4D-Lipidomics profiling of cardiolipins on heart tissue from Barth syndrome patients using timsTOF mass spectrometry

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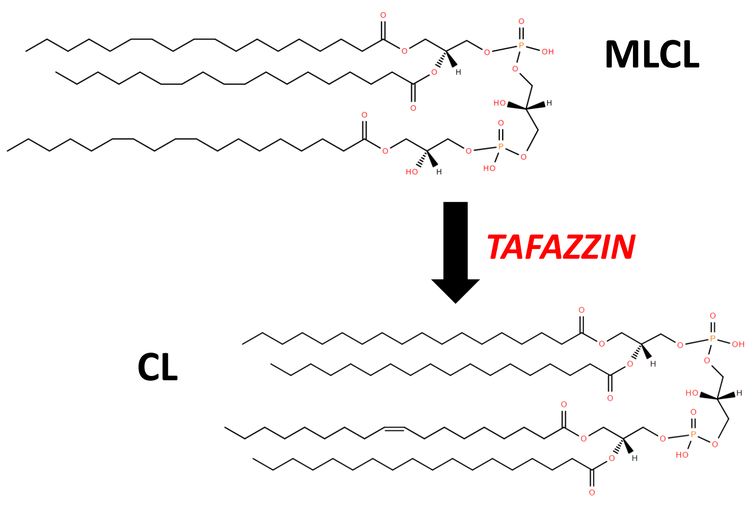
Introduction

- Barth Syndrome is caused by defects in the gene *TAFAZZIN* which is involved in the remodeling of cardiolipins (CL).
- CL are involved in the mitochondrial respiratory chain, mitochondrial dynamics, mitophagy and apoptosis.
- Defects in *TAFAZZIN* results in decreased levels of CL and increased levels of monolysocardiolipins (MLCL)



Method

- We performed 4D-Lipidomics (retention time, *m/z*, ion mobility and fragmentation) using a reversed phase HPLC-MS platform using a timsTOF Pro (Bruker) on heart tissue from Barth syndrome patients and 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® 2023 (Bruker).

Results

- Using 4D-Lipidomics we were able to generate an in-depth characterization of cardiolipins in heart tissue from Barth syndrome patients and controls.
- Our results confirm earlier findings that Barth syndrome results in elevated MLCLs and decreased CLs.
- Barth syndrome results in abnormal CL fatty acid composition which is characterized by less incorporation of unsaturated fatty acids (FA).
- PASEF enabled high speed MS/MS acquisition which in combination with MetaboScape® allowed for the characterization of the fatty acid composition of CL and MLCL.

