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CHKA, un “ marqueur-cible ” potentiel pour le CHC-MASLD à un stade précoce de fibrose : analyse par approche des “ omiques ”

Jihan Sun, Fatima Dahboul, Estelle Pujos-Guillot, Stéphanie Durand, Mélanie Pétéra, Delphine Centeno, Benoit Colsch, Guillaume Zoulim, Delphine Weil, Vincent Di Martino, et al.

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CHKA, un « marqueur-cible » potentiel pour le CHC-MASLD à un stade précoce de fibrose : analyse par approche des « omiques »

Jihan Sun¹, Fatima Dahboul¹, Estelle Pujos-Guillot², Stéphanie Durand², Mélanie Petera², Delphine Centeno², Benoit Colsch², Zoulim Guillaume¹, Delphine Weil⁴, Vincent Di Martino⁴, Aicha Demidem¹, Armando Abergel³

¹ Team ECREIN, UMR 1019, INRAE, Clermont-Auvergne Université, Clermont-Ferrand, France

² Plateforme exploration du Métabolisme, METABOHUB, INRAE, Theix, France

³ Hepato-Gastroenterology Unit, Université Hospital Estaing, Clermont-Ferrand, France

⁴ Service d'Hépatologie et de Soins Intensifs Digestifs, Hôpital Jean Minjoz, Besançon, France

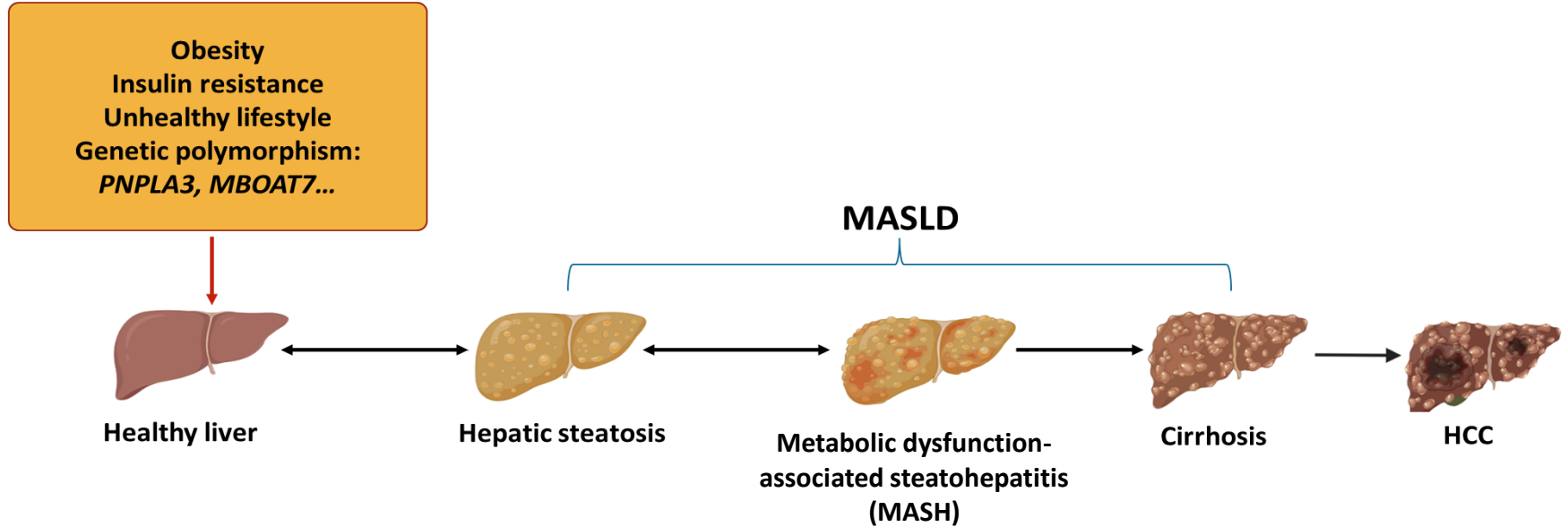
Liens d'intérêts

- L'orateur a déclaré sur le site des JFHOD ne pas avoir de liens d'intérêts.

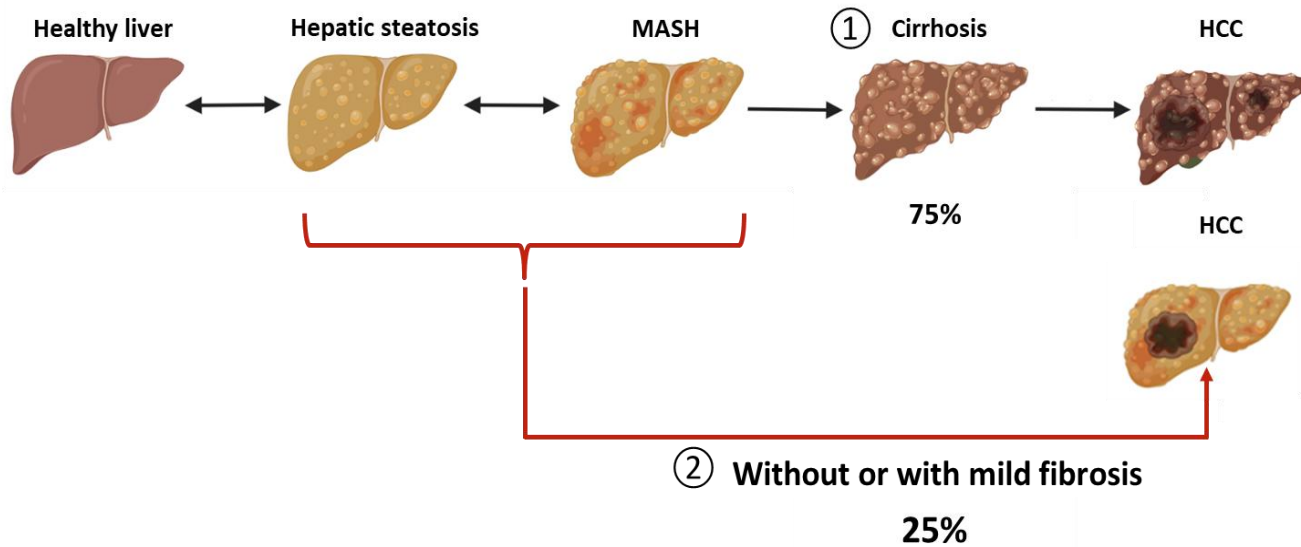
Metabolic dysfunction-associated steatotic liver disease (MASLD)

- **Old name:** non-alcoholic fatty liver disease(**NAFLD**) or metabolic dysfunction-associated fatty liver disease(**MAFLD**), changed in **June, 2023** (*Rinella et al., 2023*)
- **Diagnostic criteria:** **Hepatic steatosis** is identified by imaging or biopsy with **≥ 1 of 5 cardiometabolic risk factor**
- **MASLD** affects nearly **25% of the global** population, with the prevalence rates showing an **upward trend**

MASLD - HCC



New pathway of MASLD-HCC



Different carcinogenic pathways according to fibrosis level?

