

4D-lipidomics profiling in ALD using VIP-HESI and trapped ion mobility time-of-flight mass spectrometry

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Introduction

- Adrenoleukodystrophy (ALD) is a genetic metabolic disorder characterized by accumulation of very long-chain fatty acids (VLCFA)
- In depth characterization of the ALD lipidome is needed to elucidate the complex disease pathology and understand the highly unpredictable clinical course of ALD

Method

- We performed 4D-lipidomics (retention time, m/z, ion mobility and fragmentation) using an UHPLC-MS platform employing timsTOF Pro (Bruker) in fibroblasts from ALD patients and healthy controls.
- A vacuum-insulated probe heated ESI source was used and ions were separated and fragmented using Parallel Accumulation Serial Fragmentation (PASEF).
- Lipid Annotation was done using an in-house bioinformatics pipeline in combination with MetaboScape (Bruker).

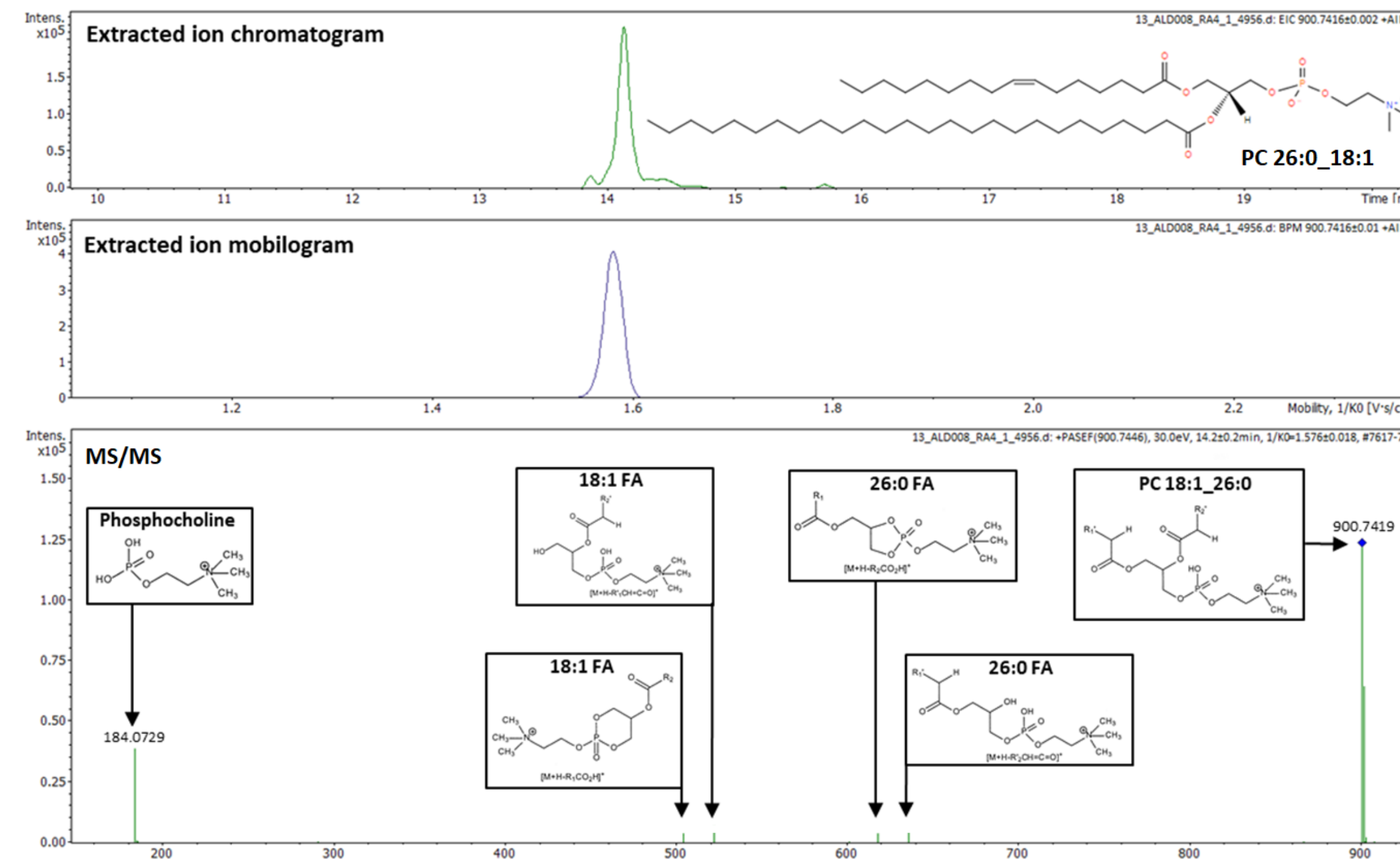
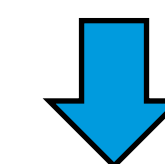
Results

