Supplementary Information

The GC-MS metabolomics signature in patients with Fibromyalgia Syndrome directs to dysbiosis as an aspect contributing factor of FMS pathophysiology

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S1: Experimental subjects symptom assessment questionnaire - Malatji et al 2017 [1]

The Fibromyalgia Impact Questionnaire (FIQR) is an internationally derived questionnaire developed by Burckhardt et al. 1991. It was developed with the aim to evaluate and understand the effects of therapy on the broad range of symptoms that manifest in FMS. As such, the questionnaire has routinely been used, since its official release in 1991, as a means to assess the progression of the disorder and any therapeutic interventions applied [3].

Questionnaire A in Table S1 shows this FIQR questionnaire that was voluntarily completed by the FMS patients who took part in this study. The in-house clinical questionnaire, Table S1B, was drawn up to identify secondary data about the patients for use in conjunction with the FIQR questionnaire.

Table S1 Fibromyalgia Impact Questionnaire (FIQR) (A) and Clinical questionnaire (B). Questionnaire A was used by the clinicians to assess the severity of the symptoms experienced by the FMS patients. Questionnaire B was used to gather supplementary information on the FMS patients

A - Fibromyalgia Impact Questionnaire (FIQR)

1. *Function domain*

Directions: For each question, place an "X" in the box that best indicates how much your fibromyalgia made it difficult to do each of the following activities during the past 7 days

Brush or comb your hair	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*
Walk continuously for 20 minutes	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*
Prepare a homemade meal	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*
Vacuum, scrub or sweep floors	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*
Lift and carry a bag full of groceries	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*
Climb one flight of stairs	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*
Change bed sheets	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*
Sit in a chair for 45 minutes	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*
Go shopping for groceries	*No difficulty*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very difficult*

2. *Overall impact domain:*

Directions: For each question, check the one box that best describes the overall impact of your fibromyalgia over the last 7 days:

Fibromyalgia prevented me from accomplishing goals for the week	*Never*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Always*
I was completely overwhelmed by my fibromyalgia symptoms	*Never*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Always*

3. *Symptoms domain:*

Directions: For each of the following 10 questions, select the one circle that best indicates the intensity of your fibromyalgia symptoms over the past 7 days

Please rate your level of pain	*No pain*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Unbearable pain*
Please rate your level of energy	*Lots of energy*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*No energy*
Please rate your level of stiffness	*No stiffness*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Severe stiffness*
Please rate the quality of your sleep	*Awoke well rested*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Awoke very tired*
Please rate your level of depression	*No depression*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very depressed*
Please rate your level of memory problems	*Good memory*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very poor memory*
Please rate your level of anxiety	*Not anxious*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very anxious*
Please rate your level of tenderness to touch	*No tenderness*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Very tender*
Please rate your level of balance problems	*No imbalance*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Severe imbalance*
Please rate your level of sensitivity to loud noises, bright lights, odors and cold	*No sensitivity*	☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9 ☐10	*Extreme sensitivity*

B - Clinical Questionnaire

1. Age: ____ Years ____ Months

2. Relationship status:

1. Married	2. Engaged	3. In a relationship, but not married or engaged	4. Single

3. Current employment status

1. Fulltime employed	2. Part-time employed	3. Home executive (housewife)	4. Retired	5. Disabled

4. How long ago did your fibromyalgia symptoms start (e.g. widespread muscle pain, poor sleep, fatigue, headaches, etc.) ____ Years ____ Months

5. How long ago were you diagnosed with fibromyalgia? ____ Years ____ Months

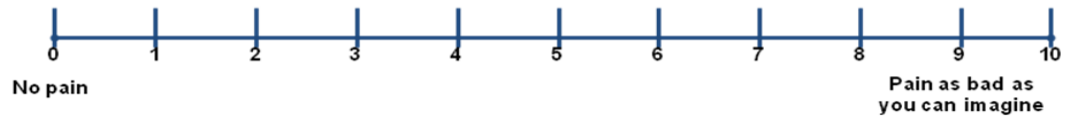
6. (a) Were your fibromyalgia symptoms triggered by? Please tick (√) (you may tick more than one block)

1. Neck injury	
2. Other injuries	
3. After surgical procedure	
4. Severe emotional stress	
5. Acute infection	
6. Spontaneous onset	
7. Uncertain	

(b)

Length	
Bodyweight	

7. Please rate your pain by circling the one number that best describes you pain at its worst in the last month. (A rating of 10 would indicate pain so severe as to prohibit all activity; the worst pain you can imagine.)



8. Please rate your pain by circling the one number that best describes you pain on the average in the last month. (A rating of 10 would indicate pain so severe as to prohibit all activity; the worst pain you can imagine.)

9. (i) Which medications are you receiving for your pain?

Please tick

Trepeline		Lyrica	
Cymbalta		Syndol	
Tramal (Tramahexal)		Myprodol	
Tenston		Mypaid	
Stilpayne		Cataflam	
Other			

(ii) How often do you take pain killers (e.g. Tramal, Tramahexal, Tramacet, Panado, Syndol., Mypaid, Cataflam, Voltaren, etc.)

± Once a week	
± Twice a week	
± Three days a week	
± Every second day	
± Daily	

10. During the past week how much did the state of your health, including any pain, interfere with the following things: choose the one number, from 0 to 4 below, that best describes your state and write them in the appropriate box (I to vi).