Fibrosis - METAVIR classification

- The METAVIR classification is currently the reference for assessing histological fibrosis level from absence of fibrosis (F0) to cirrhosis (F4)

5 degree of fibrosis :

F0 = absence

F1 = mild

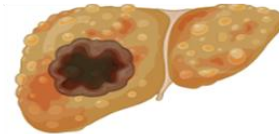
F2 = moderate

F3 = severe

F4 = cirrhosis

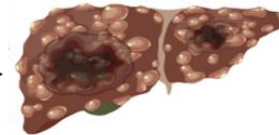
Without or with mild fibrosis

MASLD-HCC-F0F1



Cirrhosis

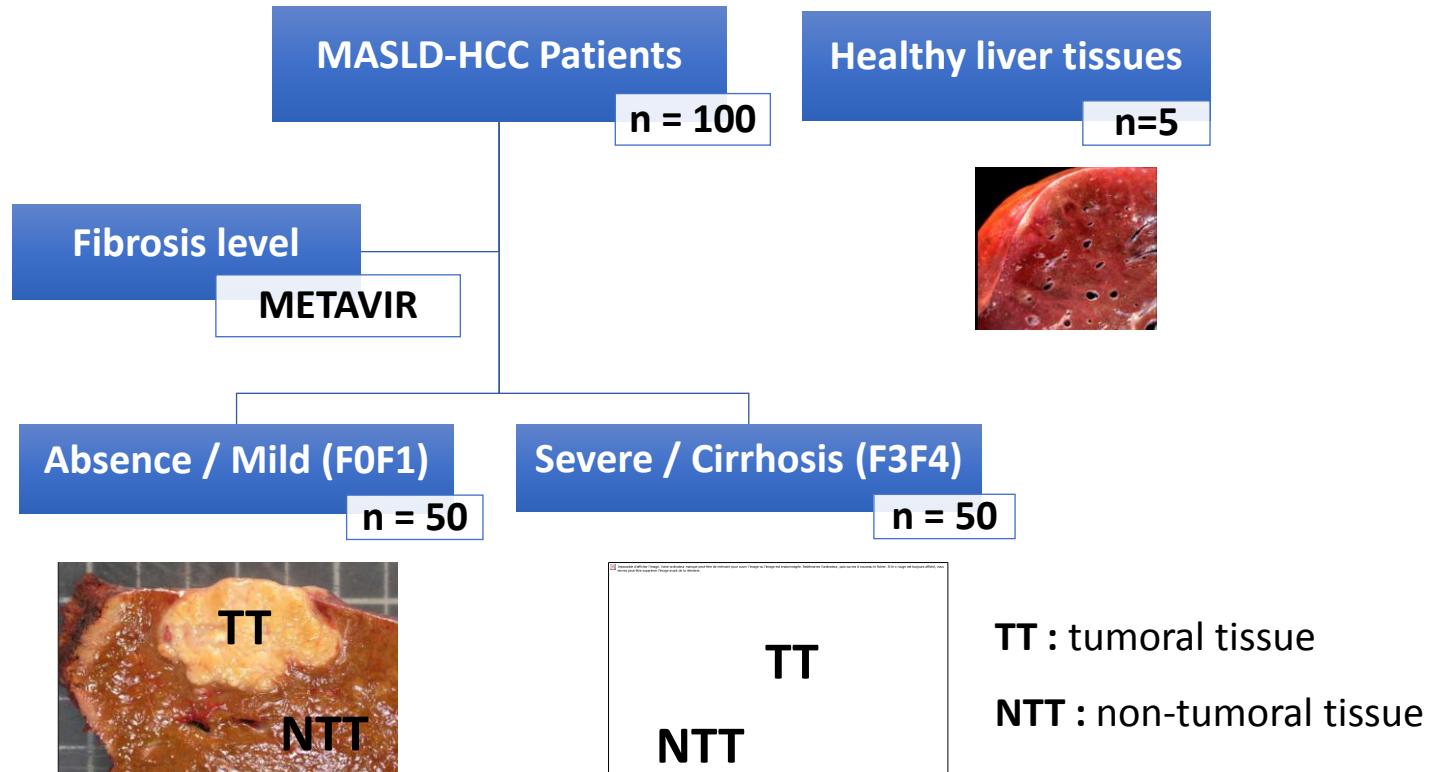
MASLD-HCC-F3F4



Purpose

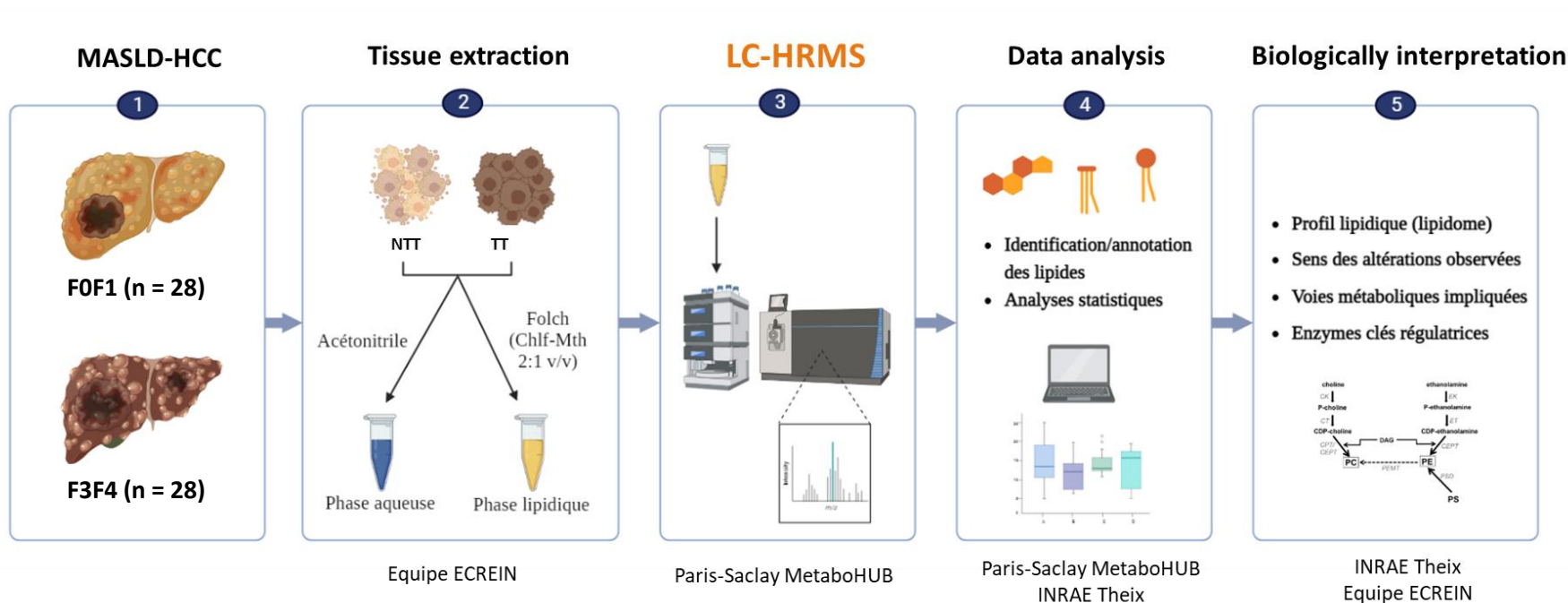
- Our previous study revealed two metabolomics phenotypes of MASLD-HCC according to fibrosis level (*Buchard et al., 2021*)
- Use combined **metabolomics and transcriptomics** analyses on **human liver tissues**, to explore **lipid metabolic pathways** and to identify potential **biomarkers** for **MASLD-HCC** according to **fibrosis level**

