	5.27	1.40	0.37	0.720	3.31	0.52	0.003	ns / **
	Glycolate	3.96	0.86	-0.19	0.044	1.35	0.20	0.113	* / ns
	Hippurate	7.57	1.40	0.60	0.0076	2.01	1.17	0.356	** / ns
	Homovanillic acid	6.94	1.51	0.19	0.004	3.72	0.74	0.000	** / ***
	Hypoxanthine	8.20	1.34	0.20	0.243	1.46	0.11	0.000	ns / ***
	Isoleucine	0.93	1.17	0.17	0.001	1.50	0.12	0.008	** / **
	Lactate	1.34	1.16	0.19	0.661	1.21	0.09	0.035	ns / *
	Methylguanidine	2.83	1.50	0.37	0.017	2.42	0.50	0.008	* / **
	Methylmalonate	1.23	0.77	-0.39	0.013	1.49	0.37	0.182	* / ns
	Methylsuccinate	1.10	0.93	-0.09	0.113	1.62	0.44	0.044	ns / *
	N,N-Dimethylformamide	3.02	1.09	0.10	0.968	1.83	0.32	0.022	ns / *
	Pyruvate	2.38	1.61	0.71	0.720	3.19	0.62	0.028	ns / *
	Succinate	2.41	1.06	0.06	0.054	2.28	0.42	0.028	ns / *
	$U_{1.47}$	1.47	1.20	0.26	0.156	2.06	0.44	0.004	ns / **
	$U_{6.50}$	6.50	1.13	0.09	0.211	1.59	0.26	0.008	ns / **
	$U_{7.69}$	7.69	1.07	0.08	0.549	2.62	0.74	0.022	ns / *
	$U_{8.01}$	8.01	0.39	-0.78	0.004	1.06	0.07	0.211	** / ns
	$U_{8.55}$	8.55	1.07	0.10	0.035	1.29	0.26	0.113	* / ns
	Valine	1.05	1.21	0.19	0.661	1.70	0.23	0.004	ns / **
CKD stages 1-2 vs Control	Dimethylamine	2.72	1.48	0.79	0.0203	1.04	0.08	0.141	* / ns
	Isoleucine	0.93	0.78	-0.26	0.031	0.55	-0.62	0.443	* / ns
	Methylguanidine	2.83	1.14	0.13	0.0009	0.85	-0.17	0.334	*** / ns
	1-Methylhistidine	7.03	1.51	0.25	0.0026	1.09	0.06	0.0203	** / *
CKD stages 3-5 vs CKD stages 1-2	1-Methylhistidine	7.03	0.33	-1.92	0.045	0.22	-2.20	0.958	* / ns
	1-Methylnicotinamide	8.98	0.38	-1.58	0.008	0.40	-1.22	0.560	** / ns
	2-Aminoadipate	2.29	0.65	-0.59	0.000	0.61	-0.59	0.263	*** / ns
	3-Hydroxybutyrate	1.21	0.22	-2.31	0.008	0.54	-0.64	0.958	** / ns
	3-Hydroxyisovalerate	1.27	0.52	-1.05	0.000	1.17	0.16	0.525	*** / ns
	3-Methyl-2-oxovalerate	0.89	0.17	-4.41	0.120	0.45	-1.06	0.029	ns / *
	Acetate	1.93	0.12	-2.19	0.025	0.26	-1.57	0.016	* / *
	α -Hydroxyisobutyrate	1.36	0.30	-2.22	0.001	0.59	-0.72	0.597	*** / ns
	Betaine	3.27	1.84	0.52	0.075	1.32	0.27	0.008	ns / **
	Citrate	2.66	1.67	0.46	0.000	1.59	0.41	0.148	*** / ns
	Ethanol	1.19	0.26	-2.24	0.0064	0.46	-1.03	0.0214	** / *
	Fumarate	6.53	0.24	-2.30	0.018	0.35	-1.37	0.066	* / ns
	D-glucoronate	5.27	0.15	-1.41	0.133	0.05	-1.01	0.003	ns / **
	Glycine	3.57	0.29	-2.10	0.002	0.45	-0.70	0.634	** / ns
	Glycolate	3.96	0.36	-1.79	0.001	0.53	-0.85	0.312	*** / ns
	Hippurate	7.57	0.57	-0.50	0.0214	0.70	-0.37	0.597	* / ns
	Histidine	7.10	0.98	-0.02	0.011	0.47	-0.73	0.200	* / ns
	Homovanillic acid	6.94	0.48	-1.13	0.001	0.60	-0.70	0.339	** / ns
	Isoleucine	0.93	0.16	-4.01	0.220	0.43	-1.07	0.039	ns / *
	Lactate	1.34	0.11	-2.44	0.001	0.29	-0.92	0.018	** / *
	Methylmalonate	1.23	0.44	-1.27	0.008	0.98	-0.02	0.491	** / ns
	Methylsuccinate	1.10	0.24	-2.57	0.005	0.67	-0.49	0.958	** / ns
	N-Phenylacetylglycine	3.70	0.23	-3.38	0.001	0.30	-1.36	0.133	** / ns
	Phenylalanine	7.43	0.69	-0.47	0.0018	0.66	-0.51	0.916	** / ns
	Pyruvate	2.38	0.15	-3.65	0.396	0.29	-1.05	0.045	ns / *
	Riboflavin	7.96	0.60	-0.60	0.0018	0.65	-0.55	0.367	** / ns
	Trigonelline	9.13	0.40	-0.78	0.263	0.45	-0.92	0.039	ns / *
	$U_{1.81}$	1.81	4.94	0.93	0.021	3.54	0.84	0.200	* / ns
	$U_{6.68}$	6.68	0.26	-1.53	0.287	#N/D	#N/D	0.029	ns / *
	$U_{7.69}$	7.69	0.28	-2.04	0.016	0.88	-0.15	0.874	* / ns

