BACKGROUND AND AIM

Hair has recently emerged as potential complementary sample type to identify biomarkers in early disease stages and retrospectively document an individual’s metabolic status due to its long detection window. However, the limited coverage of lipid profiling presented in previous studies has hindered its exploitation.

This study aimed to evaluate the lipid coverage of hair using an untargeted lipidomics platform.

Two different three-step exhaustive extraction experiment were conducted using a hair metabolomics one-phase extraction technique and the two-phase Folch extraction method, considered the gold standard for lipid extraction in biological matrices, respectively. The hair lipidome was compared with the blood and sebum lipidome to understand its role in reflecting health and disease status.

STUDY DESIGN

EXHAUSTIVE EXTRACTION 1

1. Optimized hair metabolomics extraction
   (Stavrakis et al. 2019)
   MeCN/H2O (2/8, v/v)
   16h incubation
   Lipids left?
   Extract 1 (EE1)

2. Folch
   (CHCl3/MeOH/H2O, 8/4/3, v/v/v)
   Lipids left?
   Extract 2 (EE2)

3. Ethyl acetate
   (CHCl3/MeOH/H2O, 8/4/3, v/v/v)
   Lipids left?
   Extract 3 (EE3)

EXHAUSTIVE EXTRACTION 2

4. Lipidomics extraction
   (Savastano et al. 2018)
   40min incubation
   Lipids left?
   Extract 1 (FE1)

5. Folch
   (CHCl3/MeOH/H2O, 8/4/3, v/v/v)
   Lipids left?
   Extract 2 (FE2)

6. Ethyl acetate
   (CHCl3/MeOH/H2O, 8/4/3, v/v/v)
   Lipids left?
   Extract 3 (FE3)

RESULTS 2

LIPIDOME COVERAGE

FOLCH EXTRACTION

GLYCEROLIPIDS: 11.4%
- Phosphatidylcholines (PC): 37.7%
- Glyosphingolipids (GSL): 37.7%
- Glycolipids (GL): 2.7%
- Phosphatidylethanolamines (PE): 3.2%

GLYCEROPHOSPHOLIPIDS: 4.7%
- Phosphatidylethanolamines (PE): 4.7%
- Phosphatidylcholines (PC): 4.7%
- Phosphatidylserines (PS): 0.3%

STEROID LIPIDS: 1.3%
- Sterols (ST): 0.7%
- Sterol esters: 0.6%

FATTY ACIDS: 42.4%
- Methyl esters (ME): 28.3%
- Short chain fatty acids (SCFA): 28.3%
- Medium chain fatty acids (MCFA): 1.8%
- Long chain fatty acids (LCFA): 1.8%

CONCLUSION

The Folch two-phase extraction method was superior to an optimized one-phase protocol for hair untargeted metabolomics resulting in the extraction of a higher number of lipids (n=297, covering 6 lipid categories) and a higher yield of extracted lipid (90.8%). Furthermore, N-acetyl amino acids, OxFs, FAHFAs, DGs and coenzyme Q10 were reported in hair for the first time.

Moreover, this study performed a comprehensive review of the origin of hair lipid incorporation of the annotated lipid and suggested pre-analytical decontamination, homogenization, and extraction protocols for upcoming targeted and untargeted hair analysis research. The reconstructed hair lipidome and literature-based results suggest that hair lipids originates from blood, sebum, environment or de novo formed within the hair shaft.

The results of this work offers valuable insights to contextualize untargeted hair analysis and facilitate the use of hair in translational study in the field of toxicology, dermatology and clinical research.


CONSIDERATIONS FOR RETROSPECTIVE ANALYSIS

1. Bloodstream
   - Reference matching (dot product)
   - In house (reference standards)
   - Annotations | with manual confirmation*

II. LipidMatch
   - Reference matching (dot product)
   - Annotations | with manual confirmation*

III. MS-DIAL
   - Reference matching (dot product)
   - In house (reference standards)
   - Annotations | with manual confirmation*

1. Decontamination
   1. Bloodstream
   2. Integral hair lipids
   3. de novo synthesis
   4. Environment
   5. Sebum

2. Integral hair lipids
   1. Bloodstream
   2. Integral hair lipids
   3. de novo synthesis

3. de novo synthesis
   1. Bloodstream
   2. Integral hair lipids
   3. de novo synthesis

4. Sebum
   1. Bloodstream
   2. Integral hair lipids
   3. de novo synthesis

5. Environment
   1. Bloodstream
   2. Integral hair lipids
   3. de novo synthesis

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