***Title Page***

***FNDC5* polymorphism influences the association between sarcopenia and liver fibrosis in adults with biopsy-proven nonalcoholic fatty liver disease**

**Short Title:***FNDC5*, sarcopenia and liver fibrosis

**Authors’ name**

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**Abbreviations**

ALT, alanine aminotransferase; AST, aspartate transaminase; ASM, appendicular skeletal muscle mass; ANOVA, analysis of variance; BIA, bioelectrical impedance analyzer; BMI, body mass index; CI, confidence interval; *FNDC*5, fibronectin type III domain-containing protein 5; GGT, γ-glutamyltranspeptidase; HOMA-IR, homeostasis model assessment-insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NAS-CRN, NASH-Clinical Research Network; NAS, NAFLD activity score; OR, odds ratio; TG, triglyceride; TC, total cholesterol.

**Authorship Statement:**

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All authors contributed to the manuscript for important intellectual content and approved the submission.

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**Informed consent:** Written informed consent was obtained from all participants included in the study.

**Abstract**

**Background:** The *FNDC5* gene encodes the fibronectin type III domain-containing protein 5 that is a membrane protein mainly expressed in skeletal muscle and the *FNDC5* rs3480 polymorphism may be associated with liver disease severity in nonalcoholic fatty liver disease (NAFLD). We investigated the influence of the *FNDC5* rs3480 polymorphism on the relationship between sarcopenia and the histological severity of NAFLD.

**Methods:** 370 adult individuals with biopsy-proven NAFLD were studied. Appendicular skeletal muscle mass was measured by bioelectrical impedance. The association between the key exposure sarcopenia, and the outcome liver histological severity, was investigated by binary logistic regression. Stratified analyses were undertaken to examine the impact of *FNDC5* rs3480 polymorphism on the association between sarcopenia and the severity of NAFLD histology.

**Results:** Patients with sarcopenia had more severe histological grades of steatosis and a higher prevalence of significant fibrosis and definite NASH than those without sarcopenia. There was a significant association between sarcopenia and significant fibrosis (adjusted odds ratio 2.79, 95%CI 1.31-5.95, *p*=0.008), independent of established risk factors and potential confounders. Among patients with sarcopenia, significant fibrosis occurred more frequently in the rs3480 AA genotype carriers than in those carrying the *FNDC5* rs3480 G genotype (43.8% vs. 17.2%, *p*=0.031). In the association between sarcopenia and liver fibrosis, there was a significant interaction between the *FNDC5* genotype and sarcopenia status (*p-*value for interaction=0.006).

**Conclusion:** Sarcopenia is independently associated with significant liver fibrosis, and the *FNDC5* rs3480 G variant influences the association between sarcopenia and liver fibrosis in patients with biopsy-proven NAFLD.

**KEY-WORDS:** Sarcopenia; Nonalcoholic fatty liver disease; Skeletal muscle; SNP.

**Introduction**

Sarcopenia is associated with physical inactivity, poor nutritional status, and genetic susceptibility.([1](#_ENREF_1)) Recent studies using non-invasive tests show that low skeletal muscle mass is associated with the presence of nonalcoholic fatty liver disease (NAFLD) and significant liver fibrosis.([2](#_ENREF_2); [3](#_ENREF_3); [4](#_ENREF_4); [5](#_ENREF_5)) Skeletal muscle is an important organ that affects whole-body insulin sensitivity, and low skeletal muscle mass may influence metabolic health through altered insulin-mediated glucose disposal.([6](#_ENREF_6)) Moreover, skeletal muscle may release a variety of myokines that influence other organs such as adipose tissue and liver.([7](#_ENREF_7)) It has been suggested that myokines (e.g., interleukin-6, interleukin-15, and irisin) may mediate, at least in part, the protective effects of physical exercise against chronic diseases, such as type 2 diabetes, NAFLD, and cardiovascular diseases.([8](#_ENREF_8))

Physical exercise may increase the expression of fibronectin type III domain-containing protein 5 (*FNDC5*), a membrane protein that is mainly expressed in skeletal muscle and is cleaved and released into the circulation as irisin.([9](#_ENREF_9)) Some studies have recently indicated that the *FNDC5* rs3480 G variant affects the stability and expression of *FNDC5* and may be associated with protection from clinically significant fibrosis in patients with NAFLD.([10](#_ENREF_10); [11](#_ENREF_11)) However, the impact ofthe *FNDC5* rs3480 G variant on the histologic features of NAFLD remains controversial. *Petta* et al. have reported that the *FNDC5* rs3480 G allele was associated with lower levels of significant fibrosis in NAFLD.([10](#_ENREF_10)) In contrast, *Metwally* et al. found that the *FNDC5* rs3480 G variant was associated with more severe hepatic steatosis, but not with other histological features of NAFLD.([11](#_ENREF_11))

It is known that the interaction between metabolic risk factors and genetic background plays a key role in the progression of NAFLD.([5](#_ENREF_5); [12](#_ENREF_12)) However, it is currently uncertain whether skeletal muscle-related gene polymorphisms influence the association between low skeletal muscle mass and NAFLD. Thus, the major aim of our cross-sectional study was to investigate the influence of the *FNDC5* rs3480 polymorphism on the association between sarcopenia and the histological severity of NAFLD. In addition, since sex plays an important role in the disease progression in NAFLD and may also affect muscle mass,([13](#_ENREF_13); [14](#_ENREF_14)) we have further investigated the association between *FNDC5*, sarcopenia and the severity of NAFLD in both men and women, separately.

**Materials and Methods**

***Study population and design***

We consecutively enrolled a total of 638 adults with suspected NAFLD (based on the presence of hepatic steatosis on imaging methods and/or elevated serum liver enzymes), who consecutively attended the First Affiliated Hospital of Wenzhou Medical University (China) from December 2016 to November 2018. As detailed in **Figure 1**, 268 subjects were excluded for the following reasons: (1) excessive alcohol consumption (≥ 140 g/week in men or ≥ 70g/week in women); (2) presence of viral hepatitis, autoimmune hepatitis, drug-induced liver injury, or other known chronic liver diseases; (3) incomplete clinical/biochemical or genetic data; and (4) fatty liver infiltration <5% on liver histology. As a consequence of these exclusion criteria, a sample of 370 adults with biopsy-proven NAFLD was included in the final analysis. All these patients did not have any prior history of cancer.

The study was approved by the internal review board for ethics of the First Affiliated Hospital of Wenzhou Medical University (2016-246, 1 December 2016). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration. The study protocol was registered in the Chinese Clinical Trial Registry (ChiCTR-EOC-17013562). Written informed consent was obtained from each participant enrolled in the study.

***Laboratory and clinical data***

Venous fasted blood samples were taken after a 12-hour fast in all participants. Laboratory biochemical parameters were centrally analyzed by using an automated analyzer (Abbott AxSYM). Body mass index (BMI) was calculated using the formula weight (kilograms) divided by height (meters) squared. Obesity was defined as BMI ≥25 kg/m2 or waist circumference ≥90 cm in men or ≥80 cm in women.([15](#_ENREF_15); [16](#_ENREF_16)) Hypertension was diagnosed as blood pressure ≥130/85 mmHg or the use of any anti-hypertensive drugs. Diabetes was diagnosed as either self-reported history of disease, fasting plasma glucose level ≥7.0 mmol/L, HbA1c ≥6.5% (≥48 mmol/mol) or treatment with hypoglycemic drugs. Atherogenic dyslipidemia was defined as any of the following criteria: triglycerides >1.70 mmol/L; high-density lipoprotein cholesterol (HDL-C) <1.03 mmol/L in men and <1.29 mmol/L in women; or use of any lipid-lowering drugs.([16](#_ENREF_16))

All the aforementioned anthropometric and laboratory data were obtained from participants within 24 hours of liver biopsy examinations.