Methods - Cohort design (CRB. Foie)



Metabolomics

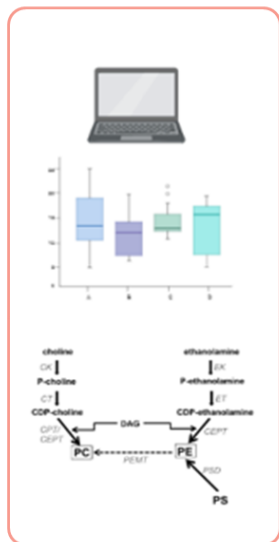
Workflow of Untargeted Lipidomics



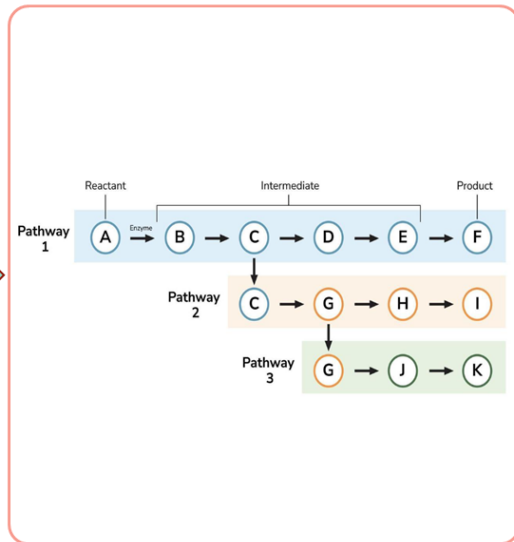
LC-HRMS: Liquid chromatography–high resolution mass spectrometry

Transcriptomics

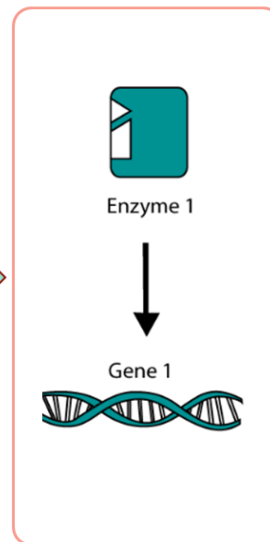
Workflow of RT-qPCR



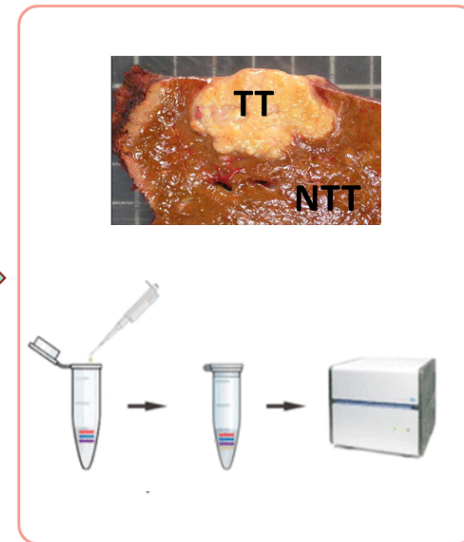
Results of LC-HRMS



Related pathways and major enzymes



Genes



RT-qPCR

Results - Metabolomics

1744 ions detected

138 ions (lipids) identified

36 discriminant lipids identified

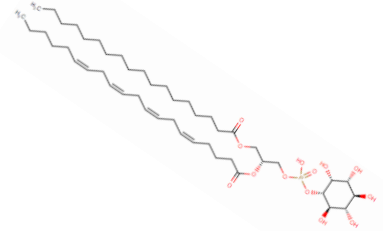
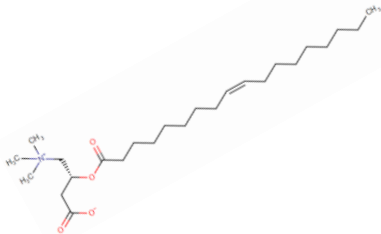
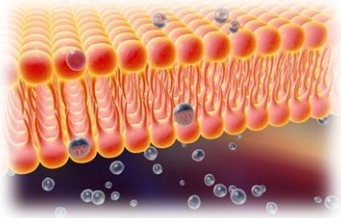
4 families:

Cholesterol ester

Glycerolipid

Sphingolipid

Glycerophospholipid



Results - Metabolomics

Intrahepatic lipids differentiating TT from NTT in the two patient groups with MASLD-HCC

NTT vs. TT

| Families / lipid compound | F0F1 NTT vs. TT | F3F4 NTT vs. TT |
|--------------------------------------|-----------------|-----------------|
| Cholesterol esters | | |
| CE (18:2) | * | |
| Glycerolipid | | |
| TAG (16:0_18:1_18:2) | | * |
| TAG (16:0_18:2_18:2) | * | * |
| TAG (16:1_18:2_18:2) | ** | * |
| TAG (18:1_18:2_18:2) | * | |
| TAG (18:1_18:2_18:3) | * | * |
| Sphingolipid | | |
| Cer (d18:1/22:0) | * | |
| Cer (d18:1/24:0) | | * |
| SM (35:1) | * | |
| SM (38:1) | | * |
| SM (39:1) | | * |
| SM (40:2) | ** | |
| SM (41:1) | * | ** |
| SM (42:3) | ** | |
| Glycerophospholipid | | |
| PC (16:0_14:0) | * | |
| PC (16:0/16:0) | * | |
| PC (16:0_20:4) | | * |
| PC (16:0_22:6) | | * |
| PC (18:0_18:1) | *** | ** |
| PC (18:0_20:3) | | * |
| PC (40:6) | | * |
| PC (40:8) | * | |
| PE (16:0_22:6) | | ** |
| PE (18:0_18:1) | ** | |
| PE (18:0_22:6) | | * |
| PE (O-16:1_20:4) or PE (P-16:0_20:4) | * | * |
| PI (16:0_18:1) | | * |
| PI (18:0_20:4) | | ** |
| LPC (18:1) | * | |
| LPE (18:1) | * | |

Increase
Decrease
Unchanged

1. Cholesterol ester (CE)

2. Glycerolipid:

TAG: triacylglycerol

3. Sphingolipid:

Cer: ceramide

SM: sphingomyelin

4. Glycerophospholipid:

PC: phosphatidylcholine

PE: phosphatidylethanolamine

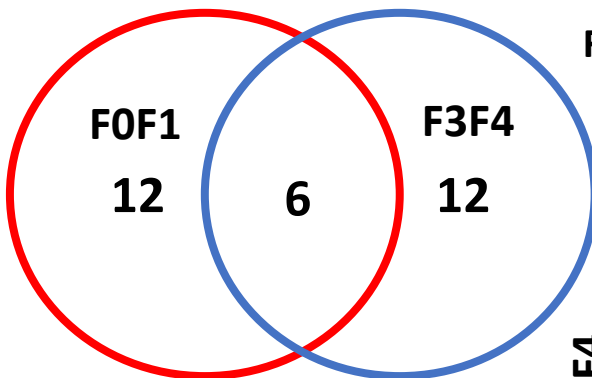
PI: phosphatidylinositol

LPC: lysophosphatidylcholine

LPE: lysophosphatidylethanolamine

FOF1: Increased metabolic contents

| | |
|---|---------------------|
| - | CE(18:2) |
| - | TAG(18:1_18:2_18:2) |
| SM (35:1) SM (40:2) SM (42:3) | Cer (d18:1/22:0) |
| PC(16:0_14:0) PC(16:0/16:0) PC(40:8) PE(18:0_18:1) LPC(18:1) LPE(18:1) | - |



F3F4: Decreased metabolic contents

| | |
|--|---|
| - | - |
| - | TAG(16:0_18:1_18:2) |
| - | Cer (d18:1/24:0) SM (38:1) SM (39:1) |
| PC(18:0_20:3) PI(16:0_18:1) Onco-metabolite (Yang et al., 2018) | PC(16:0_20:4) PC(16:0_22:6) PC(40:6) PE(16:0_22:6) PE(18:0_22:6) PI(18:0_20:4) |

PC(18:0_18:1)

SM (41:1)

PE(O-16:1_20:4)

TAG(16:0_18:2_18:2)

TAG(16:1_18:2_18:2)

