

Supporting information of:

**An integrated metabolomics approach for the research of new cerebrospinal
fluid biomarkers of multiple sclerosis**

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Table S1: Demographic and clinical variables of the patient groups.

Sample	Age at sample	Disease duration days	Phase at sample	Diagnosis	sex	Magnetic Resonance Data	Link Index (NV < 0,66)	OCB (PATTERN)	Barrier Index (NV < 5.5)	EDSS AT SAMPLE
15 MuS	48	3650	ACTIVE	RRMS	F	TYPICAL, GD-	0.66	POS (2)	5.2	4.5
08 MuS	46	1095	ACTIVE	RRMS	M	TYPICAL, GD ND	0.69	POS (2)	5.17	2.5
10 MuS	55	11315	ACTIVE	RRMS	F	TYPICAL, GD -	1.17	POS (3)	Regular	2.5
11 MuS	24	365	ACTIVE	RRMS	F	TYPICAL, GD +	2.37	POS (3)	8.6	2.5
09 MuS	54	5475	ACTIVE	RRMS	F	TYPICAL, GD -	0.65	POS (2)	2.83	1.0
12 MuS	40	1	ACTIVE	RRMS	F	ATYPICAL, GD+	0.66	POS (2)	6.56	2.5
03 MuS	20	30	ACTIVE	RRMS	F	TYPICAL, GD -	0.87	POS (2)	6.49	1.5
13 MuS	45	5475	ACTIVE	RRMS	M	TYPICAL, GD -	0.59	POS (3)	15.2	3.5
07 MuS	48	15	ACTIVE	RRMS	M	TYPICAL	1.46	POS (2)	7.5	2.0
17 MuS	49	350	ACTIVE	RRMS	M	TYPICAL, GD-	0.46	POS (3)	14.7	3.0
14 MuS	46	7	ACTIVE	RRMS	F	TYPICAL, GD -	Regular	NEG	Regular	6.0
16 MuS	36	1460	STABLE	RRMS	M	TYPICAL, GD-	0.51	POS (2)	7.1	1.0
04 MuS	46	570	ACTIVE	RRMS	M	TYPICAL, GD-	0.59	POS (2)	7.46	4.0
16 OND	45	90	ACTIVE	ASPECIFIC LEUKOENCEPHALOPATHY	M	ATYPICAL, GD-	0.45	NEG (1)	9.4	-
02 OND	54	-	STABLE	CADASIL	F	ATYPICAL, GD-	Regular	ND	Regular	-
05 OND	62	760	ACTIVE	BLEEDING FROM CAVERNOMA, VASCULITIS	F	ATYPICAL, GD-	Regular	POS (3)	Regular	-
04 OND	35	7	ACTIVE	ASPECIFIC LEUKOENCEPHALOPATHY	F	ATYPICAL, GD-	0.5	ND	4.57	-
14 OND	39	570	ACTIVE	ASPECIFIC DIPLOPIA	F	NORMAL	0.29	NEG (1)	6.5	-
12 OND	48	-	ACTIVE	STROKE	F	ATYPICAL, GD-	Regular	NEG (1)	Regular	-
09 OND	61	-	-	DEMENTIA	F	ATYPICAL, GD-	-	-	-	-
06 OND	48	-	-	AMYOTROPHIC LATERAL SCLEROSIS	M	ATYPICAL, GD-	Regular	NEG (1)	Regular	-
15 OND	22	570	ACTIVE	ASPECIFIC LEUKOENCEPHALOPATHY	F	ATYPICAL, GD-	0.6	NEG (1)	5.1	-
01 OND	59	7200	STABLE	ASPECIFIC LEUKOENCEPHALOPATHY	F	ATYPICAL, GD-	Regular	NEG (1)	Regular	-
10 OND	21	-	STABLE	CHIARI MALFORMATION TYPE 1, ANXIETY	M	ATYPICAL, GD-	0.45	NEG (1)	4.4	-
07 OND	45	3600	STABLE	ASPECIFIC LEUKOENCEPHALOPATHY	F	ATYPICAL, GD-	0.49	NEG (1)	4.6	-

RRMS= Relapsing-Remitting Multiple Sclerosis

Typical= indicates a Brain MRI suggestive of MuS, Atypical= indicates a Brain MRI not suggestive of MuS, GD+ = indicates presence of Gadolinium enhancement, GD-= indicates absence of Gadolinium enhancement, GD ND= indicates Gadolinium not done

OCB= Oligoclonal Bands are positive (POS) if the pattern is 2 or 3, are negative (NEG) if the pattern is 1 or 5.

