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Identification of pre-diagnostic lipid sets associated with liver cancer risk using untargeted lipidomics and chemical set analysis – a nested case-control study within the ATBC cohort

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Abstract

In pre-disposed individuals, a reprogramming of the hepatic lipid metabolism may support liver cancer initiation. We conducted a high-resolution mass spectrometry based untargeted lipidomics analysis of pre-diagnostic serum samples from a nested case-control study (219 liver cancer cases and 219 controls) within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study. Out of 462 annotated lipids, 158 (34.2%) were associated with liver cancer risk in a conditional logistic regression analysis at a false discovery rate (FDR) < 0.05. A chemical set enrichment analysis (ChemRICH) and co-regulatory set analysis suggested that 22/28 lipid classes and 47/83 correlation modules were significantly associated with liver cancer risk (FDR <0.05). Strong positive associations were observed for monounsaturated fatty acids (MUFA), triacylglycerols (TAGs), and phosphatidylcholines (PCs) having MUFA acyl chains. Negative associations were observed for sphingolipids (ceramides and sphingomyelins), lysophosphatidylcholines, cholesterol esters and polyunsaturated fatty acids (PUFA) containing TAGs and PCs. Stearoyl-CoA desaturase enzyme 1 (SCD1), a rate limiting enzyme in fatty acid metabolism and ceramidases seems to be

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Author contributions

Conceptualization and methodology (all authors). Data curation (DKB, JLP, AAF, SJW, KAM). Investigation (DKB, JLP, MLR, BIG, KAM). Software (DKB, OF, JLP, WAW, MLR). Formal analyses (DKB, JLP, MLR, KAM). Visualization (DKB, JLP, MLR). Resources (AAF, SJW, OF, DA, KAM). Writing-original draft (DKB, JLP, MLR, BIG, KAM). Writing-review and editing (all authors). The work reported in the paper has been performed by the authors, unless clearly specified in the text.

Conflict of interest: DKB has been a consultant for Brightseed Bio Inc, California. No potential conflicts of interest were disclosed by other authors.

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critical in this reprogramming. In conclusion, our study reports pre-diagnostic lipid changes that provide novel insights into hepatic lipid metabolism reprogramming may contribute to a pro-cell growth and anti-apoptotic tissue environment and, in turn, support liver cancer initiation.

Keywords

Liver cancer; lipidomics; ATBC study; ChemRICH; metabolic reprogramming; ceramides

1. Introduction:

Liver cancer is the sixth most frequently occurring cancer in the world and the third most common cause of cancer mortality.[1] The 5-year survival rate is poor, at only 18%.[2] In the US, liver cancer incidence rates have started to decline,[3] but liver cancer remains the sixth leading cause of cancer-related death.[2] Etiological factors that contribute to chronic hepatic inflammation are major liver cancer risk factors, including chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, consumption of aflatoxin contaminated foods, excessive alcohol consumption, smoking, and metabolic conditions, including metabolic syndrome, obesity, diabetes and non-alcoholic fatty liver disease (NAFLD).[4] Treatment options for liver cancer are limited as most cases are diagnosed at advanced stages. Curative approaches such as surgical resection, liver transplantation and tumor ablation are more suitable for early-stage liver cancer and can boost the 5-year survival rate to ~50%.[5] To improve the primary prevention strategies for liver cancer, in addition to identifying preventable risk factors, there is a need to characterize the pre-diagnostic molecular pathways that may contribute to liver cancer etiology.

Blood-based biomarkers can provide clues to liver biology prior to the development of liver cancer. Several circulating molecules, such as lipids, are routinely used as a proxy to measure liver health status and whether there are dysregulated metabolic pathways. Lipids are a class of hydrophobic or amphipathic molecules that have biological functions in energy storage, biological signaling, and cell membrane production.[6] Dysregulated lipid metabolism has been implicated in cancer development, including liver cancer.[7–9] Lipids can influence cancer risk through effects on oxidative stress, insulin signaling, and inflammation or through creating and supporting a pro-oncogenic environment.[9, 10] Hepatic lipids have also been reported to be disturbed in liver diseases[11] associated with an increased risk of HCC, such as NAFLD, alcoholic liver disease and cirrhosis, suggesting that specific lipid metabolic changes might also contribute to HCC etiology. Examination of the circulating lipidome may provide unique insights into the complex metabolic pathways and pre-diagnostic biochemical processes in liver cancer.[12]

Previous prospective epidemiologic studies have examined metabolomic panels in relation to HCC risk, identifying several associated lipids.[13–15] However, none of the panels were optimized to detect lipids, thus lacked many circulating lipid classes, such as triacylglycerols or cholesteryl esters, that are more hydrophobic and neutral.[16, 17] A comprehensive analysis of the circulating lipidome requires optimized protocols for lipid extraction, chromatography separation, mass spectrometry data collection and lipid annotation.[17, 18] To date, no

untargeted lipidomic examination of liver cancer etiology has been reported. Thus, we conducted a nested case–control study using pre-diagnostic serum samples to evaluate associations between relative concentrations of circulating lipids and liver cancer risk using an untargeted lipidomics assay and chemical set enrichment analysis.

2. Materials and methods

2.1 Study Population.

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study was a randomized controlled trial to test the effects of α-tocopherol and β-carotene on cancer incidence among male smokers.[19] Males aged 50–69 years, who smoked at least five cigarettes per day, were enrolled between 1985 and 1988 in southwestern Finland (n=29,133). Potential participants were excluded if they reported prevalent cancer (other than non-melanoma skin cancer), cirrhosis, chronic alcoholism, or other conditions that would limit their participation in the trial. At study entry, participants provided a blood sample and completed a detailed questionnaire about demographics, medical history, diet, and lifestyle factors. The trial concluded in 1993, but participants continued to be followed for cancer incidence.

