Genetic Variants and Longitudinal Changes in Liver Injury Risk in Individuals with Suspected Hepatic Steatosis

Chia-Yun Chang[1], Shun-Chun Yu[2], Hung-Hsin Chen[2]

[1]Department of Public Health, National Taiwan University, [2]Institute of Biomedical Sciences, Academia Sinica

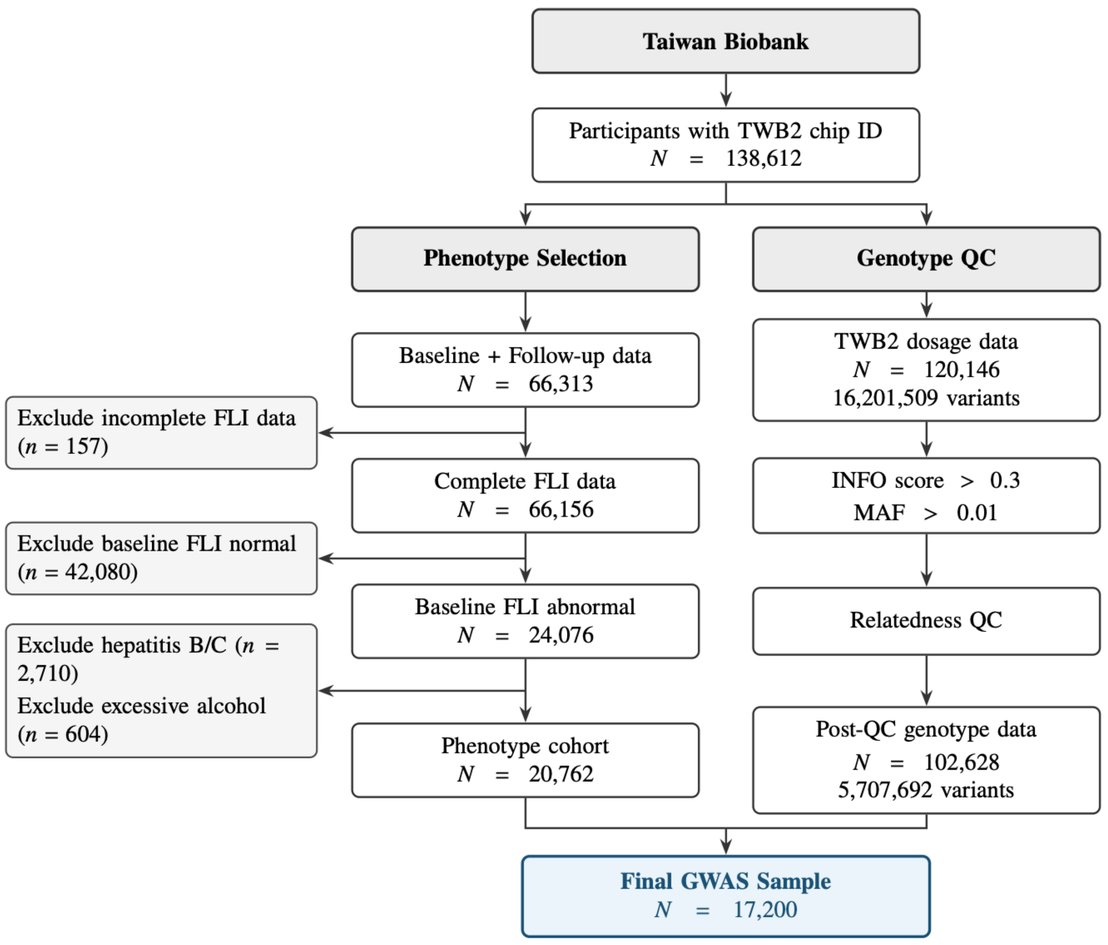
Introduction

- Metabolic dysfunction-associated steatotic liver disease (MASLD) includes a spectrum of liver disease, ranging from hepatic steatosis to steatohepatitis and fibrosis. It affects approximately 38% of adults worldwide.
- Disease progression varies across individuals, as some individuals with hepatic steatosis develop progressive liver injury while others remain stable. The heritability of MASLD is estimated at 20-50%, suggesting that genetic factors may contribute to differences in disease course. Several genetic variants, including those in *PNPLA3*, *TM6SF2*, and *HSD17B13*, have been associated with susceptibility to hepatic steatosis and MASLD. However, whether genetic factors influence longitudinal changes in liver injury risk among individuals with hepatic steatosis remains unclear.

Materials and Methods

Data Source: Longitudinal phenotype and genotype data from the Taiwan Biobank (TWB).

