BLOOD-BASED METABOLIC SIGNATURES IN ALZHEIMER'S DISEASE: SUPPLEMENTARY TEXT 1

METABOLITE ANALYSIS METHODS

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This supplementary text contains additional information on the metabolite profiling. Section 1 contains general information. Section 2 details on the profiling methods. Finally, Section 3 contains an overview of the metabolites considered detected.

1. General Information

Samples were stored at $-80°$C until used for further analysis. All samples were randomized and run in 5 batches which included a calibration line, quality control (QC) samples and blanks. QC samples were analyzed every 10 samples (or every 15 samples in the oxidative stress platform). The acquired data were evaluated using MassHunter software (Agilent) and LabSolutions software (Shimadzu). An in-house written tool was applied that uses the QC samples to compensate for shifts in the sensitivity of the mass spectrometer throughout the batches [S1.1]. Both internal standard correction and QC correction were applied to the data set before reporting results. All metabolites comply with the acceptance criteria of relative standard deviation QC ($\mathrm{RSD_{QC}}$) $< 30\%$.

2. Profiling

2.1. **Biogenic Amine Profiling.** The amine platform covers amino acids and biogenic amines employing an AccQ-tag derivatization strategy adapted from the protocol supplied by Waters. Five μL of each sample was spiked with an internal standard solution. Then proteins were precipitated by the addition of methanol. The supernatant was transferred to a new Eppendorf tube and taken to dryness in a vacuum centrifuge (speedvac). The residue was reconstituted in borate buffer (pH 8.5) with 6-aminoquinolyl-N-hydrosysuccinimidyl carbamate (AQC) reagent.

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After reaction, the vials were transferred to an autosampler tray and cooled to 10°C until the injection. One μL of the reaction mixture was injected into the ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) system.

An Agilent 1290 Infinity ultra-high performance liquid chromatography (UH-PLC) system with autosampler (Agilent, The Netherlands) was coupled online with a 6490 Triple quadrupole mass spectrometer (Agilent) operated using MassHunter data acquisition software (B.04.01; Agilent). The samples were analyzed by UPLC-MS/MS using an Accq-Tag Ultra column (Waters). The Triple quadrupole MS was used in the positive-ion electrospray mode and all analytes were monitored in dynamic Multiple Reaction Monitoring (dMRM) using nominal mass resolution [S1.2].

2.2. **Organic Acid Profiling.** This profiling platform, performed with gas chromatography-MS (GC-MS) technology, covered organic acids. Sample preparation proceeded by first doing protein precipitation of 50 μL of sample with a crash solvent (MeOH/H2O) with in situ thermal desorption (ISTD) added. After centrifugation and transferring the supernatant, the solvent was evaporated to complete dryness on the vacuum centrifuge (speedvac). Then, two-step derivatisation procedures with oximation using methoxyamine hydrochloride (MeOX, 15 mg/mL in pyridine) as first reaction and silylation using N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) as second reaction were carried out. After this final step the samples were transferred to the auto sampler vials and 1 μL was injected on GC-MS [S1.3].

The metabolites were measured by gas chromatography on an Agilent Technologies 7890A equipped with an Agilent Technologies mass selective detector (MSD 5975C) and MultiPurpose Sampler (MPS, MXY016-02A, GERSTEL). Chromatographic separations were performed on a HP-5MS UI (5% Phenyl Methyl Silox), 30m$\times$0.25m ID column with a film thickness of 25m, using helium as the carrier gas at a flow rate of 1.7 mL/min. A single-quadrupole mass spectrometer with electron impact ionization (EI, 70 eV) was used. The mass spectrometer was operated in SCAN mode mass range 50-500.

2.3. **Lipid Profiling.** The lipid platform covers Cholesteryl ester, Ceremides, Diacylglycerols, Lysophosphatidylcholines, Lysophosphatidylethanolamine, Phosphatidylcholines, Phosphatidylethanolamines, Plasmalogen Lysophosphatidylcholines, Plasmalogen Phosphatidylcholines, Plasmalogen Phosphatidylethanolamines, Sphingomyelins, and Triglycerides. Lipids were extracted with isopropyl alcohol (IPA). In short, 1000 μL IPA containing calibrant and internal standards both at C4 levels were added to 10 μL serum to precipitate proteins. After centrifugation (12,100 rpm, 10 min, at RT), supernatant containing the lipids was transferred to vials for Liquid chromatography-MS (LC-MS) analysis. In total 2.5 μL was injected for analysis.