2.2 Outcome Assessment.

For the current analysis, ATBC participants were followed through December 31, 2015, for incident cancer via linkage with the Finnish Cancer Registry. The proposed study includes 224 confirmed cases of primary liver cancer (defined based on the International Classification of Diseases, 9th revision (ICD-9); codes 155.0 and 155.1), who had an available serum sample. Controls were matched at a 1:1 ratio with cases based on age at randomization (±5 year), date of blood collection (± 30 days), number of freeze-thaw cycles, and laboratory where prior aliquoting was conducted, if applicable.[20] Controls were selected from among individuals with available serum samples who were cancer-free at the time of their matched case's diagnosis.

2.3 Laboratory Methods.

Baseline visit blood samples were collected from ATBC participants after an overnight fast (usually at least 12 hours).[19] Blood samples were centrifuged and serum was aliquoted and stored at −70°C. The lipidome analysis was performed at the West Coast Metabolomics Center (University of California Davis Genome Center), using 50μL of serum as previously described.[21] Briefly, complex lipids were semi-quantified using an untargeted approach by liquid chromatography with quadrupole time of flight mass spectrometry (LC-QTOF-MS). Internal standards were used for the calibration of retention times. A total of 462 unique, annotated lipid species were identified, after removing poorly detected and duplicate signals. Raw peak heights were normalized using systematic error removal using random forest (SERRF) to eliminate unwanted systematic variation.[22] The major lipid classes covered included acylcarnitines (AC), ceramides (CER), cholesterol esters (CE), diacylglycerols (DG), fatty acids (FA), lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), plasmalogen phosphatidylcholines (plasmPC), plasmalogen phosphatidylethanolamines (plasmPE), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylcholines (PI),

sphinhomyelins (SM), and triacylglycerols (TAG) (Figure 1). Five case-control pairs were excluded because lipidomics measurements were not available for them, resulting in a dataset of 219 case-control pairs.

2.4 Statistical Analysis.

Conditional logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of the association between lipid species and liver cancer, adjusting for education (elementary school or less, no vocational training; elementary school or less, vocational training; more than elementary school), body mass index (<25, 25 to <30, ≥30 kg/m^2), history of diabetes at baseline, age at baseline (<=54, >=55 to <=59, >=60 to <= 64, >= 65 years), cigarette smoking (<25, 25 to 34, 35 to 44, ≥45 pack-years), and alcohol intake (0, >0 to <1, 1 to <2, ≥2 drinks/day). For analysis, the lipids were modeled continuously, comparing the 90th percentile with the 10th percentile of log-transformed lipid intensity (i.e., OR = $e^{\beta(X90-X10)}$ where β is the coefficient for the lipid species modeled continuously and X_{90} and X_{10} are lipid values at the 90th and 10th percentiles, respectively). P-values were adjusted using the Benjamini-Hochberg false discovery rate (FDR) procedure for controlling the FDR at level 0.05.[23]

The ORs and unadjusted P-values of the lipid species were used as input for the chemical similarity enrichment analysis using ChemRICH.[24, 25] ChemRICH uses the structure of lipid headgroups and the degree of saturation in acyl chains [saturated fatty acids (SFA)=0, monounsaturated fatty acids (MUFA)=1, and polyunsaturated fatty acids (PUFA)≥2] to cluster lipids into non-overlapping chemical groups. At least three lipids were required to form a cluster. Lipids are poorly represented in canonical biochemical pathway databases, so a traditional pathway analysis approach is prone to ignore many lipid classes with several statistically significant lipid species. Therefore, we chose a lipid set enrichment analysis using the ChemRICH software[24] to identify the significant lipid classes that were associated with liver cancer risk. ChemRICH compares the observed p-value distribution for a lipid set against a uniform p-value distribution with an underlying hypothesis that a non-significant class will follow a uniform p-value distribution. ChemRICH uses a Kolmogorov–Smirnov (KS) test to compare these p-value distributions and does not rely on a background database, thus ChemRICH includes all the annotated lipids in the set analysis. Using this reference distribution, KS tests were used to determine lipid clusters where P-values showed sufficient evidence of departure from the null distribution.[26] ChemRICH P-values were adjusted using the Benjamini-Hochberg (BH) procedure for false discovery rate control at level 0.05.[27]

3. Results

3.1 Cohort detail

Baseline characteristics of cases and controls are shown in Table 1. Compared to controls, cases were more likely to have greater than an elementary school education, to be obese [BMI(kg/m^2) ≥30], to have a history of diabetes, and to smoke and drink more heavily. The age distribution (mean =57 years) was similar between cases and controls. The median time between the baseline and liver cancer diagnosis was 16 years.

3.2 Untargeted lipidomics dataset.

The untargeted lipidomics dataset contained 552 annotated and 2,712 unidentified lipid species. Results for only the annotated lipids are shown in this report. Because the data were generated using both electrospray ionization negative and positive modes, 75 annotated lipid species were detected redundantly. We removed those 75 duplicated lipid species based on analysis of technical reproducibility using quality control samples. We also removed an additional 15 lipid species because of their low analytical reproducibility—a relative standard deviation (RSD) less than 20% in the pooled quality control samples (n=49). Mean RSD for 24 labelled internal standards lipids was 1.7%. The filtered dataset contained 462 annotated lipids from 28 lipid classes belonging to 6 super classes (**Figure 1 and** Table S1).

3.3 Identification of individual lipids associated with liver cancer risk.

Of the total 462 annotated lipids, 158 (34.1%) were significantly associated (FDR-adjusted p-value < 0.05) with HCC risk. Out of these significant lipids, 83 were positively and 75 were negatively associated with risk. The strongest positive association was for PC 42:6 with an odds ratio (OR) of 11.3 (95% CI, 4.52–28.57). The strongest negative association was for SM d40:2 B with an OR of 0.06 (95% CI, 0.02–0.15). The top five lipid classes with the highest number of significant lipids were UFA-TAG (20, 43%), CER (15, 57.7%), UFA-SM (14, 70%), SM (12, 80%), and UFA-PC (9, 27.3%). Twenty-three lipid classes had at least two lipids that were associated with liver cancer risk (Figure 2). Table S1 contains results of conditional logistic regression analyses for all 462 annotated lipids.