***Definition of sarcopenia***

Body weight and composition were measured using the segmental multi-frequency bioelectrical impedance analyzer (BIA, Inbody 720; Inbody Japan Inc., Tokyo, Japan). BIA measured impedance in each segment, including the bilateral upper and lower limbs, providing an estimate of the appendicular skeletal muscle mass (ASM). Specifically, the ASM was calculated as the sum of skeletal muscle mass in the four limbs divided by body weight and expressed as a percentage (ASM/weigh ×100, skeletal muscle index%). The ASM-to-BMI ratio (ASM/BMI) was also calculated. According to previous studies conducted in East Asian people,([17](#_ENREF_17); [18](#_ENREF_18)) relevant to the ethnicity of our participants, sarcopenia was defined as a skeletal muscle index <29.0% in men and <22.9% in women, or ASM/BMI ratio <0.789 in men and <0.512 in women, respectively.([17](#_ENREF_17))

Liver histology

Liver histology assessment was undertaken by an experienced liver histopathologist according to the NASH-Clinical Research Network (CRN) Scoring System.([19](#_ENREF_19)) The liver histopathologist was blinded to the research measurements related to sarcopenia. NAFLD was diagnosed by the presence of hepatic steatosis in more than 5% of hepatocytes. Based on the NASH-CRN,([19](#_ENREF_19)) the NAFLD activity score (NAS) was calculated as the sum of the three histological components including steatosis (0-3), ballooning (0-2), and lobular inflammation (0-3). Liver fibrosis was not included as a component of NAS. Individuals with NAS score of 1 to 2 was defined as simple steatosis (NAFL), 3 to 4 was defined as borderline NASH, and 5 or greater was defined as definite NASH. Liver fibrosis was staged as zero to 4 according to Brunt’s criteria.([20](#_ENREF_20)) Fibrosis with 2 or greater on liver histology indicated significant fibrosis.

FNDC5 genotype

Genotyping assays for FNDC5 rs3480 A>G, *PNPLA3* rs738409 C>G, and *TM6SF2* rs58542926 C>T, on human peripheral blood leukocytes were carried out using the MassARRAY platform (Agena Bioscience, San Diego, CA, USA). Locus-specific PCR and detection primers were designed using Assay Design Suite v3.1. After the DNA samples were amplified via multiplex PCR, allele detection was performed using MALDI-TOF mass spectrometry.

Statistical analysis

Continuous variables were expressed as means ± SD or medians with inter-quartile ranges (IQRs), according to whether their distribution was normal or skewed. Categorical variables were expressed as percentages. Comparisons between the patient groups were made by the chi-square test or the Fisher exact test for categorical variables, and by the unpaired Student t-test, the Mann-Whitney U test, the one-way analysis of variance (ANOVA) or the Kruskal-Wallis test for normally and no-normally distributed continuous variables as appropriate. The chi-square test was also used to assess whether the genotypes were in Hardy-Weinberg equilibrium. *FNDC5* rs3480 associations were assessed using a dominant genetic model. The association between sarcopenia and presence of either definite NASH or significant fibrosis was assessed by binary logistic regression and the models were adjusted for potential risk factors of NASH and fibrosis (as specified in the Results section below). Stratified and interaction analyses were used to examine the impact of FNDC5 rs3480 polymorphism on the association of sarcopenia with the histological severity. Statistical analyses were two-sided and statistical significance was set at p <0.05. All statistical tests were performed using SPSS version 23.0 (SPSS Inc., Chicago, USA).

Results

***Baseline characteristics of the study population***

Among the 370 adults with biopsy-proven NAFLD, 61 adults (16.5%) were diagnosed as having sarcopenia. The mean age of the whole cohort was 41 years and 75.7% were male. The mean values of waist circumference and BMI were 92.1 cm and 26.8 kg/m2, respectively. 117 subjects (31.6%) had established type 2 diabetes, 139 (37.6%) subjects had hypertension, and 314 (84.9%) subjects had atherogenic dyslipidemia. Definite NASH was diagnosed in 131 subjects (35.4%) and significant fibrosis in 58 subjects (15.7%). Compared with those without sarcopenia, sarcopenic patients were more likely to be male and had higher levels of BMI, waist circumference, alanine aminotransferase (ALT), aspartate transaminase (AST), γ-glutamyltranspeptidase (GGT) and homeostasis model assessment-insulin resistance (HOMA-IR), more severe histological grades of hepatic steatosis and fibrosis, and higher proportions of definite NASH and significant fibrosis (Table 1).

The frequency distributions of FNDC5 rs3480, PNPLA3 rs738409, and TM6SF2 rs58542926 genotypes were in Hardy-Weinberg equilibrium (p=0.587, 0.143, and 0.609, respectively). Stratifying by the FNDC5 rs3480 polymorphism, 188 (50.8%) subjects had A/A genotype, 155 (41.9%) had A/G genotype, and 27 (7.3%) had G/G genotype, respectively. No significant differences in clinical characteristics and liver histological severity were observed among the groups of patients with different FNDC5 genotypes, except for hypertension (Supplementary Table 1). The G variant carriers had a lower percentage of hypertension (A/A: 45.5% vs. A/G: 29.7% vs. G/G 29.6%, p=0.009).

Sarcopenia is associated with histological severity of NAFLD

As shown in **Table 3**, in the unadjusted logistic regression model, patients with sarcopenia exhibited a 2-fold increase in the risk of having definite NASH (OR 1.91, 95%CI 1.06-3.43, *p*=0.031) and a 4-fold increase in the risk of having significant fibrosis (OR 3.87, 95%CI 1.94-7.69, *p*<0.001), compared to those patients without sarcopenia. In a logistic regression model with NASH/no NASH as the outcome, the association between sarcopenia and definite NASH was significant even after adjusting for age, sex, obesity, type 2 diabetes, hypertension, dyslipidemia, and smoking (model 2: OR 1.91, 95%CI 1.02-3.55, *p*=0.042). However, this association was no longer significant after further adjustment for serum liver enzymes, and HOMA-IR values (model 3: OR 1.65, 95%CI 0.86-3.18, *p*=0.130). Notably, the association between sarcopenia and significant fibrosis was slightly attenuated but remained significant in the fully adjusted regression model (model 4: OR 2.79, 95%CI 1.31-5.95, *p*=0.008).

Association between sarcopenia and the histological severity of NAFLD stratified by FNDC5 genotypes

Stratified analyses were conducted to evaluate the effect of FNDC5 rs3480 A>G variant on the severity of NAFLD histology. We observed a strong relationship between the presence of sarcopenia and more severe histological grades of ballooning and fibrosis, and a higher prevalence of both definite NASH and significant fibrosis in patients carrying the rs3480 AA genotype, but not in those carrying the rs3480 AG or GG genotypes (Figure 2 and Table 2). Moreover, sarcopenic patients had higher serum AST and GGT levels in those carrying the rs3480 AA genotype but not in those carrying the AG or GG genotypes (Table 2).

As shown in **Table 3**, in the unadjusted logistic regression model, the presence of sarcopenia was associated with a 3.3-fold increase in the risk of definite NASH (OR 3.29, 95%CI 1.50-7.20, *p*=0.003) and a nearly 7-fold increase in the risk of significant fibrosis (OR 7.31, 95%CI 3.04-17.59; *p*<0.001) in individuals with rs3480 AA genotype. However, these significant associations were not found among the rs3480 AG or GG genotype carriers.

To further explore the independent effect of sarcopenia on liver disease severity in individuals with different FNDC5 genotypes, we performed multivariable logistic regression models with potential risk factors as covariates (**Table 3**). The association between sarcopenia and the histological severity of NAFLD remained significant after adjusting for age, sex, smoking, obesity, diabetes, hypertension, dyslipidemia status, serum liver enzymes (ALT, AST, GGT), HOMA-IR, PNPLA3 rs738409, and TM6SF2 rs58542926 variants in individuals carrying the rs3480 AA genotype (adjusted OR 3.27, 95%CI 1.15-9.31, *p*=0.026 for definite NASH; adjusted OR 7.19, 95%CI 2.40-21.55, *p*<0.001 for significant fibrosis), but not in those carrying the AG or GG genotypes (adjusted OR 1.17, 95%CI 0.43-3.13, *p*=0.759 for definite NASH; OR 1.01, 95%CI 0.29-3.57, *p*=0.983 for significant fibrosis).

