Original Article

Association of lysophosphatidic acid molecules with liver fibrosis: different roles indicated

Hiroshi Tobita,[1,2,*] Hiromichi Sakai,[3,6] Akane Yamaguchi,[3,6] Yoshitomo Notsu,[4,6] Masatoshi Kataoka,[1,2] Tomotaka Yazaki,[1,2] Toru Nabika,[5,6] Shunji Ishihara,[2] and Hironori Kobayashi[4,6]

[1]Division of Hepatology, [2]Department of Internal Medicine II, [3]Interdisciplinary Center for Science Research, [4]Central Clinical Laboratory, and [6]Metabolizumo Project, Shimane University Hospital, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan
[5]School of Medicine, Shimane University, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan

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Lysophosphatidic acid is composed of lysophosphatidic acid (LPA) molecules with varied chemical forms. The present cross-sectional study was conducted to investigate the associations of various LPA molecules with liver fibrosis. Forty-six patients affected by various types of liver disease who underwent an ultrasound-guided liver biopsy were recruited for this study. Liver fibrosis was evaluated using histological grading, as well as shear wave velocity (Vs) and serum level of type IV collagen 7S (T4c7s). Serum levels of LPA molecules were determined using liquid-chromatography tandem mass-spectrometry (LC-MSMS). Total LPA showed a significant positive association with fibrosis severity evaluated based on histological grading, Vs, and T4c7s used as parameters, following adjustment for other confounding factors, including disease type, age, gender, body mass index, and high-sensitivity C-reactive protein. This association was replicated when 16:0-LPA was substituted for total LPA. In contrast, when 20:4-LPA was substituted for total LPA, no significant association with liver fibrosis was observed. In conclusion, the degree of association varied among the different LPA molecule chemical forms, suggesting different pathophysiological roles of individual LPA molecules, although total LPA concentration was shown to be associated with liver fibrosis.

***Key Words*: lysophosphatidic acid, lipid-chromatography tandem mass-spectrometry, autotaxin, liver fibrosis**

Among biochemical markers known to be associated with liver fibrosis, autotaxin (ATX) has attracted great interest from clinicians and researchers. ATX is a serum protein with lysophosphodiesterase D activity that hydrolyzes lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA).[1] Sinusoidal endothelial cells are known to uptake and degrade serum ATX,[2] and endothelial damage due to liver fibrosis increases its level in serum, thus it is considered to be a good marker of liver fibrosis.[3] In addition, ATX may play a unique role in liver fibrosis because of its production of LPA, known to be a biologically active signaling molecule, which may result in promotion of liver fibrosis as well as oncogenesis.[4] In fact, several reports have found that LPA accelerates fibrosis in various organs by stimulation of LPA receptors.[5]

Each LPA molecule has an acyl chain, though there are differences in terms of chain length and also degree of saturation. Accordingly, this group of molecules is known to have different chemical forms that may demonstrate various biological roles.[6] Nevertheless, the structural variety among LPA molecules has not been well considered in most investigations related to liver disease.

In the present study, a method for quantification of different chemical forms of LPA, as well as LPC, lysophosphatidylethanolamine (LPE), and lysophosphatidylinositol (LPI), using liquid-chromatography tandem mass-spectrometry (LC-MSMS) was established for examination of their potentially different roles in progression of liver fibrosis. The results showed that while total LPA was correlated with severity of liver fibrosis as well as serum ATX level, some forms of LPA molecules did not demonstrate such correlations. The importance of these observations is also discussed.

Materials and Methods

Patients. All patients who underwent an ultrasound-guided liver biopsy examination at Shimane University Hospital between April 2017 and March 2020 were invited to participate, of whom 46 agreed and provided written informed consent. The biopsy results led to a diagnosis of non-alcoholic fatty liver disease (NAFLD) in 16, autoimmune hepatitis (AIH) with or without primary biliary cholangitis (PBC) in 5, PBC in 3, alcoholic steatohepatitis (ASH) in 3, primary sclerosing cholangitis in 1, chronic viral hepatitis type B in 1, drug-induced liver injury in 6, and no diagnosis in 11 patients. Because of the limited number of participants, they were divided into three groups based on disease types, i.e., NAFLD/ASH (n = 19), AIH/PBC (n = 8), and others (n = 19). Each underwent ultrasound elastography on the same day of the liver biopsy. This retrospective observational study was performed at Shimane University Hospital (Shimane-Izumo, Japan) after receiving approval (Study number. 4509 and 4831) from the local ethics committee. The study protocol fulfilled ethical requirements noted in the Declaration of Helsinki. Reporting of the findings of this study conforms to the STROBE guidelines.[7]