3.4 Identification of lipid sets associated with liver cancer risk.

Next, we sought to conduct a functional and structural set enrichment analysis to interpret the statistically significant individual lipids. ChemRICH analysis revealed that, out of 28 total lipid classes, 17 were significantly associated with liver cancer risk at an FDR cutoff of 0.05. The top five significantly associated lipid classes were UFA-TAG (p=2.66e-15), SM (p=3.18e-12), UFA-SM (p=5.81e-12), SFA-TAG (p=1.03e-11), and MUFA-TAG (p=3.29e-11). Table 2 contains the results of the ChemRICH analysis and Figure 3 shows the individual lipids within each class. UFA-TAG and UFA-PC were the most positive while CER and UFA-SM were the most negatively associated with liver cancer risk.

3.5 Identification of co-regulatory lipid sets associated with liver cancer risk.

We observed that there were associations in both positive and negative directions within few lipid classes in the ChemRICH enrichment analysis, indicating a sub-class level regulation of lipid metabolic pathways. To identify these specifically coregulated lipids, we conducted a co-regulatory set analysis[28] using a pair-wise correlation matrix among all the individual lipids. A correlation module is a group of lipids whose levels show stronger and distinct inter-chemical correlation patterns, reflecting a sub-class. It complements the chemical structure-based grouping of lipids. A total of 83 co-regulatory lipid modules were detected by this approach. Of those, 47 modules were significantly associated (FDR < 0.05) with liver cancer risk in a set analysis using the same p-value distribution comparison approach, based on use of a KS-test, that was used for the ChemRICH analysis (Table 3).

3.6 Sensitivity analysis:

Results for individual, chemical class and co-regulatory set analyses did not change after excluding the cases diagnosed within 5 years of study randomization (Table S1–S3).

4. Discussion

Improved understanding of the hepatic metabolic reprogramming that may predispose individuals to liver cancer initiation can enable novel prevention strategies.[29, 30] In our analysis, we found that several lipid classes were strongly and significantly associated with the development of liver cancer, suggesting that a pro-cancerous modification in lipid biochemistry may increase the initiation of hepatocarcinogenesis. Overall, we have found that smoking men who go on to develop liver cancer have more saturated and mono-unsaturated triacylglycerols and phospholipids and fewer sphingolipids in circulation. These patterns can support a pro-proliferation state in the liver which may, in turn, support liver cancer initiation. These results, for the first time, implicate stearoyl-CoA desaturase (SCD1) and the ceramidase enzymes in pro-hepatocarcinogenesis metabolic programming in liver.

4.1 Epidemiologic evidence:

In comparison to prior epidemiology studies,[14, 29–41] the majority of which have utilized generic and broad metabolic extraction and data collection protocols, this study, to the best of our knowledge, is the first liver cancer study to characterize the pre-diagnostic circulating lipidome using an untargeted lipidomics method. We tested over 400 annotated lipids from 28 lipid classes using chemical set enrichment analysis methods to identify structural and functional lipid sets that were associated with liver cancer. In prior prospective cohort studies from Europe and Asia, higher pre-diagnostic levels of bile acids[13, 14, 34, 38, 39] and triglycerides[35, 36] have been shown to increase risk, suggesting changes in liver metabolic pathways are perturbed to create a pro-carcinogenic environment.

A recent report from an international, multicenter study used a broad metabolomic profile, comprising 1,295 metabolites, in 295 patients to examine differences between NAFLD-associated HCC (n=43), alcohol and viral hepatitis-associated HCC (n=32), NAFLD cases (n=130) and healthy controls (n=44).[37] The study reported that NAFLD-associated HCC was characterized by a rearrangement of the serum lipidome, which distinguished NAFLD-associated HCC from the other patients. Further, the study showed serum PUFA depletion in the NAFLD-associated HCC cases.[37] Herein, we documented an inverse association between PUFA lipids (acylglycerols, cholesterol esters and phospholipids) and liver cancer risk.

Our study also observed a notable link between sphingolipid metabolism and liver cancer risk. In prior epidemiologic studies, ceramides and sphingomyelins have been positively associated with the risk of NAFLD,[42, 43] CVD,[42, 43] and diabetes.[44, 45] Two prior studies from the European Prospective Investigation into Cancer and Nutrition cohort identified an inverse association between sphingomyelin levels and liver cancer risk, using an untargeted metabolomic platform (118 metabolites, n=121 HCC cases)[40] and a targeted platform (132

metabolites, n=147 HCC cases).[41] These results suggest that a disturbed sphingolipids metabolism may be a critical event in hepatocarcinogenesis.

4.2 Mechanistic evidence:

Mechanistically, our results can be interpreted for lipids that promote cell survival and growth and that suppress cell death mechanisms (Figure 4).

4.2.1 Cell growth and survival promotion.—A cancer cell needs building blocks to support its rapid growth, thus a tissue microenvironment with higher de-novo lipid production can favor growth.[9, 46] In this study, we observed that MUFA and associated lipids are positively linked with liver cancer. This pattern points toward an increase in activity of the Stearoyl-CoA desaturase 1 (SCD1) enzyme in liver cells to convert SFA to MUFA, which are then converted into PUFA to create lipids that support membrane fluidity (Figure 4). MUFA fatty acids are the preferred substrate for membrane lipid synthesis. SCD1 is a rate limiting enzyme and has been shown to be critical for rapid cell growth, cancer cell initiation, cell survival and malignant transformation.[47] Inhibition of SCD1 by aramchol, a targeted inhibitor that is currently in a phase 3 trial, or other candidate drugs, has been shown to slow lipid production in the livers of persons with NAFLD and the most severe form of NAFLD, non-alcoholic steatohepatitis (NASH).[48, 49] SCD1 inhibition may also promote cell death by increasing cellular stress imposed by SFA and associated lipids.[50] This evidence base, and our results, underscore the roles of SCD1 in hepatic metabolic programming that may support liver cancer initiation.

