Gut and Liver
https://doi.org/10.5009/gnl210545
pISSN 1976-2283 eISSN 2005-1212

Original Article

Impact of Evolutionary Changes in Nonalcoholic Fatty Liver Disease on Lung Function Decline

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Article Info
Received November 26, 2021
Revised February 23, 2022
Accepted February 24, 2022
Published online May 25, 2022

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Background/Aims: A relationship between fatty liver and lung function impairment has been identified, and both are independently associated with metabolic dysfunction. However, the temporal relationship between changes in fatty liver status and lung function and their genome-wide association remain unclear.

Methods: This longitudinal cohort consisted of subjects who received serial health check-ups, including liver ultrasonography and spirometry, for ≥3 years between 2003 and 2015. Lung function decline rates were classified as "slow" and "accelerated" and compared among four different sonographic changes in steatosis status: "normal," "improved," "worsened," and "persistent." A genome-wide association study was conducted between the two groups: normal/improved steatosis with a slow decline in lung function versus worsened/persistent steatosis with an accelerated decline in lung function.

Results: Among 6,149 individuals, the annual rates of decline in forced vital capacity (FVC) and forced expiratory volume measured in the first second of exhalation (FEV_1) were higher in the worsened/persistent steatosis group than in the normal/improved steatosis group. In multivariable analysis, persistent or worsened status of fatty liver was significantly associated with accelerated declines in FVC (persistent status, odds ratio [OR]=1.22, 95% confidence interval [CI]=1.04–1.44; worsened status, OR=1.30, 95% CI=1.12–1.50), while improved status of fatty liver was significantly associated with slow declines in FEV_1 (OR=0.77, 95% CI=0.64–0.92). The *PNPLA3* risk gene was most strongly associated with steatosis status change and accelerated declines in FVC (rs12483959, $p=2.61\times10^{-7}$) and FEV_1 (rs2294433, $p=3.69\times10^{-8}$).

Conclusions: Regression of fatty liver is related to lung function decline. Continuing efforts to improve fatty liver may preserve lung function, especially for subjects with a high genetic risk. **(Gut Liver 2023;17:139-149)**

Key Words: Non-alcoholic fatty liver disease; Disease progression; Pulmonary function test; Genome-wide association study; Longitudinal study

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as a condition of excessive intrahepatic fat accumulation ≥5% of total liver weight. Liver ultrasonography has been widely used for the diagnosis of NAFLD given its easy accessibility, low levels of discomfort, and reasonable cost.[1] In addition, NAFLD severity can be semiquantitatively as-

sessed by liver ultrasonography.[2] NAFLD is bidirectionally associated with metabolic syndrome and partly accounts for cardiovascular, endocrinologic, and renal complications through systemic inflammation.[3] Moreover, NAFLD with high metabolic risk has been reported to significantly increase the risk for overall mortality.[4]

Recent evidence has indicated an association between lung function impairment and metabolic syndrome. After adjusting for body mass index (BMI) and physical activity, lung function impairment was independently associated with metabolic syndrome and showed a strong correlation with systemic inflammation.[5] Indeed, the forced expiratory volume measured in the first second of exhalation (FEV_1) and forced vital capacity (FVC) were correlated with metabolic syndrome after multivariable adjustment, and abdominal obesity was the most significant predictor of lung function impairment among metabolic factors.[6]

NAFLD is related to lung function impairment, and both conditions are independently associated with metabolic syndrome.[7] Notably, subjects with NAFLD showed significant impairment in FVC and FEV_1.[8] Furthermore, FVC and FEV_1 showed an inverse correlation with the severity of hepatic steatosis or fibrosis in multivariable analyses.[9] When analyzing histological data from NAFLD patients, the relationship between hepatic steatosis and lung function impairment might be substantially confounded by factors related to metabolic syndrome; however, fibrosis severity was independently associated with FVC impairment.[10] Therefore, the association between lung function impairment and NAFLD may be partly explained by the mechanisms underlying metabolic syndrome or hepatic fibrosis. However, since lung function is a dynamic variable that changes over time, the results of any cross-sectional analysis of the relationship between lung function and NAFLD should be interpreted with caution.

Herein, the current longitudinal observational cohort study investigated whether the decline in lung function is associated with the longitudinal change in fatty liver status and explored potential genetic clues as a shared mechanism using a genome-wide association study (GWAS).

MATERIALS AND METHODS

Our study was reported based on the standard guidance of the Strengthening the Reporting of Observational Studies in Epidemiology statement.[11]

1. Study design and eligibility criteria

The current observational study analyzed the Gene-Environment Interaction and Phenotype cohort[12] consisting of examinees who received regular health check-ups from October 2003 to September 2015 at the Seoul National University Hospital Healthcare System Gangnam Center. The inclusion criteria were adults (1) who were ≥18 years old and (2) who underwent two or more health check-ups at intervals of ≥1 year. The following exclusion criteria were employed: (1) no available medical records on liver ultrasonography described by an experienced radiologist; (2) no available results on lung function assessed by spirometric examination; (3) hepatitis B or C virus infection; and (4) excessive alcohol consumption (male >30 g/day, female >20 g/day) assessed using a validated questionnaire, namely, the Korean revised version of the Alcohol Use Disorder Identification Test.[13]

2. Clinical and laboratory assessments

At the first visit, we acquired the baseline medical information of the study subjects from anthropometric assessments and several questionnaires on past medical and social history. Detailed basic medical information and results of laboratory blood tests, abdominal ultrasonography, and spirometric testing were evaluated. Hepatic fibrosis was evaluated using the fibrosis-4 index (FIB-4) stratified by age. The lower cutoff was 1.3 in those <65 years old and 2.0 in those ≥65 years old.[14] Spirometric testing measured prebronchodilator FVC, L (% of predicted value), prebronchodilator FEV_1, L (% of predicted value), and FEV_1/FVC. At the last visit, the liver ultrasonography results and the measurements from spirometric testing were evaluated.