Regular= indicates a value of Link or Barrier Index inferior the normal value (NV)

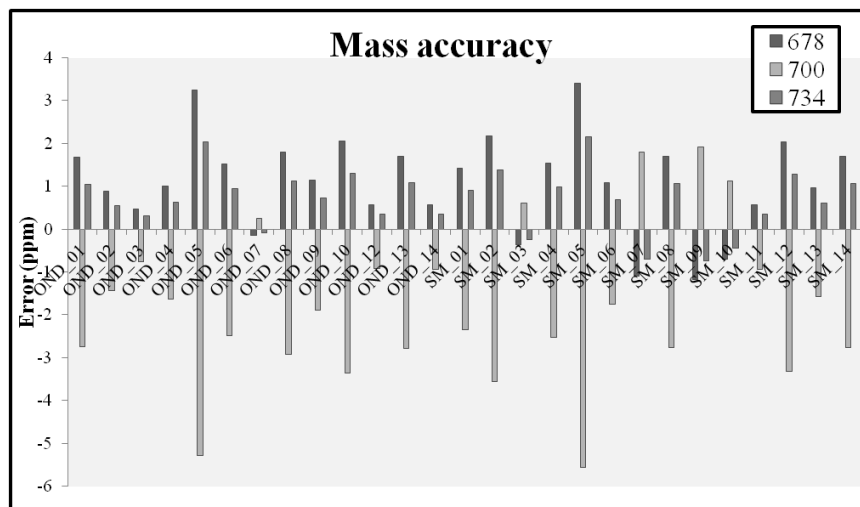
CADASIL= Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy.

ND = not definable.

MALDI-TOF-MS analysis of CSF lipids

Figure S1:

A) mass accuracy (ppm) measured for DMPC standard at $m/z=678,5069$ Da for the $M+H^+$ ion and $m/z= 700,4888$ Da For low signal of the $M+Na^+$ ion and for an endogenous signal identified as PC (see Table 2) at $m/z= 734,5695$ Da for the $M+H^+$ ion, measured in each CSF sample analyzed. As reported in the figure in all samples acquired the mass error was below 10 ppm.



B) linearity response, expressed as signal to noise (S/N), of the MALDI-MS method on standard DMPC. Data were obtained by a CSF pool fortified with DMPC extracted and analysed with the described method at 2.5, 5, 10, 25 $\mu\text{g/mL}$. Data were acquired in triplicate obtaining an RSD % below 20% of variability. A) DMPC signal at $m/z= 678.5$ [$M+H^+$]; B) DMPC signal at $m/z= 700.5$ [$M+Na^+$]. These m/z regions not contain endogenous signals (data not shown).

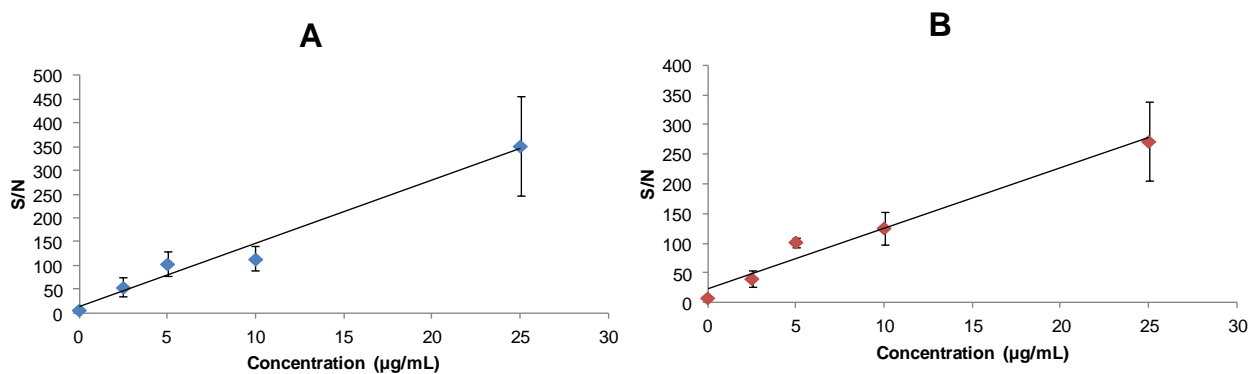


Figure S2

MS/MS spectrum obtained by fragmentation of signal at $m/z=522$ Da identified as Lysophosphatidylcholine (LPC). The fragment at $m/z=184$, 104 and 86 Da are specific of the polar head phosphocoline.