4D-Lipidomics

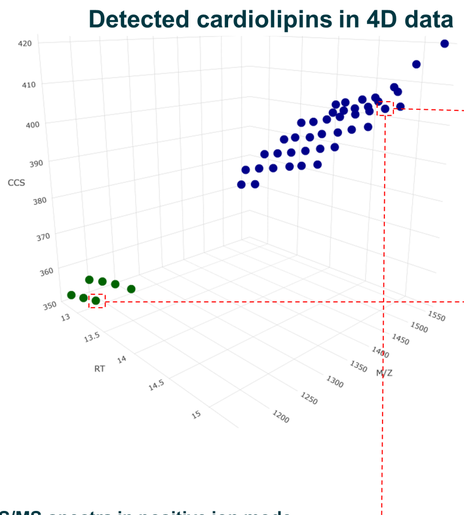
MetaboScape 2023

Feature Table

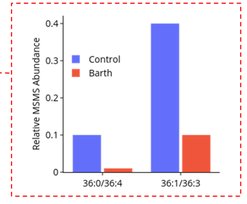
	RT [min]	m/z meas.	M meas.	Δm/z [ppm]	CCS (Å²)	mSigma	Ions	MS/MS	Name	Molecular Formula	Annotations	AQ
70	13.73	1258.88166	1240.84769	-0.042	349.7	13.8			CL 28:0/28:0	$C_{65}H_{126}O_{17}P_2$	LS	
71	13.95	1386.94370	1368.90987	0.417	364.2	32.0			CL 30:2_36:4	$C_{75}H_{134}O_{17}P_2$	LS SL	
72	14.12	1388.96006	1370.92624	0.119	365.4	21.9			CL 32:2_34:3	$C_{75}H_{136}O_{17}P_2$	LS SL	
73	14.38	1416.99697	1398.96315	4.933	369.5	28.4			CL 32:2_36:3	$C_{77}H_{140}O_{17}P_2$	LS SL	
74	14.03	1412.95968	1394.92585	0.075	367.7	22.4			CL 32:3_36:4	$C_{77}H_{136}O_{17}P_2$	LS SL	
75	14.82	1423.03840	1405.00458	2.016	375.1	32.4			CL 34:1/34:1	$C_{77}H_{146}O_{17}P_2$	LS SL	
76	14.88	1449.05388	1431.02005	0.772	378.3	23.7			CL 34:1_36:2	$C_{79}H_{148}O_{17}P_2$	LS SL	
77	14.73	1447.03978	1429.00703	1.441	376.9	13.0			CL 34:1_36:3	$C_{79}H_{146}O_{17}P_2$	LS SL	
78	14.58	1445.02456	1426.99273	1.862	374.8	35.9			CL 34:2_36:3	$C_{79}H_{144}O_{17}P_2$	LS SL	
79	14.43	1443.01095	1424.97599	3.899	373.0	41.8			CL 34:3_36:3	$C_{79}H_{142}O_{17}P_2$	LS SL	
80	14.26	1440.99529	1422.96098	4.718	372.4	7.5			CL 34:3_36:4	$C_{79}H_{140}O_{17}P_2$	LS SL	
81	14.12	1438.97637	1420.94254	1.424	371.2	38.7			CL 34:3_36:5	$C_{79}H_{138}O_{17}P_2$	LS SL	
82	14.94	1475.06836	1457.03454	-0.417	381.3	72.9			CL 36:2/36:2	$C_{81}H_{150}O_{17}P_2$	LS SL	
83	14.78	1473.05516	1455.02175	1.529	380.1	25.3			CL 36:2_36:3	$C_{81}H_{148}O_{17}P_2$	LS SL	
84	14.64	1471.04133	1453.00677	2.704	378.9	28.5			CL 36:3/36:3	$C_{81}H_{146}O_{17}P_2$	LS SL	

m/z value fit
retention time fit
isotopic pattern fit (mSigma)
MS/MS spectrum fit
CCS value fit

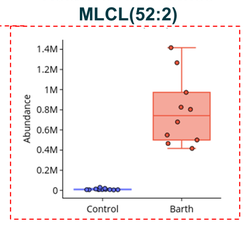
Detected cardiolipins in 4D data



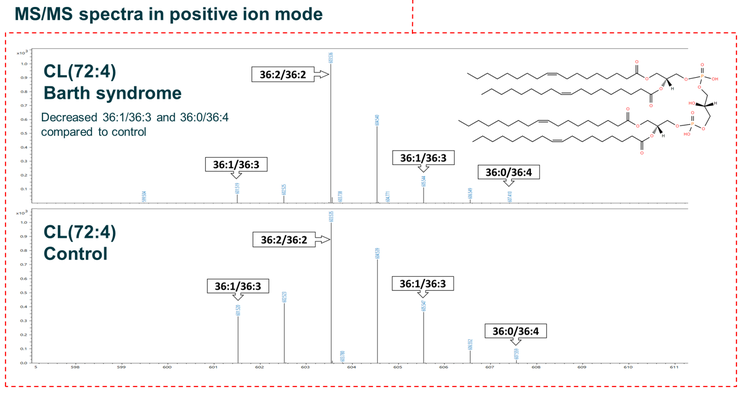
Relative abundance of FA combinations in CL(72:4)