The details of the methodologic information on ultrasonographic and spirometric assessment were described in a previous study.[9] In brief, experienced radiologists performed liver ultrasonography without knowing the spirometric information of the individuals. Ultrasonographic diagnosis of fatty liver was based on increased echogenicity in the liver compared to the kidney, vessel blurring, or deep beam attenuation. Well-trained technicians performed computerized spirometry based on the official guidelines for the standardization of spirometry published by the American Thoracic Society and European Respiratory Society.

3. Subject allocation according to ultrasonographic changes in fatty liver status

Ultrasonographic findings were categorized as no fatty liver, mild fatty liver and moderate to severe fatty liver. If the presence of a fatty liver was noted on the initial exam, the participants were classified into three groups based on the last exam compared to the initial finding: (1) fatty liver, improved; (2) fatty liver, persistent; and (3) fatty liver, worsened. Based on the initial and last liver ultrasonographic description, we classified the study population into

the following four groups: (1) normal, unchanged; (2) fatty liver, improved; (3) fatty liver, persistent; and (4) fatty liver, worsened. The "fatty liver, improved" or "fatty liver, worsened" group was determined when fatty liver was found in the initial liver ultrasonographic assessment and the severity of fatty liver improved or worsened in the last liver ultrasonographic assessment. The "fatty liver, persistent" group was determined when the severity of fatty liver was not changed in the last liver ultrasonographic assessment compared to baseline liver ultrasonographic assessment. Among individuals with no steatosis at baseline, those developing incident fatty liver during the follow-up period were also included in the "fatty liver, worsened" group, assuming that those with incident fatty liver shared similar pathophysiology with those with worsening fatty liver. We briefly described "normal, unchanged" as the "normal" group, "fatty liver, improved" as the "improved" group, "fatty liver, worsened" as the "worsened" group, and "fatty liver, persistent" as the "persistent" group.

4. Subject allocation according to spirometric changes in lung function

The annual decline rates of FVC and FEV_1 were estimated by serial spirometric testing performed over more than 3 years for each individual. The annual rates of lung function decline were classified into two categories according to the median value:[15] the median FVC decline rate was 37 mL/yr, and the median FEV_1 decline rate was 43 mL/yr. Annual decline rates of FVC <37 mL/yr or FEV_1 <43 mL/yr were defined as slow decline rates, and annual decline rates of FVC ≥37 mL/yr or FEV_1 ≥43 mL/yr were defined as accelerated decline rates.

5. Statistical analyses

Analysis of variance or the Kruskal-Wallis test was used to compare continuous variables among the different groups. The chi-square test or the Fisher exact test was used for statistical evaluation of categorical variables. Univariable logistic regression analysis was conducted with clinically important variables related to accelerated lung function decline. To clarify an independent relationship between fatty liver or hepatic fibrosis change and lung function change, we performed multivariable logistic regression analysis adjusted for statistically significant variables in the unadjusted analysis. A variance inflation factor <4.0 was considered to indicate significant multicollinearity. Here, $p<0.05$ was considered statistically significant. For statistical analyses, R statistical software, version 3.6.1 (2018; R Core Team, Vienna, Austria) was used.

A GWAS was performed to evaluate the relationship between fatty liver change and lung function decline. Variants were filtered by minor allele frequency (<0.05), missing genotype (>0.03), and Hardy-Weinberg equilibrium ($p\geq0.0001$), and samples with high identity-by-descent (≥0.10) and ambiguous sex were excluded. Imputation was performed by SHAPEIT-IMPUTE2 on 1000 Genomes Phase 3. The imputed variants were filtered under the same conditions described above. Variables including age,