***The FNDC5*** ***rs3480 G variant provided a protective effect for significant fibrosis in patients with*** sarcopenia

The aforementioned analyses suggested that the *FNDC5* rs3480 variant might have a protective effect on the severity of NAFLD histology. However, there were no significant differences in the histological severity of NAFLD among the groups of patients with different *FNDC5* genotypes (**Supplementary Table 1**). As reported in **Figure 3A**, patients with sarcopenia carrying the G variant genotype appeared to have a lower proportion of definite NASH than those carrying the A/A genotype, but the effect was not statistically significant (48.3% vs. 59.4%, *p*=0.385). Among patients without sarcopenia, there was no difference in the percentage of definite NASH between patients with the A/A genotype and those with the G variant genotype (30.8% vs. 32.7%, *p*=0.718). As shown in **Figure 3B**, there was a significantly lower proportion of patients with significant fibrosis in sarcopenic patients with the G variant genotype, than in those with the A/A genotype (17.2% vs. 43.8%, *p*=0.031). However, among patients without sarcopenia, there was no significant difference in the percentage of significant fibrosis between the two genotypes (15.7% vs. 9.6%, *p*=0.108). Notably, there was a significant interaction between *FNDC5* genotype and sarcopenia, with significant fibrosis (*p-*value for interaction=0.006).

***Stratified analyses according to sex differences***

We further explored the association between sarcopenia, *FNDC5* genotypes, and liver fibrosis both in men and in women. As shown in **Table 4**, in the unadjusted model, the presence of sarcopenia was associated with an increased risk of significant fibrosis in both sexes (men: OR 3.85, 95% CI 1.82-8.14; women: OR 8.82, 95% CI 1.41-55.35). After adjustment for potential confounding factors (fully adjusted model 3), the association between sarcopenia and significant fibrosis remained statistically significant in men (adjusted OR 2.57, 95%CI 1.10-5.98, *p*=0.029). We also observed a significant association between sarcopenia and significant fibrosis in women, even after adjustment for age, obesity, diabetes, hypertension, and dyslipidemia (adjusted OR 11.74, 95%CI 1.43-96.11, *p*=0.022). Further adjustment for other potential confounding variables was not feasible due to the relatively small number of women included in the study.

As shown in **Figure 4**, in NAFLD patients carrying the rs3480 AA genotype, both men and women with sarcopenia had a higher proportion of significant fibrosis than those without sarcopenia (men: 37.04% vs. 7.27%, *p*<0.001; women: 80.0% vs. 15.22%, *p*=0.006). However, in those carrying the AG or GG genotype carriers, the proportion with significant fibrosis was not significantly different between patients with and without sarcopenia irrespective of sex (men: 17.86% vs. 10.43%, *p*=0.276; women: 0% vs. 31.58%, *p*=0.999). In addition, as shown in **Table 4**, the association between sarcopenia and significant fibrosis remained significant after full adjustment for potential confounders only in the men with rs3480 AA genotype (adjusted OR 4.47, 95%CI 1.26-15.79, *p*=0.020), but not in men with AG or GG genotypes (OR 1.05, 95%CI 0.24-4.57, *p*=0.950). This fully adjusted regression model was not feasible in women due to their small sample size.

**Discussion**

Our novel results show that in patients with biopsy-confirmed NAFLD, sarcopenia is independently associated with significant liver fibrosis, and in this association, there is a significant interaction between *FNDC5* genotype and sarcopenia status. In addition, when our NAFLD patients were stratified by sex, both men and women carrying the rs3480 AA genotype who had sarcopenia exhibited a significantly higher proportion of significant fibrosis than those without sarcopenia. However, in NAFLD patients carrying the rs3480 AG or GG genotypes, neither female nor male patients with sarcopenia had a higher proportion of significant fibrosis than their counterparts without sarcopenia. These results remained essentially unchanged in men even after adjustment for potential confounding variables.

FNDC5 has effects in several organs, including a role in the liver and muscle.([21](#_ENREF_21)) A recent study found that the hepatic expression of *FNDC5* in NAFLD could dampen hepatocyte fat accumulation, insulin resistance and liver injury.([21](#_ENREF_21)) These beneficial effects could occur in both liver and muscle as irisin mediates adipose tissue thermogenesis, and may regulate carbohydrate and lipid metabolism. To our knowledge, no previous studies have explored the potential influence of skeletal muscle-related gene FNDC5 polymorphisms on the relationship between sarcopenia and NAFLD. Therefore, we analyzed the association between sarcopenia and the severity of NAFLD histology in subjects stratified by *FNDC5* rs3480 polymorphism. Our stratified analyses revealed that these associations became statistically not significant among individuals with the *FNDC5* rs3480 G variant genotype.

In recent years, the relationship between sarcopenia and NAFLD has attracted attention.([22](#_ENREF_22)) However, most published studies have used non-invasive tests to evaluate the severity of liver steatosis and fibrosis([2](#_ENREF_2); [3](#_ENREF_3); [4](#_ENREF_4); [5](#_ENREF_5)) and, importantly, the use of non-invasive tests may result in misclassification of disease status.([23](#_ENREF_23)) There is still a lack of reliable non-invasive tests for the assessment of NAFLD severity and liver biopsy remains, to date, the ‘gold standard’ for diagnosing NASH and fibrosis. In this study, we showed that patients with NAFLD and sarcopenia have higher levels of serum liver enzymes and more severe liver histological features compared to patients without sarcopenia. Even after adjusting for age, sex, anthropometric, biochemical, and genetic risk factors, the presence of sarcopenia remained significantly associated with approximately a two-fold increased risk of significant fibrosis. This association is in agreement with the results from other Asian and European studies.([17](#_ENREF_17); [24](#_ENREF_24)) Moreover, we also showed a strong relationship between sarcopenia and both obesity and insulin resistance, which is consistent with findings reported in other clinical settings.([17](#_ENREF_17); [24](#_ENREF_24))

Contrary to our expectation, patients with sarcopenia were younger than those without sarcopenia in the present study. This unexpected finding might in part be related to the associated metabolic factors that were more adversely affected in patients with sarcopenia than in those without sarcopenia (e.g. increased central obesity and insulin resistance). Moreover, the diagnosis of sarcopenia was not only affected by the muscle mass but also by body weight and BMI. Using the age cut-off of 60 years to define the older and the younger patients, we observed that the former not only had lower muscle mass (20.6 ± 3.8 vs. 23.5 ± 4.3 kg), but also lower body weight (70.2 ± 9.3 vs. 77.7 ± 13.8 kg) and lower BMI (25.8 ± 2.4 vs. 27.1 ± 3.5 kg/m2) compared with the younger patients. The observation that the younger patients were heavier and have a higher BMI than the older patients may influence the association between sarcopenia and age in our cohort of NAFLD patients. The occurrence of NAFLD parallels the high rates of obesity in young individuals.([25](#_ENREF_25)) Moreover, the participants in our cohort were selected from patients who were referred with suspected NAFLD rather than the general population. Thus, our findings are applicable only to patients with NAFLD.

Low muscle mass has a strong negative prognostic impact in obese individuals and may lead to increased morbidity and mortality.([26](#_ENREF_26)) Maintaining skeletal muscle mass in obesity is important and therefore the term ‘sarcopenic obesity’ has been proposed. Our study also demonstrated a high proportion of sarcopenic obesity in NAFLD. When obesity was defined by a BMI ≥25 kg/m2, 16.2% (60/370) of our NAFLD patients met the criteria for sarcopenic obesity. When obesity was defined by a BMI ≥30 kg/m2, 5.7% (21/370) of our NAFLD patients met the criteria for sarcopenic obesity.