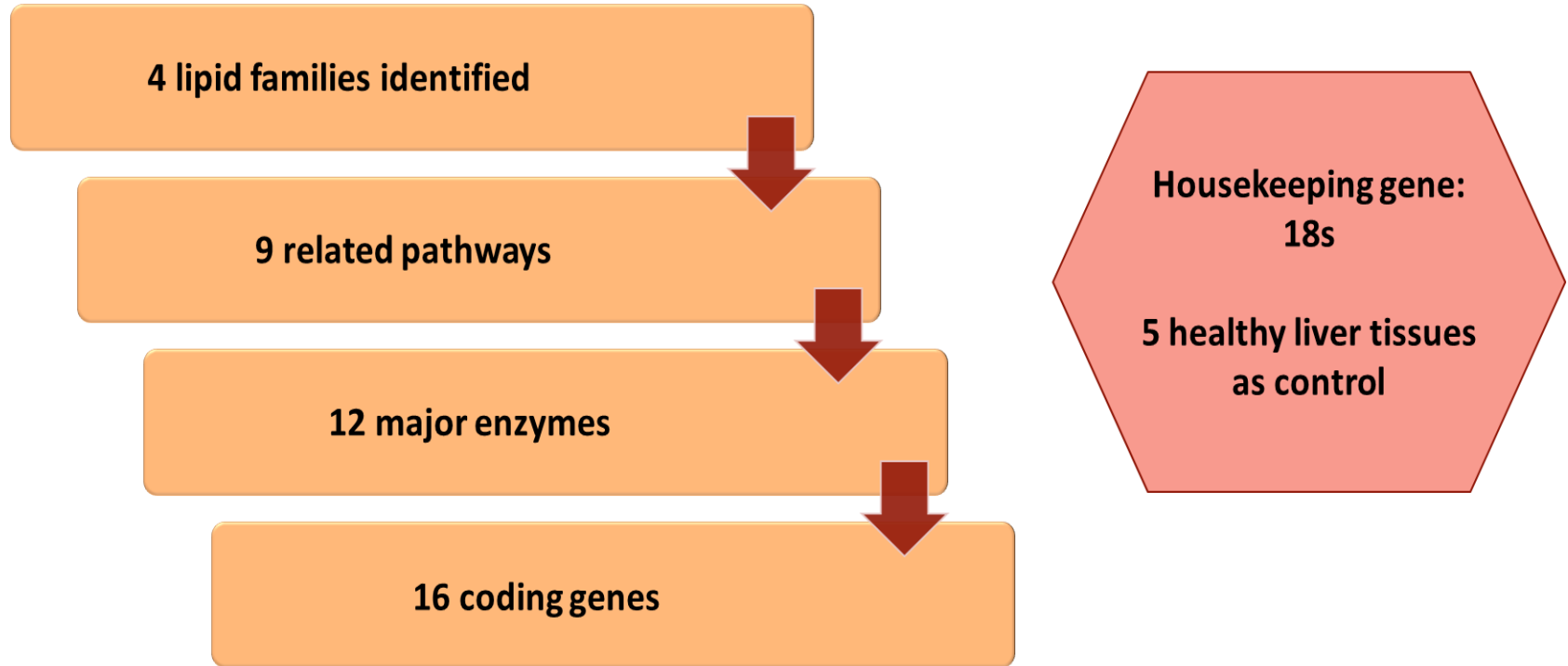
TAG(18:1_18:2_18:3)

Amplified anabolism

LC-HRMS analysis confirmed the existence of **two metabolomics**

phenotypes of MASLD-HCC according to fibrosis level

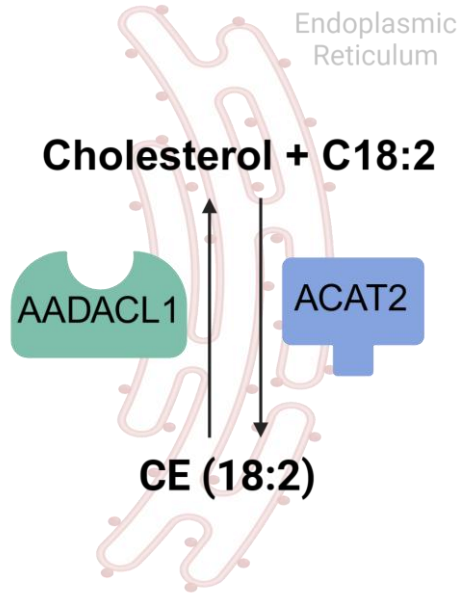
Results - Transcriptomics



| | | |
|---------------------------------------|------------|---|
| Family 1 - Cholesterol ester | 1.ACAT2 | Cholesterol esterification |
| | 2.AADACL1 | Cholesterol ester hydrolysis |
| Family 2 - Glycerolipid | 3. DGAT1 | DAG \longrightarrow TAG |
| | 4. DGAT2 | |
| Family 3 - Sphingolipid | 5. SPTLC1 | Ceramide <i>De Novo</i> synthesis pathway |
| | 6. SPTLC2 | |
| | 7. SGMS1 | Ceramide \longrightarrow Sphingomyelin |
| | 8. SGMS2 | |
| | 9. SMPD1 | Sphingomyelin \longrightarrow Ceramide |
| | 10. SMPD3 | |
| Family 4 - Glycerophospholipid | 11. MBOAT7 | Remodeling of PI |
| | 12. ACOX1 | Synthesis of Docosahexaenoic acid (C22:6) (DHA) |
| | 13.PEMT | Kennedy pathway (PE,PC synthesis) |
| | 14. CHPT1 | |
| | 15. PCYT1A | |
| | 16. CHKA | |

Family 1 - Cholesterol ester

AADACL1 and ACAT2



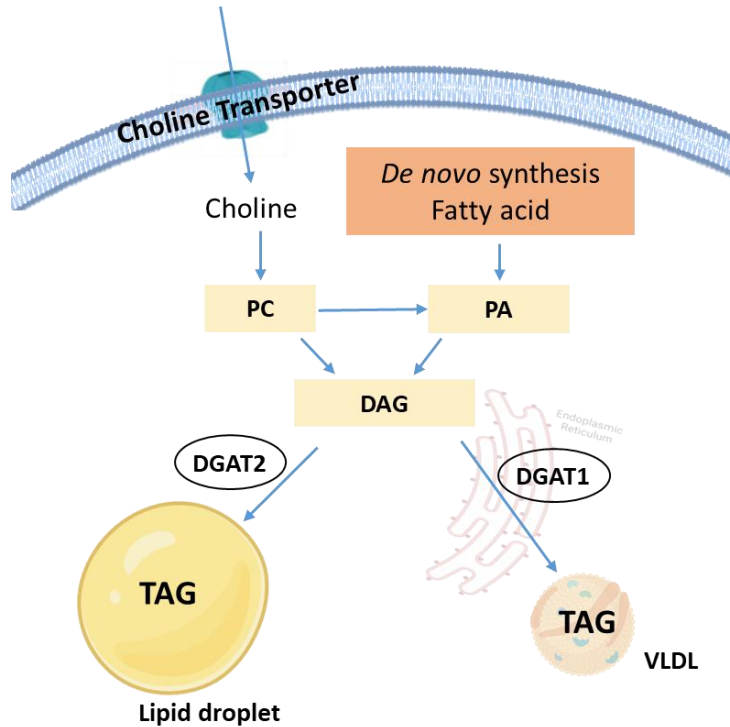
ACAT2: Acetyl-CoA Acetyltransferase 2

AADACL1: ArylAcetamide DeAcetyLase 1

The expression of the gene coding for **ACAT2** is **exclusively up-regulated** in MASLD-HCC-F0F1