Study Population:



Phenotype Definition:

- Fatty Liver Index (FLI)
  A non-invasive index for estimating hepatic steatosis based on BMI, waist circumference, triglycerides (TG), and γ-glutamyl transferase (GGT), with cutoffs of ≥ 35 for males and ≥ 20 for females based on a Taiwanese validation study. Baseline FLI abnormality was used to identify individuals with suspected hepatic steatosis.
- Fibrotic NASH Index (FNI)
  A predicted probability score for fibrotic nonalcoholic steatohepatitis (NASH) based on aspartate aminotransferase (AST), high-density lipoprotein cholesterol (HDL-C), and hemoglobin A1c (HbA1c), with values ranging from 0 to 1. Fibrotic NASH was defined as NASH with NAFLD Activity Score (NAS) ≥ 4 and fibrosis stage ≥ 2 based on liver biopsy.
- Gholam score
  A non-invasive score for estimating the likelihood of NASH based on AST and type 2 diabetes mellitus, originally developed against liver biopsy-defined NASH.

Genome-wide Association Analysis

- Genome-wide association analyses were performed for FNI and Gholam scores using a longitudinal regression model, adjusting for baseline score, follow-up time, age, sex, alcohol use, and the first 10 principal components.
- LD (linkage disequilibrium) clumping was performed (index SNP $P < 5\times10^{-8}$, $r^2 > 0.1$, 500kb window)

Discussion and Limitations

- Our findings suggest that genetic variation within the *PNPLA3* locus may be associated with longitudinal changes in liver injury risk among individuals with hepatic steatosis, providing a longitudinal perspective on a locus previously studied primarily in relation to hepatic steatosis susceptibility.
- The genome-wide significant variant identified in this study, rs738408, differs from the previously reported rs738409 variant associated with hepatic steatosis, although both are located within the same *PNPLA3* locus and are in linkage disequilibrium. Further fine-mapping is needed to clarify whether rs738408 or other correlated variants within the locus may account for the observed signal.
- FNI and Gholam score are non-invasive prediction scores rather than direct histological measures of liver injury, and hepatic steatosis was identified using FLI rather than imaging- or biopsy-confirmed measures. In addition, only two time points were available, limiting assessment of longer-term disease trajectories.

Results

Table 1. Baseline and Follow-up Characteristics of the Study Population (N = 17,200)

Variable	Baseline	Follow-up
Age, years	51.1 (10.4)	56.2 (10.4)
Gender, female	10237 (59.5%)	-
Follow-up time, years	5.14 (1.47)	-
BMI, kg/m², male	28.04 (3.24)	27.96 (3.58)
BMI, kg/m², female	27.09 (3.57)	27.01 (3.87)
Waist circumference, cm, male	94.9 (7.9)	95.1 (8.9)
Waist circumference, cm, female	89.5 (8.4)	89.2 (9.3)
Fatty Liver Index (FLI)	50.6 (20.8)	47.8 (24.2)
Aspartate Aminotransferase (AST), U/L	27.2 (13.0)	27.2 (12.2)
Alanine Aminotransferase (ALT), U/L	31.6 (23.9)	30.9 (26.0)
High-Density Lipoprotein Cholesterol (HDL-C), mg/dL	48.2 (10.8)	50.2 (11.7)
Low-Density Lipoprotein Cholesterol (LDL-C), mg/dL	128.3 (32.7)	122.7 (35.8)
Triglycerides (TG), mg/dL	170.6 (118.0)	160.8 (125.1)
Gamma-Glutamyl Transferase (GGT), U/L	34.9 (35.2)	31.7 (34.7)
Hemoglobin A1c (HbA1c), %	6.02 (0.97)	6.16 (1.03)
Fibrotic NASH Index (FNI)	0.208 (0.180)	0.211 (0.178)
Gholam score	8.89 (1.22)	9.06 (1.23)
Diabetes Mellitus	2948 (17.1%)	4216 (24.5%)
Hypertension	6517 (37.9%)	8194 (47.6%)
Alcohol use, current	1036 (6.0%)	1020 (5.9%)
Smoking, current	1747 (10.2%)	1577 (9.2%)

Data are presented as mean (SD), or n (%).