The role of *FNDC5* on the progression of NAFLD is controversial. Our results showed that there were no significant differences in the prevalence of sarcopenia and severity of liver histology among individuals with different genotypes. One recent study has found that the *FNDC5* rs3480 G variant was associated with lower levels of significant fibrosis,([10](#_ENREF_10)) although another study has found that the G variant was only associated with more severe steatosis.([11](#_ENREF_11)) Differences in baseline characteristics might, at least in part, explain these conflicting results. As our study found, the presence of sarcopenia influenced the effect of the *FNDC5* gene on NAFLD, and the *G* variant only provided a protective effect on patients with sarcopenia. The underlying mechanism is uncertain. However, what is certain is that *FNDC5* rs3480 G variant may affect the stability and expression of *FNDC5*,([11](#_ENREF_11)) which is cleaved as irisin. Irisin has been shown to have favorable metabolic effects on metabolic diseases, including NAFLD.([27](#_ENREF_27); [28](#_ENREF_28)) Zhang et al. also reported that increased serum irisin levels were associated with lower serum liver enzymes and decreased hepatic triglyceride content in obese Chinese adults.([29](#_ENREF_29))

The underlying mechanisms explaining the association between sarcopenia and significant fibrosis are not fully understood. The widely accepted mechanism is that loss of muscle mass reduces a key cellular target for insulin, contributing to systemic insulin resistance and insulin resistance is very strongly associated with NAFLD.([30](#_ENREF_30)) Skeletal muscle is responsible for the majority of the body’s postprandial glucose disposal, and insulin mediates GLUT-4 glucose uptake in skeletal muscle. Therefore, the potential mechanisms linking sarcopenia to NAFLD may involve skeletal muscle insulin resistance.([31](#_ENREF_31)) The findings of our research also suggest another possible mechanism. Sarcopenia might contribute to liver damage via a reduced production of myokines, e.g. interleukin-6 and irisin, and the expression of *FNDC5* directly affects irisin levels. This suggests that irisin may play an important role in the association between *FNDC5* variants, sarcopenia with liver fibrosis.

There are some important limitations to our study. Firstly, owing to the cross-sectional design of the study, it is not possible to draw any conclusion about causality. However, the genetic variant is inherited and, therefore, reverse causation does not apply. Secondly, skeletal muscle mass was measured by BIA. BIA is an instrument for screening low skeletal muscle mass in NAFLD.([32](#_ENREF_32)) Previous studies have confirmed that the use of BIA (instead of magnetic resonance imaging or dual-energy X-ray absorptiometry) for estimating ASM is appropriate.([33](#_ENREF_33)) Thirdly, all participants in our study are of Asian ethnicity and, therefore, our findings need to be verified in other ethnic groups. Finally, it is well known that muscle mass is affected by sex and reproductive status. Unfortunately, no detailed information was available on pre-menopausal and post-menopausal status in our cohort. Future larger cohorts of NAFLD patients with available data on reproductive status are needed to better examine the association between FNDC5, sarcopenia and liver fibrosis in NAFLD.

In conclusion, the results of our study show that sarcopenia is independently associated with significant fibrosis and there is a significant interaction between *FNDC5* genotype and sarcopenia status with significant fibrosis in a well-characterized cohort of patients with biopsy-proven NAFLD.

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**Conflict of interest statement:**

All authors: nothing to declare.

REFERENCES

1. Marty E, Liu Y, Samuel A *et al.* (2017) A review of sarcopenia: Enhancing awareness of an increasingly prevalent disease. *Bone* **105**, 276-286.

2. Lee Y-h, Kim SU, Song K *et al.* (2016) Sarcopenia is associated with significant liver fibrosis independently of obesity and insulin resistance in nonalcoholic fatty liver disease: Nationwide surveys (KNHANES 2008-2011). *Hepatology (Baltimore, Md)* **63**, 776-786.

3. Hong HC, Hwang SY, Choi HY *et al.* (2014) Relationship between sarcopenia and nonalcoholic fatty liver disease: the Korean Sarcopenic Obesity Study. *Hepatology (Baltimore, Md)* **59**, 1772-1778.

4. Peng T-C, Wu L-W, Chen W-L *et al.* (2019) Nonalcoholic fatty liver disease and sarcopenia in a Western population (NHANES III): The importance of sarcopenia definition. *Clin Nutr* **38**, 422-428.

5. Xia M-F, Chen L-Y, Wu L *et al.* (2019) The PNPLA3 rs738409 C>G variant influences the association between low skeletal muscle mass and NAFLD: the Shanghai Changfeng Study. *Alimentary pharmacology & therapeutics* **50**, 684-695.

6. Mesinovic J, Zengin A, De Courten B *et al.* (2019) Sarcopenia and type 2 diabetes mellitus: a bidirectional relationship. *Diabetes Metab Syndr Obes* **12**, 1057-1072.

7. Karstoft K, Pedersen BK (2016) Skeletal muscle as a gene regulatory endocrine organ. *Curr Opin Clin Nutr Metab Care* **19**, 270-275.

8. Gomarasca M, Banfi G, Lombardi G (2020) Myokines: The endocrine coupling of skeletal muscle and bone. *Adv Clin Chem* **94**, 155-218.

9. Perakakis N, Triantafyllou GA, Fernández-Real JM *et al.* (2017) Physiology and role of irisin in glucose homeostasis. *Nat Rev Endocrinol* **13**, 324-337.

10. Petta S, Valenti L, Svegliati-Baroni G *et al.* (2017) Fibronectin Type III Domain-Containing Protein 5 rs3480 A>G Polymorphism, Irisin, and Liver Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *J Clin Endocrinol Metab* **102**, 2660-2669.

11. Metwally M, Bayoumi A, Romero-Gomez M *et al.* (2019) A polymorphism in the Irisin-encoding gene (FNDC5) associates with hepatic steatosis by differential miRNA binding to the 3'UTR. *Journal of hepatology* **70**, 494-500.

12. Hu DS, Zhu SH, Liu WY *et al.* (2020) PNPLA3 polymorphism influences the association between high-normal TSH level and NASH in euthyroid adults with biopsy-proven NAFLD. *Diabetes Metab* **doi:10.1016/j.diabet.2020.02.001**.

13. Lonardo A, Suzuki A (2020) Sexual Dimorphism of NAFLD in Adults. Focus on Clinical Aspects and Implications for Practice and Translational Research. *Journal of clinical medicine* **9**.

14. Capozza RF, Cointry GR, Cure-Ramírez P *et al.* (2004) A DXA study of muscle-bone relationships in the whole body and limbs of 2512 normal men and pre- and post-menopausal women. *Bone* **35**, 283-295.

15. Goda A, Masuyama T (2016) Obesity and Overweight in Asian People. *Circulation journal : official journal of the Japanese Circulation Society* **80**, 2425-2426.

16. Alberti KGMM, Eckel RH, Grundy SM *et al.* (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* **120**, 1640-1645.

17. Koo BK, Kim D, Joo SK *et al.* (2017) Sarcopenia is an independent risk factor for non-alcoholic steatohepatitis and significant fibrosis. *Journal of hepatology* **66**, 123-131.

18. Kim Y-S, Lee Y, Chung Y-S *et al.* (2012) Prevalence of sarcopenia and sarcopenic obesity in the Korean population based on the Fourth Korean National Health and Nutritional Examination Surveys. *J Gerontol A Biol Sci Med Sci* **67**, 1107-1113.

19. Kleiner DE, Brunt EM, Van Natta M *et al.* (2005) Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology (Baltimore, Md)* **41**, 1313-1321.

20. Brunt EM, Janney CG, Di Bisceglie AM *et al.* (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *The American journal of gastroenterology* **94**, 2467-2474.

21. Canivet CM, Bonnafous S, Rousseau D *et al.* (2020) Hepatic FNDC5 is a potential local protective factor against Non-Alcoholic Fatty Liver. *Biochimica et biophysica acta Molecular basis of disease* **1866**, 165705.

22. Cai C, Song X, Chen Y *et al.* (2020) Relationship between relative skeletal muscle mass and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Hepatology international* **14**, 115-126.

23. Castera L, Friedrich-Rust M, Loomba R (2019) Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* **156**.

24. Petta S, Ciminnisi S, Di Marco V *et al.* (2017) Sarcopenia is associated with severe liver fibrosis in patients with non-alcoholic fatty liver disease. *Alimentary pharmacology & therapeutics* **45**, 510-518.