Clinical and laboratory assessments. Clinical data were collected on the day of the liver biopsy, while blood samples were taken on the same day after overnight fasting. Serum levels of type IV collagen 7S (T4c7s),[8] ATX,[9] and high-sensitivity C-reactive protein (hs-CRP)[10] were determined based on chemiluminescent enzyme immunoassay, fluoroenzyme immunoassay, and latex agglutination assay findings, respectively.

Ultrasound elastography. Point shear wave elastography (pSWE) and real-time tissue elastography (RTE) examinations, developed for evaluation of liver fibrosis in patients with liver injury, were performed using an ultrasound device (ARIETTA S70; Hitachi, Tokyo, Japan) with an EUP-C251 convex type probe (1–5 MHz, 50-mm radius scan width, 75° field of view scan angle) (Hitachi). Liver fibrosis in each patient was evaluated based on both shear wave velocity (Vs) shown by pSWE and

*To whom correspondence should be addressed.
E-mail: ht1020@med.shimane-u.ac.jp

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liver fibrosis index (LFI) shown by RTE, as previously reported.[11]

Liver biopsy and histological analysis. Using a 16-gauge cutting biopsy needle, a liver biopsy sample was obtained from the same area that was subjected to ultrasound elastography, based on a previously reported method.[11] Biopsied liver specimens were fixed in 10% formalin, then stained with hematoxylin-eosin or Masson's trichrome. Fibrotic stage was evaluated as a routine pathological diagnostic procedure by pathologists blinded to the results of ultrasound elastography and other biochemical markers for liver fibrosis. Staging of liver fibrosis was done using the Brunt classification system[12] for NAFLD/ASH cases and METAVIR scoring system[13] for other categories of liver disease. Because of the low number of cases, fibrotic stages shown by both systems were combined and the patients were categorized as stage 0, 1, or ≥2. Although the Brunt and METAVIR systems employ different criteria for evaluation of fibrosis, this categorization was considered feasible because stage 0, 1, and ≥2 could be considered to represent the same concept; i.e., no, minimal, and more than minimal fibrosis, respectively (see Discussion).

LC-MSMS analysis of lysophospholipids. Lysophospholipids were extracted using previously reported methods, with minor modifications.[14,15] Each serum sample (15 µl) was mixed with 10 mM ammonium formate in methanol (85 µl) containing four internal standards (0.1 µM of 17:0-LPA and 17:0-LPC, 0.2 µM of 13:0-LPE, and 0.3 µM of 17:1-LPI; Avanti Polar Lipids, Alabaster, AL). Samples were then sonicated for three minutes, followed by centrifugation at 13,000 rpm for 10 min at 4°C. Supernatants (10 µl) were obtained and used for LC–MSMS analysis, which was performed as previously described, with some modifications.[14,15] A Nexera X2 system HPLC and an LC-8030 coupled to a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Shimadzu, Kyoto, Japan) were used. The source parameters were as follows: heat block temperature, 400°C; flow rates for nebulizing and drying gas, 2 and 15 L/min, respectively; dissolved-line temperature, 250°C. Analysis was performed in negative ion mode for each molecular species of LPA, LPC, LPE, and LPI using multiple reaction monitoring (MRM) mode, with the MRM transitions set according to previous reports.[14,15] LC separation was done using an InertSustain C18 UHPLC PEEK column (3 mm, i.d. 150 × 2.1 mm, GL Sciences, Tokyo, Japan). Mobile phase A and B consisted of 5 mM ammonium formate in water (pH 4.0) and 5 mM ammonium formate in 95% (v/v) acetonitrile (pH 4.0), respectively. The gradient elution program was as follows: 60% of B for 3 min, 60–85% of B for 12 min, and 100% of B for 7 min. The flow rate was 0.2 ml/min and chromatography was performed at 35°C.

Data were analyzed using the LabSolutions software package (Shimadzu). Quantification of LPA, LPC, LPE, and LPI were performed with calibration curves for 16:0-LPA, 16:0-LPC, 16:0-LPE, and 16:0-LPI (Avanti Polar Lipids), respectively, with a lower quantification limit of 0.625, 1.0, 5.0, and 5.0 nM, respectively. Total LPC, LPA, LPE, and LPI values were calculated as the sum of all molecules of different chemical forms. Representative images showing raw traces of LPA molecules are presented in Fig. 1.