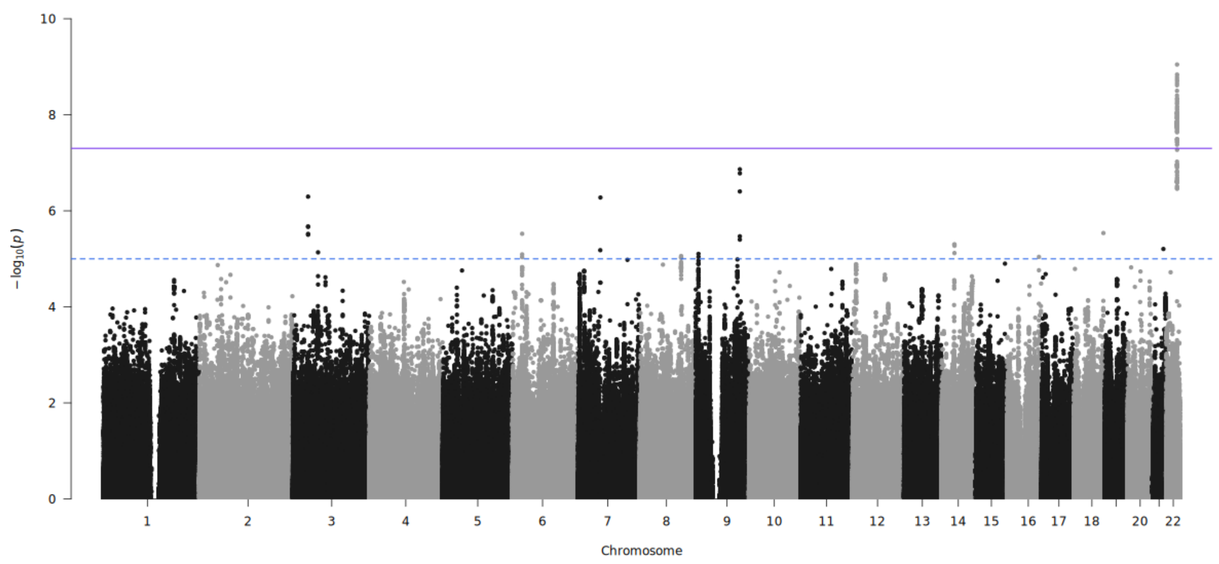


Figure 1. Manhattan plot for longitudinal changes in FNI

Table 2. Genome-wide significant locus for longitudinal changes in FNI

Chr	Position	SNP	Gene	Risk Allele	Allele Frequency	BETA	SE	P-value	SNPs in clump
22	43,928,850	rs738408	PNPLA3	T	0.381	0.0103	0.00168	8.99×10^{-10}	157

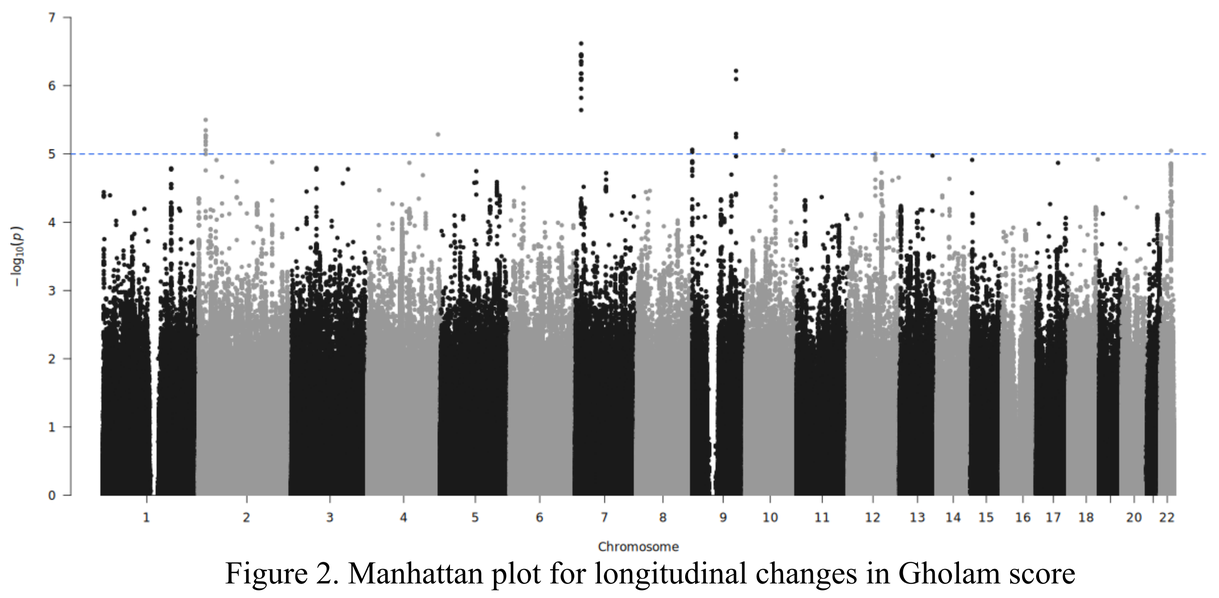


Figure 2. Manhattan plot for longitudinal changes in Gholam score

- FNI: 78 SNPs reached genome-wide significance ($P < 5 \times 10^{-8}$), corresponding to one independent locus within the *PNPLA3* region after LD clumping.
- Gholam score: No SNP reached genome-wide significance. However, rs738408 within the *PNPLA3* locus showed a suggestive association with Gholam score ($\beta = 0.0475$, $P = 2.01 \times 10^{-5}$), with an effect direction concordant with the FNI analysis.

Conclusion

Genetic variation within the *PNPLA3* locus may be associated with longitudinal changes in liver injury risk among individuals with hepatic steatosis.