- Using 4D-lipidomics we were able to generate an in-depth characterization of the ALD lipidome in a four-dimensional data space consisting of >1300 lipids.
- Our results demonstrate that VLCFA are incorporated into multiple complex lipid species including (lyso)phosphatidylcholines and neutral lipids
- Acquisition of MS/MS spectra using PASEF enabled the identification of individual fatty acid moieties of VLCFA incorporated complex lipids.

Data analysis

Bruker MetaboScape

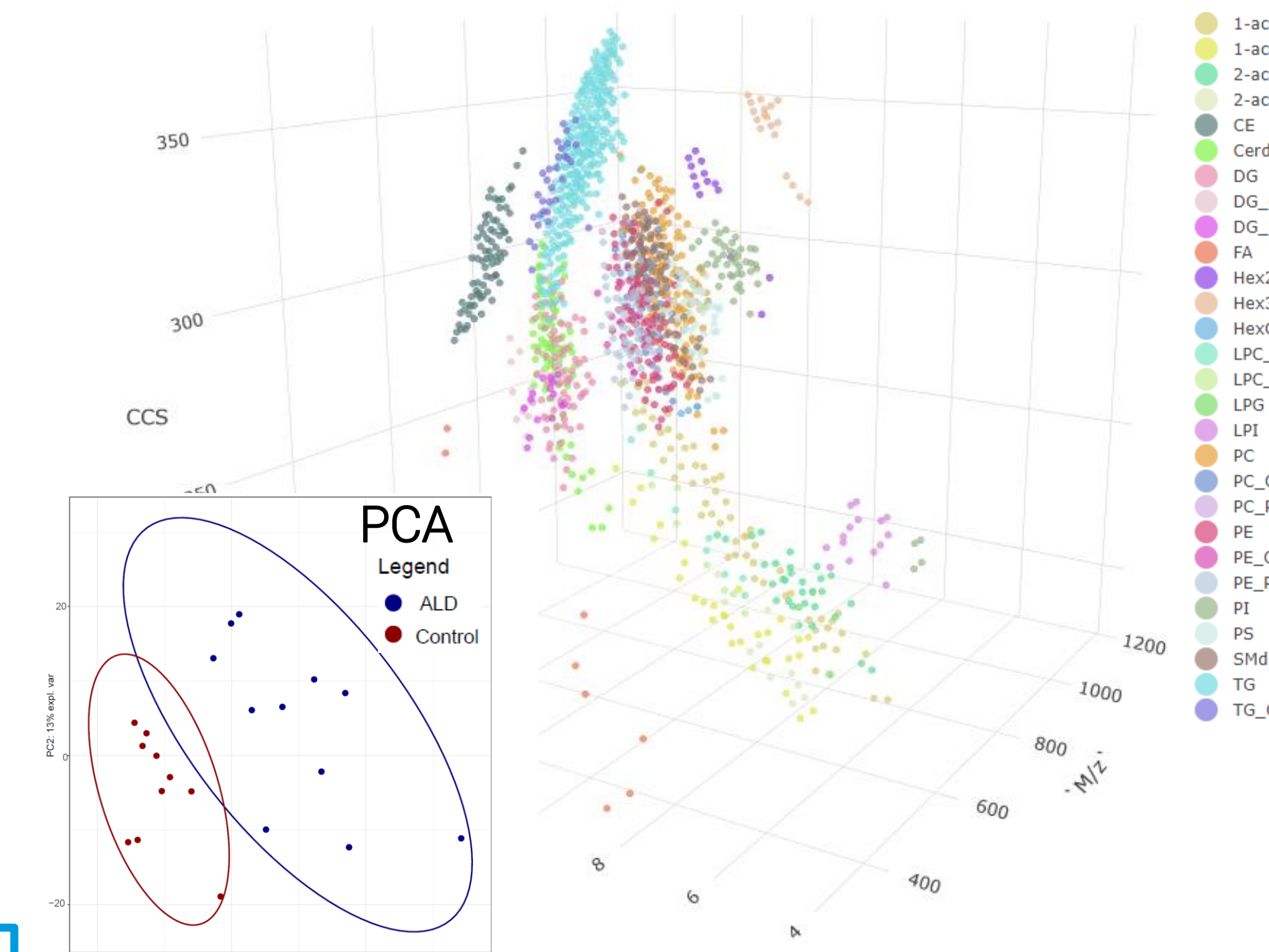
RT [min]	CCS (Å ²)	m/z meas.	M meas.	Ions	MS/MS	Name	Molecular For...	Annotations	AQ
267	7.04	239.4	572.37015	571.36288	±	2-acyl LPC(22:4)	C ₃₀ H ₅₄ NO ₇ P	[LIP] [LPC]	[M]
268	7.16	225.0	530.32363	529.31635	±	2-acyl LPE(22:4)	C ₂₇ H ₄₈ NO ₇ P	[LIP] [LPE]	[M]
269	9.14	252.8	628.43299	627.42571	±	1-acyl LPC(26:4)	C ₃₄ H ₆₂ NO ₇ P	[LIP] [LPC]	[M]
270	9.65	255.5	618.44841	617.44113	±	1-acyl LPC(25:2)	C ₃₃ H ₆₄ NO ₇ P	[LIP] [LPC]	[M]
271	10.05	259.0	632.46380	631.45653	±	1-acyl LPC(26:2)	C ₃₄ H ₆₆ NO ₇ P	[LIP] [LPC]	[M]
272	11.05	282.6	730.54061	729.53334	±	PC 16:1_16:1	C ₄₀ H ₇₆ NO ₈ P	[LIP] [PC]	[M]