Statistical analysis. Concentrations of lysophospholipids, ATX, T4c7s, hs-CRP, Vs, and LFI were log-transformed before analysis. All analyses were performed using SPSS, ver. 28 (IBM Japan, Tokyo, Japan).

Results

Subject demographic data. Demographic data for the subjects after dividing into three groups according to severity of fibrosis are presented in Table 1. All clinical parameters of liver fibrosis examined, i.e., Vs, LFI, T4c7s, and ATX, showed highly significant differences for level among the fibrosis stages. Furthermore, trend analysis results indicated that these parameters had linear associations with severity. Disease type also demonstrated a significant association with severity of fibrosis, with more cases of NAFLD/ASH found to have a higher degree of fibrosis. Of note, among the four lysophospholipids studied, only LPA was found to be associated with fibrosis.

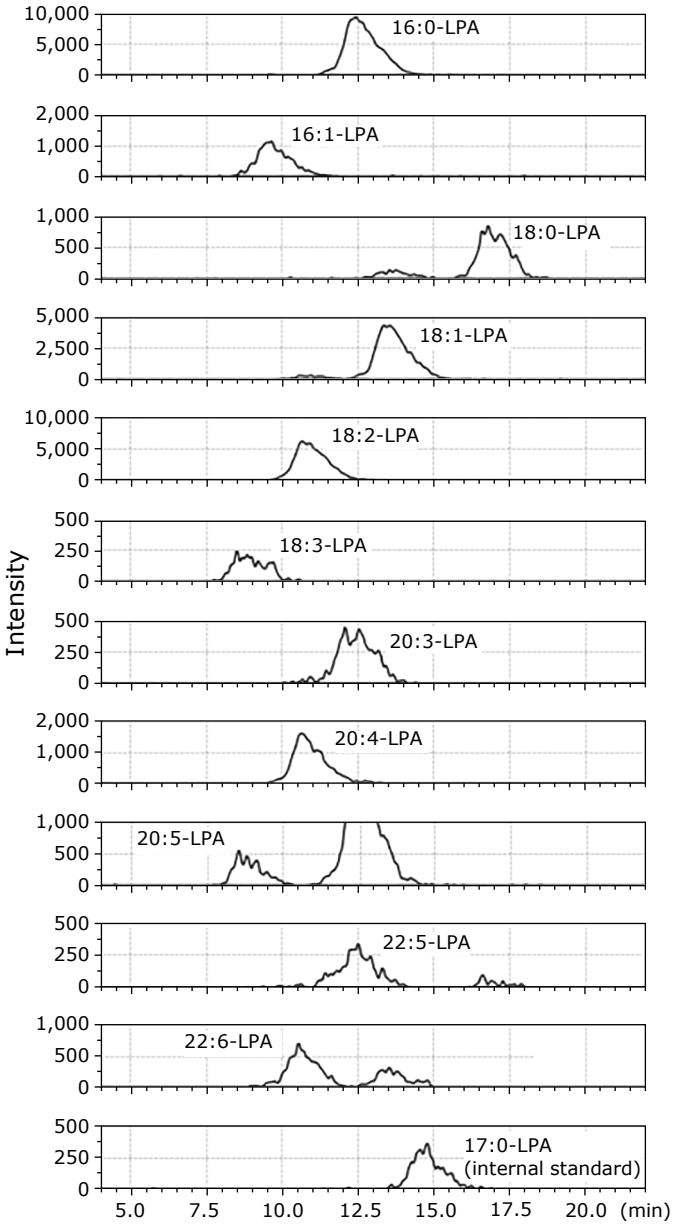

Fig. 1. Raw peaks of each LPA molecule in LC-MSMS analysis. LPA molecules in serum were detected by LC-MSMS and raw traces are presented.

Effects of LPA on liver fibrosis. The association of LPA with liver fibrosis was examined using ordered logistic regression analysis, with disease type, age, gender, body mass index (BMI), and high sensitivity C-reactive protein (hs-CRP) used as confounding factors. Total LPA showed a significant positive association with severity of fibrosis following adjustment for other confounding factors (Table 2A). This result was replicated when 16:0-LPA, one of the major forms of LPA, was substituted for total LPA (Table 2B), though when 20:4-LPA was substituted for total LPA, no significant association was observed (Table 2C).