0 Not at all
1 A little bit
2 Moderately
3 Quite a bit
4 Extremely

i.	Mood	
ii.	Relations with other people	
iii.	Walking ability	
iv.	Sleep	
v.	Normal Work (includes both work outside the home and housework)	
vi.	Enjoyment of life	

11. Have you ever been diagnosed and treated for depression ☐ Yes ☐ No

12. Do you suffer from regular headaches? ☐ Yes ☐ No

13 Irritable bowel syndrome (IBS) is known to commonly affect patients with fibromyalgia. It is characterized by abdominal pain and cramps as well as bloating, flatulence, diarrhea and/or constipation

Have you ever been diagnosed with IBS? ☐ Yes ☐ No

14 Restless leg syndrome (RLS) is characterized by uncomfortable sensations in the lower legs and an uncontrollable urge to move them so as to provide relief. Some of the sensations felt in RLS include burning, creeping or a crawling feeling inside the legs.

Have you ever experienced symptoms of RLS? ☐ Yes ☐ No

15. Have you suffered from anxiety since being diagnosed with fibromyalgia or thereafter? (Symptoms such as feeling nervous most of the time, not able to control worrying, etc.) ☐ Yes ☐ No

16. If yes, have you been diagnosed and treated for anxiety? ☐ Yes ☐ No

17. Have you ever suffered from a sleep disturbance? ☐ Yes ☐ No

If yes, please tick the appropriate block(s)
(you may tick more than one block)

- Problem with sleep initiation ☐
- Problem with maintaining sleep ☐
- Early morning awakening ☐
- Waking up feeling unrefreshed ☐

18. Dysmenorrhoea is defined as painful menstruation often associated with cramps for mostly 1–3 days after beginning of menstruation.

Were you treated before or are you currently being treated for dysmenorrhoea?

- Yes ☐
- No ☐

Table S2 Summary of the supplementary data collected based on the in-house questionnaire (Table S1B) completed by the FMS patient group.

Variable	**Mean (SD)**	**%**
1. Socio-demographic information		
Age (years)	45.5	n/a
Marital status		
Single	2	11
Separated/Divorced/Widow	0	0
In permanent relationship	2	11
Married	15	78
Employment		
Disabled/Retired	2	11
Housewife	6	32
Part-time	1	5
Full-time	10	52
2. Pain experience		
Worst pain experience	7,7	n/a
Recent past pain average	5,7	n/a
Pain specific medication		
Trepiline (antidepressant for neuropathic pain)	12	63
Cymbalta (antidepressant for chronic pain)	10	52
Tramal (analgesic for moderate to severe pain)	9	47
Myprodol (relief of pain of inflammatory origin)	6	32
Patients using also other medication against pain	11	58
3. Levels of emotional experiences affected by FMS		n/a
Mood	2.3	
Relations with other people	2.2	
Enjoyment of life	2.1	
Normal work	2.4	

0 = Not at all **1** = A little bit **2** = Moderately **3** = Quite a bit **4** = Extremely

S2: Standard Operating Protocol for organic acid extraction

In this section, the laboratory standard operating protocol (SOP) is presented.

General organic acid analysis of urine by GC/MS:

Overview

The 3 principal steps for this method are:

1. Isolation of the organic acids from physiological fluids
2. Formation of volatile derivatives
3. GC/MS analysis.

Organic acids are isolated from physiological fluids with ethyl acetate and diethyl ether extractions. The organic acid extract is evaporated to dryness under nitrogen; volatile trimethylsilyl (TMS) derivatives of the extracted organic acids are formed by heating with N,O-bis-(trimethylsilyl)trifluoraceteamine (BSTFA). The TMS derivatives are less than ideal products for some classes of compounds such as acylglycine, which form mono and di-TMS derivatives, yet they are the most useful and versatile compounds for the wide range of functional groups in organic acids. The derivatives are analysed on GC/MS.

GC/MS is able to separate the highly volatile organic acids using gas chromatography, followed by detection of individual components by means of mass spectroscopy. This procedure permits rapid identification and quantification of constituent organic acids with a high degree of sensitivity and chromatographic resolution

Reagents

NOTE: refer to chemical information sheet below for descriptions and precautions/hazards of chemicals used.

Internal standard (3-phenylbutyric acid solution) prepared by measuring precisely 26.25 mg 3-phenylbutyric acid, adding 3 drops of 1M sodium hydroxide (NaOH) to dissolve and adding 50 ml distilled H_2O (dH_2O). Other reagents include: 5M hydrochloric acid (HCl); ethyl acetate (HPLC grade), distilled once to purify further; diethyl ether (HPLC grade), distilled once to purify further; anhydrous sodium sulphate (Na_2SO_4); bis(trimethylsilyl)-trifluoracetamide (BSTFA); trimethylchlorosilane (TMCS); pyridine and hexane.

Instrument Settings

Gas chromatography (GC)

GC	Agilent 7890A
Autosampler	Agilent 7693
Oven program	50°C for 1 min; then 20°C/min to 60°C for 0 min; then 5°C/min to 120°C for 0 min; then 7°C/min to 280°C for 4 min
Run time	40.35 min
Post run	1 min at 300°C
Injection volume	1 µl
Pre-injection washes	Solvent A: 2 x 4 µl Solvent B: 0
Post-injection washes	Solvent A: 1 x 4 µl Solvent B: 2 x 4 µl
Front inlet	Heater: 280°C Carrier gas: Helium Total flow: 15.29 ml/min Split ratio: 12:1 Split flow: 11.34ml/min
Column	DB-1MS 340°C: 30 m x 250 µm x 0.25 µm

Mass spectrometry (MS)