273	11.70	287.7	746.57245	745.56460	±	PC 15:0_18:1	C ₄₁ H ₈₀ NO ₈ P	[LIP] [PC]	[M]
274	12.10	295.4	786.60353	785.59625	±	PC 18:1_18:1	C ₄₄ H ₈₄ NO ₈ P	[LIP] [PC]	[M]
275	12.35	294.4	762.60210	761.59451	±	PC 16:0_18:0	C ₄₂ H ₈₄ NO ₈ P	[LIP] [PC]	[M]
276	12.47	297.6	788.61947	787.61219	±	PC 18:0_18:1	C ₄₄ H ₈₆ NO ₈ P	[LIP] [PC]	[M]
277	14.15	321.0	900.74297	899.73660	±	PC 26:0_18:1	C ₆₂ H ₁₂₂ NO ₈ P	[LIP] [PC]	[M]
278	14.66	291.6	724.68163	706.64785	±	DG 16:0_26:1	C ₄₅ H ₈₆ O ₅	[LIP] [DG]	[M]
279	14.99	297.3	752.71402	734.68093	±	DG 26:0_18:1	C ₄₇ H ₉₀ O ₅	[LIP] [DG]	[M]
280	16.05	332.5	906.84995	888.81630	±	TG 18:0_18:0_1...	C ₅₇ H ₁₀₆ O ₆	[LIP] [TG]	[M]
281	16.22	335.0	908.86589	890.83165	±	TG 18:0_18:0_1...	C ₅₇ H ₁₁₀ O ₆	[LIP] [TG]	[M]
282	14.09	321.9	871.76465	870.75737	±	SM(d46:1)	C ₅₁ H ₁₀₃ N ₂ O ₆ P	[LIP] [SM]	[M]
283	10.60	219.3	678.50534	677.49807	±	PC(28:0) (S)	C ₅₆ H ₇₂ NO ₈ P	[LIP] [PC]	[M]
284	13.70	316.5	843.73274	842.72522	±	SM(d44:1)	C ₄₉ H ₉₉ N ₂ O ₆ P	[LIP] [SM]	[M]
285	15.95	314.8	810.79088	792.75771	±	TG O-16:0_16:0...	C ₅₁ H ₁₀₂ O ₅	[LIP] [TG]	[M]
286	16.54	348.7	990.94251	972.90958	±	TG 16:0_18:0_2...	C ₆₃ H ₁₂₀ O ₆	[LIP] [TG]	[M]



