Application of Lipidomics to identify new phospholipid disorders

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One year old today!
Phospholipids, lipidomics and applications

- Phospholipid structure and measurement with emphasis on cardiolipin and Barth syndrome (3-MG Auria type II)
- Recent developments: Lipidomics pipeline
- Another 3-MG Auria: MEGDEL syndrome, the functional defect elucidated using a Lipidomics approach
Phospholipids

- Phospholipids are important membrane components but also are involved in signal transduction/signaling

  - **Head groups:**
    - Choline
    - Ethanolamine
    - Serine
    - Inositol
    - Glycerol
    - ...

  - **Fatty acids:**
    - Palmitic acid (16:0)
    - Stearic acid (18:0)
    - Palmitoleic acid (16:1)
    - Oleic acid (18:1)
    - Linoleic acid (18:2)
    - ...

Two Side-chains
Technique

- Extraction of lipids followed by HPLC MS
- Two modes; negative and positive scan

- HPLC profile of negative scan; “total ion current”
Phosphatidylinositol (PI)

Technique: negative scan (MS)

HPLC trace

Spectrum

34:1 = 16:1 / 18:0

36:1 = 18:1 / 18:0

Phosphatidylinositol (PI)

Mass/charge = m/z
Technique: negative scan (MS)

Spectrum

HPLC trace

Phosphatidylglycerol
Cardiolipin

Mass/charge = m/z
Cardiolipin (CL)

- Mitochondrial phospholipid with an unusual structure

“Regular” phospholipid structure

Cardiolipin

Phosphate

Head group

Two fatty acid Side-chains

Glycerol
Functions of cardiolipin

- Important constituent of mitochondrial membrane

- Mitochondrial energy metabolism
  - Essential for oxidative phosphorylation

- Mitochondrial protein import

- Type II (mitochondria-mediated) apoptosis
Cardiolipin in the human heart

- Cardiolipin contains almost exclusively linoleic acid
- How to get the specific composition of cardiolipin?
Cardiolipin synthesis and remodeling

- Cardiolipin is actively remodelled to achieve the mature acylcomposition.
- This remodeling is deficient in Barth syndrome.
Barth syndrome patients have:

- Lower CL levels
- Higher MLCL levels
- Altered CL and MLCL composition
Cardiolipins in Barth syndrome

Indeed: lower CL, higher MLCL, altered composition!
Is there something wrong with phospholipids?

• Frequently asked question
• Analysis is relatively simple
• Data-analysis, however, is labor intensive and biased
• Development of a “pipeline” to analyze this data, fast and in an unbiased manner
• But what is a pipeline? How does it work?
The pipeline

Current Data Analysis Scheme

<table>
<thead>
<tr>
<th>Input files conversion</th>
<th>Pre-Processing</th>
<th>Reporting</th>
<th>Additional Analysis</th>
<th>Visualization</th>
<th>Statistical Analysis</th>
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<td>3</td>
<td>4</td>
<td>4a</td>
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<td>Packages from Perry &amp; Isthiaq (Galaxy Platform)</td>
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</table>

- Only for positive data sets

Biobase:
- bash-script for mzlevel correction
- mzxmlParser.jar (channel separation)

XCMS Package:
- Peak Extraction
- Time alignment
- Peak grouping
- Filling missing peaks in the group
- generating final peak-list

• But what does the pipeline really do?
HPLC profile (TIC)
Mass spectrum of 6.06 – 6.59 min.

CL IS

CLs

Mass spectrum of 6.06 – 6.59 min.

PG / BMP
HPLC trace of m/z 619
The pipeline

• In essence, the pipeline does this for every m/z value.
• Then it integrates the area of the chromatographic peak
• Generates a “peaklist” listing retention time, m/z value and area
## The peaklist

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<th>mzmin</th>
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Visualization in extracted ion-chromatographic overlays of data sets per $m/z$ value and $R_t$

**Extracted Ion Chromatogram:** 796.42 - 796.6 m/z

- **CONTROL**
- **PATIENTS**

**Extracted Ion Chromatogram:** 932.57 - 932.69 m/z

- **CONTROL**
- **PATIENTS**
Visualization by Box & Whiskers plots of data sets of peaks per $m/z$ value and $R_t$
Phospholipid analysis/pipeline

• Analysis of major phospholipid classes:
  – PE, PC, PI, PG, CL, SM etc.
  – Determination of molecular composition of the species (fatty acid side-chains)

• The pipeline allows fast and unbiased quantification of all ions in the analysis.