MS	Agilent 5975C VL MSD
Solvent delay	7 min
Acquisition mode	scan
Scan parameters	Low mass: 50.0 High mass: 600.0 Threshold: 15
MS source	230°C (max 250°C)
MS quad	150°C (max 200°C)

Organic acid extraction

1. Add 1 ml sample to large kimax test tube
2. Add 6 drops 5M HCl to adjust to pH 1 (using glass pipette)
3. Add 100 µl internal standard
4. Add 6 ml ethyl acetate
5. Cap test tubes and check there is no leakage by inverting test tube (quality control step to ensure no sample is lost during next step)
6. Mix for 30 min in Roto-torque
7. Centrifuge at 3000 RPM for 3 min
8. Aspirate the organic phase into clean large kimax test tube (using glass pipette) and set aside
9. Add 3 ml diethyl ether to the aqueous (lower) phase
10. Cap test tubes and check there is no leakage by inverting test tube
11. Mix for 10 min in Roto-torque
12. Centrifuge at 3000 RPM for 3 min
13. Aspirate the organic phase and add to the ethyl acetate phase (using glass pipette)
14. Discard lower aqueous phase into appropriate organic waste container
15. Add two level spatula scoops of anhydrous Na_2SO_4

16. Cap and invert test tube several times (or vortex for 5 seconds) to ensure good mixing (proper dispersion of Na_2SO_4 ensures all water molecules removed from organic phase as water reverses chemical process of silylation, thereby reducing the efficiency of derivatization)
17. Centrifuge at 3000 RPM for 1min
18. Pour/decant the organic phase into a clean small kimax tube
19. Evaporate to dryness in heating block at 37°C under nitrogen gas (~1 hour)
20. Use Hamilton glass syringe to add 40 µl BSTFA, 8 µl TMCS and 8 µl pyridine.

NOTE: Hamilton glass syringe is kept clean with pyridine and approximately 100 µl hexane is withdrawn into syringe and discarded (five times) between the addition of each reagent (quality control step to ensure syringe is clean and avoid cross-contamination)

21. Cap test tubes and incubate at 60°C for 1 hour (45 min at 70°C)
22. Set up and label GC/MS vials, with insert and cap
23. Transfer approximately 100 µl sample to GC/MS vial

NOTE: clean glass syringe with hexane (five times) after each transfer

24. Cap GC/MS vial and place in autosampler and process via GC/MS.

General AMDIS settings (amino acids and organic acids):

- 80% minimum match factor
- Type of analysis: use internal standards for RI (show standards)
- Resolution: medium
- Sensitivity: medium
- Shape requirements: medium.

Feature/metabolite identification is done by comparing each feature's/metabolite's MS-spectral pattern with customised spectral library specific to the urine sample under investigation.

Chemical Information Sheet

- 3-Phenylbutyric acid – $C_{10}H_{12}O_2$; Mw: 164.21 g/mol; supplier: Fluka (25 g) (index no.78243). Precaution/ hazard: avoid contact with skin and eyes.
- Sodium hydroxide – NaOH; Mw: 40.00 g/mol; supplier: Merck (500 g). Precaution/ hazard: corrosive (causes severe burns).

- Hydrochloric acid (32%) – HCl; Mw: 36.36 g/mol; supplier: Merck (2.5 l). Precaution/ hazard: corrosive (causes severe burns); irritating to respiratory system.

- Ethyl acetate – $CH_3COOC_2H_5$; Mw: 88.11 g/mol; supplier: Merck (2.5 l) (index no. 607-022-00-5). Precaution/ hazard: highly flammable; causes drowsiness/dizziness; causes eye irritation; repeated exposure causes skin dryness/cracking.

- Diethyl ether – $(C_2H_5)_2O$; Mw: 74.12 g/mol; supplier: Merck (2.5 l) (index no. 602-022-00-4). Precaution/ hazard: extremely flammable; harmful if swallowed; causes drowsiness/dizziness; repeated exposure causes skin dryness/cracking; may form explosive peroxides.

- n-Hexane – $CH_3(CH_2)_4CH_3$; Mw: 86.18 g/mol; supplier: Merck (2.5 l) (index no. 601-037-00-0). Precaution/ hazard: highly flammable; fatal if swallowed; causes skin irritation; toxic to aquatic life; causes drowsiness/dizziness; may cause infertility or damage to unborn child; may cause damage to organs through prolonged or repeated exposure.

- Sodium sulphate – Na_2SO_4; Mw: 142.04 g/mol; supplier: Merck (500 g).

- Chlorotrimethylsilane (TMCS) – C_3H_9ClSi; Mw: 108.64 g/mol; supplier: Flukka Analytical (100 ml) (index no. 92360). Precaution/ hazard: highly flammable; corrosive (causes severe burns); reacts violently with water; harmful by inhalation/contact to skin; irritating to respiratory system.

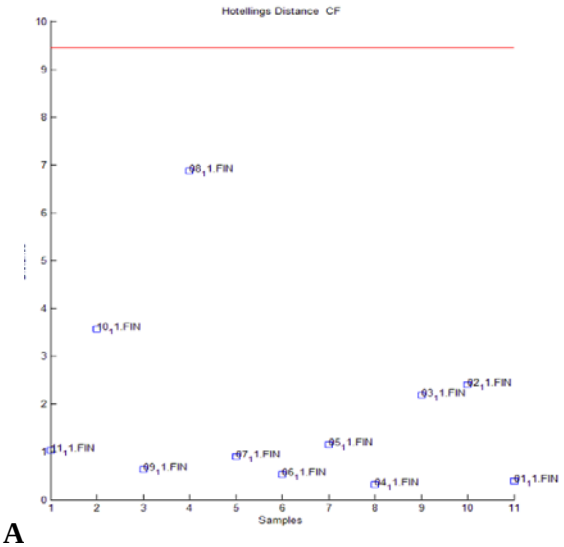
- Pyridine – C_5H_5N; Mw: 79.10 g/mol; supplier: Flukka Analytical (1 l) (index no. 82703). Precaution/ hazard: highly flammable; harmful if inhaled or swallowed; harmful to skin.