Chromatographic separation was achieved on an ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 $\times$ 100mm) with a flow of 0.4 mL/min over a 16 min gradient. The lipid analysis is performed on a UPLC-ESI-Q-TOF (Agilent 6530, Jose, CA, USA) high resolution mass spectrometer using reference mass correction. Lipids were detected in full scan in the positive ion mode [S1.4].

2.4. **Oxidative Stress Profiling.** The oxidative stress platform covers isoprostanes, prostaglandins, nitro-fatty acids, lyso-sphingolipids, lysophosphatidic

acids, alkyl-lysophosphatidic acids and cyclic-phosphatidic acids. One hundred and fifty μL of each sample was spiked with an internal standard solution. The metabolite extraction is performed via liquid-liquid extraction. To extract the compounds from the aqueous phase, butanol and ethylacetate are used. After collection, the organic phase is concentrated by first drying and then reconstitution in a smaller volume. After reconstitution, the extract is divided in two vials (one for each chromatography) and used for injection on UPLC-MS/MS. The oxidative stress platform is divided in two chromatographic methods: low and high pH. In the low pH method, isoprostanes, prostaglandins, nitro-fatty acids and lyso-sphingolipids are analyzed. The high pH method covers lyso-sphingolipids, lysophosphatidic acids, alkyl-lysophosphatidic acids and cyclic-phosphatidic acids.

A Shimadzu system with three high pressure pumps (LC-30AD), a controller (CBM-20Alite), an autosampler (SIL-30AC) and an oven (CTO-30A) from Shimadzu Benelux, was coupled online with a LCMS-8050 Triple quadrupole mass spectrometer (Shimadzu) operated using LabSolutions data acquisition software (Version 5.72, Shimadzu). The samples were analyzed by UPLC-MS/MS using a Kromasil Eternity XT C18 column (Akzo Nobel) for high pH and an Acquity BEH C18 column (Waters) for the low pH method. The Triple quadrupole MS was used in polarity switching mode and all analytes were monitored in dynamic Multiple Reaction Monitoring (dMRM).

3. Detected Compounds

After QC correction, 53 amine compounds, 22 organic acid compounds, 120 lipid compounds, and 40 oxidative stress compounds are detected, respectively. The detected compounds are listed in the tables below. These tables make use of the following abbreviations: HMDB = Human Metabolome Database; ID = identifier; InChI = International Union of Pure and Applied Chemistry (IUPAC) International Chemical Identifier; Lipid Maps = LIPID Metabolites and Pathways Strategy [S1.5]. Detected amine, organic acid, lipid, and oxidative stress compounds are listed in Tables S1.1, S1.2, S1.3, and S1.4, respectively.

References

[S1.1] van der Kloet, F.M., Bobeldijk, I., Verheij, E.R., & Jellema, R.H. (2009). Analytical error reduction using single point calibration for accurate and precise metabolomic phenotyping. Journal of Proteome Research, 8: 5132–5141.

[S1.2] Noga, M.J., Dane, A., Shi, S., Attali, A., van Aken, H., Suidgeest, E., Tuinstra, T., Muilwijk, B., Coulier, L., Luider, T., Reijmers, T.H., Vreeken, R.J., & Hankemeier, T. (2012). Metabolomics of cerebrospinal fluid reveals changes in the central nervous system metabolism in a rat model of multiple sclerosis. Metabolomics, 8: 253–263.

[S1.3] Koek, M.M., van der Kloet, F.M., Kleemann, R., Kooistra, T., Verheij, E.R., & Hankemeier, T. (2011). Semi-automated non-target processing in GC $\times$ GCMS metabolomics analysis: applicability for biomedical studies. Metabolomics, 7: 1–14.

[S1.4] Hu, C., van Dommelen, J., van der Heijden, R., Spijksma, G., Reijmers, T.H., Wang, M., Slee, E., Lu, X., Xu, G., van der Greef, J., & Hankemeier, T. (2008). RPLC-ion-trap-FTMS method for lipid profiling of

plasma: method validation and application to p53 mutant mouse model. Journal of Proteome Research, 7: 4982–4991.

[S1.5] http://www.lipidmaps.org/.

TABLE S1.1. Detected amine compounds.