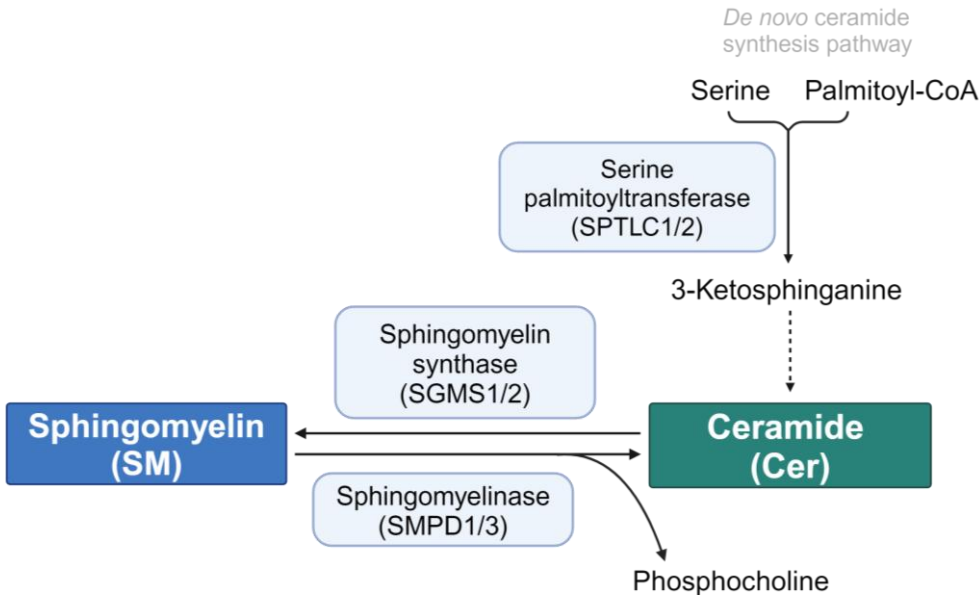
Family 2 - Glycerolipid

DGAT1 and DGAT2



The expression of the two isoforms of gene coding for **DGAT1 and 2** is **exclusively up-regulated** in **MASLD-HCC-F0F1**

Family 3 – Sphingolipid



| Gene | Control: healthy liver tissues | |
|--------|--------------------------------|-----------------|
| | FOF1 NTT vs. TT | F3F4 NTT vs. TT |
| SGMS1 | ↗ | ↗ |
| SGMS2 | ↗ | ↗ |
| SMPD1 | ↗ | ↗ |
| SMPD3 | ns | ns |
| SPTLC1 | ↗ | ↗ |
| SPTLC2 | ↗ | ↗ |

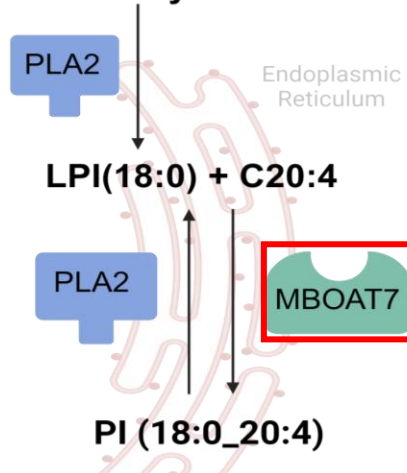
The expression of 6 genes coding for **SPTLC1/2**, **SGMS1/2** and **SMPD1/3** shows **no difference** between the two groups of MASLD-HCC

Family 4 - Glycerophospholipid

| | |
|------------|---|
| 11. MBOAT7 | Remodeling of PI |
| 12. ACOX1 | Synthesis of Docosahexaenoic acid (C22:6) (DHA) |
| 13. PEMT | Kennedy pathway (PE, PC synthesis) |
| 14. CHPT1 | |
| 15. PCYT1A | |
| 16. CHKA | |

MBOAT7: Membrane Bound O-AcylTransferase 7

De novo synthesised PI

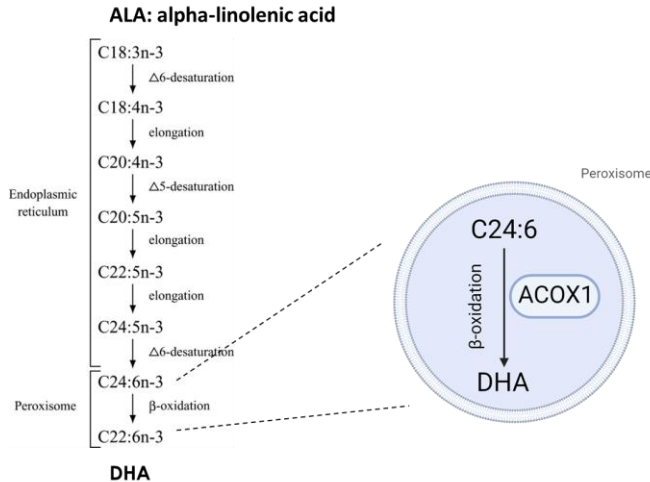


The expression of gene coding for **MBOAT7** is **up-regulated** in both groups of MASLD-HCC

Family 4 - Glycerophospholipid

| | |
|------------|---|
| 11. MBOAT7 | Remodeling of PI |
| 12. ACOX1 | Synthesis of Docosahexaenoic acid (C22:6) (DHA) |
| 13. PEMT | Kennedy pathway (PE,PC synthesis) |
| 14. CHPT1 | |
| 15. PCYT1A | |
| 16. CHKA | |

| Families / lipid compound | F0F1 NTT vs. TT | F3F4 NTT vs. TT |
|--------------------------------------|-----------------|-----------------|
| Glycerophospholipid | | |
| PC (16:0_14:0) | * | |
| PC (16:0/16:0) | * | |
| PC (16:0_20:4) | | * |
| PC (16:0_22:6) | → | * |
| PC (18:0_18:1) | *** | ** |
| PC (18:0_20:3) | | * |
| PC (40:6) | | * |
| PC (40:8) | * | |
| PE (16:0_22:6) | → | ** |
| PE (18:0_18:1) | ** | |
| PE (18:0_22:6) | → | * |
| PE (O-16:1_20:4) or PE (P-16:0_20:4) | * | * |



The expression of gene coding for **ACOX1** is exclusively **up-regulated** in MASLD-HCC-F0F1