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Table 1. Demographic data

	Fibrosis			p[2]	p for trend[3]
	0	1	≥2		
Female/male[1]	7/7	10/7	7/7	0.8	1.0
Age (years)	62 (54, 70)	61 (54, 68)	60 (58, 74)	0.6	0.46
BMI (kg/m^2)	21.0 (18.5, 23.4)	24.6 (22.3, 26.8)	26.1 (23.6, 28.5)	0.015	0.005
Disease type[1]					
NAFLD/ASH	1	9	9		
AIH/PBC	4	2	2		
Others	9	6	3		
Vs (m/s)	1.38 (1.16, 1.61)	1.71 (1.51, 1.92)	2.05 (1.82, 2.28)	<0.001	<0.001
LFI	1.80 (1.37, 2.24)	2.68 (2.29, 3.07)	2.86 (2.42, 3.29)	0.002	0.002
T4c7s (ng/ml)	4.21 (3.59, 4.94)	5.65 (4.67, 6.53)	7.28 (6.21, 8.55)	<0.001	<0.001
hs-CRP, mg/dl	0.049 (0.025, 0.087)	0.095 (0.052, 0.17)	0.26 (0.14, 0.51)	0.003	0.001
ATX (mg/L)	0.74 (0.64, 0.86)	0.95 (0.82, 1.09)	1.12 (0.96, 1.31)	0.002	<0.001
Total LPC (μM)	69.2 (55.0, 87.1)	79.4 (64.6, 97.7)	63.1 (50.1, 79.4)	0.32	0.53
Total LPA (μM)	1.23 (0.97, 1.57)	1.91 (1.53, 2.38)	2.15 (1.69, 2.74)	0.005	0.004
Total LPI (μM)	0.26 (0.19, 0.36)	0.26 (0.19, 0.34)	0.27 (0.20, 0.37)	0.98	0.82
Total LPE (μM)	0.76 (0.63, 0.91)	0.69 (0.59, 0.82)	0.24 (0.57, 0.83)	0.7	0.87

Vs, T4c7s, hs-CRP, ATX, total LPC, total LPA, total LPI, and total LPE were log-transformed before subjecting to analysis. Values representing 95% confidential intervals are shown in parentheses. [1]Contingency table analysis was used. ANOVA was employed for the other analyses. [2]p value determined by either contingency table analysis or ANOVA. [3]p value determined by either Somer's D or Jonckheere–Terpstra analysis.

Table 2. Effects of LPA on liver fibrosis

A. Total LPA

	B	SE	Wald	p
Disease type vs others				
NAFLD/ASH	2.1	0.9	4.93	0.03
AIH/PBC	–1.2	1.0	1.49	0.2
Age	0.05	0.03	2.66	0.1
Gender, female vs male	–0.04	0.7	0.003	0.95
BMI	0.03	0.08	0.12	0.7
Log hsCRP	2.0	0.7	8.35	0.004
Log total LPA	3.8	1.9	4.18	0.04

B. 16:0-LPA

	B	SE	Wald	p
Disease type vs others				
NAFLD/ASH	2.0	0.9	4.59	0.03
AIH/PBC	–1.3	1.0	1.67	0.2
Age	0.05	0.03	2.92	0.09
Gender, female vs male	–0.07	0.7	0.01	0.9
BMI	0.04	0.08	0.19	0.7
Log hsCRP	2.0	0.7	7.59	0.006
Log 16:0-LPA	2.9	1.4	4.27	0.04

C. 20:4-LPA

	B	SE	Wald	p
Disease type vs others				
NAFLD/ASH	2.2	0.9	6.05	0.01
AIH/PBC	–1.3	0.9	1.85	0.2
Age	0.05	0.03	3.58	0.06
Gender, female vs male	0.4	0.7	0.40	0.5
BMI	0.03	0.08	0.15	0.7
Log hsCRP	2.1	0.7	9.4	0.002
Log 20:4-LPA	1.2	1.7	0.47	0.5

Ordered logistic analysis was performed with total LPA (A), 16:0-LPA (B), or 20:4-LPA (C) included as an independent factor.