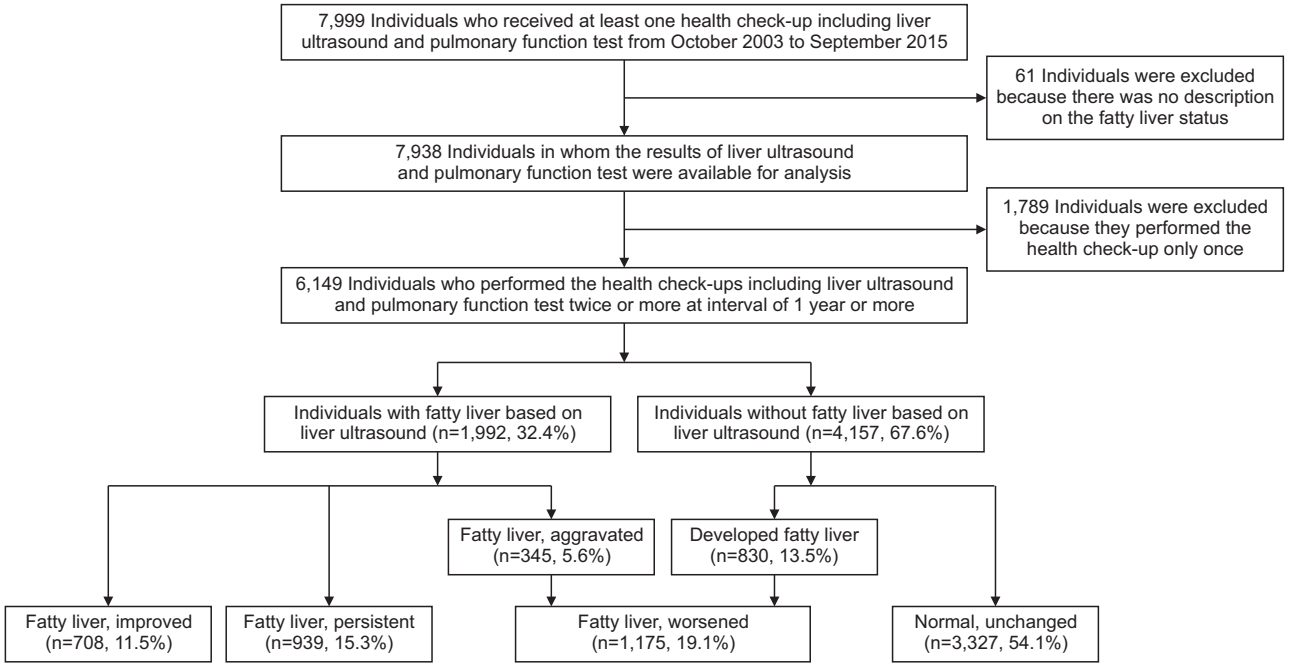

Fig. 1. Flow diagram for inclusion and classification based on the change in fatty liver status.

Table 1. Baseline Characteristics and Clinical Parameters at Initial Assessment According to Different Sonographic Changes in Fatty Liver Status

Characteristics	Normal, unchanged (n=3,327)	Fatty liver, improved (n=708)	Fatty liver, persistent (n=939)	Fatty liver, worsened (n=1,175)
Age, yr	45.17±9.37	48.56±9.03	47.83±8.66	46.48±8.29
Male sex	1,490 (44.8)	557 (78.7)	777 (82.7)	812 (69.1)
Body weight, kg	60.23±10.26	71.75±10.91	72.75±10.38	68.14±10.74
Body mass index, kg/cm^2	21.91±2.52	25.08±2.87	25.39±2.72	24.12±2.64
Waist circumference, cm	79.72±7.28	89.00±7.40	89.33±7.09	86.12±7.13
Smoking history				
Never smoker	2,300 (69.1)	324 (45.8)	394 (42.0)	598 (50.9)
Ex-smoker	612 (18.4)	243 (34.3)	318 (33.9)	289 (24.6)
Current smoker	415 (12.5)	141 (19.9)	227 (24.2)	288 (24.5)
Alcohol history				
Frequency of alcohol consumption				
1–3 drinks/mo	2,824 (84.9)	589 (83.2)	717 (76.4)	992 (84.4)
1–2 drinks/wk	203 (6.1)	28 (4.0)	84 (8.9)	55 (4.7)
3–5 drinks/wk	154 (4.6)	40 (5.6)	57 (6.1)	70 (6.0)
Daily	130 (3.9)	50 (7.1)	73 (7.8)	53 (4.5)
Missing	16 (0.5)	1 (0.1)	8 (0.9)	5 (0.4)
Quantity of alcohol consumption				
≤3 drinks/wk	2,905 (87.3)	594 (83.9)	728 (77.5)	1,003 (85.4)
4–7 drinks/wk	121 (3.6)	28 (4.0)	45 (4.8)	41 (3.5)
8–10 drinks/wk	92 (2.8)	35 (4.9)	52 (5.5)	43 (3.7)
>10 drinks/wk	182 (5.5)	48 (6.8)	103 (11.0)	82 (7.0)
Missing	27 (0.8)	3 (0.4)	11 (1.2)	6 (0.5)
Underlying disease				
Hypertension	228 (9.4)	114 (21.6)	162 (22.8)	147 (16.2)
Diabetes	45 (1.9)	34 (6.5)	53 (7.5)	45 (5.0)
Blood test				
Platelet, 10^3/μL	240.80±53.08	247.88±54.12	249.41±55.25	251.15±55.30
Fasting glucose, mg/dL	92.47±12.45	102.32±16.59	103.30±21.79	98.31±16.03
Cholesterol, mg/dL	189.33±32.59	204.96±34.01	203.36±36.00	196.28±32.48
Albumin, g/dL	4.38±0.25	4.46±0.26	4.48±0.25	4.41±0.25
AST, IU/L	21.57±10.95	26.15±10.65	26.65±10.94	23.91±13.35
ALT, IU/L	19.61±13.98	32.34±20.31	34.82±22.73	26.50±17.59
GGT, IU/L	25.45±24.77	45.82±42.75	49.23±46.90	38.32±34.06
Creatinine, mg/dL	0.92±0.22	1.01±0.20	1.00±0.18	0.98±0.19
Triglyceride, mg/dL	85.54±45.25	147.11±93.01	154.04±94.18	126.58±79.49
HDL cholesterol, mg/dL	58.13±12.89	49.59±11.05	48.49±10.40	51.08±11.69
LDL cholesterol, mg/dL	114.53±29.47	132.09±31.01	131.62±31.27	124.97±29.74
HbA1c, %	5.54±0.38	5.79±0.60	5.82±0.69	5.68±0.53
Fatty liver index				
<30	2,812 (85.1)	243 (34.3)	280 (29.9)	617 (52.6)
≥60	90 (2.7)	182 (25.8)	286 (30.6)	186 (15.9)
Lung function				
FEV_1, L	3.09±0.69	3.27±0.68	3.30±0.65	3.29±0.70
FEV_1, % of predicted value	103.97±12.74	102.44±13.08	101.97±13.15	103.68±12.13
FVC, L	3.72±0.84	4.02±0.80	4.07±0.76	4.02±0.83
FVC, % of predicted value	95.81±11.19	95.16±10.84	95.00±10.93	96.26±10.26
FEV_1/FVC, %	83.34±7.14	81.46±6.57	81.16±6.29	82.04±6.50