- Bis(trimethylsilyl)-trifluoracetamide (BSTFA) – $CF_3C=NSi(CH_3)_3OSi(CH_3)_3$; Mw: 257.40 g/mol; supplier: Supelco Analytical (25 ml) (index no. 3-3027). Precaution/ hazard: flammable; irritant to eyes and skin; causes burns.

S3: Case reduction analyses

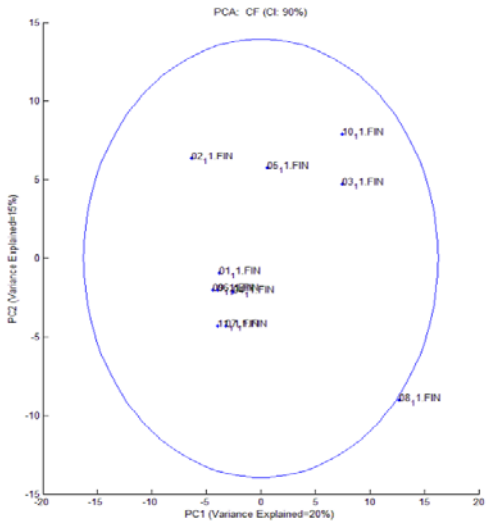
Using the original variable data, case reduction was first applied to all four experimental groups. Outliers were identified based on the presence of suspicious metabolites (including due to medication) and statistical by using a 95% confidence region in a Hotelling's T^2 test in conjunction with the respective PCA score plots with 90% confidence regions. Cases that were identified as outliers by either method were removed. Figure S1 shows the results of these case reduction analyses.

Outlier Detection Hotellings Distances



A

Outliers (CI90) PCA



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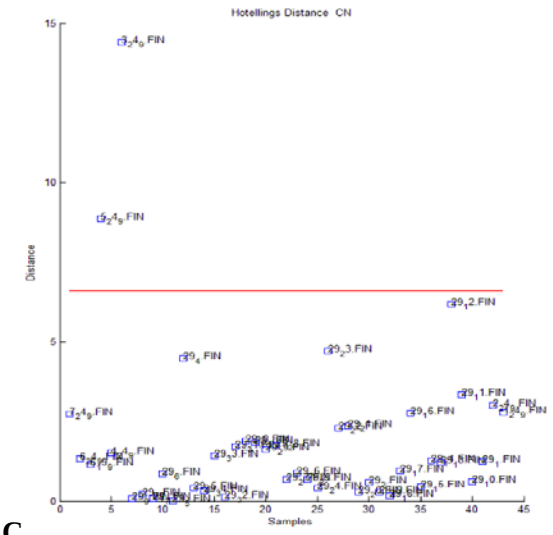

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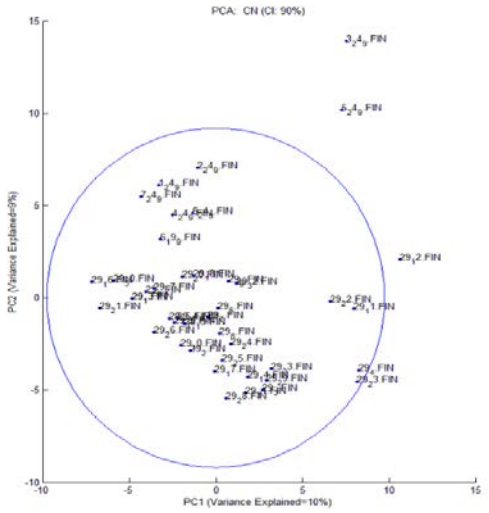