• Example of the power of the analysis/pipeline: MEGDEL syndrome
MEGDEL syndrome

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Bioinformatica
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3-Methylglutaconic aciduria syndromes

• Type II: **Barth syndrome** (X-linked, DCM, cyclic neutropenia, hypotonia, normal cognitive function, *TAZ*)

• Type IV: “unclassified” “mitochondrial dysfunction” (*TMEM70, POLG, RYR1, SUCLA2.....*)
New subtype of type IV

- Termed MEGDEL syndrome:
  - 3-MEthylGlutaconic aciduria
    (30-300 umol/mmol creatinine, n<20)
  - Hypotonia/spasticity/dystonia
  - Deafness
  - Encephalopathy, psychomotor retardation
  - Leigh like syndrome/disease
  - OXPHOS dysfunction
    (mild decrease in ATP production and complex I)
  - Lactic acidemia (predominantly neonatal)
  - Liver dysfunction (predominantly neonatal)
MEGDEL syndrome
Exome sequencing (Nijmegen)

- 2 patients exome sequencing $\rightarrow$ both mutation in \textit{SERAC1}
- All other patients (n=16) also found to have mutation in \textit{SERAC1}
- Function unknown
- \textit{SERAC1} subcellular localization?

Nature Genetics, Wortmann et al, epub 10 June 2012
SERAC1 is localized to MAM’s

- SERAC1 is localized to mitochondria associated membranes (protK studies and IF).

Nature Genetics, Wortmann et al, epub 10 June 2012
SERAC1 characterization

- SERAC1 protein has an acyltransferase/lipase domain
- MEGDEL patients have:
  - Methylglutaconic aciduria
  - Mitochondrial disease
- Phospholipids?

Nature Genetics, Wortmann et al, epub 10 June 2012
MEGDEL lipidomics experiment

• 5 MEGDEL fibroblast lines
• 10 control fibroblast lines
  [all cultured simultaneously, same medium, FCS etc…]
• Phospholipid analysis + bioinformatics pipeline
Structures of “the players”

- **Phosphatidylglycerol (PG)**

- **Cardiolipin (CL)**

- **Bismonoacylglycerolphosphate (BMP)**

BMP is a structural isomer of PG
Functions

- **Cardiolipin** (CL) and **phosphatidylglycerol** (PG) are mitochondrial phospholipids that are important for mitochondrial function.

- **Bismonoacylglycerolphosphate** (BMP) is an endosomal phospholipid involved in the transport and breakdown of lipids and cholesterol.
PG is a precursor of CL and BMP

PG → CL

PG → BMP → remodeling → PI, LPI?

BMP
Differences found by the pipeline

Differences in:
- PG
- BMP
- CL
- (specific) LysoPI

BMP remodeling

PI

LPI?
- Accumulation of PG34:1
- Deficiency of PG36:1
• Lower BMP levels
• Normal/same molecular composition of BMP
Cardiolipin

Control

Patient

Control

Patient

**CL(66:4) (m/z 699.5) p<0.0001**

**CL(66:3) (m/z 686.4) p<0.0001**

**CL(70:7) (m/z 710.5) p=0.24**

**CL(70:6) (m/z 711.5) p=0.85**

**CL(72:8) (m/z 723.5) p=0.85**

**CL(72:7) (m/z 724.5) p=0.85**

**CL(70:5) (m/z 712.5) p=0.28**

**CL(70:4) (m/z 713.5) p=0.02**

**CL(72:5) (m/z 713.5) p=0.54**

**CL(74:8) (m/z 736.5) p=0.50**

**CL(74:7) (m/z 737.5) p=0.37**

**CL(74:6) (m/z 738.5) p=0.41**

**Concentration (pmol/mg protein)**

**Concentration (pmol/mg protein)**
The surprise of the pipeline

Control (fibroblast)

LPI(20:4)
The surprise of the pipeline

Patient (fibroblast)

no LPI(20:4)
Hypothesized function of SERAC1

SERAC1 is a PG transacylase involved in the formation of PG36:1, which is a precursor of BMP.
SERAC1 function in cellular context

Mitochondrion
Lysosome/late endosome

PG34:1
PG36:1
PG34:1

MAM
BMP function

- BMP is needed to export cholesterol from lysosomes.
- Accumulation of cholesterol in MEGDEDEL syndrome?
- BMP deficiency results in cellular cholesterol accumulation.
Conclusions (1)

• MEGDEL syndrome is new subtype of 3-methylglutaconic aciduria

• MEGDEL syndrome is, like Barth syndrome, a defect in phospholipid metabolism

• A lot of “loose ends”
Conclusions (2)

• Phospholipid analysis in combination with automated analysis of the raw data is a powerful research/diagnostic tool

• This combined approach elucidated the functional defect in MEGDEL syndrome
Thank you for your attention!

Questions?
SERAC1 expression profile

Fetal tissues

Adult tissues
Mitochondria (arrows)
lysosomes with fat droplets (asterisks)
membranous remnants (arrowheads)

bar = 0.2µm