

Supplementary information

Thinking outside the box: non-canonical targets in multiple sclerosis

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Supplementary table | Overview of selected studies analysing metabolic profile in MS.

Sample type	Sample size	Key Metabolite/pathways analyzed	Method/Technology	Results/Changes in MS patients*	Ref
Serum	RRMS (209) SPMS (136) PPMS (51)	NAD ⁺ ; NADH	Thiazolyl blue assay	↓ in MS: NAD ⁺ , NAD ⁺ /NADH ratio	1
Serum	MS (23)	glucose, valine, selenium	¹ H-NMR	↑ in MS: glucose; ↓ in MS: valine, selenium	2
Serum	RRMS (47) NMO (44)	scyllo-inositol, glutamine, glutamate, acetate, lactate, lysine	¹ H-NMR	↑ in MS: scyllo-inositol, glutamine ↑ in NMO: acetate, glutamate, lactate, lysine	3
Plasma	RRMS (61) PMS (12)	tryptophan metabolism, energy metabolism	¹ H-NMR	↑ in MS: 3-hydroxybutyrate, acetoacetate, acetone, alanine, choline ↓ in MS: glucose, tryptophan, 5-hydroxytryptophan	4
Serum	RRMS (50) SPMS (20) PPMS (17)	kynurenine pathway, tryptophan metabolism	UHPLC GC-MS	↑ in MS: kynurenine, K/T ratio, picolinic acid ↓ in MS: NAD ⁺ ; ↑ in RRMS: kynurenic acid ↑ in PPMS: quinolinic acid	5
Serum	RRMS (360) SPMS (132) PPMS (26)	mitochondrial energy metabolism	HPLC	↑ in MS: lactate, creatinine, uracil, β-pseudouridine, uridine, hypoxanthine, xanthine, inosine, uric acid, Σ oxypurines	6
Plasma	RRMS (22)	amino acids and acylcarnitines	LC-MS/MS	↑ in MS: glutamate; ↓ in MS: leucine, isoleucine, decenoylcarnitine	7
Serum	RRMS (151) PMS (100)	ceramides	HPLC-MS/MS	↑ in MS: Cer16:0; Cer22:1, Hex-Cer24:1, Lac-Cer24:1, Lac-Cer22:0, DH-Cer20:0, DH-Cer24:0; ↓ in MS: Hex-Cer16:1, Lac-Cer20:1, DH-HexCer26:0	8
Plasma	RRMS (28)	plasma metabolome including amino acids	NMR	↓ in MS: arginine, citrate, isoleucine, serine, histidine, phenylalanine, methionine, asparagine, myo-inositol	9
Serum	RRMS (24)	phosphatidylcholine phosphatidylethanolamine	HILIC-MS	phospholipids can differentiate between HC and MS especially: PC (34:3), PC (36:6), PE (40:10), PC (38:1)	10
Serum	RRMS (56) PMS (51)	bile acid metabolism	UPLC-MS/MS LC-MS/MS	↓ in PMS: primary bile acid metabolites ↓ in RRMS and PMS: secondary bile acid metabolites	11
Serum	MS (514)	plasma metabolome including amino acids	GC-MS LC-MS/MS	↓ in MS with higher EDSS score: 3-(4-hydroxyphenyl) lactate (tyrosine metabolism) ↓ in MS: AAA metabolites (ILA, imidazole lactate, PLA, kynurenine, kynurenate, tryptophan, and phenylalanine)	12
Plasma, CSF	MS (32)	tryptophan, amino acids	IEC	↓ in MS: tryptophan, leucine, isoleucine, valine, tyrosine, phenylalanine	13
Serum, CSF	RRMS (5) SPMS (9) NMO (9)	nitric oxide, nitrate, nitrite, l-arginine, asymmetric dimethylarginine	GC-MS/MS	↑ in MS/NMO: asymmetric dimethylarginine; ↑ in NMO: nitrate, nitrite ↓ in NMO: l-arginine	14
Serum, CSF	RRMS (22) PPMS (12)	lipids, biogenic amines, amino acids	NMR GC-MS/ LC-MS	differentiate RRMS vs PPMS; <u>CSF</u> : nitrogen, arginine and ornithine metabolism, branched chain amino acid biosynthesis; <u>Serum</u> : glutathione, nitrogen, arginine and proline met.	15
CSF	MS (19)	lactate, fructose, creatinine, phenylalanine	MRS	↑ in MS: lactate, fructose; ↓ in MS: creatinine, phenylalanine	16
CSF	RRMS (26) SPMS (6)	kynurenic acid	HPLC	↓ in MS: kynurenic acid	17
CSF	RRMS (15)	NO metabolites, glutathione	WB, CLIA	↑ in MS: nitrotyrosine, nitrate, oxidized glutathione and S-nitrosothiols	18
CSF	CIS (33)	glutamine, b-hydroxyisobutyrate (BHIB), fructose, acetate, creatinine, β-glucose	¹ H-NMR	↑ in CIS: BHIB, glutamine; fructose; creatinine, β-glucose; ↓ in CIS: acetate	19
CSF	RRMS (85); SPMS (54)	sorbitol, fructose, lactate, glucose metabolism	GC-MS	↑ in MS: sorbitol, fructose, lactate	20
CSF	RRMS (11); SPMS (3) CIS (1)	energy, biogenic amine, phospholipid metabolism	¹ H-NMR	↑ in MS: citrate, mannose, 3-hydroxybutyrate, phenylalanine, 2-hydroxyisovalerate; ↓ in MS: choline, myo-inositol, threonate	21
CSF	RRMS (13)	Lipidomics, acylcarnitines, aminoacids	MALDI-TOF-MS LCMS/MS	↑ in MS: glutamate, lysophosphatidylcholine (18:1; 18:0), lysophosphatidylinositol 16:0, phosphatidylcholine, phosphatidylinositol ↓ in MS: phosphatidic acid	22