25. Doycheva I, Watt KD, Alkhouri N (2017) Nonalcoholic fatty liver disease in adolescents and young adults: The next frontier in the epidemic. *Hepatology (Baltimore, Md)* **65**, 2100-2109.

26. Barazzoni R, Bischoff S, Boirie Y *et al.* (2018) Sarcopenic Obesity: Time to Meet the Challenge. *Obesity facts* **11**, 294-305.

27. Polyzos SA, Anastasilakis AD, Efstathiadou ZA *et al.* (2018) Irisin in metabolic diseases. *Endocrine* **59**, 260-274.

28. Shanaki M, Moradi N, Emamgholipour S *et al.* (2017) Lower circulating irisin is associated with nonalcoholic fatty liver disease and type 2 diabetes. *Diabetes Metab Syndr* **11 Suppl 1**, S467-S472.

29. Zhang H-J, Zhang X-F, Ma Z-M *et al.* (2013) Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. *Journal of hepatology* **59**, 557-562.

30. Cleasby ME, Jamieson PM, Atherton PJ (2016) Insulin resistance and sarcopenia: mechanistic links between common co-morbidities. *J Endocrinol* **229**, R67-R81.

31. Montalcini T, Pujia A, Donini LM *et al.* (2020) A Call to Action: Now Is the Time to Screen Elderly and Treat Osteosarcopenia, a Position Paper of the Italian College of Academic Nutritionists MED/49 (ICAN-49). *Nutrients* **12**, E2662.

32. Kim G, Lee S-E, Lee Y-B *et al.* (2018) Relationship Between Relative Skeletal Muscle Mass and Nonalcoholic Fatty Liver Disease: A 7-Year Longitudinal Study. *Hepatology (Baltimore, Md)* **68**, 1755-1768.

33. Bosy-Westphal A, Jensen B, Braun W *et al.* (2017) Quantification of whole-body and segmental skeletal muscle mass using phase-sensitive 8-electrode medical bioelectrical impedance devices. *Eur J Clin Nutr* **71**, 1061-1067.

**FIGURE LEGENDS**

**Figure 1**. The flow chart for the study.

**Figure 2.** Association between sarcopenia and histological features of NAFLD, stratified by *FNDC5* rs3480 genotypes.

**Figure 3.** Association between *FNDC5* rs3480 genotypes and the histological severity of NAFLD, stratified bysarcopenia.

**Figure 4.** Association between sarcopenia, *FNDC5* genotypes and significant fibrosis, stratified by sex.

**Table 1. Baseline characteristics of study participants, stratified by sarcopenia status.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Overall population**  **(n=370)** | **Without sarcopenia**  **(n=309)** | **With sarcopenia**  **(n=61)** | ***P* value** |
| **Demographics** |  |  |  |  |
| Age, years | 41.0 ± 11.9 | 42.0 ± 11.7 | 36.0 ± 11.9 | <0.001 |
| Male sex, n (%) | 280 (75.7%) | 225 (72.8%) | 55 (90.2%) | 0.004 |
| **Metabolic factors** |  |  |  |  |
| BMI, kg/m2 | 26.8 ± 3.3 | 26.1 ± 2.8 | 30.1 ± 3.4 | <0.001 |
| Waist circumference, cm | 92.1 ± 8.7 | 90.7 ± 7.7 | 99.1 ± 10.0 | <0.001 |
| Skeletal muscle index†, % | 30.0 ± 3.1 | 30.5 ± 3.0 | 27.3 ± 2.2 | <0.001 |
| ASM/BMI ratio | 0.86 ± 0.15 | 0.87 ± 0.15 | 0.79 ± 0.11 | <0.001 |
| Type 2 diabetes, n (%) | 117 (31.6%) | 100 (32.4%) | 17 (27.9%) | 0.490 |
| Hypertension, n (%) | 139 (37.6%) | 117 (37.9%) | 22 (36.1%) | 0.791 |
| Dyslipidemia, n (%) | 314 (84.9%) | 260 (84.1%) | 54 (88.5%) | 0.383 |
| Cigarette smoking, n (%) |  |  |  | 0.706 |
| Never | 247 (66.8%) | 209 (67.6%) | 38 (62.3%) |  |
| Ever | 37 (10.0%) | 30 (9.7%) | 7 (11.5%) |  |
| Current | 86 (23.2%) | 70 (22.7%) | 16 (26.2%) |  |
| **Laboratory parameters** |  |  |  |  |
| ALT, IU/L | 54 (33-92) | 49 (31-87) | 87 (53-150) | <0.001 |
| AST, IU/L | 34 (26-54) | 33 (25-50) | 52 (33-81) | <0.001 |
| γ-GT, IU/L | 53 (33-82) | 49 (31-80) | 66 (49-96) | <0.001 |
| Albumin, g/dL | 4.6 ± 0.4 | 4.6 ± 0.4 | 4.7 ± 0.4 | 0.168 |
| Bilirubin, μmol/L | 12 (10-16) | 12 (10-16) | 14 (11-17) | 0.157 |
| Fasting glucose, mmol/L | 5.6 ± 1.5 | 5.7 ± 1.5 | 5.4 ± 1.2 | 0.277 |
| Fasting insulin, mIU/L | 15.3 (10.5-21.8) | 14.6 (9.6-21.2) | 19.3 (13.0-26.9) | <0.001 |
| HbA1c, % | 6.1 ± 1.4 | 6.2 ± 1.4 | 6.0 ± 1.1 | 0.303 |
| HOMA-IR | 3.4 (2.4-5.1) | 3.3 (2.3-4.8) | 4.2 (3.0-6.1) | 0.004 |
| Prothrombin time, s | 12.8 ± 0.7 | 12.8 ± 0.7 | 12.8 ± 0.6 | 0.949 |
| Platelet count, ×109/L | 245 ± 57 | 246 ± 58 | 242 ± 56 | 0.691 |
| Triglycerides, mmol/L | 2.3 ± 1.4 | 2.2 ± 1.4 | 2.4 ± 1.0 | 0.535 |
| Total cholesterol, mmol/L | 5.0 ± 1.1 | 4.9 ± 1.1 | 5.3 ± 1.1 | 0.027 |
| HDL-cholesterol, mmol/L | 1.0 ± 0.2 | 1.0 ± 0.2 | 1.0 ± 0.3 | 0.981 |
| LDL-cholesterol, mmol/L | 3.0 ± 0.9 | 3.0 ± 0.9 | 3.3 ± 0.9 | 0.022 |
| **Genotypes, n (%)** |  |  |  |  |
| *FNDC5* rs3480 |  |  |  | *0.564* |
| A/A | 188 (50.8%) | 156 (50.5%) | 32 (52.5%) |  |
| A/G | 155 (41.9%) | 132 (42.7%) | 23 (37.7%) |  |
| G/G | 27 (7.3%) | 21 (6.8%) | 6 (9.8%) |  |
| *PNPLA3* rs738409 |  |  |  | 0.558 |
| C/C | 106 (28.65%) | 92 (29.77%) | 14 (22.95%) |  |
| C/G | 170 (45.95%) | 140 (45.31%) | 30 (49.18%) |  |
| G/G | 94 (25.41%) | 77 (24.92%) | 17 (27.87%) |  |
| TM6SF2 rs58542926 |  |  |  | 0.786 |
| C/C | 310 (83.78%) | 259 (83.82%) | 51 (83.61%) |  |
| C/T | 56 (15.14%) | 47 (15.21%) | 9 (14.75%) |  |
| T/T | 4 (1.08%) | 3 (0.97%) | 1 (1.64%) |  |
| **Liver histology** |  |  |  |  |
| Fibrosis stage, n (%) |  |  |  | <0.001 |
| F0 | 149 (40.3%) | 134 (43.4%) | 15 (24.6%) |  |
| F1 | 163 (44.1%) | 136 (44.0%) | 27 (44.3%) |  |
| F2 | 45 (12.2%) | 30 (9.7%) | 15 (24.6%) |  |
| F3 | 10 (2.7%) | 8 (2.6%) | 2 (3.3%) |  |
| F4 | 3 (0.8%) | 1 (0.3%) | 2 (3.3%) |  |
| Steatosis grade, n (%) |  |  |  | 0.001 |
| S1 | 147 (39.7%) | 134 (43.4%) | 13 (21.3%) |  |
| S2 | 147 (39.7%) | 120 (38.8%) | 27 (44.3%) |  |
| S3 | 76 (20.5%) | 55 (17.8%) | 21 (34.4%) |  |
| Ballooning grade, n (%) |  |  |  | 0.192 |
| B0 | 66 (17.8%) | 60 (19.4%) | 6 (9.8%) |  |
| B1 | 237 (64.1%) | 195 (63.1%) | 42 (68.9%) |  |
| B2 | 67 (18.1%) | 54 (17.5%) | 13 (21.3%) |  |
| Lobular inflammation grade, n (%) |  |  |  | 0.243 |
| L0 | 47 (12.7%) | 39 (12.6%) | 8 (13.1%) |  |
| L1 | 236 (63.8%) | 203 (65.7%) | 33 (54.1%) |  |
| L2 | 80 (21.6%) | 61 (19.7%) | 19 (31.1%) |  |
| L3 | 7 (1.9%) | 6 (1.9%) | 1 (1.6%) |  |
| NAS score | 4 (3-5) | 4 (3-5) | 5 (3-5) | <0.001 |
| **Definite NASH** | 131 (35.4%) | 98 (31.7%) | 33 (54.1%) | <0.001 |
| **Significant fibrosis** | 58 (15.7%) | 39 (12.6%) | 19 (31.2%) | <0.001 |