4.2.2 Anti-apoptotic mechanisms.—Our observations that ceramides are inversely associated with liver cancer risk may be related to their pro-apoptotic properties. It is more favorable for cancer cells when the generation of pro-apoptotic molecules are suppressed. Ceramides can be converted to sphingosine 1 phosphate (S1P) and sphingosine via ceramide catabolic pathway that involves N-acylsphingosine amidohydrolase (ASAHs) and Alkaline ceramidases (ACERs) enzymes.[51] They are also precursors for sphingomyelins biosynthesis. Ceramides are known to induce apoptotic mechanisms[52] whereas S1P promotes cell growth.[53] Negative associations with both ceramides and sphingomyelins suggest that ceramides are probably diverted to the catabolic pathway to generate more pro-growth signaling molecules. Blocking of key enzymes, such as sphingosine kinase 2 (SphK2)[54] and S1P lyase (SPL)[55] in sphingolipids metabolism,[53] has also been shown to block liver cancer growth in cell and animal bioassays. Our results point to a higher activity of ceramidases in liver that can serve to both promote cell survival and to suppress the ceramide dependent apoptotic cascade.

4.3. Limitations and strengths:

Our study had notable strengths, a major one being that it is the largest and most comprehensive lipidomics study to date on liver cancer risk. The ATBC cohort collected blood prospectively from all participants after an overnight fast. For studying endogenous lipids in particular, fasting samples are preferred, as dietary factors are known to affect detected lipid metabolite levels[56] and reproducibility of results.[57] In addition, for the current analysis, individuals enrolled in the cohort were followed up to 30 years, which

has yielded a substantial number of liver cancer cases (n=224). Blood samples and covariate data were collected from the cohort at baseline, when height and weight were measured by a trained staff member. As we proposed to determine if ceramides accounted for the increased risk of liver cancer, having accurate anthropometry measures was crucial given the known relationships between obesity and lipid metabolism.[51, 58] We utilized a chemical set enrichment analysis approach which is a more sensitive approach in comparison to a traditional pathway analysis to facilitate the interpretation of all significant lipid associations with liver cancer risk. By utilizing this approach for a comprehensive, untargeted lipidomics dataset in a nested case-control study, we have identified the perturbed lipid pathways in the hepatic lipid metabolism reprogramming that play a significant role in liver cancer etiology. In addition to these strengths, there were also several limitations. The study only included smoking men and the liver cancer cases were identified using ICD codes so the findings could not be associated with liver cancer histologic subtypes. In addition, we did not have access to clinical data, although men with self-reported cirrhosis were not eligible to participate in the trial.

5. Conclusions

In this prospective study of male smokers, we observed significant, inverse associations between sphingolipids and direct associations between MUFA-containing lipids and liver cancer development. These results suggest that a hepatic lipid metabolism reprogramming, resulting in lower circulating levels of sphingolipids and higher MUFA-containing lipids, may predispose individuals to liver cancer. The underlying biochemical reactions and enzymes involved in this reprogramming expands our understanding of roles of endogenous molecular pathways in liver cancer etiology. Although our results are strong in terms of effect size, replications of these findings in other nested case control studies using the untargeted lipidomics methods will increase the strength of evidence to implicate hepatic metabolic reprogramming in liver cancer etiology. Future studies are also needed to detangle the pre-diagnostic lipid metabolic reprogramming specific to different subtypes of liver cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement:

The mass spectrometry-based raw lipidomics data generated in this study are available in the Metabolomics WorkBench database (www.metabolomicsworkbench.org) with accession

number ST002764. Other data that support the findings of this study are available from the corresponding author upon request and approval of a research proposal, and subsequent completion of a Data Transfer Agreement. Proposals can be submitted here: https://atbcstudy.cancer.gov/ptsa/

Abbreviations

AC	Acylcarnitine
ALD	Alcoholic Liver Disease
ATBC	Alpha-Tocopherol, Beta-Carotene Cancer Prevention study
CE	Cholesterol esters
CER	Ceramides
CVD	Cardiovascular disease
DG	Diacylglycerols
FA	Fatty acids
HCC	Hepatocellular carcinoma
LC-QTOF-MS	Liquid Chromatography quadrupole time-of-flight mass spectrometry
LPC	Lysophosphatidylcholines
LPE	Lysophosphatidylethanolamines
MUFA	Monounsaturated Fatty Acids
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
PC	Phosphatidylcholines
PE	Phosphatidylethanolamines
PI	Phosphatidylinositol
p-PC	Plasmalogen phosphatidylcholines
p-PE	Plasmalogen phosphatidylethanolamines
PUFA	Polyunsaturated Fatty Acids
RSD	Relative Standard Deviation
SCD1	Stearoyl-CoA desaturase 1
SERRF	Systematic error removal by random forest

SFA	Saturated Fatty Acids
SM	Sphingomyelins
TAG	Triacylglycerols
UFA	Unsaturated Fatty Acids

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What's new?