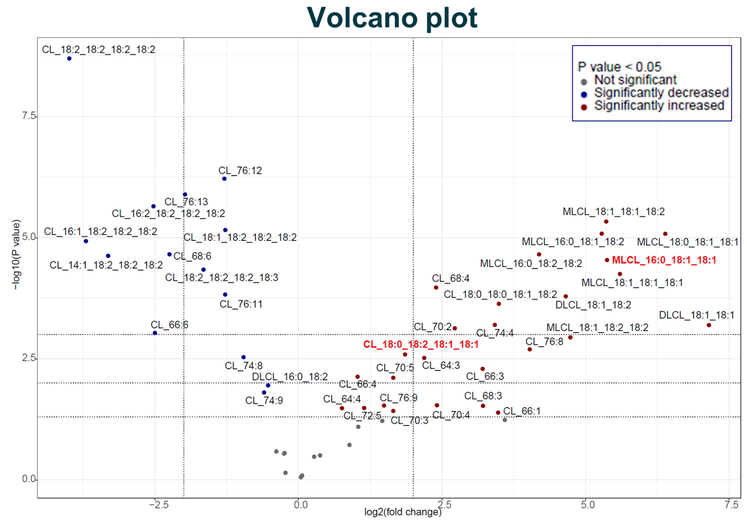
Relative Abundance MLCL(52:2)



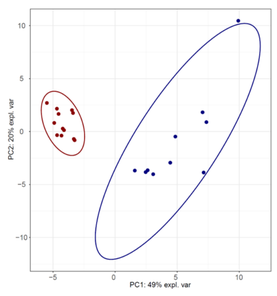
MS/MS spectra in positive ion mode



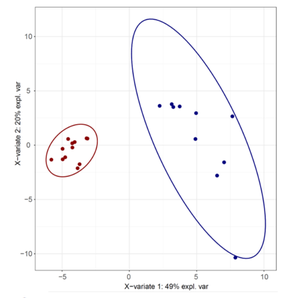
Volcano plot



PCA

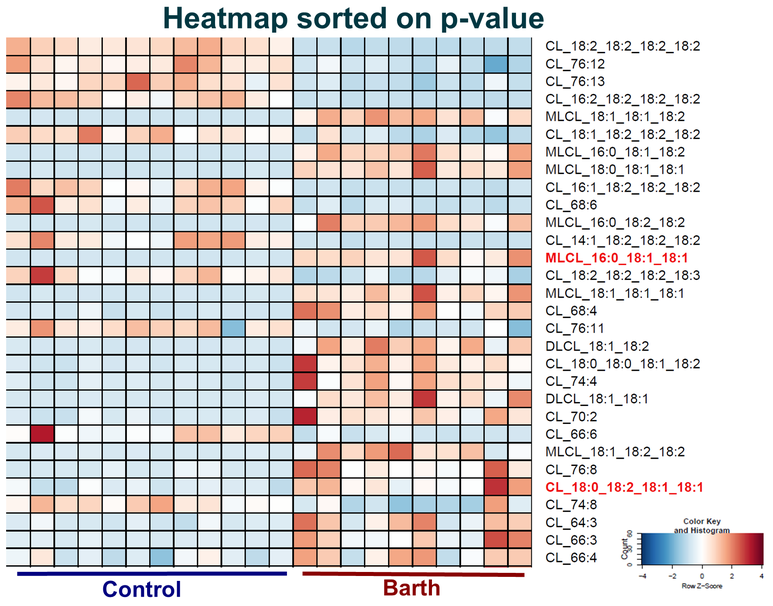


PLS-DA



Legend
Barth
Ctrl

Heatmap sorted on p-value



Highlights

- 4D-lipidomics using PASEF is a promising technique for in-depth characterization of cardiolipins in complex samples.
- PASEF enables high speed acquisition of MSMS spectra.
- Barth syndrome results in significant changes in cardiolipin levels. Further investigations are needed to analyze whether these systemic lipidomic changes can be tied to the pathology of Barth syndrome.

4D-Lipidomics