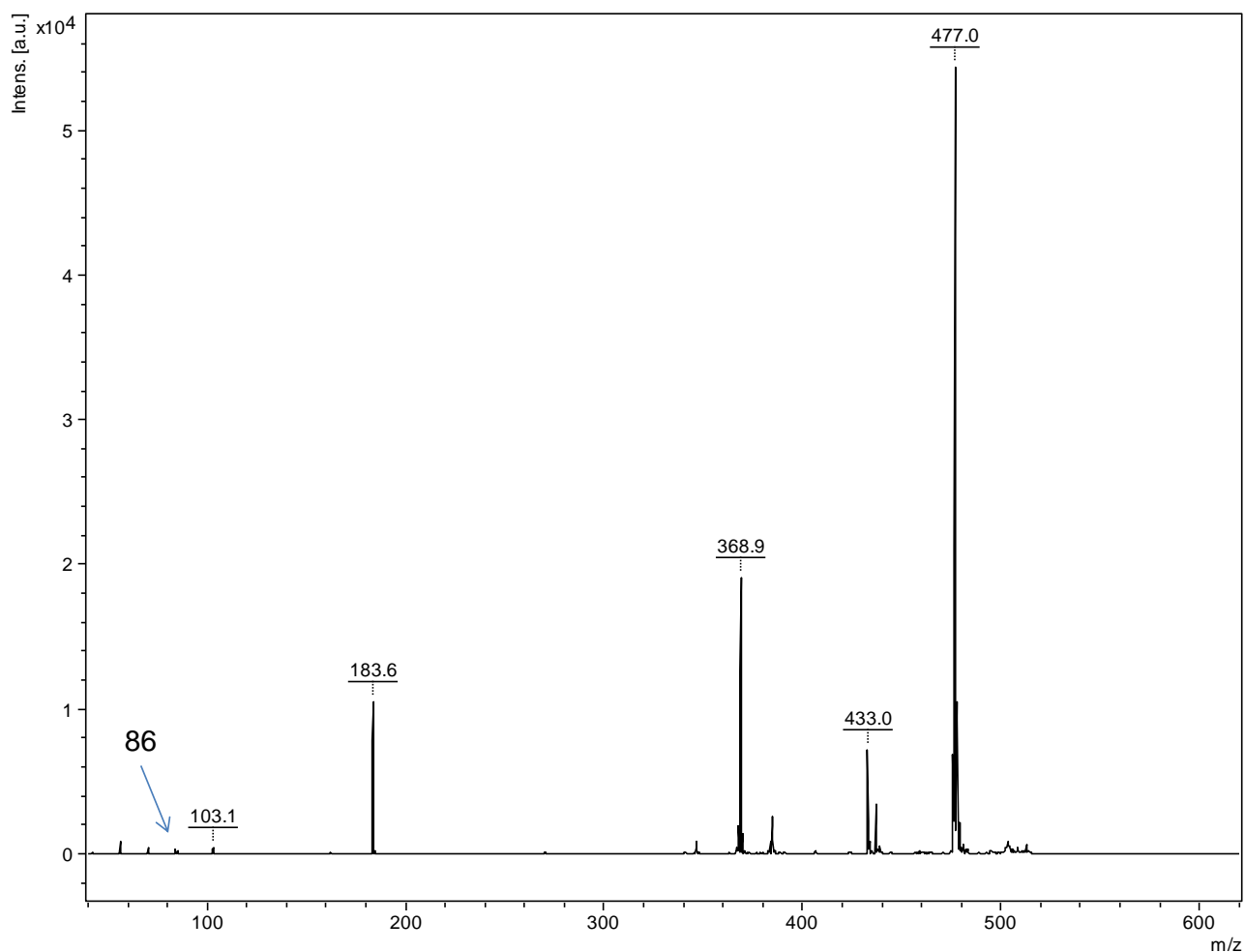


Figure S3

MS/MS spectrum obtained by fragmentation of signal at $m/z = 524$ Da identified as Lysophosphatidylcholine (LPC). The fragment at $m/z = 184$, 104 and 86 Da are specific of the polar head phosphocoline.