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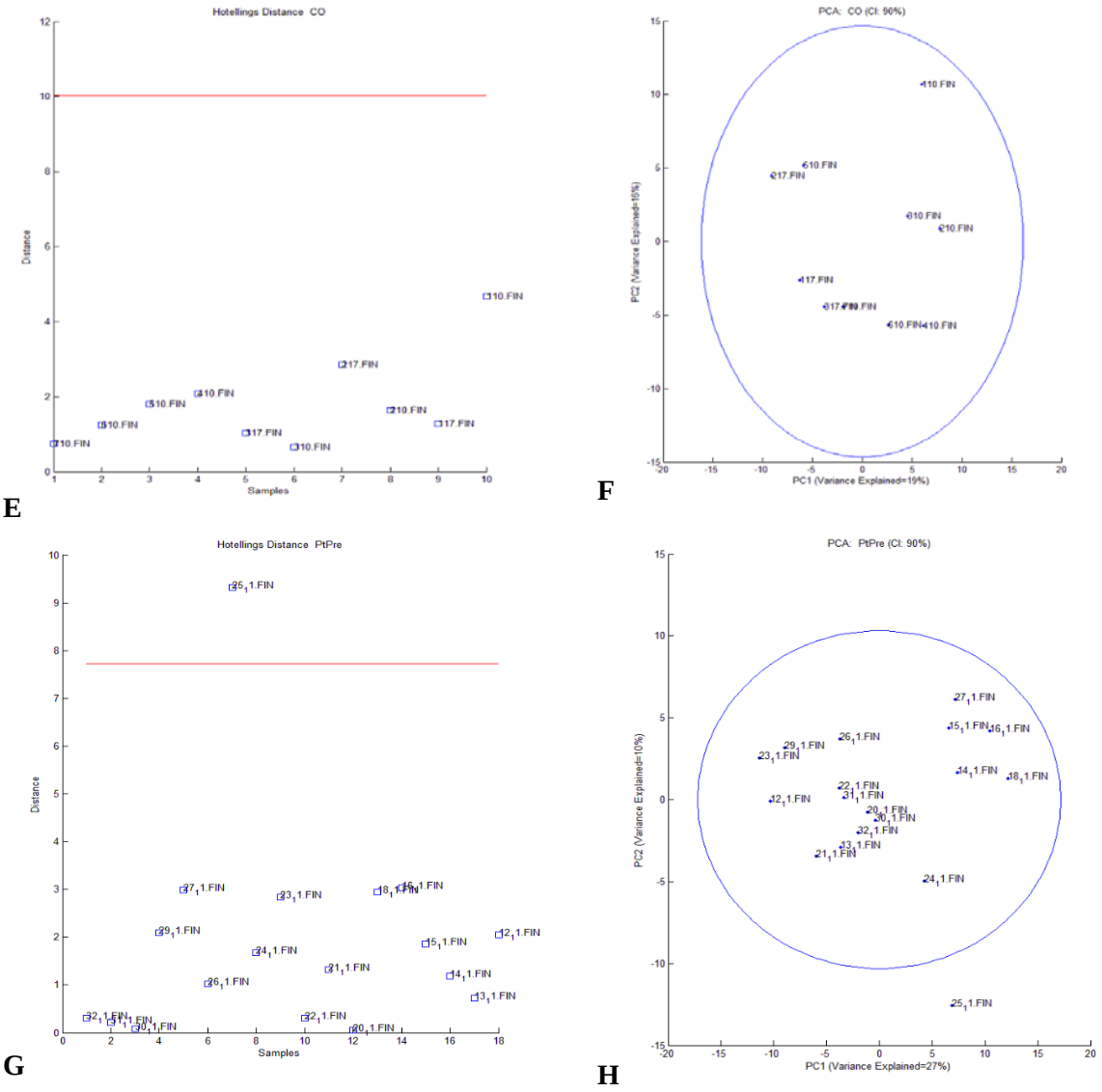


Fig. S1 Case reduction analyses, using Hotelling's T2 and a PCA test. These tests were used to detect outliers in the controls (CF (A to B), CO (C to D) and CN (E to F)) and patients (G to H). Red (Hotelling's) and blue (PCA) lines indicate the threshold where a sample is considered an outlier.

S4: Variable lists indicating metabolite groupings

In this section we show the metabolite lists used to obtain the results in Fig. 4 of the main text namely (1) gut-host metabolites with a focus on benzene derivatives of poly-phenolic dietary origin (54 metabolites), (2) metabolites of energy and intermediary metabolism (36 metabolites), (3) carbohydrates and related metabolites (30 metabolites), and (4) the remaining metabolites. Note: We regard assignment as relative as a certain metabolite may actually be classified to more than one group, while each metabolite was classified here in one group only.

<u>Benzenes:</u>

1,2,3,5-Tetramethylbenzene
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
1,2-Benzenedicarboxylic-acid
1,2-Dihydroxybenzene
2,3,4-Trihydroxybenzoic-Acid
2,3-Dihydroxybenzoic-Acid
2,4-Dihydroxybenzoic-Acid
2,5-Dihydroxybenzoic acid
2,6-Dihydroxybenzoic-Acid
2-Aminobenzoic-Acid
2-Hydroxy-5-Methoxybenzoic-Acid
2-Hydroxybenzoic-Acid
2-Hydroxyhippuric-Acid
2-Hydroxyphenylacetic-Acid
3,4-Dihydroxybenzoic-Acid
3,4-Dihydroxycinnamic-Acid
3,4-Dihydroxyphenylacetic-Acid
3,4-Dihydroxyphenylpropionic-Acid
3,5-Dihydroxybenzoic-Acid
3-Hydoxybenzoic-Acid
3-Hydroxyhippuric-Acid
3-Hydroxyphenylacetic-Acid
3-Hydroxyphenylhydracrylic-Acid
3-Hydroxyphenylpropionic-Acid
3-Methoxy-4-hydroxycinnamic-acid
3-Methoxy-4-Hydroxyphenylhydracrylic-Acid
3-Methoxy-4-Hydroxyphenyllactic-Acid
3-Methoxy-4-Hydroxyphenylpropionic-Acid
4-Hydroxbenzoic-Acid
4-Hydroxybenzeneacetic-Acid
4-Hydroxybutyric-Acid
4-Hydroxycinnamic-Acid

4-Hydroxycyclohexylacetic-Acid
4-Hydroxyhippuric-Acid
4-Hydroxymandelic-Acid
4-Hydroxyphenyllactic-Acid
4-Methoxy-3-Hydroxycinnamic-Acid
4-Methylmandelic-Acid
4-Phenol
Benzamide, N-(trimethylsilyl)-
Benzoic-Acid
Butylated Hydroxytoluene
Hippuric-Acid
Homovanillic-Acid
Hydroxymethoxybenzoylglycine
Mandelic-acid
N-Acethyl-4-Phenol
N-ACETYLTYROSINE
Phenylacetic-Acid
Phenylacetylglutamine
Phenyllactic-Acid
p-Tolylglucuronide
Vanillic-Acid
Vanillylmandelic-Acid