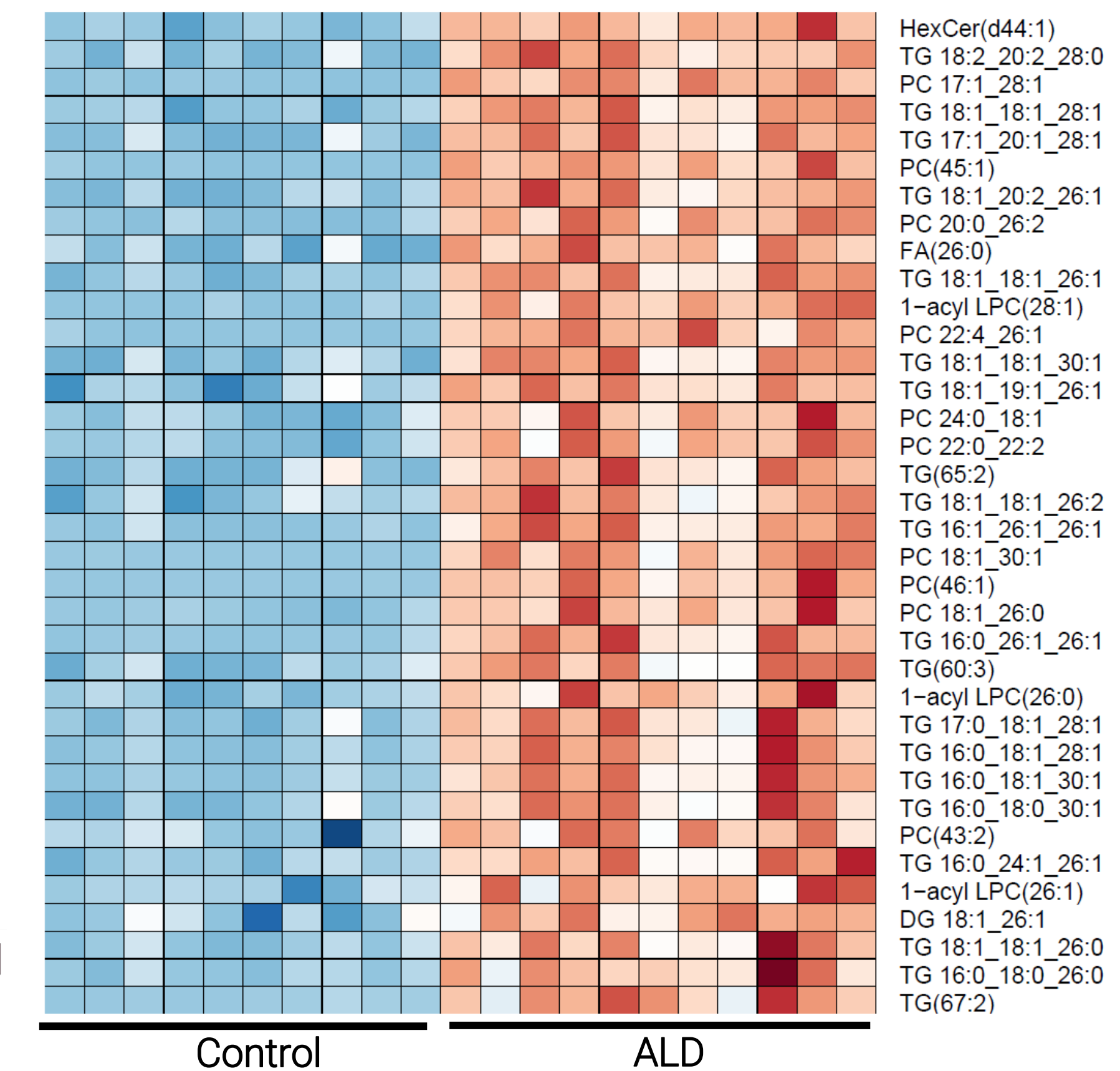
Note: Fatty acid annotation with the highest MS/MS score is shown.