Hepatic lipid metabolic reprogramming is a critical hallmark in liver cancer etiology. In this study, untargeted lipidomics, paired with lipid set enrichment analysis found several circulating lipid classes and functional modules that are associated with liver cancer risk in the ATBC cohort. MUFA-containing lipids showed a strong positive association and PUFA-containing lipids showed an inverse association with liver cancer risk. Sphingolipids (ceramides and sphingomyelins) were also negatively associated with liver cancer risk, an opposite trend than observed for metabolic diseases (diabetes, non-alcoholic fatty liver disease, and cardiovascular disease). These lipids newly associated with liver cancer may advance our understanding about pro-hepatocarcinogenesis lipid perturbations and can be useful for improving the primary prevention strategies for liver cancer.

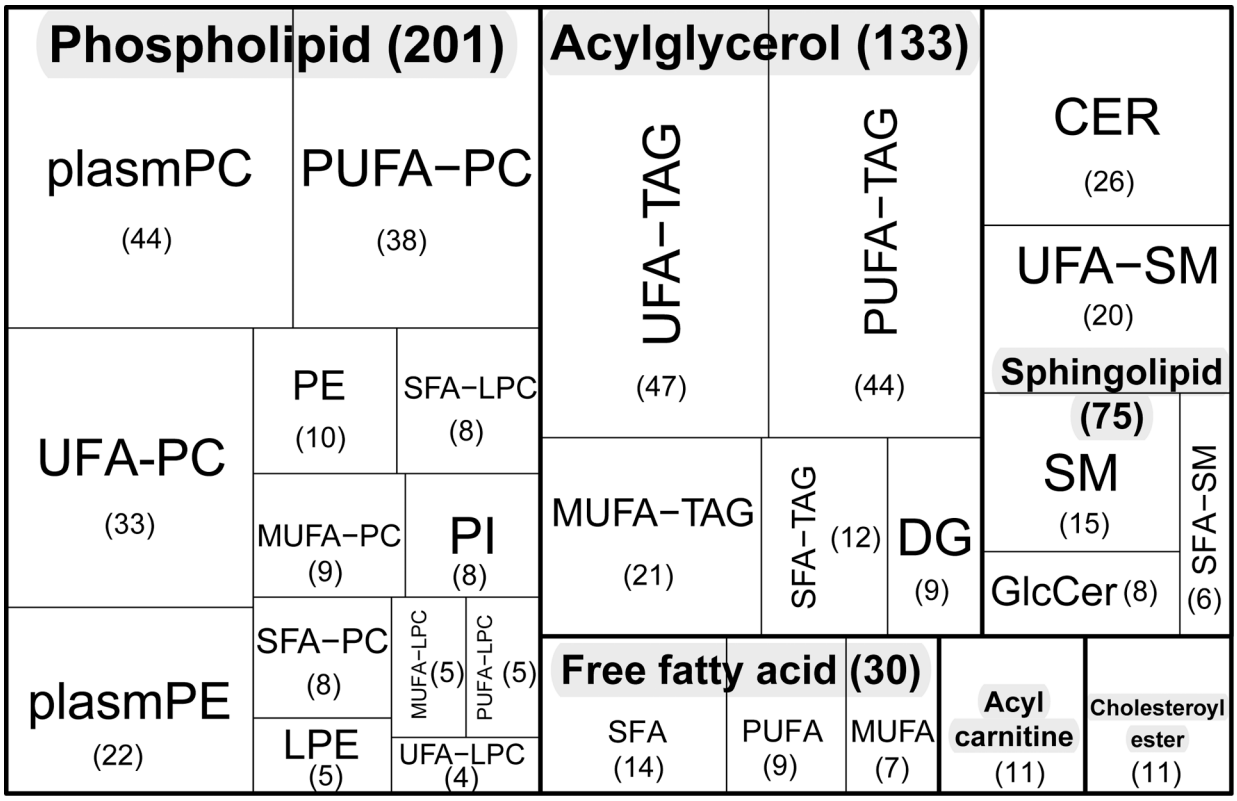

Figure 1.

Overview of detected lipid classes by the untargeted lipidomics assay. Shaded labels are major lipid categories. The number is parentheses indicate the lipid species count. UFA-TAG (Triacylglycerol) ,SM (Sphingomyelin),UFA-SM (Sphingomyelin),SFA-TAG (Triacylglycerol),MUFA-TAG (Triacylglycerol),CER (Ceramide),CE (Cholesterol ester),UFA-PC (Phosphatidylcholine),plasmPC (Plasmalogen phosphatidylcholine),PUFA-TAG (Triacylglycerol),UFA-LPC (Lysophosphatidylcholine),MUFA (Monounsaturated fatty acid),MUFA-PC(Phosphatidylcholine),PUFA-PC (Phosphatidylcholine),PUFA-LPC (Lysophosphatidylcholine),SFA-LPC (Lysophosphatidylcholine),PE (Phosphatidylethanolamine),AC (Acylcarnitine),SFA-PC (Phosphatidylcholine),DG (Diglyceride),PI (Phosphatidylinositol), GlcCer (Glucosylceramide), MUFA(monounsaturated fatty acid), PUFA(polyunsaturated fatty acid), SFA(saturated fatty acid), UFA(unsaturated fatty acid).