	$U_{8.01}$	8.01	1.37	0.25	0.001	1.35	0.28	0.001	*** / **
	$U_{8.53}$	8.53	0.96	-0.05	0.000	1.16	0.16	0.200	*** / ns
	$U_{8.55}$	8.55	0.32	-1.83	0.004	0.48	-0.86	0.426	** / ns
	$U_{8.67}$	8.67	1.75	0.48	0.001	1.77	0.49	0.066	*** / ns
CKD stages 3-5 vs Control	1-Methylhistidine	7.03	1.08	0.06	0.1130	1.59	0.70	0.0435	ns / *
	1-methylnicotinamide	8.98	1.29	0.38	0.0030	1.98	1.56	0.356	** / ns
	2-Aminoadipate	2.29	0.72	-0.70	0.0220	1.12	0.36	0.905	* / ns
	3-Hydroxybutyrate	1.21	1.68	0.86	0.0220	2.38	1.95	0.905	* / ns
	3-Hydroxyisovalerate	1.27	0.42	-1.61	0.0000	0.62	-0.96	0.113	*** / ns
	α-Hydroxyisobutyrate	1.36	0.65	-0.67	0.0076	1.00	-0.01	0.549	** / ns
	Citrate	2.66	0.58	-0.85	0.0003	0.89	-0.27	0.0279	*** / *
	Formate	8.47	0.45	-1.07	0.0133	0.62	-0.63	0.72	* / ns
	Fumarate	6.53	2.44	1.74	0.0220	3.59	4.06	0.0535	* / ns
	D-glucoronate	5.27	25.35	9.96	0.1130	38.71	22.55	0.00299	ns / **
	Glycine	3.57	0.92	-0.22	0.0030	1.36	0.88	0.905	** / ns
	Glycolate	3.96	0.62	-0.79	0.0007	0.94	-0.14	0.278	*** / ns
	Histidine	7.10	0.54	-0.79	0.0133	0.80	-0.33	0.278	* / ns
	Homovanillic acid	6.94	0.69	-0.46	0.0435	1.02	0.04	0.905	* / ns
	Isoleucine	0.93	1.15	0.16	0.022	1.66	0.96	0.079	* / ns
	Methylmalonate	1.23	0.62	-0.62	0.0057	0.97	-0.07	0.113	** / ns
	Methylsuccinate	1.10	0.77	-0.40	0.0435	1.20	0.45	0.842	* / ns
	N-Phenylacetylglycine	3.70	1.06	0.08	0.0172	1.61	1.08	0.315	* / ns
	Phenylalanine	7.43	0.99	-0.03	0.0021	1.54	1.28	0.497	** / ns
	Riboflavin	7.96	0.53	-0.54	0.0076	0.90	-0.16	0.78	** / ns
	$U_{8.01}$	8.01	0.11	-1.18	0.0030	0.17	-1.14	0.0041	** / **
	$U_{8.53}$	8.53	0.39	-1.26	0.0220	0.57	-0.89	0.133	* / ns
	$U_{8.67}$	8.67	0.56	-1.18	0.0279	0.90	-0.31	0.661	* / ns