Table 3. Effects of LPA on Vs

A. Total LPA

	B	SE	Wald	*p*
Disease type vs others				
NAFLD/ASH	0.09	0.04	5.95	0.02
AIH/PBC	0.05	0.04	1.39	0.2
Age	0.003	0.001	6.15	0.01
Gender, female vs male	0.02	0.03	0.62	0.4
BMI	–0.006	0.003	2.8	0.09
Log hsCRP	0.1	0.03	8.35	0.004
Log total LPA	0.12	0.02	5.03	0.03

B. 16:0-LPA

	B	SE	Wald	*p*
Disease type vs others				
NAFLD/ASH	0.08	0.04	4.37	0.04
AIH/PBC	0.05	0.04	1.65	0.2
Age	0.003	0.001	5.94	0.002
Gender, female vs male	0.008	0.03	0.08	0.8
BMI	–0.005	0.003	1.95	0.2
Log hsCRP	0.06	0.03	5.59	0.02
Log 16:0-LPA	0.12	0.05	4.65	0.03

C. 20:4-LPA

	B	SE	Wald	*p*
Disease type vs others				
NAFLD/ASH	0.09	0.04	5.95	0.02
AIH/PBC	0.05	0.04	1.39	0.2
Age	0.003	0.001	6.15	0.01
Gender, female vs male	0.02	0.03	0.62	0.4
BMI	–0.006	0.003	2.8	0.09
Log hsCRP	0.08	0.03	8.35	0.004
Log 20:4-LPA	0.12	0.07	2.66	0.1

Vs was log-transformed, then GLM analysis was performed.

To confirm the association of LPA molecules with liver fibrosis, the relationships of LPA with Vs and T4c7s, quantitative parameters known to represent liver fibrosis severity, were examined using the same set of independent variables. These two parameters were chosen because they were measured by different methods, i.e., ultrasound elastography and biochemical analysis of serum protein, respectively. GLM analysis showed that total LPA and 16:0-LPA had a significant positive association with both Vs and T4c7s, while 20:4-LPA did not have an association with either (Table 3 and 4).

Next, the same analysis used to obtain the findings shown in Table 2 was applied to other molecules of LPA to determine whether the association with liver fibrosis could be replicated when those were substituted for total LPA. The results are summarized in Fig. 2, together with findings showing the serum concentration of each LPA molecule. Among the four major LPA molecules with the highest concentrations (>0.1 μM), only 20:4-LPA alone did not show a nominally significant association. On the other hand, 20:3-LPA demonstrated a significant association even though the serum level was 50 times lower than the level of the other major LPA molecules. The levels of 18:3- and 22:5-LPA, the two molecules with the lowest concentrations, could be measured in only 27 and 21 samples, respectively, which might have affected the results of association analysis (grey squares in Fig. 2).

Correlation between LPA and ATX. Correlation coefficients of various forms of LPA with ATX are presented in Table 5. Many of the LPA molecules as well as total LPA were found to be significantly correlated with serum ATX level. However, it is of interest that 20:4- and 20:5-LPA did not correlate with ATX level in spite of their relatively high concentrations as compared with the other LPA molecules (Fig. 2). Additionally, while those did not show a significant correlation with the level of ATX, the concentrations of 18:3- and 22:5-LPA were the lowest, which might have affected the results, as noted above.

Discussion

A method for quantification of different chemical forms of lysophospholipids by LC-MSMS was established in the present study. In a previous report using LC-MSMS, measurements of taurine (TU), hypotaurine (HTU), and glutathione levels in hepatic tissue samples of patients with acute liver failure suggest the HTU-TU pathway is enhanced for anti-oxidative defense mechanisms.[16] Among the four lysophospholipids examined, i.e., LPC, LPA, LPE, and LPI, only LPA showed a significant positive association with liver fibrosis. Our findings seem to be robust, as the association was also observed when Vs or T4c7s, two quantitative parameters of liver fibrosis evaluated by different methodologies, were substituted for histological grading

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Table 4. Effects of LPA on T4c7s

A. Total LPA

	B	SE	Wald	*p*
Disease type vs others				
NAFLD/ASH	0.05	0.04	1.48	0.2
AIH/PBC	0.08	0.05	3.20	0.07
Age	0.001	0.001	0.37	0.6
Gender, female vs male	0.006	0.03	0.03	0.9
BMI	–0.005	0.004	1.75	0.2
Log hsCRP	0.16	0.03	26.2	<0.001
Log total LPA	0.23	0.08	7.53	0.006