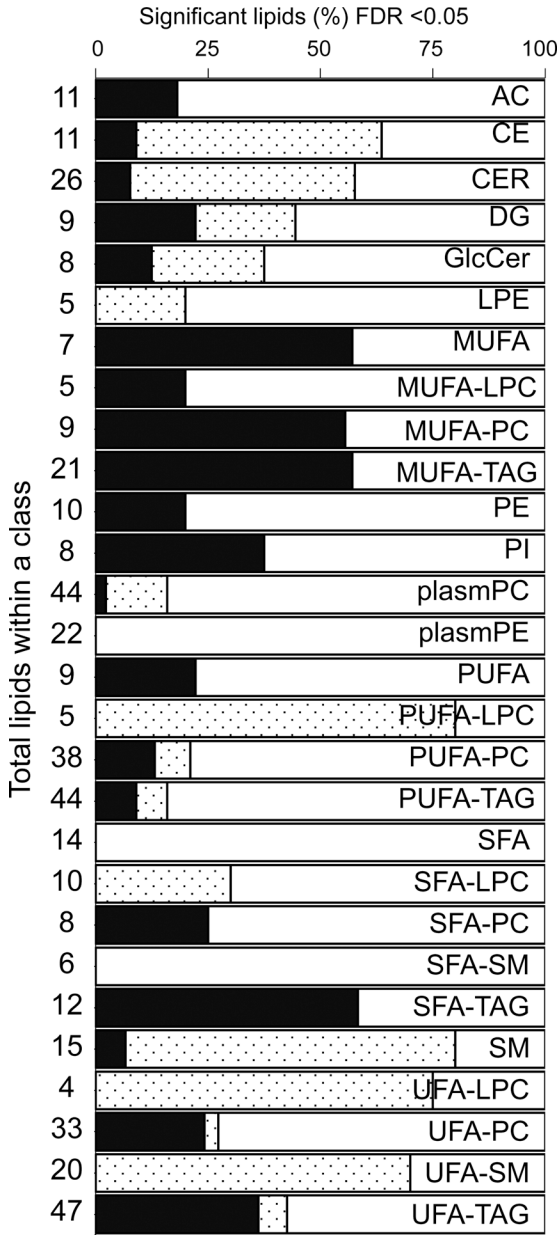

Figure 2.

Proportion of lipids significantly associated (FDR-adjusted p-value < 0.05) with liver cancer risk within each lipid class. Black means positive, dotted means negative, and white means null associations.

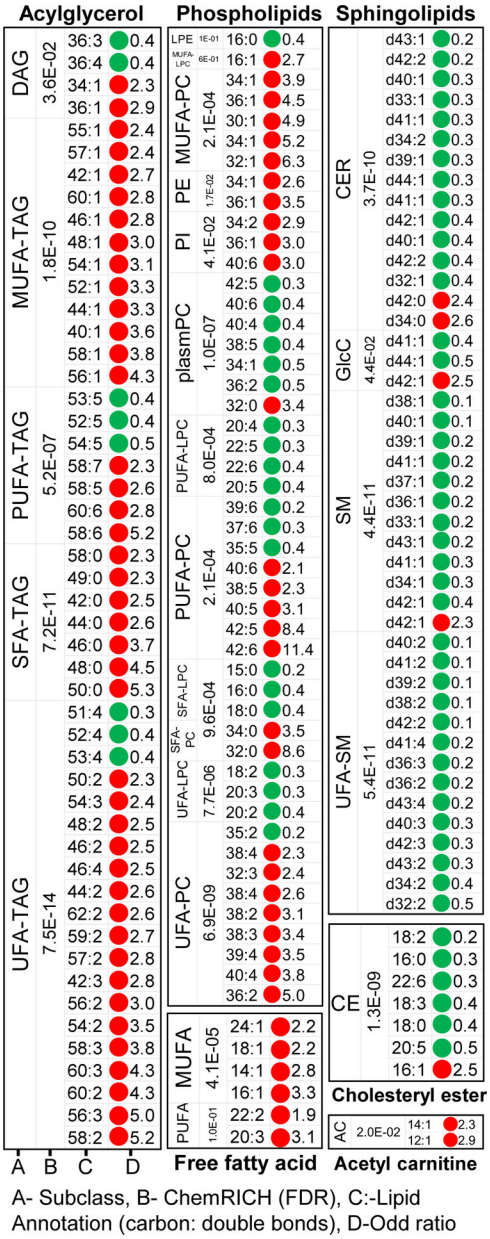

Figure 3.

Significantly associated individual lipids within classes. Red color indicates positive, and the green color indicates negative associations.