Data are expressed as means ± SD, medians and inter-quartile ranges or percentages.

*Abbreviations*: ALT, alanine aminotransferase; AST, aspartate transaminase; ASM, appendicular skeletal muscle mass; BMI, body mass index; GGT, γ-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment-insulin resistance; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NAS, NAFLD activity score.

†Skeletal muscle index was defined as appendicular skeletal muscle mass (ASM) divided by body weight.

**Table 2. Baseline characteristics of study participants stratified by both *FNDC5* rs3480 genotypes and sarcopenia status.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **A/A genotype** | |  |  | **A/G + G/G genotype** | |  |
| **No sarcopenia**  **(n=156)** | **Sarcopenia**  **(n=32)** | ***P* value** |  | **No sarcopenia**  **(n=153)** | **Sarcopenia**  **(n=29)** | ***P* value** |
| **Demographics** |  |  |  |  |  |  |  |
| Age, years | 42.8 ± 12.0 | 36.4 ± 14.0 | 0.008 |  | 41.1 ± 11.3 | 35.5 ± 9.4 | 0.013 |
| Male sex, n (%) | 110 (70.5%) | 27 (84.4%) | 0.129 |  | 115 (75.2%) | 28 (96.6%) | 0.007 |
| **Metabolic factors** |  |  |  |  |  |  |  |
| BMI, kg/m2 | 25.9 ± 2.8 | 29.4 ± 3.2 | <0.001 |  | 26.3 ± 2.8 | 30.8 ± 3.6 | <0.001 |
| Waist circumference, cm | 90.8 ± 7.9 | 97.2 ± 8.3 | <0.001 |  | 90.6 ± 7.5 | 101.3 ± 11.2 | <0.001 |
| Skeletal muscle index, % | 30.6 ± 3.0 | 27.0 ± 2.6 | <0.001 |  | 30.4 ± 3.1 | 27.6 ± 1.7 | <0.001 |
| ASM/BMI ratio | 0.87 ± 0.15 | 0.78 ± 0.13 | 0.002 |  | 0.87 ± 0.15 | 0.79 ± 0.09 | 0.008 |
| Type 2 diabetes, n (%) | 52 (33.3%) | 8 (25.0%) | 0.357 |  | 48 (31.4%) | 9 (31.0%) | 0.971 |
| Hypertension, n (%) | 76 (48.7%) | 9 (28.1%) | 0.033 |  | 41 (26.8%) | 13 (44.8%) | 0.051 |
| Dyslipidemia, n (%) | 129 (82.7%) | 26 (81.2%) | 0.803 |  | 131 (85.6%) | 28 (96.6%) | 0.133 |
| Cigarette smoking, n (%) |  |  | 0.414 |  |  |  | 0.350 |
| Never | 102 (65.4%) | 20 (62.5%) |  |  | 107 (69.9%) | 18 (62.1%) |  |
| Ever | 13 (8.3%) | 5 (15.6%) |  |  | 17 (11.1%) | 2 (6.9%) |  |
| Current | 41 (26.3%) | 7 (21.9%) |  |  | 29 (19.0%) | 9 (31.0%) |  |
| **Laboratory parameters** |  |  |  |  |  |  |  |
| ALT, IU/L | 48 (29-79) | 86 (54-146) | <0.001 |  | 50 (32-90) | 88 (45-153) | 0.003 |
| AST, IU/L | 32 (25-49) | 54 (35-97) | <0.001 |  | 33 (25-56) | 43 (32-69) | 0.054 |
| γ-GT, IU/L | 48 (32-78) | 72 (51-102) | 0.002 |  | 51 (31-81) | 59 (46-93) | 0.064 |
| Albumin, g/dL | 4.6 ± 0.4 | 4.7 ± 0.4 | 0.136 |  | 4.7 ± 0.3 | 4.7 ± 0.3 | 0.731 |
| Bilirubin, μmol/L | 12 (10-16) | 15 (13-17) | 0.141 |  | 12 (10-16) | 13 (11-17) | 0.589 |
| Fasting glucose, mmol/L | 5.7 ± 1.4 | 5.5 ± 1.4 | 0.568 |  | 5.7 ± 1.7 | 5.4 ± 0.9 | 0.343 |
| Fasting insulin, mIU/L | 14.3 (9.6-21.3) | 18.4 (12.8-22.7) | 0.037 |  | 15.0 (10.0-20.8) | 21.5 (15.6-28.7) | 0.003 |
| HbA1c, % | 6.2 ± 1.4 | 6.0 ± 1.3 | 0.484 |  | 6.1 ± 1.4 | 5.9 ± 0.9 | 0.444 |
| HOMA-IR | 3.2 (2.2-4.7) | 3.8 (3.0-5.3) | 0.071 |  | 3.3 (2.3-5.1) | 5.0 (3.1-6.8) | 0.017 |
| Prothrombin time, s | 12.8 ± 0.7 | 12.9 ± 0.6 | 0.439 |  | 12.8 ± 0.6 | 12.7 ± 0.6 | 0.415 |
| Platelet count, ×109/L | 251 ± 59 | 237 ± 60 | 0.241 |  | 240 ± 56 | 248 ± 51 | 0.497 |
| Triglycerides, mmol/L | 2.3 ± 1.7 | 2.3 ± 1.1 | 0.977 |  | 2.1 ± 1.1 | 2.4 ± 0.9 | 0.263 |
| Total cholesterol, mmol/L | 4.9 ± 1.1 | 5.3 ± 1.1 | 0.080 |  | 5.0 ± 1.1 | 5.3 ± 1.1 | 0.177 |
| HDL-cholesterol, mmol/L | 1.0 ± 0.2 | 1.0 ± 0.3 | 0.805 |  | 1.0 ± 0.2 | 1.0 ± 0.2 | 0.745 |
| LDL-cholesterol, mmol/L | 2.9 ± 0.9 | 3.3 ± 0.9 | 0.053 |  | 3.1 ± 0.8 | 3.3 ± 0.9 | 0.194 |
| **Genotypes, n (%)** |  |  |  |  |  |  |  |
| *PNPLA3* rs738409 |  |  | 0.066 |  |  |  | 0.454 |
| C/C | 48 (30.77%) | 4 (12.50%) |  |  | 44 (28.76%) | 10 (34.48%) |  |
| C/G | 68 (43.59%) | 20 (62.50%) |  |  | 72 (47.06%) | 10 (34.48%) |  |
| G/G | 40 (25.64%) | 8 (25.00%) |  |  | 37 (24.18%) | 9 (31.03%) |  |
| *TM6SF2* rs58542926 |  |  | 0.354 |  |  |  | 0.614 |
| C/C | 128 (82.05%) | 24 (75.00%) |  |  | 131 (85.62%) | 27 (93.10%) |  |
| C/T | 26 (16.67%) | 7 (21.88%) |  |  | 21 (13.73%) | 2 (6.90%) |  |
| T/T | 2 (1.28%) | 1 (3.12%) |  |  | 1 (0.65%) | 0 (0.00%) |  |
| **Liver histology** |  |  |  |  |  |  |  |
| Fibrosis stage, n (%) |  |  | <0.001 |  |  |  | 0.548 |
| F0 | 68 (43.6%) | 7 (21.9%) |  |  | 66 (43.1%) | 8 (27.6%) |  |
| F1 | 73 (46.8%) | 11 (34.4%) |  |  | 63 (41.2%) | 16 (55.2%) |  |
| F2 | 14 (9.0%) | 11 (34.4%) |  |  | 16 (10.5%) | 4 (13.8%) |  |
| F3 | 1 (0.6%) | 1 (3.1%) |  |  | 7 (4.6%) | 1 (3.4%) |  |
| F4 | 0 (0.0%) | 2 (6.2%) |  |  | 1 (0.7%) | 0 (0.0%) |  |
| Steatosis grade, n (%) |  |  | 0.030 |  |  |  | 0.032 |
| S1 | 66 (42.3%) | 7 (21.9%) |  |  | 68 (44.4%) | 6 (20.7%) |  |
| S2 | 61 (39.1%) | 13 (40.6%) |  |  | 59 (38.6%) | 14 (48.3%) |  |
| S3 | 29 (18.6%) | 12 (37.5%) |  |  | 26 (17.0%) | 9 (31.0%) |  |
| Ballooning grade, n (%) |  |  | 0.021 |  |  |  | 0.994 |
| B0 | 34 (21.8%) | 1 (3.1%) |  |  | 26 (17.0%) | 5 (17.2%) |  |
| B1 | 101 (64.7%) | 24 (75.0%) |  |  | 94 (61.4%) | 18 (62.1%) |  |
| B2 | 21 (13.5%) | 7 (21.9%) |  |  | 33 (21.6%) | 6 (20.7%) |  |
| Lobular inflammation grade, n (%) |  |  | 0.569 |  |  |  | 0.081 |
| L0 | 19 (12.2%) | 6 (18.8%) |  |  | 20 (13.1%) | 2 (6.9%) |  |
| L1 | 105 (67.3%) | 18 (56.2%) |  |  | 98 (64.1%) | 15 (51.7%) |  |
| L2 | 30 (19.2%) | 7 (21.9%) |  |  | 31 (20.3%) | 12 (41.4%) |  |
| L3 | 2 (1.3%) | 1 (3.1%) |  |  | 4 (2.6%) | 0 (0.0%) |  |
| **NAS score** | 4 (3-5) | 5 (4-5) | 0.011 |  | 4 (3-5) | 4 (3-6) | 0.028 |
| **Definite NASH** | 48 (30.8%) | 19 (59.4%) | 0.003 |  | 50 (32.7%) | 14 (48.3%) | 0.110 |
| **Significant fibrosis** | 15 (9.6%) | 14 (43.8%) | <0.001 |  | 24 (15.7%) | 5 (17.2%) | 0.834 |