Data are presented as mean±SD or number (%).
AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c; FEV_1, forced expiratory volume measured in the first second of exhalation; FVC, forced vital capacity.

sex, BMI, and diabetes were adjusted for the GWAS. Here, $p<5\times10^{-8}$ was considered a statistically significant threshold in the GWAS.

6. Ethics

Our study followed the ethical guidelines of the Declaration of Helsinki. The Institutional Review Board Com-

mittee of Seoul National University Hospital approved the study protocol (IRB number: 2005-019-1121) and waived the requirement for informed consent from study subjects for access to the electronic medical records.

RESULTS

Of the 7,999 individuals in our cohort, we excluded 61 individuals without a description of fatty liver status and 1,789 individuals without a follow-up health check-up. We analyzed 6,149 individuals who underwent serial liver ultrasonography and pulmonary function tests yearly (Fig. 1). At the baseline sonographic exam, 1,992 individuals (32.4%) had fatty liver, while 4,157 individuals (67.6%) did not. The study subjects underwent an average of 4.75 (±0.69) health check-ups during a median of 63.0 (interquartile range, 52.0) months of observation. NAFLD never developed in 3,327 patients (54.1%, normal group) during the observation period. Of the study subjects who had been diagnosed with NAFLD during the observation period, 708 (11.5%, improved group) showed improvement of fatty liver status, 1,175 (19.1%, worsened group) showed aggravation of fatty liver status, and 939 (15.3%, persistent group) maintained a constant status of fatty liver.

1. Baseline characteristics of study subjects

At baseline, the normal group without NAFLD was younger and predominantly female, had a smaller proportion of obese subjects, and had fewer ever smokers than the improved and persistent groups with NAFLD (Table 1). Metabolic diseases, such as hypertension and diabetes, were more frequently observed among the subjects who had ever been diagnosed with NAFLD. Metabolic risk, as assessed by fasting glucose, cholesterol, triglycerides, low-density lipoprotein cholesterol, and hemoglobin A1c, was higher in the improved and persistent groups than in the normal group. As the subjects in the worsened group included individuals with and without fatty liver at baseline, they showed intermediate levels of baseline characteristics and clinical parameters. There were no significant differences in liver and kidney function among the four different steatosis change groups. In baseline fatty liver severity, a lower fatty liver index was found in the "normal" group compared with the other three groups.

2. Lung function decline rate and the change in fatty liver status

We found significant differences in the lung function decline rate according to the change in fatty liver status (Fig. 2A). Accelerated FVC decline was observed in 46.9%, 48.4%, 55.1%, and 56.9% of the subjects in the normal, improved, persistent, and worsened fatty liver groups, re-