Energy:

1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
1H-Indole-3-Acetic-Acid
2-HYDROXYGLUTARIC-ACID
2-Hydroxyphenylacetic-Acid
3-(4-Hydroxy-2,5-Dioxoimidazolidin-4-yl)propanoic-Acid
3,4-Dihydroxybenzoic-Acid
3,4-Dihydroxycinnamic-Acid
3-Hydroxypyridine
3-Methoxy-4-Hydroxyphenylpropionic-Acid
4-Hydroxybenzeneacetic-Acid
4-Hydroxybutyric-Acid
4-Hydroxyphenyllactic-Acid
4-Phenol
5-Hydroxyindoleacetic-Acid
Aconitic-Acid
Butylated Hydroxytoluene
Dodecanoic-Acid
ETHYLMALONIC-ACID
Furoylglycine

GLUTARIC-ACID
GLYCOLIC-ACID
Hexanoic-Acid
Levulinic-Acid
Maleic-Acid
Malic-Acid
METHYLMALONIC-ACID
METHYLSUCCINIC-ACID
Monohexadecanoylglycerol
Monostearylglycerol
N-ACETYLASPARTIC-ACID
N-ACETYLTYROSINE
N-TIGLYLGLYCINE
Octadecanoic-Acid
OXALIC-ACID
Palmitic-Acid
Phosphoric-Acid
SUCCINIC-ACID
Tiglic-Acid

Sugars:

1,2-Dihydroxyethane
2,3,4,5-Tetrahydroxypentanoic-Acid-1,4-Lactone
2,3,4-Trihydroxybutyric-Acid
2,3,4-Trihydroxybutyric-Acid-Lactone
2,4-Dihydroxybutyric-Acid
2-Deoxy-3,5-Dihydroxypentonic-Acid-G-Lactone
2-Keto-l-gluconic-Acid
2-Methyl,2,3-Dihydroxypropanoic-Acid
3,4,5-Trihydroxypentanoic-Acid
3,4,5-trihydroxyvaleric-Acid-Lactone
3,4-Dihydroxybutyric-Acid
3-Deoxy-erythro-Pentonic-Acid
3-Deoxy-ribohexonic acid
Arabinose
D-Erythronic acid τ-lactone
Erythro-Pentonic-Acid
Fructopyranose
Fucono-G-Lactone
Galactonic-Acid-Gamma-Lactone
Galactonic-Acidlactone
Galactopyranose-2-Deoxy
Galactopyranose-Alpha-D
Glycerol

Mannonic-Acid
Mannose
Rhamnose
Sorbose
Tagatofuranose
Tagatose
Threonic-Acid

Other

1,2-Butanediol
1,2-Dihydroxypropane
1,6-Dihydroxyhexane
1H-Indole-1-acetic-Acid
2-(Furan-2-yl)-2-Hydroxyacetic-Acid
2,2-Dihydroxyacetic-Acid
2,3,5-Trihydroxyvaleric-Acid-Lactone
2,3-Dihydroxybutane
2,3-Dihydroxybutanoic-Acid
2,5-Furandicarboxylic-Acid
2,6-Dihydroxy-4-Pyrimidinecarboxylic-Acid
2-Ethyl-3-Hydroxypropionic-Acid
2-Hexenoic-Acid
2-Hydroxy-3-Methylbutryic-Acid
2-Hydroxy-3-Methylvaleric-Acid
2-Hydroxyadipic-Acid
2-Hydroxybutyric-Acid
2-Hydroxyisobutyric-Acid
2-Hydroxysebacic-Acid
2-Keto-3-Methylbutyric-Acid
2-Ketobutyric-Acid
2-Ketoglutaric-Acid
2-Methyl-2-Hydroxybutyric-Acid
2-Methyl-3-Hydroxybutyric-Acid
2-Octenoic-Acid
3-Hydroxy-3-methylglutaric-Acid
3-Hydroxyglutaric-Acid
3-Hydroxyisobutyric-Acid
3-Hydroxyisovaleric-Acid
3-Hdrocypropionic-Acid
3-Hydroxysebacic-Acid
3-Methyl-2-pentenedioic-Acid
3-Methyladipic-Acid
3-Methylglutaconic-Acid
3-Methylglutaric-Acid

4-Hydroxy-3-Penten-2-One
4-Hydroxycyclohexanecarboxylic-Acid
4-Ketovaleric-Acid
4-Pyridinecarboxylic-Acid
5-(Hydroxymethyl)Furan-2-Carboxylic-Acid
5-Hydroxyhydantoin
5-Hydroxyvaleric-Acid
6-Hydroxyhexanoic-Acid
Acetoacetic-Acid
Acetylaminophenylglucopyranosiduronic-Acid
Adipic-Acid
Altro-2-Heptulose
Azelaic-Acid
Citraconic-Acid
Citramalic-Acid
Citric-acid
Erythronic-Acid
Fumaric-Acid
Glucopyranose
Glucopyrorono-(6-1)Lactone
Glucuronic-Acid
Glutaconic-Acid
GLYOXYLIC-ACID
Hydantoinpropionic-Acid
Isocitric-Lactone
Lactic-Acid
Malonic-Acid
Methylcitric-Acid
Methylmaleic-Acid
N-Acetylanthranilic-Acid,
N-Acetylisoleucine
N-Acetyltrheonine
N-Hexanoylglycine
N-Isobutyrylglycine
N-Isovalerylglycine
Nonanoic-Acid
Octenedioic-Acid
Oleic-Acid
Pantothenic-Acid
Parabanic-Acid
Pimelic-Acid
Pyroglutamic-Acid
Pyrrole-2-Carboxylic-Acid
Pyruvic-Acid
Quinolinic-Acid
Ribonic-G-Lactone