[1]Significance: ns: not significant; * p<0,05; **: p<0,01; ***: p<0,001

Figure S3. Multivariate and univariate analysis comparing primary mitochondrial disease (PMD) patients and controls.

A. PLS-DA biplot based on short list of identified metabolist (one peak per metabolite) illustrating the separation between PMD patients (orange) and controls (green). Arrows represent the direction and relative contribution of individual metabolites to group discrimination. **B.** Volcano plot showing the distribution of metabolites according to $\log_2$ fold change (x-axis) and $-\log_{10}$(p-value) (y-axis). Dotted lines indicate the thresholds for $|\log_2(FC)| > 1$ and $p < 0.05$. **C.** Variable Importance in Projection (VIP) plot from the PLS-DA model, listing the top discriminating metabolites with corresponding heatmap profiles per group. All panels were generated using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca).

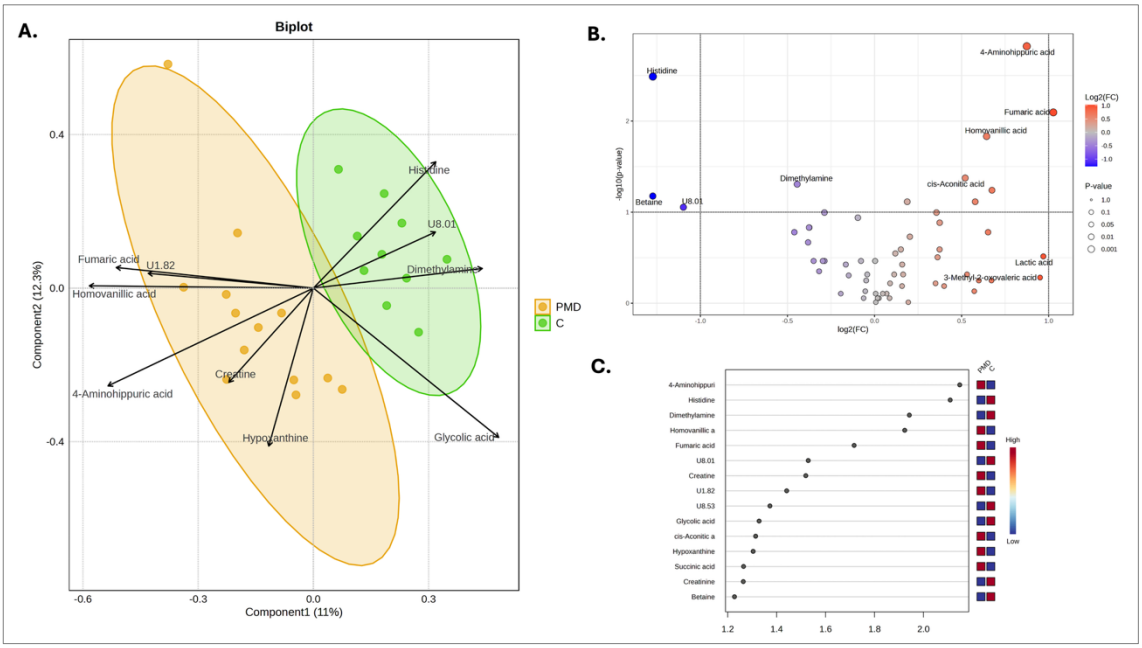

Figure S4. Correlation analysis among urinary metabolites in PMD versus controls.