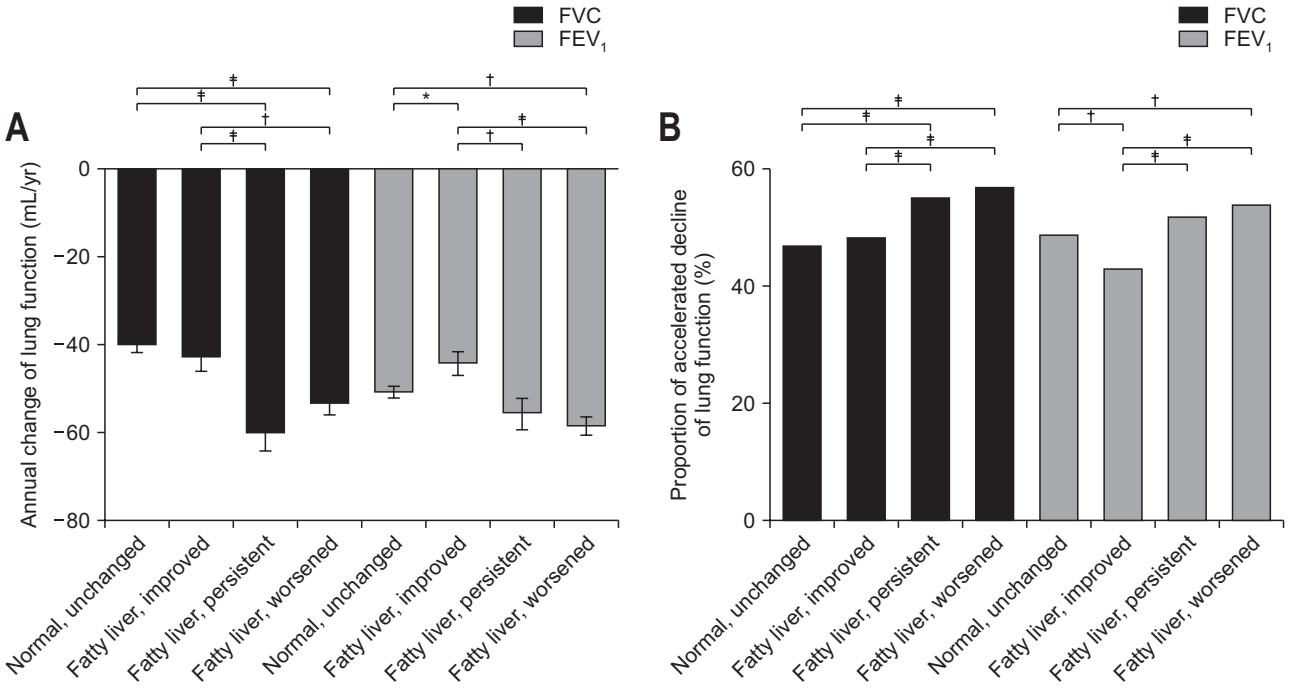

Fig. 2. Annual lung function decline rates (A) and proportion of accelerated lung function decline (B) according to different sonographic changes in fatty liver status.
FVC, forced vital capacity; FEV_1, forced expiratory volume measured in the first second of exhalation. *p<0.05, †p<0.01, ‡p<0.001.

Table 2. Univariable and Multivariable Logistic Regression Analyses to Evaluate the Risk of Accelerated Lung Function Decline According to the Change in Fatty Liver Status

Variable	Univariable analysis		Multivariable analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Accelerated FVC decline				
Age	1.04 (1.03–1.04)	<0.001	1.03 (1.02–1.04)	<0.001
Sex, male	1.59 (1.43–1.76)	<0.001	1.42 (1.25–1.61)	<0.001
Annual change in waist circumference (cm/yr)	0.99 (0.95–1.03)	0.798	1.12 (1.06–1.19)	<0.001
Annual change in BMI	0.78 (0.67–0.89)	<0.001	0.56 (0.46–0.69)	<0.001
Current smoker	1.34 (1.17–1.53)	<0.001	1.17 (1.01–1.36)	0.039
Initial FVC, % of predicted value	1.06 (1.05–1.06)	<0.001	1.06 (1.05–1.06)	<0.001
Change in fatty liver status				
Normal, unchanged (reference)	1.00	-	-	-
Fatty liver, improved	1.06 (0.90–1.25)	0.460	0.94 (0.78–1.12)	0.468
Fatty liver, persistent	1.39 (1.20–1.60)	<0.001	1.22 (1.04–1.44)	0.014
Fatty liver, worsened	1.49 (1.30–1.70)	<0.001	1.30 (1.12–1.50)	<0.001
Accelerated FEV_1 decline				
Age	1.01 (1.01–1.02)	<0.001	1.00 (1.00–1.01)	0.121
Sex, male	1.50 (1.35–1.66)	<0.001	1.67 (1.48–1.89)	<0.001
Annual change of waist circumference (cm/yr)	0.91 (0.87–0.95)	<0.001	1.06 (1.00–1.12)	0.045
Annual change of BMI	0.53 (0.46–0.62)	<0.001	0.45 (0.37–0.55)	<0.001
Current smoker	1.34 (1.17–1.52)	<0.001	1.14 (0.98–1.32)	0.093
Initial FEV_1, % of predicted value	1.04 (1.03–1.04)	<0.001	1.04 (1.04–1.05)	<0.001
Change in fatty liver status				
Normal, unchanged (reference)	1.00	-	-	-
Fatty liver, improved	0.79 (0.67–0.93)	0.005	0.77 (0.64–0.92)	0.004
Fatty liver, persistent	1.13 (0.97–1.30)	0.108	1.00 (0.85–1.17)	0.996
Fatty liver, worsened	1.23 (1.07–1.40)	0.003	1.03 (0.89–1.19)	0.690

OR, odds ratio; CI, confidence interval; FVC, forced vital capacity; BMI, body mass index; FEV_1, forced expiratory volume measured in the first second of exhalation.

Multivariable logistic regression analysis was adjusted for covariates, including age, sex, body weight, current smoking status, and initial FVC or FEV_1 (% of predicted value). Waist circumference was not included in multivariable logistic regression analysis because multicollinearity (variance inflation factor >4) was identified. The median FVC decline rate was 37 mL/yr in all study subjects. The accelerated FVC decline rate was defined as a FVC decline rate of ≥37 mL/yr.

spectively (p for trend <0.001) (Fig. 2B). There was also a significant increasing trend in the incidence of accelerated FEV_1 decline as the sonographic fatty liver status worsened: 48.8%, 42.9%, 51.8%, and 53.9% (p for trend=0.010) (Fig. 2B). For the GWAS, the normal and improved steatosis groups were combined into the control group, whereas the persistent and worsened steatosis groups were combined into the case group.

In univariable logistic regression analysis, old age, male sex, annual change in BMI, current smoking, initial FVC % of predicted value, and worsened or persistent status were associated with accelerated declines in FVC (Table 2). Old age, male sex, annual change in waist circumference or BMI, current smoking, initial FEV_1% of predicted value, and worsened or persistent status were associated with accelerated declines in FEV_1. In multivariable analysis, persistent or worsened status of fatty liver was significantly associated with accelerated declines in FVC (persistent status, odds ratio=1.22, p=0.014; worsened status, odds ratio=1.30, p<0.001). In addition, improved status of fatty liver was significantly associated with slow declines in FEV_1 (odds ratio=0.77, p=0.004). When assessed using the FIB-4, FVC and FEV_1 decline rates were faster in subjects with persistent hepatic fibrosis than in normal subjects without hepatic fibrosis in multivariable analysis (Supplementary Fig. 1).