Sorbic-Acid
Suberic-Acid
Threitol
Uracil

S5: Heat map analysis of FMS and CO controls

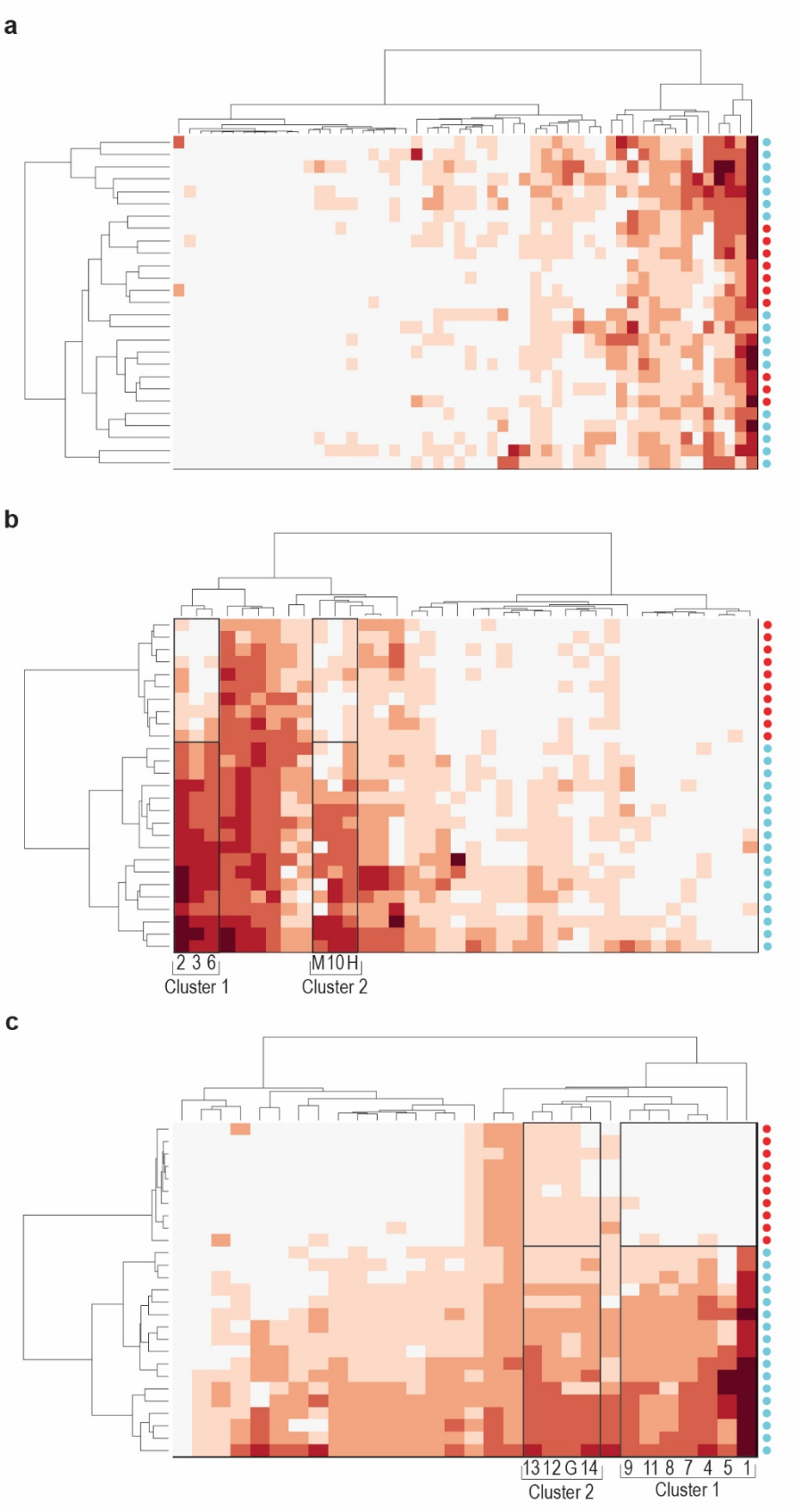


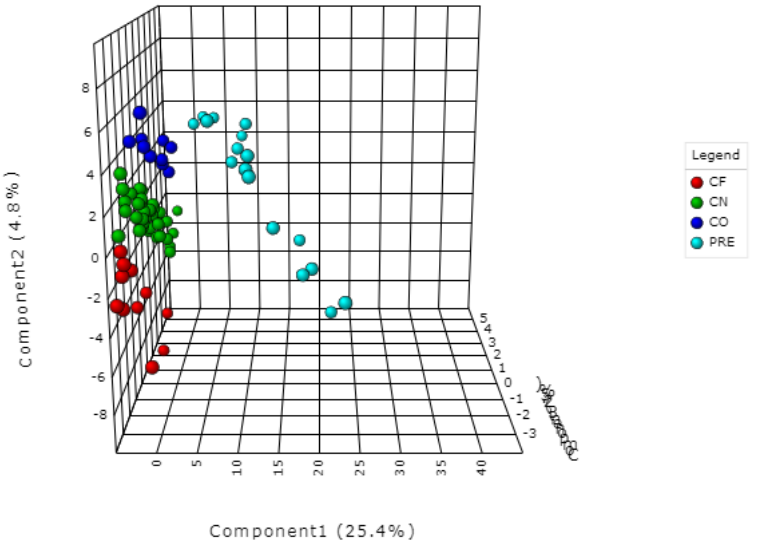
Fig. S2 Heat map analysis of FMS and CO controls. A clustered analysis of metabolites, expressed as quantified values, representing (a) 54 gut-host metabolites, (b) 36 energy-related metabolites and (c) 30 carbohydrates and their metabolites, determined in urine from the FMS patients and controls. Indicated clusters which differentiate between FMS (17 blue dots) and CO (10 red dots) controls are: (a) none; (b) Cluster 1: 2 = phosphoric acid; 3 = glutaric acid; 6 = oxalic acid; Cluster 2: M = malic acid; 10 = 4-hydroxybutyric acid; H= 2-hydroxy-glutaric acid. (c) Cluster 1: 1 = sorbose; 5 = tagatose; 4 = threonic acid; 7 = erythropentonic acid; 8 = rhamnose; 11 = 2,3,4-trihydroxybutyl-lactone; 9 = arabinose; Cluster 2: 14 = 3-deoxy-ribohexonic acid; G = galactonic acid-lactone; 12: 2-keto-gluconic acid; 13 = 2-deoxy-3,5-dihydroxy-pentanoic acid-lactone