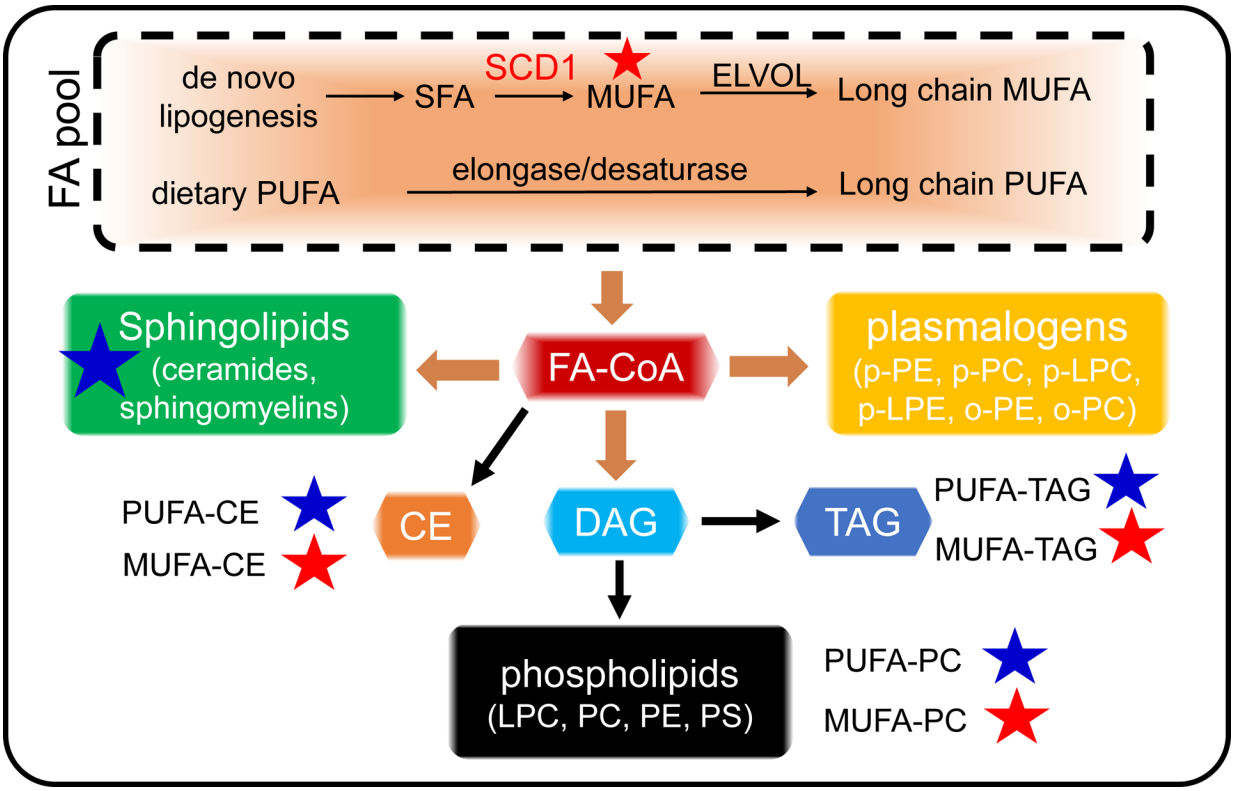

Figure 4.

An overview of major lipid metabolic pathways in liver. Blue stars show the lipid classes negatively associated with liver cancer risk whereas red stars are positive ones.

Table 1.

Participant characteristics in a nested case-control study of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) cohort.

	Cases	Controls
	(n=219)	(n=219)
Age at randomization (%)		
≤ 54	61 (27.9)	65 (29.7)
55 – 59	90 (41.1)	99 (45.2)
60 – 64	48 (21.9)	42 (19.1)
≥ 65	20 (9.1)	13 (5.9)
Body mass index (kg/m^2) (%)		
< 25	61 (27.8)	84 (38.3)
25 – <30	108 (49.3)	106 (48.4)
≥ 30	50 (22.8)	29 (13.2)
Education (%)		
Elementary school or less, no vocational training	45 (20.5)	69 (31.5)
Elementary school or less, vocational training	112 (51.1)	104 (47.9)
More than elementary school	62 (28.3)	45 (20.5)
Cigarette smoking (pack-years) (%)		
< 25	52 (23.7)	70 (31.9)
25 – 34	44 (20.0)	51 (23.2)
35 – 44	49 (22.3)	52 (23.7)
≥ 45	74 (33.7)	46 (21)
Drinks of alcohol (per day) (%)		
0	14 (6.3)	14 (6.3)
>0 – <1	68 (31.0)	105 (47.9)
1 – <2	60 (27.4)	54 (24.7)
≥ 2	62 (28.3)	35 (16.4)
missing	15 (6.8)	10 (4.6)
Diabetes (%)		
No	198 (90.4)	214 (97.7)
Yes	21 (9.6)	5 (2.3)

Table 2.

ChemRICH set enrichment analysis to identify lipid set associated with liver cancer risk.

Lipid Class	p-value (ChemRICH)	FDR (ChemRICH)	Significant lipids (FDR < 0.05)	Significant lipids (%)	Set Size	Positive associations (FDR <0.05)	Negative Associations (FDR < 0.05)
UFA-TAG (Triacylglycerol)	2.7E-15	7.5E-14	20	43%	47	17	3
SM (Sphingomyelin)	3.2E-12	4.4E-11	12	80%	15	1	11
UFA-SM (Sphingomyelin	5.8E-12	5.4E-11	14	70%	20	0	14
SFA-TAG (Triacylglycerol)	1.0E-11	7.2E-11	7	58%	12	7	0
MUFA-TAG (Triacylglycerol)	3.3E-11	1.8E-10	12	57%	21	12	0
CER (Ceramide)	7.9E-11	3.7E-10	15	58%	26	2	13
CE (Cholesterol ester)	3.2E-10	1.3E-09	7	64%	11	1	6
UFA-PC (Phosphatidylcholine	2.0E-09	6.9E-09	9	27%	33	8	1
plasmPC (Plasmalogen phosphatidylcholine)	3.2E-08	1.0E-07	7	16%	44	1	6
PUFA-TAG (Triacylglycerol)	1.9E-07	5.2E-07	7	16%	44	4	3
UFA-LPC (Lysophosphatidylcholine)	3.0E-06	7.7E-06	3	75%	4	0	3
MUFA (Monounsaturated fatty acid)	1.8E-05	4.1E-05	4	57%	7	4	0
MUFA-PC(Phosphatidylcholine)	9.8E-05	2.1E-04	5	56%	9	5	0
PUFA-PC (Phosphatidylcholine)	1.1E-04	2.1E-04	8	21%	38	5	3
PUFA-LPC (Lysophosphatidylcholine)	4.3E-04	8.0E-04	4	80%	5	0	4
SFA-LPC (Lysophosphatidylcholine)	5.5E-04	9.6E-04	3	30%	10	0	3
PE (Phosphatidylethanolamine)	1.0E-02	1.7E-02	2	20%	10	2	0
AC (Acylcarnitine)	1.3E-02	2.0E-02	2	18%	11	2	0
SFA-PC (Phosphatidylcholine)	2.2E-02	3.3E-02	2	25%	8	2	0
DG (Diglyceride)	2.6E-02	3.6E-02	4	44%	9	2	2
PI (Phosphatidylinositol)	3.1E-02	4.1E-02	3	38%	8	3	0
GlcCer (Glucosylceramide)	3.5E-02	4.4E-02	3	38%	8	1	2

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid, UFA, unsaturated fatty acid.

Table 3.

Co-regulatory lipid modules associated with liver cancer risk.