Metabolite	Chemical formula	HMDB ID	InChI Key
1-Methylhistidine	C7H11N3O2	HMDB00001	BRMWTNUJHUMWMS-LURJTMIESA-N
2-Aminoadipic acid	C6H11NO4	HMDB00510	OYIFNHCXNCRBQI-UHFFFAOYSA-N
3-Aminoisobutyric acid	C4H9NO2	HMDB00452	QWCKQJZIFLGMSD-VKHMYHEASA-N
3-Methoxytyramine	C9H13NO2	HMDB00022	DIVQKHQLANKJQO-UHFFFAOYSA-N
3-Methoxytyrosine	C10H13NO4	HMDB01434	PFDUUKDQEHURQC-UHFFFAOYSA-N
3-Methylhistidine	C7H11N3O2	HMDB00479	JDHILDINMRGULE-LURJTMIESA-N
4-Hydroxyproline	C5H9NO3	HMDB00725	PMMYEEVYMWASQN-DMTCNVIQSA-N
5-Hydroxylysine	C6H14N2O3	HMDB00450	YSMODUONRAFBET-UHNVWZDZSA-N
ADMA	C8H18N4O2	HMDB01539	YDGMGEXADBMOMJ-LURJTMIESA-N
Alanine	C3H7NO2	HMDB00161	QNAYBMKLOCPYGJ-REOHCLBHSA-N
Alpha-aminobutyric acid	C4H9NO2	HMDB03911	QCHPKSFMDHPSNR-UHFFFAOYSA-N
Arginine	C6H14N4O2	HMDB00517	ODKSFYDXXFIFQN-BYPYZUCNSA-N
Asparagine	C4H8N2O3	HMDB00168	DCXYFEDJOCDNAF-REOHCLBHSA-N
Aspartic acid	C4H7NO4	HMDB00191	CKLJMWTZIZZHCS-REOHCLBHSA-N
Carnosine	C9H14N4O3	HMDB00033	CQOVPNPJLQNMDC-ZETCQYMHSA-N
Citrulline	C6H13N3O3	HMDB00904	RHGKLRLOHDJJDR-BYPYZUCNSA-N
Cysteine	C3H7NO2S	HMDB00574	XUJNEKJLAYXESH-REOHCLBHSA-N
Dopamine	C8H11NO2	HMDB00073	VYFYYTLLBUKUHU-UHFFFAOYSA-N
Ethanolamine	C2H7NO	HMDB00149	HZAXFHJVJLSVMW-UHFFFAOYSA-N
Gamma-aminobutyric acid	C4H9NO2	HMDB00112	BTCSSZJGUNDROE-UHFFFAOYSA-N
Gamma-glutamylalanine	C8H14N2O5	HMDB06248	WQXXXVRAFAKQJM-WHFBIAKZSA-N
Glutamic acid	C5H9NO4	HMDB00148	WHUUTDBJXJRKMK-VKHMYHEASA-N
Glutamine	C5H10N2O3	HMDB00641	ZDXPYRJPNDTMRX-VKHMYHEASA-N
Glutathione	C10H17N3O6S	HMDB00125	RWSXRVCMGQZWBV-WDSKDSINSA-N
Glycine	C2H5NO2	HMDB00123	DHMQDGOQFOQNFH-UHFFFAOYSA-N
Glycylglycine	C4H8N2O3	HMDB11733	YMAWOPBAYDPSLA-UHFFFAOYSA-N
Histamine	C5H9N3	HMDB00870	NTYJJOPFIAHURM-UHFFFAOYSA-N
Histidine	C6H9N3O2	HMDB00177	HNDVDQJCIGZPNO-YFKPBYRVSA-N
Homoserine	C4H9NO3	HMDB00719	UKAUYVFTDYCKQA-VKHMYHEASA-N
Isoleucine	C6H13NO2	HMDB00172	AGPKZVBTJJNPAG-WHFBIAKZSA-N
Kynurenine	C10H12N2O3	HMDB00684	YGPSJZOEDVAXAB-QMMMGPOBSA-N
Leucine	C6H13NO2	HMDB00687	ROHFNLRQFUQHCH-YFKPBYRVSA-N
Lysine	C6H14N2O2	HMDB00182	KDXKERNSBIXSRK-YFKPBYRVSA-N
Methionine	C5H11NO2S	HMDB00696	FFEARJCKVFRZRR-BYPYZUCNSA-N
Methionine sulfoxide	C5H11NO3S	HMDB02005	QEFRNWWLZKMPFJ-UHFFFAOYSA-N
Methyldopa	C10H13NO4	HMDB11754	CJCSPKMFHVPWAR-JTQLQIEISA-N
N6,N6,N6-Trimethyllysine	C9H20N2O2	HMDB01325	MXNRLFUSFKVQSK-QMMMGPOBSA-N
O-Acetylserine	C5H9NO4	HMDB03011	VZXPDPZARILFQX-BYPYZUCNSA-N
O-Phosphoethanolamine	C2H8NO4P	HMDB00224	SUHOOTKUPISOBE-UHFFFAOYSA-N
Ornithine	C5H12N2O2	HMDB00214	AHLPHDHHMVZTML-BYPYZUCNSA-N
Phenylalanine	C9H11NO2	HMDB00159	COLNVLDHVKWLRT-QMMMGPOBSA-N
Pipecolic acid	C6H11NO2	HMDB00716	HXEACLLIILLPRG-YFKPBYRVSA-N
Proline	C5H9NO2	HMDB00162	ONIBWKKTOPOVIA-BYPYZUCNSA-N
Putrescine	C4H12N2	HMDB01414	KIDHWZJUCRJVML-UHFFFAOYSA-N
Sarcosine	C3H7NO2	HMDB00271	FSYKKLYZXJSNPZ-UHFFFAOYSA-N
SDMA	C8H18N4O2	HMDB03334	HVPFXCBJHIIJGS-LURJTMIESA-N
Serine	C3H7NO3	HMDB00187	MTCFGRXMJLQNBG-REOHCLBHSA-N
Serotonin	C10H12N2O	HMDB00259	QZAYGJVTTNCVMB-UHFFFAOYSA-N
Taurine	C2H7NO3S	HMDB00251	XOAAWQZATWQOTB-UHFFFAOYSA-N
Threonine	C4H9NO3	HMDB00167	AYFVYJQAPQTCCC-GBXIJSLDSA-N
Tryptophan	C11H12N2O2	HMDB00929	QIVBCDIJIAJPQS-VIFPVBQESA-N
Tyrosine	C9H11NO3	HMDB00158	OUYCCCASQSFEME-QMMMGPOBSA-N
Valine	C5H11NO2	HMDB00883	KZSNJWFQEVHDMF-BYPYZUCNSA-N