S6: PLS-DA Model validation

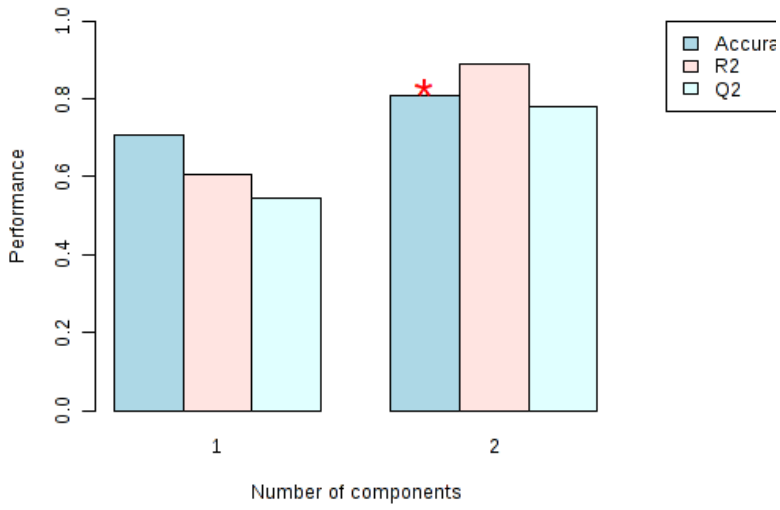
It is well-known that PLS-DA models are prone to over-fit and so lack generalizability. To further assess the finding reported in the main paper, MetaboAnalyst[4], an online software tool, was used to construct and validate a global model incorporating all experimental groups.

Figure S3 below displays the resulting scores plot and validation statistics. The scores plot (Fig S3a) confirms the separation evident in the main paper, while the validation graphs confirm a high prediction accuracy (Fig S3b) in addition to a significant model p-value (Fig S3c).

a



b



c

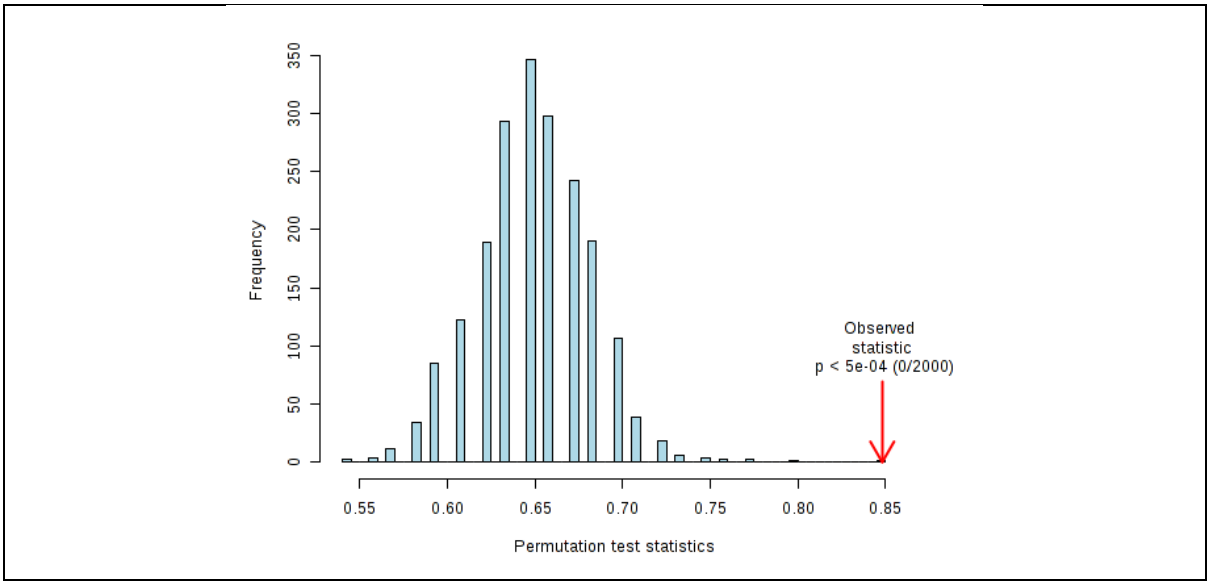


Fig. S3 PLS-DA model and validation summary.

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