3. Genetic pleiotropic effects on changes in lung function and fatty liver status

Next, we classified the study subjects into two groups according to lung function decline and the change in fatty liver status: subjects with normal or improved steatosis and slow lung function decline rates (group 1, control) and subjects with worsened or persistent steatosis and accelerated lung function decline rates (group 2, case) (Fig. 3). A multivariable (age, sex, BMI, and diabetes mellitus)-adjusted GWAS for steatosis status change and lung function (e.g., FVC and FEV_1) decline was conducted between groups 1 (control) versus 2 (case). Forty-seven different single-nucleotide polymorphisms (SNPs) for FVC decline

A Subjects for FVC decline rate analysis

Change in fatty liver \ FVC decline rate	Q1 or Q2	Q3 or Q4
"Normal" or "Improved"	Group 1 (n=1,803)	
"Worsened" or "Persistent"		Group 2 (n=1,018)

B Subjects for FEV_1 decline rate analysis

Change in fatty liver \ FEV_1 decline rate	Q1 or Q2	Q3 or Q4
"Normal" or "Improved"	Group 1 (n=1,771)	
"Worsened" or "Persistent"		Group 2 (n=951)

Fig. 3. Two-by-two table based on the lung function (A: FVC, B: FEV_1) decline rate and the change in fatty liver status. The included subjects were classified according to two different dichotomous variables: slow versus accelerated lung function decline groups and "improved" or "normal" versus "worsened" or "persistent" steatosis groups.
FVC, forced vital capacity; FEV_1, forced expiratory volume measured in the first second of exhalation; Q1, 1st quartile; Q2, 2nd quartile; Q3, 3rd quartile; Q4, 4th quartile.

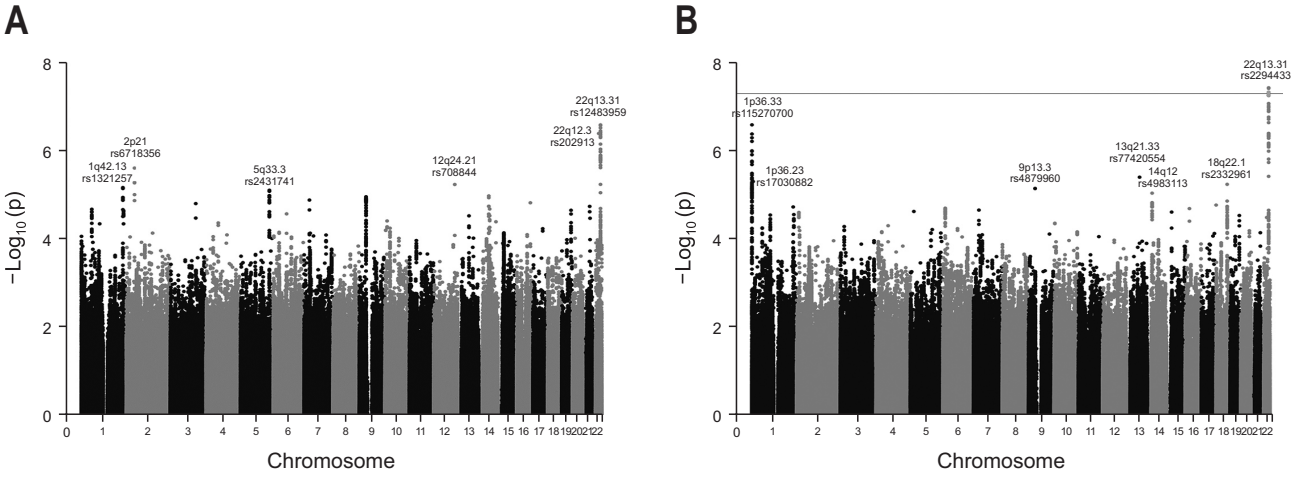

Fig. 4. Manhattan plot of the lung function decline rate and the change in fatty liver status. (A) Manhattan plot of the accelerated forced vital capacity (FVC) decline rate: population with a slow decline rate and improved or normal steatosis (control) versus population with an accelerated decline rate and worsened or persistent steatosis. (B) Manhattan plot of accelerated forced expiratory volume measured in the first second of exhalation (FEV_1) decline rate: population with slow decline rate and improved or normal steatosis (control) versus population with an accelerated decline rate and worsened or persistent steatosis.

and 40 different SNPs for FEV_1 decline were discovered in the Manhattan plot (Fig. 4, Supplementary Table 1). The most notable SNPs were rs12483959 ($p=2.61\times10^{-7}$) in the analysis of FVC decline and rs2294433 ($p=3.69\times10^{-8}$) in the analysis of FEV_1 decline, both of which showed strong linkage disequilibrium with a SNP locus, rs738409 in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene on chromosome 22q13.31, which is already known as a major risk variant of NAFLD (Table 3, Fig. 5). Only rs2294433 reached the threshold for genome-wide statistical significance in the analysis of FEV_1 decline according to the change in fatty liver status.