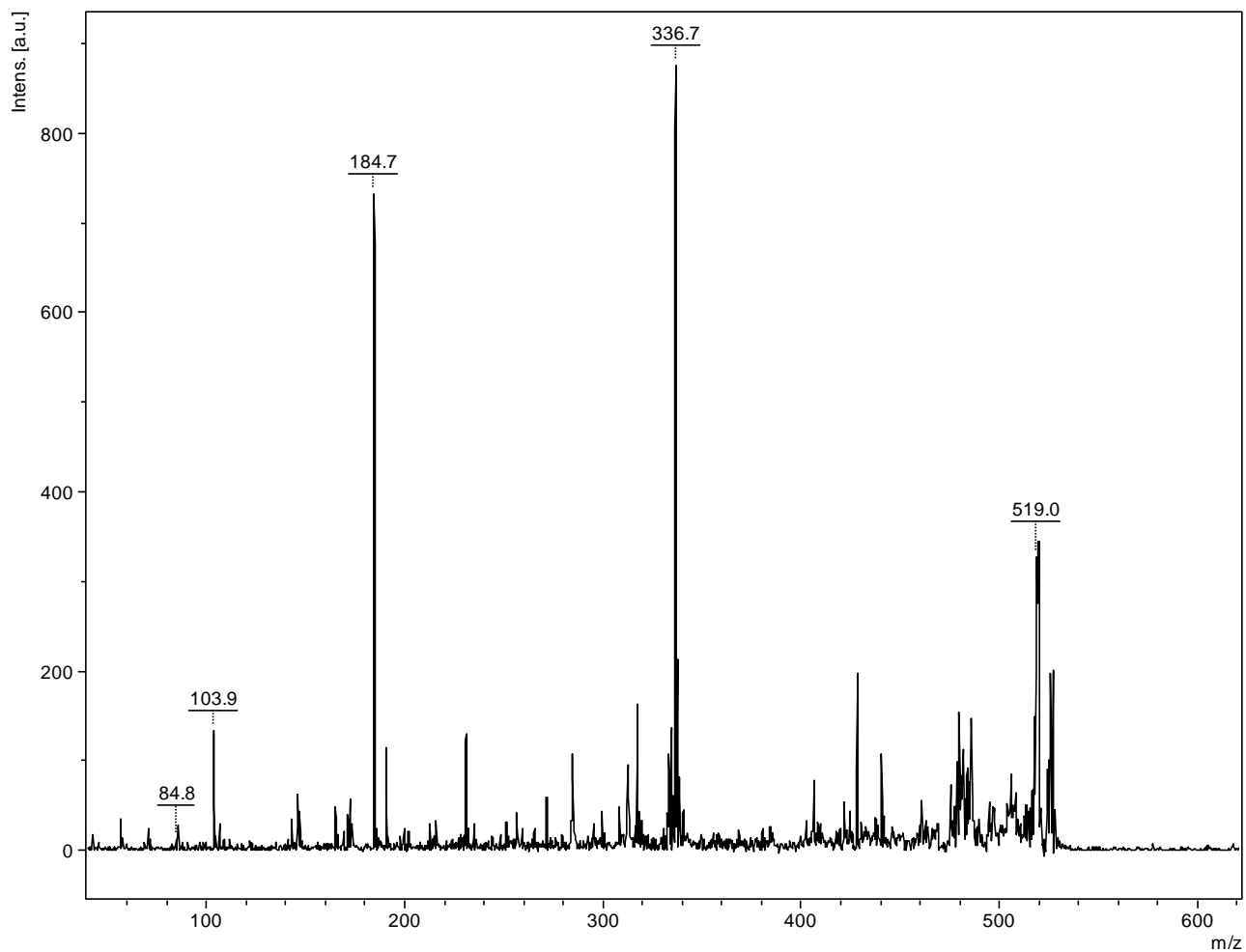


Figure S4

MS/MS spectrum obtained by fragmentation of signal at $m/z=734$ Da identified as a Phosphatidylcholine (PC). The fragment at $m/z=184$, 104 and 86 Da are specific of the polar head phosphocoline.