*Abbreviations*: ALT, alanine aminotransferase; AST, aspartate transaminase; ASM, appendicular skeletal muscle mass; BMI, body mass index; GGT, γ-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment-insulin resistance; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NAS, NAFLD activity score.

**Table 3. Associations between presence of sarcopenia (as the exposure variable) and definite NASH or significant fibrosis (as the outcome measures) in participants with different *FNDC5* genotypes.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Total (n=370)** | |  | **A/A (n=188)** | |  | **A/G+G/G (n=182)** | |
|  | **OR (95% CI)** | ***P* value** |  | **OR (95% CI)** | ***P* value** |  | **OR (95% CI)** | ***P* value** |
| **Definite NASH** |  |  |  |  |  |  |  |  |
| Unadjusted model | 1.91 (1.06, 3.43) | 0.031 |  | 3.29 (1.50, 7.20) | 0.003 |  | 1.92 (0.86, 4.29) | 0.111 |
| Adjusted model 1 | 2.21 (1.21, 4.04) | 0.010 |  | 2.71 (1.12, 6.58) | 0.028 |  | 1.91 (0.82, 4.46) | 0.134 |
| Adjusted model 2 | 1.91 (1.02, 3.55) | 0.042 |  | 2.98 (1.14, 7.78) | 0.026 |  | 1.48 (0.60, 3.64) | 0.392 |
| Adjusted model 3 | 1.65 (0.86, 3.18) | 0.130 |  | 2.93 (1.09, 7.87) | 0.034 |  | 1.16 (0.43, 3.07) | 0.773 |
| Adjusted model 4 | 1.58 (0.82, 3.06) | 0.175 |  | 3.27 (1.15, 9.31) | 0.026 |  | 1.17 (0.43, 3.13) | 0.759 |
| **Significant fibrosis** |  |  |  |  |  |  |  |  |
| Unadjusted model | 3.87 (1.94, 7.69) | <0.001 |  | 7.31 (3.04, 17.59) | <0.001 |  | 1.12 (0.39, 3.22) | 0.834 |
| Adjusted model 1 | 4.37 (2.18, 8.79) | <0.001 |  | 9.50 (3.61, 25.01) | <0.001 |  | 1.77 (0.57, 5.50) | 0.325 |
| Adjusted model 2 | 3.32 (1.61, 6.85) | 0.001 |  | 8.20 (2.93, 22.89) | <0.001 |  | 0.97 (0.29, 3.21) | 0.962 |
| Adjusted model 3 | 2.85 (1.35, 6.04) | 0.006 |  | 7.42 (2.59, 21.28) | <0.001 |  | 0.86 (0.25, 2.94) | 0.810 |
| Adjusted model 4 | 2.79 (1.31, 5.95) | 0.008 |  | 7.19 (2.40, 21.55) | <0.001 |  | 1.01 (0.29, 3.57) | 0.983 |

Data are expressed as odds ratio (OR) and 95% confidence intervals (CI) tested by logistic regression analysis.

Model 1: adjusted for age and sex.

Model 2: adjusted for age, sex, obesity, type 2 diabetes, hypertension, dyslipidemia, and smoking history.

Model 3: adjusted for covariates included in model 2 *plus* serum ALT, AST, GGT, and HOMA-IR levels.

Model 4: adjusted for covariates included in model 3 *plus* the *PNPLA3* rs738409*,* and *TM6SF2* rs58542926 variants*.*

**Table 4. Associations between presence of sarcopenia (as the exposure variable) and significant fibrosis (as the outcome measure) in participants with different *FNDC5* genotypes, stratified by sex.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Total** | |  | **A/A** | |  | **A/G+G/G** | |
|  | **OR (95% CI)** | ***P* value** |  | **OR (95% CI)** | ***P* value** |  | **OR (95% CI)** | ***P* value** |
| **Men** | **n=280** |  |  | **n=137** |  |  | **n=143** |  |
| Unadjusted model | 3.85 (1.82, 8.14) | <0.001 |  | 7.50 (2.59, 21.69) | <0.001 |  | 1.87 (0.60, 5.82) | 0.276 |
| Adjusted model 1 | 2.74 (1.23, 6.08) | 0.013 |  | 4.79 (1.53, 15.05) | 0.007 |  | 1.11 (0.32, 3.79) | 0.871 |
| Adjusted model 2 | 2.55 (1.11, 5.87) | 0.027 |  | 4.96 (1.50, 16.37) | 0.009 |  | 0.90 (0.22, 3.71) | 0.880 |
| Adjusted model 3 | 2.57 (1.10, 5.98) | 0.029 |  | 4.47 (1.26, 15.79) | 0.020 |  | 1.05 (0.24, 4.57) | 0.950 |
| **Women** | **n=90** |  |  | **n=51** |  |  | **n=39** |  |
| Unadjusted model | 8.82 (1.41, 55.35) | 0.020 |  | 22.29 (2.16, 230.06) | 0.006 |  | —§ | 0.999 |
| Adjusted model 1 | 11.74 (1.43, 96.11) | 0.022 |  | —§ | 0.010 |  | —§ | 0.999 |
| Adjusted model 2 | —§ |  |  | —§ |  |  | —§ |  |

Data are expressed as odds ratio (OR) and 95% confidence intervals (CI) tested by logistic regression analysis.