4D-Lipidomics

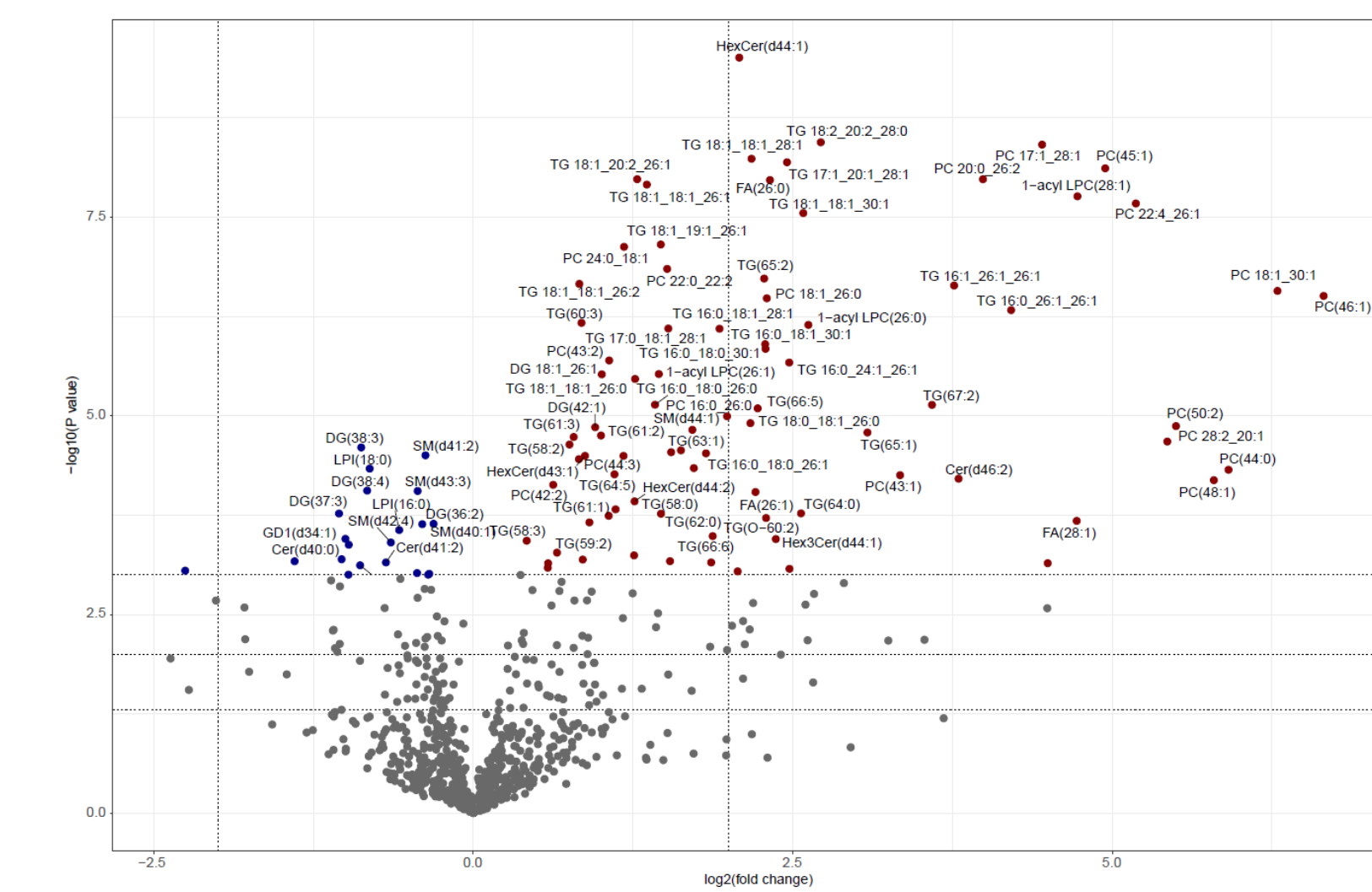
Detected lipids



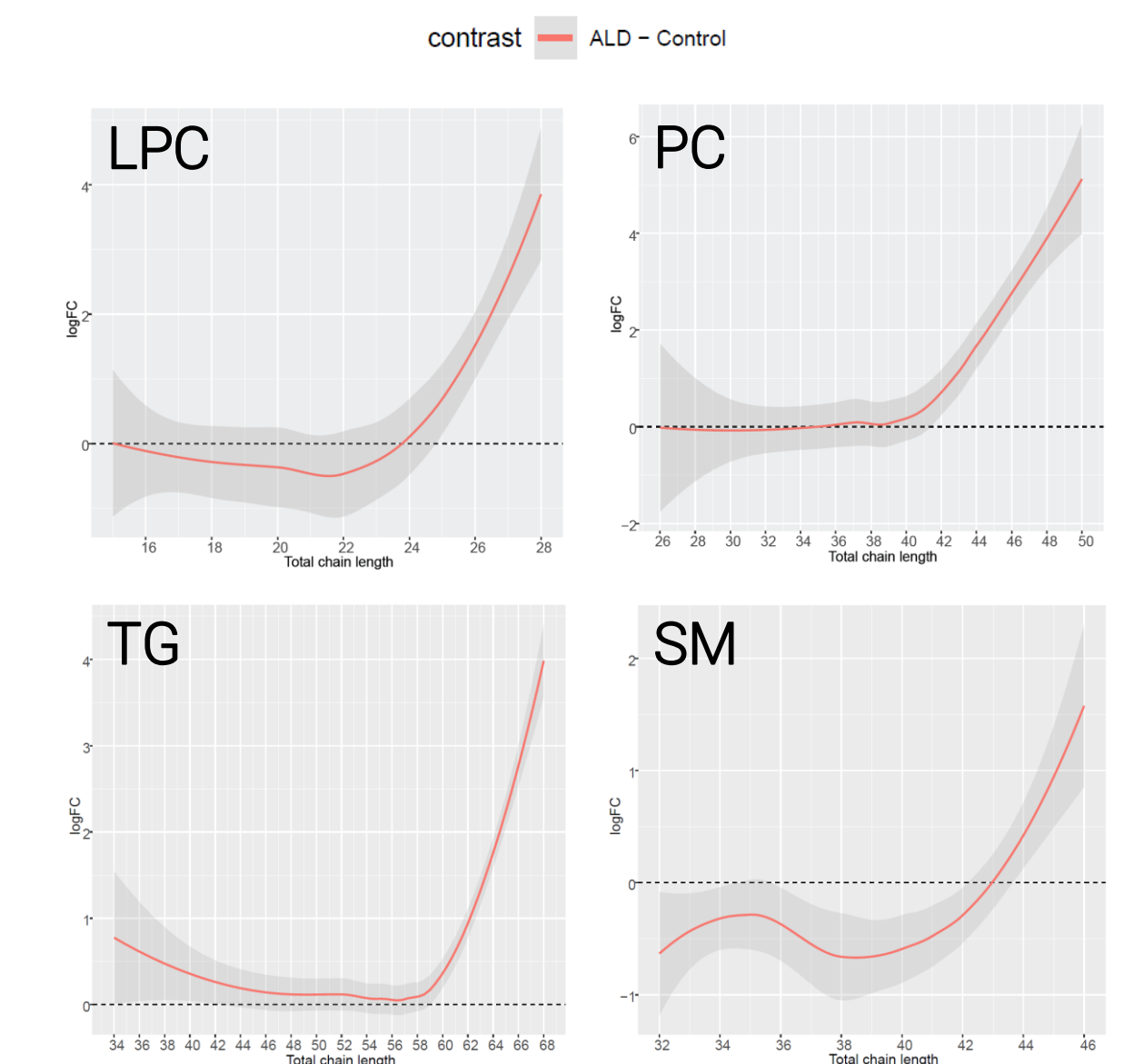
Heatmap sorted on VIP score



Volcano plot



Trend: logFC vs Chain length



Conclusion

- 4D-lipidomics using PASEF is a promising technique to enhance the characterization of the lipidome.
- VLCFA are incorporated into multiple complex lipid species thereby profoundly altering the ALD lipidome.
- Further investigation is needed to analyse whether these systemic lipidome changes can be tied to the multiform pathology of ALD.

Technology