Module	Size	FDR	Lipids	DIR
M10	9	1.8E-18	SM (36:1,37:1,38:1,39:1,40:1,41:1,41:2,41:4,43:4)	↓
M52	4	9.2E-15	SM (36:3,38:2,40:2 B,40:3)	↓
M54	4	1.0E-12	Cer 43:1 SM (43:1, 43:2,41:1)	↓
M18	7	6.1E-12	DG (34:1,36:1) TAG(52:1,54:1,54:2,56:2,56:3)	↑
M36	5	7.1E-11	TAG (44:0,44:1,46:0,48:0,50:0,40:0)	↑
M12	9	1.2E-10	TAG(54:0,55:1,57:1,57:2,58:0,59:2,59:3,60:1,62:2)	↑
M11	9	1.7E-10	TAG (56:1,58:1,58:2,58:3,58:4,58:5,60:2,60:3,60:4)	↑
M25	7	9.0E-08	PC (40:5 B,38:2,40:4,42:5,42:6) TAG (46:5,60:6)	↑
M61	4	2.1E-07	Cer 34:2 SM(32:2,34:2,36:2)	↓
M69	4	3.2E-07	Cer 44:1, GlcCer14:14E/20:02OH,SM(42:1, 42:2 B)	↓
M13	8	9.7E-07	CE 16:1, LPC 16:1, PC (32:1,34:1,36:1,36:4 B,38:4 B) PI 36:1	↑
M9	9	1.0E-06	Cer (38:1,39:1,40:1,40:2,41:1,38:1,40:1,41:1) SM 40:4	↓
M43	5	5.3E-06	PC 28:0,PC 30:0,PC 30:1,PC 32:0,PC 32:3	↑
M20	7	9.3E-06	FA (14:0,14:1,16:0,16:1,18:1,20:1,20:3)	↑
M2	10	1.4E-05	DG (36:3,36:4 A) TAG(51:5,52:3,52:4,52:5,54:4,54:5 A,54:6 A,54:7 A)	↓
M65	4	1.4E-05	plams PC(34:1e,38:4e,32:0,32:0)	↑
M26	6	1.6E-05	TAG(40:0,40:1,42:0,42:1,42:2,44:2)	↑
M73	3	1.0E-04	plasmPC(34:2,34:2,36:2)	↓
M31	6	3.5E-04	CE(16:0,18:1,18:2,18:3,20:3,20:4)	↓
M59	4	9.4E-04	LPC(15:0,16:0), plasmLPC(16:0,18:0)	↓
M72	3	9.4E-04	Cer (34:0,40:0,42:0)	↑
M14	8	9.4E-04	Cer (32:1,33:1) SM (30:1,32:0,32:1,33:1,40:2 A,41:2 A)	↓
M45	5	1.1E-03	PC(35:2,33:2,35:2 A,35:2 B,37:2)	↓
M7	10	1.1E-03	plasmPC (38:3,40:3,40:4,34:0e,38:2,38:3,40:3,40:4,42:3) GlcCer d34:1	↓
M17	7	1.1E-03	TAG(45:1,53:1,47:0, 47:0,49:0,50:1,51:0, 51:0,51:1,53:1)	↑
M37	5	1.1E-03	TAG (51:3,51:4,53:3,53:4,53:5)	↓
M40	5	1.5E-03	plasmPC (34:1,36:1,36:2,34:1,36:1)	↓
M83	3	3.7E-03	TAG (62:1, 62:3, 62:4)	↑
M8	9	4.0E-03	TAG(46:1,46:2,47:2, 47:2,48:1,48:2,49:1,49:2,49:3,50:2)	↑
M48	5	7.7E-03	PC 34:0,PC 35:4,PC 37:4,PC 39:4,TAG 55:5 TAG 17:0–18:1–20:4	↑
M60	4	1.1E-02	Cer (42:1,42:2 B,42:1,42:2 B)	↓
M76	3	1.1E-02	PC(37:6,39:6,42:6)	↓
M67	4	1.1E-02	CE 22:6 plasmPC(38:5,38:6,40:6)	↓
M79	3	1.2E-02	PC(34:2,34:3 A,36:3 A)	↑
M3	10	1.3E-02	DG 36:2 PC (35:1,37:3,38:4 A) TAG (51:2,52:2, 52:2,53:2,54:3,55:2,55:3)	↑
M27	6	1.4E-02	AC(10:0,12:0,12:1,14:1,16:0,18:0)	↑
M19	7	1.6E-02	PI 34:2 PC (34:3 C,36:2,36:3 B,38:3,38:5,38:5,38:6 C)	↑

Module	Size	FDR	Lipids	DIR
M71	4	1.7E-02	SM d39:2 CE(18:0,22:2) TAG(52:2,52:2)	↓
M4	10	2.3E-02	TAG(47:1,54:8,56:7 B,56:8, B,56:9,56:9,58:10,58:8,58:9,60:11)	↓
M23	7	2.5E-02	PE (34:1,36:2,36:3,38:4 B,34:2,36:1,38:4)	↑
M1	10	2.6E-02	CE 20:5 LPC 20:5 PC(36:5 B, 34:5,35:5,36:5,36:5D,37:5,38:5 B,38:7)	↓
M53	4	2.6E-02	plasmPC(36:5,38:5 B,36:5,38:5 B)	↓
M39	5	3.8E-02	TAG(42:3,46:3 A,46:4 A,48:4 A,48:5)	↑
M15	7	4.0E-02	LPC(18:0 A,18:0 B,20:1, 20:0, 20:1, 20:2,18:0)	↓
M6	10	4.0E-02	TAG (50:6,50:6,52:6,52:6,53:5,54:6 C,54:7,55:6,58:10,58:7)	↑
M80	3	4.7E-02	PC(40:7,40:7 B,42:7)	↑
M46	5	4.7E-02	LPC (17:1,22:6,18:1,18:3,22:5)	↓

Note: DIR column shows the overall direction of most lipids in the correlation module.