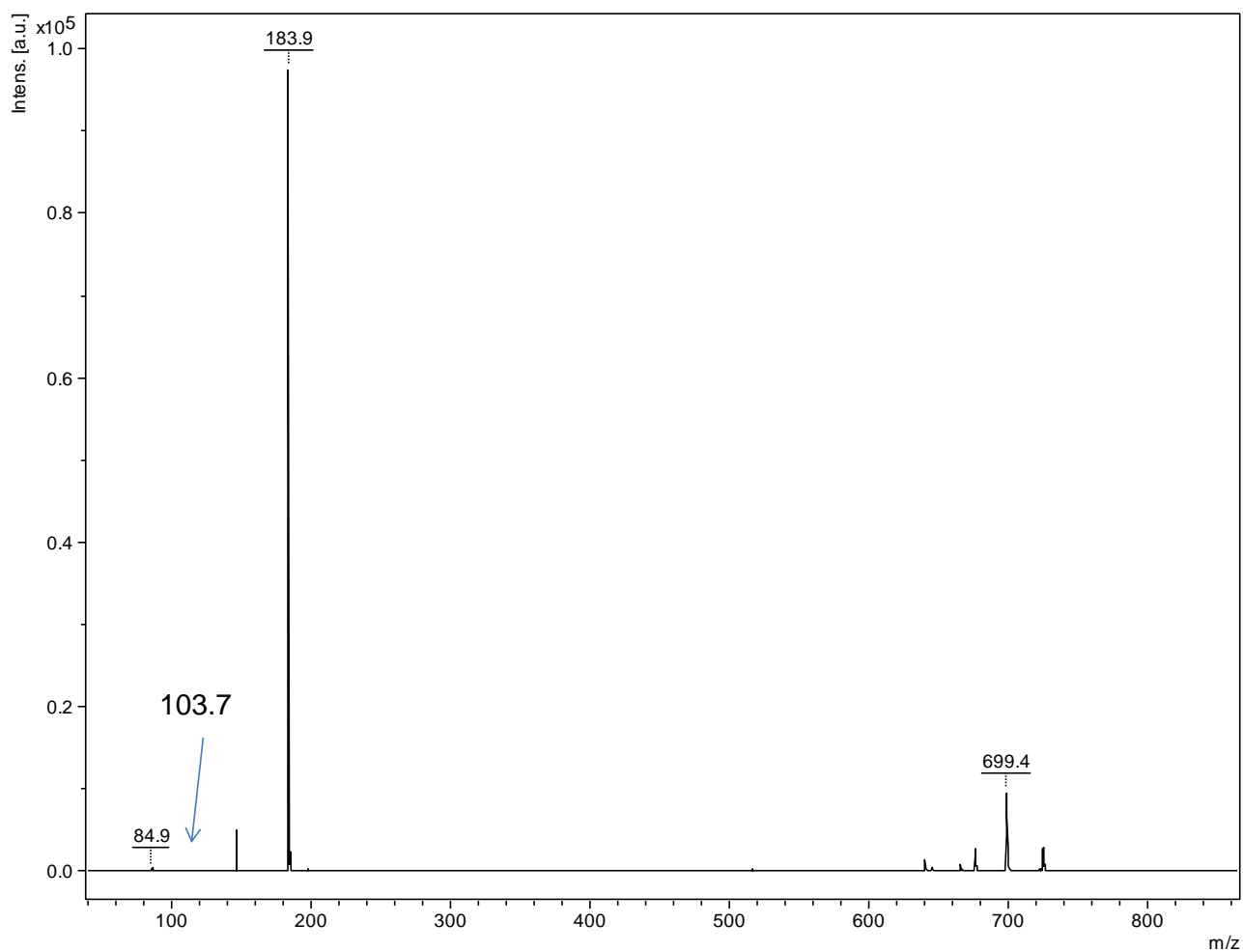


Figure S5

Relative distribution between sexes of the highlighted discriminant compounds in CSF. Data were presented as means (error bars: 95% CI for mean) of normalized intensity within each clinical group investigated. No significant difference ($p > 0.05$) females (F) vs males (M) was obtained for each signal examined. Panel A) OND; Panel B) MuS.