DISCUSSION

The current observational cohort study, including 6,149 health check-up examinees, identified significant differences in the rates of lung function decline according to the change in fatty liver status over ≥3 years of follow-up. We demonstrated that an aggravated or persistent status of fatty liver was significantly related to accelerated annual declines in FVC, whereas an improved fatty liver status was significantly associated with alleviation of annual declines in FEV_1, even after adjusting for major confounding factors, including age, sex, annual change in waist circumference or BMI, current smoking status, and initial lung function. Similarly, the FVC decline rate was faster in those with worsened hepatic fibrosis compared to improved hepatic fibrosis. Of note, the improved steatosis group had unfavorable metabolic profiles at baseline but showed a slower decline in lung function than the worsened steatosis group. In the GWAS, regarding both the change in fatty liver status and lung function decline, the most notable

Table 3. SNPs That Are Most Significantly Associated with the Change in Fatty Liver Status and Accelerated Lung Function Decline

	Rank	Chromosome	Position (bp)	SNP	Risk allele	Nearest genes	OR*	95% CI	p-value
Accelerated FVC decline	1	22	44325996	rs12483959	A	*PNPLA3*	1.39	1.22–1.57	2.61×10^{-7}
	2	22	34683751	rs202913	A	*Z68323.1*	0.70	0.61–0.80	4.10×10^{-7}
	3	2	45425655	rs6718356	A	*LINC01121*	1.65	1.34–2.04	2.50×10^{-6}
	4	12	116308724	rs708844	A	*LINC02463*	0.58	0.45–0.73	5.88×10^{-6}
	5	1	230305312	rs1321257	A	*AL136988.2*	1.37	1.20–1.58	6.91×10^{-6}
	6	5	159537803	rs2431741	T	*PWWP2A*	1.35	1.18–1.53	7.95×10^{-6}
Accelerated FEV_1 decline	1	22	44329275	rs2294433	A	*PNPLA3*	1.43	1.26–1.63	3.69×10^{-8}
	2	1	1185524	rs115270700	C	*AL162741.1*	1.51	1.29–1.76	2.57×10^{-7}
	3	22	44342325	rs2294919	T	*PNPLA3*	0.73	0.64–0.84	3.82×10^{-6}
	4	13	71421690	rs77420554	T	*MTCL1P1*	0.64	0.53–0.77	3.98×10^{-6}
	5	1	7505217	rs17030882	T	*CAMTA1-AS1*	1.49	1.26–1.77	4.99×10^{-6}
	6	18	62028617	rs2332961	T	*LINC01924*	1.35	1.19–1.54	5.79×10^{-6}
	7	9	36203718	rs4879960	C	*CLTA*	0.53	0.41–0.70	7.17×10^{-6}

SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; FVC, forced vital capacity; FEV_1, forced expiratory volume measured in the first second of exhalation.
*Multivariable analysis was adjusted for age, sex, body mass index, and diabetes mellitus.

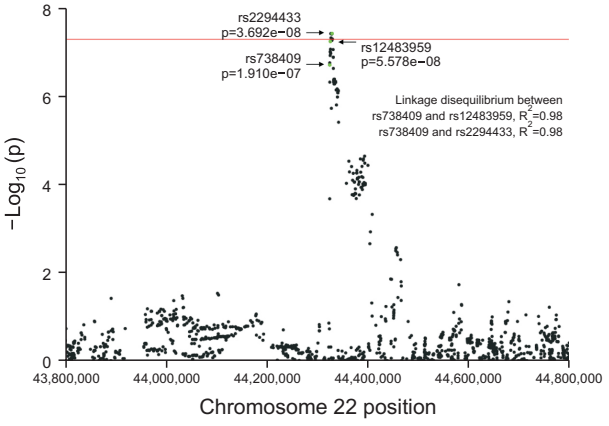

Fig. 5. Regional linkage disequilibrium between two newly identified SNPs (rs2294433 and rs12483959) and rs738409 in *PNPLA3* gene associated with nonalcoholic fatty liver disease. Statistical significance is marked by a horizontal line.

SNPs were found in the *PNPLA3* gene, which is related to NAFLD and insulin resistance.[16] Therefore, lifestyle modifications that reverse such metabolic derangements may not only improve fatty liver status but also alleviate lung function decline.

Growing evidence indicates that lung function impairment is related to the presence of NAFLD or the histological severity of NAFLD (i.e., fibrosis severity).[10] Many cross-sectional studies have reported that NAFLD has a significant correlation with lung diseases with obstructive or restrictive physiology.[17] In a recent cohort study, the FEV_1% of the predicted value was classified into quartiles, and the incidence of NAFLD was higher in the group with a lower quartile of FEV_1.[8] The most plausible hypothesis is that one of the extrahepatic metabolic complications of NAFLD may be lung function impairment. Lung function impairment may be caused by obesity, insulin resistance, and systemic inflammation, all of which have a close pathophysiological linkage with NAFLD. However, there have been no studies analyzing lung function changes according to changes in fatty liver status in a longitudinal setting. In the current study, the worsening of hepatic steatosis was accompanied by accelerated lung function decline. Conversely, the improvement of hepatic steatosis was accompanied by slow lung function decline. Thus, our results revealed the temporal relationship between the change in fatty liver status and lung function decline.