CSF	MS (50) NMO (57)	2-hydroxybutyrate, acetone, formate, citrate, lactate, pyroglutamate, acetate, glucose	¹ H-NMR	↑ in MS/NMO: 2-hydroxybutyrate, acetone, formate, pyroglutamate; ↓ in MS/NMO: acetate, glucose; ↑ in MS: citrate; ↑ in NMO: lactate	23
CSF	SPMS (12)	glycerophospholipids, glycerides, acylcarnitines, sphingolipids, amino acids, biogenic amines, cholesteryl- esters, hexoses	LC-HRMS/ FIA-HRMS	↑ in SPMS: glycine, asymmetric dimethylarginine, glycerophospholipid PC-O (34:0), hexoses	24
Urin	RRMS (8), NMO (9)	ketone bodies, amino acids, propionate, pyruvate, tricarboxylic acid cycle, glycolysis	NMR	↑ in MS: 3-hydroxyisovalerate, oxaloacetate; ↓ in MS: creatinine; ↑ in NMO: oxaloacetate; ↓ in NMO: creatinine, 3-hydroxybutyrate, methylmalonate	25
Urin	RRMS (47)	tryptophan metabolism, kynurenine pathway	HPLCMS/MS	↓ in MS: kynurenine, K/T ratio; ↓ in K/A ratio with recent relapse	26
Retina	RRMS (124) CIS (7) PPMS (9) SPMS (3)	NADH, N-acetyl-aspartate, amyloid-beta	Raman spectroscopy	↑ in MS: amyloid-beta; ↓ in MS: NADH, NAA	27
MRI	non-lesional MS (15) lesional MS (20)	Glutamate, Glutamine, N-acetylaspartate, creatine, myo-Inositol, choline	qMRI	↑ in MS: glutamate, glutamine; ↓ in lesional MS: N-acetylaspartate, N-acetylaspartate-glutamate	28
Microbiome/ Serum	MS (129/90)	Gut microbiome, plasma metabolome including lipids and amino acids	LC/MS	↓ in MS: indolepropionate, indolelactate, sphingosine 1-phosphate, β-hydroxyasparagine, carnitine ↓ in MS: levels of microboitic species involved in butyrate production	29

* Limited to the most relevant findings of the publication

Abbreviations: CLIA chemiluminescence assay; FIA-HRMS flow-injection analysis-HRMS; GC-MS gas chromatography-mass spectrometry; HILIC-MS hydrophilic interaction chromatography-mass spectrometry; ¹H-NMR – ¹H nuclear magnetic resonance; HPLC high-performance liquid chromatography; HRMS high-resolution mass spectrometry; IEC ion-exchange chromatography; LC-MS liquid chromatography-mass spectrometry; MALDI-TOF-MS matrix-assisted laser desorption ionization-time of flight - mass spectrometry; MS multiple sclerosis; NAD nicotinamide adenine dinucleotide; NMO neuromyelitis optica; PMS progressive multiple sclerosis; PPMS primary progressive multiple sclerosis; RRMS relapsing remitting multiple sclerosis; SPMS secondary progressive multiple sclerosis; UHPLC ultra-high-pressure liquid chromatography; WB western blot; qMRI quantitative magnetic resonance imaging.

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