B. 16:0-LPA

	B	SE	Wald	*p*
Disease type vs others				
NAFLD/ASH	0.05	0.04	1.28	0.3
AIH/PBC	0.08	0.05	2.86	0.09
Age	0.001	0.001	0.23	0.6
Gender, female vs male	0.007	0.03	0.06	0.8
BMI	–0.005	0.004	1.39	0.2
Log hsCRP	0.15	0.03	23.8	<0.001
Log 16:0-LPA	0.17	0.06	7.35	0.007

C. 20:4-LPA

	B	SE	Wald	*p*
Disease type vs others				
NAFLD/ASH	0.08	0.05	2.75	0.1
AIH/PBC	0.08	0.05	2.30	0.1
Age	–0.00001	0.001	0.005	0.1
Gender, female vs male	0.02	0.03	0.47	0.5
BMI	–0.005	0.04	1.44	0.2
Log hsCRP	0.17	0.03	28.1	<0.001
Log 20:4-LPA	0.06	0.09	0.41	0.5

T4c7s was log-transformed, then GLM analysis was performed.

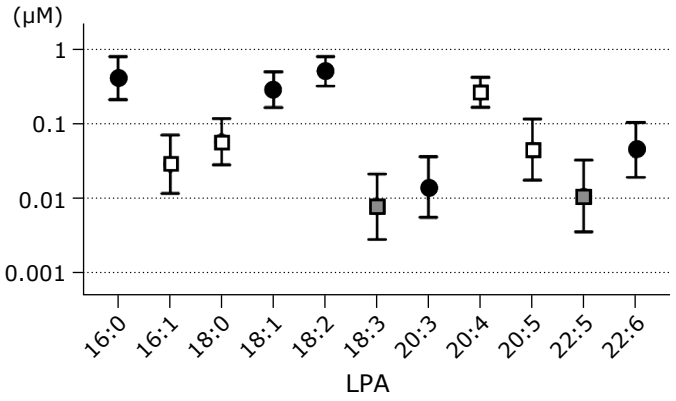

Fig. 2. Summary of serum LPA levels and their association with liver fibrosis. The level of each LPA molecule is shown. Error bars indicate SD. The association of each LPA molecule with liver fibrosis was examined using ordered logistic analysis. Closed circles indicate nominally significant ($p<0.05$) and open squares non-significant association with liver fibrosis. Grey squares indicate that less than 30 samples had a detectable peak of LPA molecules (see Results).

Table 5. Correlation between LPA and ATX

	R	*p*
Total LPA	0.55	<0.001
16:0-LPA	0.51	<0.001
16:1-LPA	0.49	<0.001
18:0-LPA	0.42	0.004
18:1-LPA	0.56	<0.001
18:2-LPA	0.41	0.005
18:3-LPA	0.31	0.10
20:3-LPA	0.36	0.019
20:4-LPA	0.19	0.20
20:5-LPA	0.12	0.43
22:5-LPA	0.29	0.20
22:6-LPA	0.37	0.012

Pearson's correlation coefficient (R) between log LPA and log ATX was calculated. LPA molecules with *p* value >0.05 are shown in italics. Serum levels of 18:3 and 22:5 LPA were very low, with a detectable peak observed in only 27 and 21 samples, respectively.

of fibrosis. The present results confirmed the unique biological role of LPA in liver fibrosis, as previously reported.[17]

As for specific LPA molecules that have influence on liver fibrosis, it has been reported that 18:1-LPA promoted proliferation of hepatic stellate cells *in vitro*.[18] In the present study, 18:1-LPA showed a significant correlation with ATX level, thus implicating its involvement in liver fibrosis (see Table 5). Furthermore, we found that two other LPA molecules, 20:4- and 20:5-LPA, were not associated with either liver fibrosis or ATX level, which may be a significant observation when considering the various roles of LPA as a signaling molecule.