TABLE S1.2. Detected organic acid compounds.

Metabolite	Identifier
2-hydroxybutyric acid	HMDB00008
Citric acid	HMDB00094
Glutamic acid	HMDB00148
Glycolic acid	HMDB00115
L-Lactic acid	HMDB00190
Malic acid	HMDB00744
2-Ketoglutaric acid	HMDB00208
Succinic acid	HMDB00254
Fumaric acid	HMDB00134
Pyruvic acid	HMDB00243
Methylmalonic acid	HMDB00202
Pyroglutamic acid	HMDB00267
Isocitrate	HMDB00193
3-hydroxybutyric acid	HMDB00357
3-Phosphoglyceric acid	HMDB00807
Aspartic acid	HMDB00191
Iminodiacetate	HMDB11753
S-3-Hydroxyisobutyric acid	HMDB00023
3-Hydroxyisovaleric acid	HMDB00754
Glyceric acid	HMDB00139
Uracil	HMDB00300
Cis-Aconitic acid	HMDB00072

TABLE S1.3. Detected lipid compounds.

Lipid class	Lipidmaps	Metabolite species
Cholesteryl ester (CE)	ST0102	CE(18:1); CE(18:2)
Ceremides (Cer)	SP02	Cer(d18:1/24:0)
Diacylglycerol (DG)	GL0201	DG(36:2); DG(36:3)
Lysophosphatidylcholine (LPC)	GP0105	LPC(14:0); LPC(16:0); LPC(16:1); LPC(18:0); LPC(18:1); LPC(18:2); LPC(18:3); LPC(20:3); LPC(20:4); LPC(20:5); LPC(22:6)
Lysophosphatidylethanolamine (LPE)	GP0205	LPE(18:0)
Phosphatidylcholine (PC)	GP0101	PC(32:0); PC(32:1); PC(32:2); PC(34:1); PC(34:2); PC(34:3); PC(34:4); PC(36:1); PC(36:2); PC(36:3); PC(36:4); PC(36:5); PC(36:6); PC(38:2); PC(38:3); PC(38:5); PC(38:6); PC(40:4); PC(40:5); PC(40:7)
Phosphatidylethanolamine (PE)	GP0201	PE(38:2); PE(38:4)
Plasmalogen Lysophosphatidylcholine (pLPC)	GP0106	LPC(O-16:0); LPC(O-16:1); LPC(O-18:1)
Plasmalogen Phosphatidylcholine (pPC)	GP0102	PC(O-34:1); PC(O-34:2); PC(O-34:3); PC(O-36:4); PC(O-36:5); PC(O-36:6); PC(O-38:4); PC(O-38:5); PC(O-38:6); PC(O-44:5)
Plasmalogen Phosphatidylethanolamine (pPE)	GP0202	PE(O-36:5); PE(O-38:5); PE(O-38:7)
Sphingomyelins (SM)	SP0301	SM(d18:1/14:0); SM(d18:1/15:0); SM(d18:1/16:0); SM(d18:1/16:1); SM(d18:1/18:0); SM(d18:1/18:1); SM(d18:1/18:2); SM(d18:1/20:0); SM(d18:1/20:1); SM(d18:1/21:0); SM(d18:1/22:0); SM(d18:1/22:1); SM(d18:1/23:0); SM(d18:1/23:1); SM(d18:1/24:0); SM(d18:1/24:1); SM(d18:1/24:2); SM(d18:1/25:0)
Triglycerides (TG)	GL0301	TG(42:0); TG(44:0); TG(44:1); TG(46:0); TG(46:1); TG(46:2); TG(48:0); TG(48:1); TG(48:2); TG(48:3); TG(50:0); TG(50:1); TG(50:2); TG(50:3); TG(50:4); TG(51:1); TG(51:2); TG(51:3); TG(52:0); TG(52:1); TG(52:2); TG(52:3); TG(52:4); TG(52:5); TG(54:0); TG(54:1); TG(54:2); TG(54:3); TG(54:4); TG(54:5); TG(54:6); TG(56:0); TG(56:1); TG(56:2); TG(56:3); TG(56:6); TG(56:7); TG(56:8); TG(57:2); TG(58:1); TG(58:10); TG(58:2); TG(58:3); TG(58:8); TG(58:9); TG(60:2); TG(O-50:0)