Model 1: adjusted for age, obesity, type 2 diabetes, hypertension, dyslipidemia, and smoking history;

Model 2: adjusted for covariates included in model 2 *plus* serum ALT, AST, GGT, and HOMA-IR levels;

Model 3: adjusted for covariates included in model 3 *plus* the *PNPLA3* rs738409*,* and *TM6SF2* rs58542926 variants*.*

§ The adjusted model failed due to the small sample size.

**Supplementary Table 1. Baseline characteristics of study participants, stratified by *FNDC5* rs3480 genotypes.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **A/A**  **(n=188)** | **A/G**  **(n=155)** | **G/G**  **(n=27)** | ***P* value** |
| **Demographics** |  |  |  |  |
| Age, years | 41.7 ± 12.5 | 40.1 ± 11.3 | 40.4 ± 10.3 | 0.454 |
| Male sex, n (%) | 137 (72.9%) | 121 (78.1%) | 22 (81.5%) | 0.411 |
| **Metabolic factors** |  |  |  |  |
| BMI, kg/m2 | 26.5 ± 3.2 | 27.0 ± 3.4 | 27.5 ± 3.2 | 0.201 |
| Waist circumference, cm | 91.9 ± 8.3 | 92.2 ± 9.0 | 92.8 ± 9.1 | 0.857 |
| Skeletal muscle index, % | 30.0 ± 3.2 | 30.0 ± 2.9 | 29.8 ± 3.8 | 0.926 |
| ASM/BMI ratio | 0.85 ± 0.15 | 0.86 ± 0.14 | 0.85 ± 0.18 | 0.778 |
| Sarcopenia, n (%) | 32 (17.02%) | 23 (14.84%) | 6 (22.22%) | 0.564 |
| Type 2 diabetes, n (%) | 60 (31.9%) | 50 (32.3%) | 7 (25.9%) | 0.802 |
| Hypertension, n (%) | 85 (45.2%) | 46 (29.7%) | 8 (29.6%) | 0.009 |
| Dyslipidemia, n (%) | 155 (82.4%) | 135 (87.1%) | 24 (88.9%) | 0.407 |
| Cigarette smoking, n (%) |  |  |  | 0.873 |
| Never | 122 (64.9%) | 106 (68.4%) | 19 (70.4%) |  |
| Ever | 18 (9.6%) | 16 (10.3%) | 3 (11.1%) |  |
| Current | 48 (25.5%) | 33 (21.3%) | 5 (18.5%) |  |
| **Laboratory parameters** |  |  |  |  |
| ALT, IU/L | 54 (32-88) | 54 (34-101) | 51 (30-120) | 0.861 |
| AST, IU/L | 35 (26-52) | 35 (25-59) | 32 (27-50) | 0.940 |
| γ-GT, IU/L | 52 (34-82) | 52 (33-82) | 54 (35-102) | 0.815 |
| Albumin, g/dL | 4.6 ± 0.4 | 4.7 ± 0.3 | 4.6 ± 0.4 | 0.433 |
| Bilirubin, μmol/L | 12 (10-16) | 12 (10-16) | 11 (10-18) | 0.789 |
| Fasting glucose, mmol/L | 5.6 ± 1.4 | 5.7 ± 1.6 | 5.5 ± 1.2 | 0.830 |
| Fasting insulin, mIU/L | 14.8 (10.2-21.3) | 15.6 (10.7-21.9) | 17.6 (11.9-24.3) | 0.665 |
| HbA1c, % | 6.2 ± 1.4 | 6.1 ± 1.4 | 6.1 ± 1.2 | 0.875 |
| HOMA-IR | 3.4 (2.4-5.0) | 3.4 (2.4-5.6) | 4.0 (2.8-5.3) | 0.679 |
| Prothrombin time, s | 12.8 ± 0.7 | 12.8 ± 0.6 | 12.6 ± 0.6 | 0.149 |
| Platelet count, ×109/L | 248 ± 59 | 244 ± 56 | 228 ± 49 | 0.199 |
| Triglycerides, mmol/L | 2.3 ± 1.6 | 2.1 ± 1.0 | 2.4 ± 1.1 | 0.327 |
| Total cholesterol, mmol/L | 5.0 ± 1.1 | 5.0 ± 1.0 | 5.0 ± 1.3 | 0.939 |
| HDL-cholesterol, mmol/L | 1.0 ± 0.2 | 1.0 ± 0.2 | 0.9 ± 0.2 | 0.148 |
| LDL-cholesterol, mmol/L | 3.0 ± 0.9 | 3.1 ± 0.8 | 3.0 ± 1.0 | 0.300 |
| **Genotypes, n (%)** |  |  |  |  |
| *PNPLA3* rs738409 |  |  |  | 0.691 |
| C/C | 52 (27.66%) | 45 (29.03%) | 9 (33.33%) |  |
| C/G | 88 (46.81%) | 73 (47.10%) | 9 (33.33%) |  |
| G/G | 48 (25.53%) | 37 (23.87%) | 9 (33.33%) |  |
| *TM6SF2* rs58542926 |  |  |  | 0.536 |
| C/C | 152 (80.85%) | 133 (85.81%) | 25 (92.59%) |  |
| C/T | 33 (17.55%) | 21 (13.55%) | 2 (7.41%) |  |
| T/T | 3 (1.60%) | 1 (0.65%) | 0 (0.00%) |  |
| **Liver histology** |  |  |  |  |
| Fibrosis stage, n (%) |  |  |  | 0.180 |
| F0 | 75 (39.9%) | 67 (43.2%) | 7 (25.9%) |  |
| F1 | 84 (44.7%) | 62 (40.0%) | 17 (63.0%) |  |
| F2 | 25 (13.3%) | 17 (11.0%) | 3 (11.1%) |  |
| F3 | 2 (1.1%) | 8 (5.2%) | 0 (0.0%) |  |
| F4 | 2 (1.1%) | 1 (0.6%) | 0 (0.0%) |  |
| Steatosis grade, n (%) |  |  |  | 0.947 |
| S1 | 73 (38.8%) | 63 (40.6%) | 11 (40.7%) |  |
| S2 | 74 (39.4%) | 61 (39.4%) | 12 (44.4%) |  |
| S3 | 41 (21.8%) | 31 (20.0%) | 4 (14.8%) |  |
| Ballooning grade, n (%) |  |  |  | 0.559 |
| B0 | 35 (18.6%) | 27 (17.4%) | 4 (14.8%) |  |
| B1 | 125 (66.5%) | 94 (60.6%) | 18 (66.7%) |  |
| B2 | 28 (14.9%) | 34 (21.9%) | 5 (18.5%) |  |
| Lobular inflammation grade, n (%) |  |  |  | 0.308 |
| L0 | 25 (13.3%) | 17 (11.0%) | 5 (18.5%) |  |
| L1 | 123 (65.4%) | 101 (65.2%) | 12 (44.4%) |  |
| L2 | 37 (19.7%) | 33 (21.3%) | 10 (37.0%) |  |
| L3 | 3 (1.6%) | 4 (2.6%) | 0 (0.0%) |  |
| NAS score | 4 (3-5) | 4 (3-5) | 4 (3-5) | 0.849 |
| **Definite NASH** | 67 (35.6%) | 54 (34.8%) | 10 (37.0%) | 0.972 |
| **Significant fibrosis** | 29 (15.4%) | 26 (16.8%) | 3 (11.1%) | 0.794 |

*Abbreviations*: ALT, alanine aminotransferase; AST, aspartate transaminase; ASM, appendicular skeletal muscle mass; BMI, body mass index; GGT, γ-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment-insulin resistance; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NAS, NAFLD activity score.