LPA is produced from LPC by ATX, thus it may simply function as a biochemical marker of liver fibrosis by reflecting the activity of ATX. However, several studies have suggested that LPA might have an active role in various pathological processes through LPA receptors (LPAR1 to 6).[19] Those six LPA receptors are characterized by their distribution, signal transduction systems, and functional roles, with LPAR1 in particular suggested to promote fibrosis in various organs.[20] It is therefore possible that LPA molecules with different acyl chain lengths as well as degree of saturation in the acyl chain show different levels of affinity to the six LPARs, and may have different functional roles. In this context, it is of interest that two of the LPA molecules examined in the present study, 20:4- and 20:5-LPA, were found to not have a correlation with liver fibrosis severity or serum level of ATX. Among all four of the examined LPA molecules, 20:4-LPA had the highest serum concentration (Fig. 2), thus the lack of correlation was not due to a technical limitation related to the measurement method, as noted with 18:3- and 22:5-LPA, which had very low concentrations. Since the other LPA molecules showed a high correlation with ATX level, it is considered that 20:4- and 20:5-LPA could be unique in that they are mainly produced through pathways other than ATX. In this context, it is notable that 20:4-LPA has been reported to be increased in patients with as compared to without hepatocellular carcinoma (HCC).[21] It is interesting to speculate that 20:4-LPA may not play a role in development of liver fibrosis but rather in oncogenesis of HCC. On the other hand, neither 16:1- nor 18:0-LPA showed a significant association with liver fibrosis in spite of having a significant correlation with ATX level (Fig. 2, Table 5). Presently, we do not have a plausible explanation for why these two LPA molecules were not found to be associated with liver fibrosis, though that suggests heterogeneity among LPA molecules in regard to their pathophysiological roles.

For evaluation of liver fibrosis histological stage in the present study, the Brunt scoring system was used for NAFLD/ASH cases and the METAVIR system for other types of liver disease, because the starting region of fibrosis is known to vary among the different types.[13] However, both scoring systems share a common concept, as no, minimal, and greater than minimal fibrosis are classified in each as stage 0, 1, and ≥2, respectively, thus it was considered reasonable to categorize liver fibrosis into those groups. Additionally, quantitative parameters for liver fibrosis, such as Vs and T7c4s, were found to have a linear association with those three categories (Table 1), thus supporting the feasibility of the categorization employed in this study. Nevertheless, in the future it will be necessary to analyze a greater number of cases to more fully elucidate the role of LPA molecules in NAFLD alone using a single scoring system.

An additional limitation is that the participants were recruited from a single center, which might have caused a sampling bias. Furthermore, since the sample size was rather small, the disease types among the participants were heterogeneous, including 11 cases with unknown etiology. To obtain more details to determine the precise association of LPA molecules with individual types of liver disease, a greater number of subjects will be necessary.

In conclusion, among the four lysophospholipids examined, only LPA concentration was associated with liver fibrosis. Furthermore, the degree of association differed among the different chemical forms of LPA molecules. While it is interesting to speculate that particular LPA molecules may have a functional role in liver fibrosis, it will be necessary to conduct biological and/or biochemical cellular studies. Nevertheless, the present results indicate the importance of analyzing individual LPA molecules, for which LC-MSMS can be useful. In the future, we intend to continue the search for particular LPA molecules causally related to the pathology of NAFLD and also develop specific treatments that target those molecules.

Author Contributions

Study coordination and design: HT, HS, TN, and HK. Data collection: HT. Statistical analysis: TN. Critical review of the manuscript: AY, YN, TN, and IS. Approval of final version of the manuscript: All authors. Writing and approval of final version of the manuscript: HT, HS, TN, IS, and HK.

Disclosures and Ethical Statements

Approval of research protocol: The present study was approved by the ethics committee of Shimane University Faculty of Medicine. Informed consent: All participants provided written informed consent prior to participation in this study. Registry and registration no. of study/trial: 20200721-3. Animal studies: N/A. Research involving recombinant DNA: N/A.

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Abbreviations

AIH	autoimmune hepatitis
ASH	alcoholic steatohepatitis
ATX	autotaxin
ESI	electrospray ionization
hs-CRP	high-sensitivity C-reactive protein
LC-MSMS	liquid-chromatography tandem mass-spectrometry
LFI	liver fibrosis index
LPA	lysophosphatidic acid
LPC	lysophosphatidylcholine
LPE	lysophosphatidylethanolamine
LPI	lysophosphatidylinositol
MRM	multiple reaction monitoring
NAFLD	non-alcoholic fatty liver disease
PBC	primary biliary cholangitis
pSWE	point shear wave elastography
RTE	real-time tissue elastography
T4c7s	type IV collagen 7S
Vs	shear wave velocity

Conflict of Interest

No potential conflicts of interest were disclosed.

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