TABLE S1.4. Detected oxidative stress compounds.

Compound name	Compound class	Lipid Maps ID
2,3-dinor-8-iso-PGF2a	Isoprostane	LMFA03110010
5-iPF2a VI	Isoprostane	LMFA03110010
8-iso-PGF2a (15-F2t-IsoP)	Isoprostane	LMFA03110001
8,12-iPF2a IV	Isoprostane	-
aLPA C16:1	Alkyl-lyso-phosphatidic acid	-
aLPA C18:1	Alkyl-lyso-phosphatidic acid	-
cLPA C16:0	Cyclic-lyso-phosphatidic acid	LMGP00000057
cLPA C18:0	Cyclic-lyso-phosphatidic acid	LMGP00000055
cLPA C18:1	Cyclic-lyso-phosphatidic acid	LMGP00000056
cLPA C18:1	Cyclic-lyso-phosphatidic acid	-
cLPA C18:2	Cyclic-lyso-phosphatidic acid	-
cLPA C20:3	Cyclic-lyso-phosphatidic acid	-
cLPA C20:4	Cyclic-lyso-phosphatidic acid	-
iPF2a-Unknown	-	-
LPA C14:0	Lyso-phosphatidic acid	LMGP10050007
LPA C16	Lyso-phosphatidic acid	LMGP10050006
LPA C16:1	Lyso-phosphatidic acid	LMGP10050016
LPA C18	Lyso-phosphatidic acid	LMGP10050005
LPA C18:1	Lyso-phosphatidic acid	LMGP10050008
LPA C18:2	Lyso-phosphatidic acid	LMGP10050017
LPA C18:3	Lyso-phosphatidic acid	LMGP10050023
LPA C20:1	Lyso-phosphatidic acid	LMGP10050026
LPA C20:3	Lyso-phosphatidic acid	LMGP10050028
LPA C20:4	Lyso-phosphatidic acid	LMGP10050013
LPA C20:5	Lyso-phosphatidic acid	LMGP10050033
LPA C22:4	Lyso-phosphatidic acid	LMGP10050020
LPA C22:5	Lyso-phosphatidic acid	-
LPA C22:6	Lyso-phosphatidic acid	LMGP10050019
NO2-aLA (C18:3)	Nitro-Fatty acid	-
NO2-LA (C18:2)	Nitro-Fatty acid	LMFA01120001/2
NO2-OA (C18:1)	Nitro-Fatty acid	LMFA01120003/4
PAF C16:0	Platelet activating factor	LMGP01020046
PGA2	Prostaglandins	LMFA03010035
PGD2	Prostaglandins	LMFA03010004
PGE2	Prostaglandins	LMFA03010003
PGF2a	Prostaglandins	LMFA03010002
S1P C18:1	Lyso-sphingolipid	LMSP01050001
SPH C18:1	Lyso-sphingolipid	LMSP01010001
SPHA C18:0	Lyso-sphingolipid	LMSP01020001
SPHA-1-P C18:0	Lyso-sphingolipid	LMSP01050002

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