A. Correlation network plot showing Pearson correlations between urinary metabolites (threshold: |r| > 0.5, p < 0.05). Positive correlations are shown in red and negative in blue. Highly interconnected nodes include Methylsuccinate, Methylmalonate, Lactate, and 3-hydroxyisobutyrate. **B.** Top 25 metabolites correlated with 4-Aminohippurate, revealing strong positive associations with cis-Acotinate, homovanillic acid, and Succinate, and negative correlations with histidine, betaine, and dimethylamine. **C.** Top 25 metabolites correlated with homovanillic acid, including positive correlations with Fumarate, 4-hydroxyphenylacetate, and 1-methylnicotinamide, and negative correlations with Glycolate and creatinine. Pathway enrichment analysis. All panels were generated using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca).

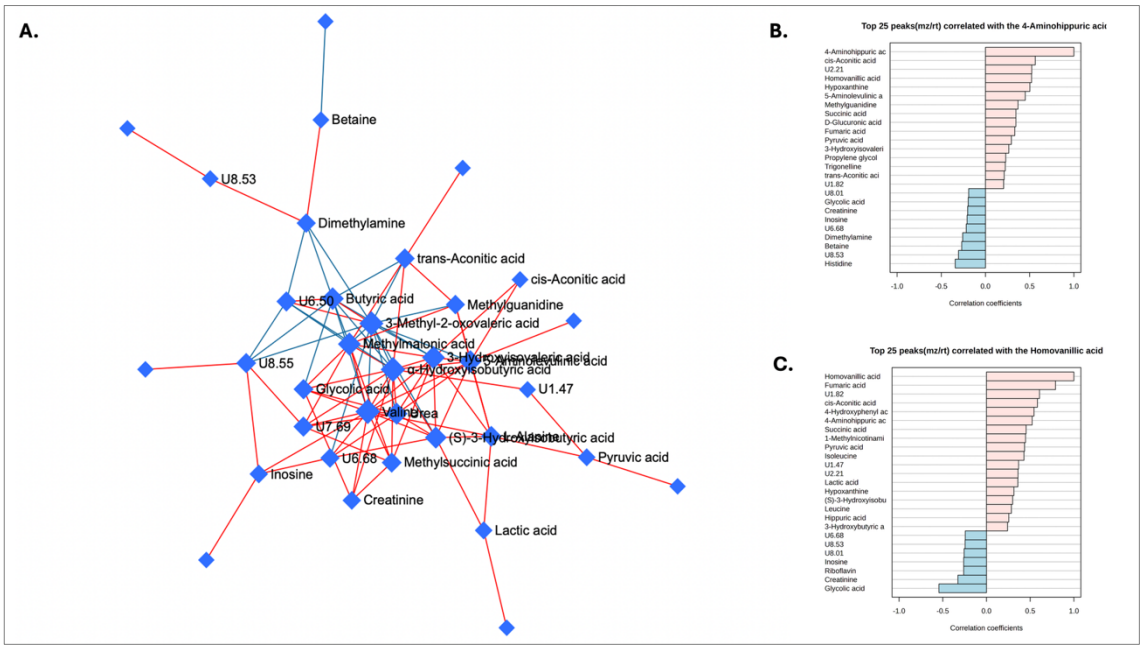

Figure S5. Quantitative enrichment pathway analysis of PMD versus controls

A. Enrichment results based on the Small Molecule Pathway Database (SMPDB) library; **B.** Enrichment results based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) library. Analyses were performed using Quantitative Enrichment Analysis (QEA) in MetaboAnalyst 5.0. Pathways are ranked by adjusted p-value and enrichment ratio. Node color represents statistical significance (p-value), and node size represents pathway impact.