Family 4 - Glycerophospholipid

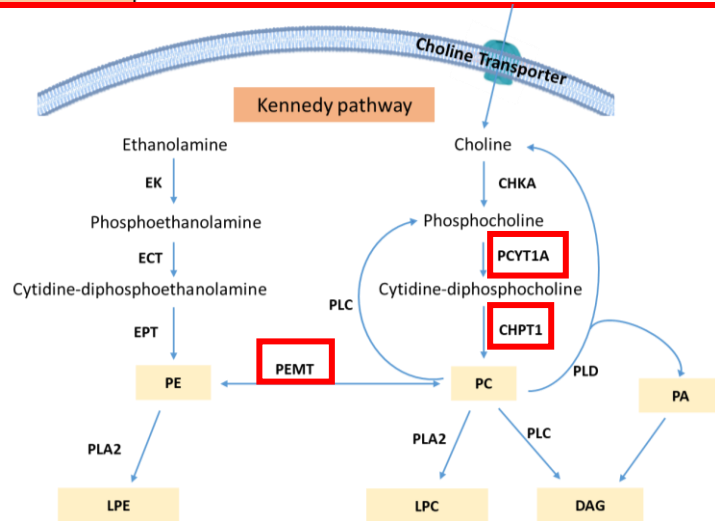
| | |
|------------|---|
| 11. MBOAT7 | Remodeling of PI |
| 12. ACOX1 | Synthesis of Docosahexaenoic acid (C22:6) (DHA) |
| 13. PEMT | Kennedy pathway (PE,PC synthesis) |
| 14. CHPT1 | |
| 15. PCYT1A | |
| 16. CHKA | |

PEMT: PhosphatidylEthanolamine N-MethylTransferase

CHPT1: Choline PhosphoTransferase 1

PCYT1A: Phosphocholine CytidylylTransferase

CHKA: Choline Kinase Alpha

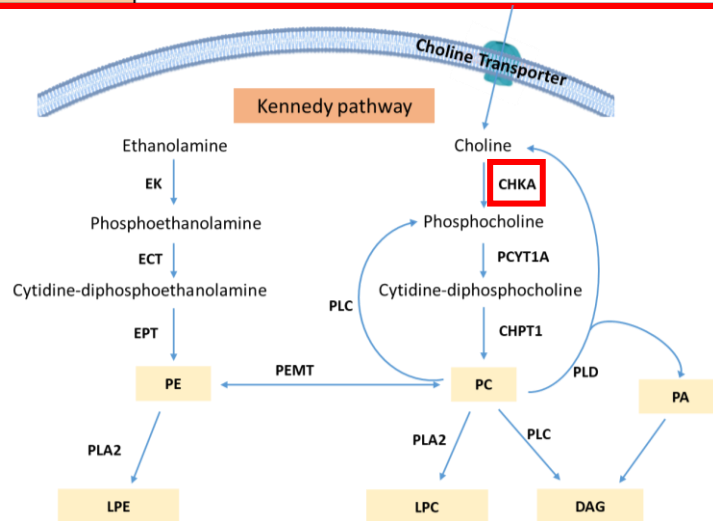


| Family | Gene | Control: healthy liver tissues | |
|---------------------|--------|--------------------------------|-----------------|
| | | FOF1 NTT vs. TT | F3F4 NTT vs. TT |
| Glycerophospholipid | PEMT | ns | ns |
| | CHPT1 | ↗ | ↗ |
| | PCYT1A | ↗ | ↗ |

The expression of 3 genes coding for **PEMT**, **CHPT1** and **PCYT1A** shows **no difference** between the two groups of MASLD-HCC

Family 4 - Glycerophospholipid

| | |
|------------|---|
| 11. MBOAT7 | Remodeling of PI |
| 12. ACOX1 | Synthesis of Docosahexaenoic acid (C22:6) (DHA) |
| 13. PEMT | Kennedy pathway (PE,PC synthesis) |
| 14. CHPT1 | |
| 15. PCYT1A | |
| 16. CHKA | |



The expression of gene coding for **CHKA** is exclusively **up-regulated** in MASLD-HCC-F0F1

| Family | Gene | Control: healthy liver tissues | |
|---------------------|---------|--------------------------------|-----------------|
| | | F0F1 NTT vs. TT | F3F4 NTT vs. TT |
| Cholesterol ester | ACAT2 | ↗ | ns |
| | AADACL1 | ↗ | ↗ |
| Glycerolipid | DGAT1 | ↗ | ns |
| | DGAT2 | ↗ | ns |
| Sphingolipid | SGMS1 | ↗ | ↗ |
| | SGMS2 | ↗ | ↗ |
| | SMPD1 | ↗ | ↗ |
| | SMPD3 | ns | ns |
| | SPTLC1 | ↗ | ↗ |
| | SPTLC2 | ↗ | ↗ |
| Glycerophospholipid | ACOX1 | ↗ | ns |
| | MBOAT7 | ↗ | ↗ |
| | PEMT | ns | ns |
| | CHPT1 | ↗ | ↗ |
| | PCYT1A | ↗ | ↗ |
| | CHKA | ↗ | ns |



Transcriptomics analysis of **16 genes** revealed:

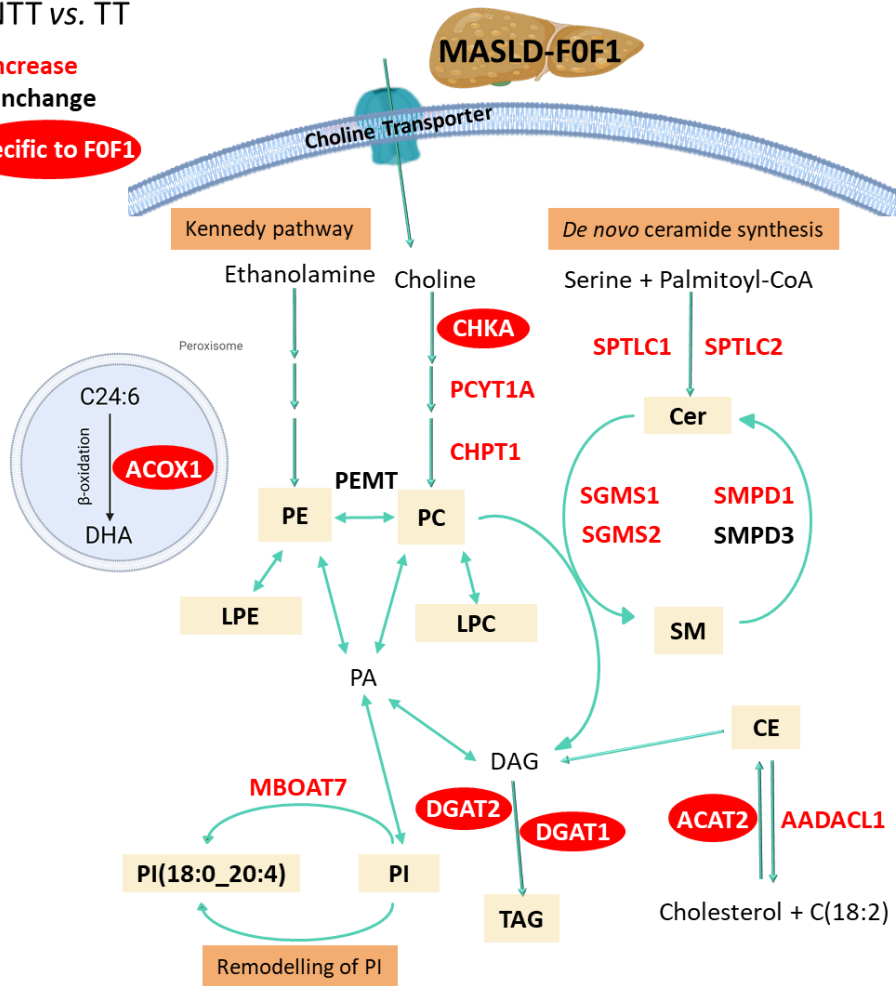
- The upregulation of 9 genes is common in the two groups
- **MASLD-HCC-F0F1**: five genes are specifically **up-regulated**
- **MASLD-HCC-F3F4**: none of the gene is changed

NTT vs. TT

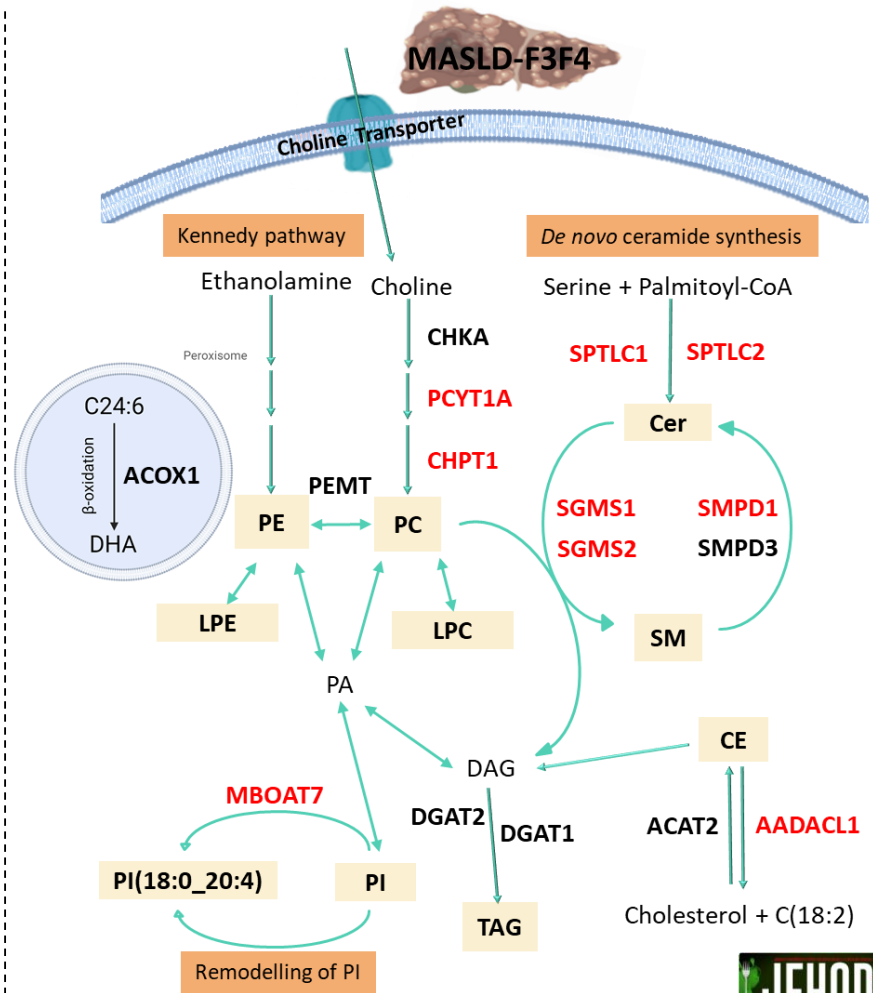
Increase
Unchange

Specific to FOF1

MASLD-FOF1



MASLD-F3F4



Conclusions

Combined metabolomics and transcriptomics analyses allow us to discriminate MASLD-HCC based on fibrosis level

- **Metabolomics** analysis revealed that in **MASLD-HCC-F0F1**, **sphingolipid** and **glycerophospholipid** contents **increase**; while these metabolites contents **decrease** in **MASLD-HCC-F3F4**
- **Transcriptomics** analysis revealed that the expression of 5 genes coding for: **ACAT2**, **DGAT1**, **DGAT2**, **ACOX1** and **CHKA** is **up-regulated** exclusively in **MASLD-HCC-F0F1**

CHKA - Clinical application

Labeled choline may be favored as a **more effective** tracer for PET scanning in HCC patients with **minimal fibrosis**

Due to:

- MASLD-HCC-F0F1 mainly **overexpressed** choline derivatives contents (*Buchard et al., 2021*)
- CHKA is up-regulated exclusively in MASLD-HCC-F0F1

Thank you for your attention

Thanks again for Dr. Aicha Demidem, Dr. Fatima Dahboul, and Pr. Armand Abergel



The study was approved by the Ethic Committee Sud-Est VI Clermont-Ferrand (Agreement number AU887, 04/03/2011)

Fare 2022