Metabolic risk factors, such as obesity, low-grade inflammation, and insulin resistance, may cause lung function impairment through the activation of lung fibrosis or bronchial inflammation and the inhibition of airway smooth muscle.[18] In several cross-sectional studies, homeostasis model assessment of insulin resistance, an indicator of insulin resistance, showed an inverse correlation with FEV_1 and FVC.[19] In several observational studies, the use of antidiabetic drugs that reduce insulin resistance (i.e., insulin sensitizers) was related to a reduced risk of exacerbation of airway inflammation in chronic obstructive lung disease patients.[20] There have been a few similar hypotheses, such as dysregulation of airway smooth muscle receptors by insulin resistance, although the specific pathway by which insulin resistance and lung function impairment are interrelated has not been well clarified.[21] In addition, several inflammatory pathways in adipose tissue, liver, and skeletal muscle contribute to the pathogenesis of insulin resistance.[22] Many clinical studies have evaluated serum high-sensitivity C-reactive protein as a systemic inflammatory marker that is related to reduced lung function.[23] In particular, obstructive lung disease patients showed a higher systemic inflammatory level than restrictive lung

disease patients.[24]

We conducted a GWAS as a new approach to explore the pleiotropic effect of common genetic factors shared between NAFLD progression and lung function impairment. As shown in the current study, genetic predisposing factors, such as the *PNPLA3* risk variant, may account for the pleiotropic effect of a shared genetic trait on multiple organs, including the liver (i.e., NAFLD) and lungs (i.e., obstructive lung disease). The *PNPLA3* polymorphism rs738409[G], encoding I148M, is a well-known risk factor for NAFLD development and progression, independent of obesity and insulin resistance.[16,25] The *PNPLA3* I148M polymorphism also reportedly increased the risk of insulin resistance, obesity, and systemic inflammation.[26-28] Indeed, previous studies demonstrated that accelerated lung function decline is attributed to insulin resistance.[19] The associations among lung function, BMI, and systemic inflammation have been well studied.[29] Considering these findings, we speculate that the *PNPLA3* I148M polymorphism may have a pleiotropic effect on NAFLD progression and accelerated lung function decline. Therefore, it is conceivable that potential therapeutic agents targeting *PNPLA3* I148M may have dual beneficial effects on liver and lung function in patients with NAFLD carrying *PNPLA3* risk variants.

Our study has several limitations. First, we detected longitudinal changes in fatty liver severity through serial liver ultrasonography, but it was difficult to quantify the absolute change in liver fat quantity. Although a misclassification bias might be noted in some patients, a previous meta-analysis reported that hepatic ultrasound provided a good measure of the presence of hepatic steatosis with good intra- and inter-observer agreement.[30] In addition, all ultrasonographic examinations in this study were performed by board-certified radiologists using the same standard protocols to reduce intra- or inter-observer diagnostic variations. Second, there are several limitations in interpreting the GWAS results. The number of subjects included in the GWAS might not be sufficient for a robust genome-wide analysis. Large-scale genome-wide research is needed to clarify the specific functional pathways that link the change in fatty liver status to lung function decline. In addition, the generalizability of our findings is limited because we used an operational definition regarding the changes in lung function and fatty liver status. The results of GWAS analysis can vary according to different definitions of outcome variables. Third, further long-term observational studies are warranted to determine whether symptomatic lung diseases actually develop in the long run, although the lung function decline rate is higher in the 'worsened' steatosis group. Fourth, the development or regression of NAFLD is largely associated with the worsening or improvement of metabolic traits, which is related to residual confounding factors, such as lifestyle modifications and changes in medical treatment or health status. Unfortunately, it could not be evaluated in this study. Since the effects of lifestyle and diet may surpass those of SNPs, further studies are needed to take into account changes in lifestyle and diet to confirm the actual association between lung functions and changes in fatty liver. Fifth, although the FIB-4 has been often used to assess fibrotic burden in patients with NAFLD, our study assessed advanced fibrosis in the included subjects with and without NAFLD.[31,32] However, recent studies showed that the FIB-4 exhibits acceptable accuracy in predicting long-term mortality risk among the general population, not only for patients with NAFLD,[33,34] and that the FIB-4 can identify individuals at risk of severe liver disease among the general population.[34] Thus, we believe that the assessment of the FIB-4 in our study subjects might have clinical implications.

With these caveats in mind, a temporal relationship between the change in fatty liver status and lung function decline over 5 years of follow-up was evident. The GWAS results suggest insulin resistance and systemic inflammation as potential linked mechanisms mediating fatty liver status change and lung function decline. Given that lung function decline is one of the major extrahepatic metabolic manifestations of NAFLD, lifestyle modifications, such as increased physical activity and hypocaloric diet, may help preserve lung function in patients with NAFLD, especially those carrying high genetic risk, such as *PNPLA3* risk variants.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MEST) (2021R1A2C2005820 and 2021M3A9E4021818), the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea (HI21C0538), and the Research Supporting Program of the Korean Association for the Study of the Liver and the Korean Liver Foundation (KASLKLF2021-02).

AUTHOR CONTRIBUTIONS

Study concept and design: H.W.L., G.E.C., B.K.K., D.K.K., W.K. Data acquisition: G.E.C., S.H.C. Data analysis and interpretation: H.S., M.C. Drafting of the manuscript; critical revision of the manuscript for important intellectual content: H.W.L., D.H.L., S.H.K., D.K.K., W.K. Statistical analysis: H.W.L. Obtained funding: W.K. Administrative, technical, or material support; study supervision: D.K.K., W.K. Approval of final manuscript: all authors.

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SUPPLEMENTARY MATERIALS

Supplementary materials can be accessed at https://doi.org/10.5009/gnl210545.

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