Lung function and nonalcoholic fatty liver disease: a Mendelian randomization study

Abstract

Introduction

Associations of pulmonary function as evaluated by forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) with non-alcoholic fatty liver disease (NAFLD) have been reported in observational studies. Nevertheless, observational studies are susceptible to bias and reverse causality, making it difficult to infer the existence and direction of causality. We aimed to evaluate the causal effect of pulmonary function on NAFLD using the Mendelian randomization (MR) method.

Material and methods

We performed univariate MR, multivariate MR, and bidirectional two-sample MR analyses to jointly assess the causal relationship between pulmonary function and NAFLD. In addition to the inverse variance weighting method as the primary MR analysis, three complementary methods were also performed. A series of sensitivity analyses were carried out to rule out pleiotropy.

Results

We found that each genetically predicted (standard deviation) SD increase in FEV1 and FVC was associated with decreased NAFLD risk. However, after adjusting for height in the multivariate MR, only the effect of FEV1 on NAFLD risk remained significant. Furthermore, we found no causal effect of NAFLD on lung function in the reverse MR analysis.

Conclusions

Our findings indicated that reduced lung function, especially FEV1, is causally associated with the risk of NAFLD. Although the mechanism remains unclear, FEV1 could be considered when assessing NAFLD risk and as a potential target for NAFLD prevention.

Title page

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Introduction

69   Nonalcoholic fatty liver disease (NAFLD), which is one of the most common

70 forms of chronic liver disorder, has an estimated prevalence of 20-35% in the general

71 population [1]. It is defined as a diffuse accumulation of triglycerides (TGs) in

72 hepatocytes after excluding other causes of hepatic steatosis (including other causes of

73 liver disorder, excessive alcohol intake, and other conditions that could contribute to

74 hepatic steatosis) [2]. Almost 20% of patients progress over time to nonalcoholic

75 steatohepatitis (NASH), liver necrosis, cirrhosis, and ultimately hepatocellular

76 carcinoma [3, 4]. Indeed, NAFLD is considered as a hepatic manifestation of metabolic

77 syndrome, and substantial evidence suggests that NAFLD has side effects that extend

78 well beyond the liver [5]. Multiple metabolic abnormalities and extrahepatic

79 complications are associated with NAFLD, such as insulin resistance, cardiac metabolic

80 illness, and chronic kidney disease, through enhanced low-grade systemic

81 inflammatory mechanisms, which also exerts a causal role in the occurrence and

82 development of NAFLD [6-12].

83   Lung function, as evaluated by forced expiratory volume in 1 second (FEV1) and

84 forced vital capacity (FVC), was usually used to forecast morbidity, mortality, and all-

85 cause mortality related to different airway illnesses [13-17]. Recent evidence has suggested

86 that lung function impairment is linked with increased risk of metabolic syndrome,

87 cardiovascular disorders, diabetes, as well as systemic inflammation [18, 19]. Thus, the

4

damage of lung function may link to an increased risk of NAFLD, which has been reported in several observational studies [20-22]. Clarifying the nature and direction of the association between pulmonary function and NAFLD can help guide primary prevention. However, due to the limitations of study design, traditional epidemiological studies are usually susceptible to residual confounding, selection bias and other factors, resulting in difficulty in distinguishing causal effects from spurious associations [23]. Hence, it remains unclear whether the observed relationship between lung function and the risk of NAFLD is causal.

Mendelian randomization (MR) is an emerging and potent epidemiological technique, which employs genetic variants that are robustly associated with underlying modifiable risk factors to ascertain their causal contribution to disorder risk [24]. Since these variants are randomly assigned from parents to offspring at conception, MR studies are less vulnerable to reverse causality, confounding, and measurement biases [25]. Given that previous epidemiological studies have not clearly established a causal relationship between lung function and NAFLD, bidirectional two-sample MR approach may help to clarify the complex causal relationship between the two conditions. Herein, we used published results from genome-wide association studies (GWASs) to investigate whether there is a causal association between lung function and NAFLD, and to assess and analyze the causal effects.

Materials and methods

Study design

To evaluate the association of FEV1 (per SD) as well as FVC (per SD) with

NAFLD, we conducted univariate MR, multivariate MR, and bidirectional two-sample MR analyses, using publicly available genetic data derived from the worldwide genetic consortium. No additional informed consent was required. Single nucleotide polymorphisms (SNPs) were served as instrumental variables (IVs) according to three key assumptions. Firstly, the chosen genetic variants proposed as IVs should be strongly correlated with the exposure. Herein, R^2 and F statistic were applied to represent the strength of the association between genetic variants and exposure. The latest and most rigorous methods of calculation were used. $F = R^2(n-k-1)/k(1-R^2)$. In this formula, R^2 is equal to the cumulative explanatory variance of the selected SNPs on exposure, where k refers to the total number of SNPs selected and n is the sample size. An F value greater than 10 indicates that the correlation is robust enough to avoid bias due to weak IVs. Secondly, the genetic variants need to be independent of potential confounding factors. Thirdly, the genetic variants should affect the risk of outcome merely via the exposure, not through other alternative pathways, which would require no horizontal pleiotropy effect between IVs and outcome.

SNP-FEV1 and FVC association estimates

In this study, genetic IVs for FEV1 (SD, GWAS ID: ukb-b-19657) and FVC (SD, GWAS ID: ukb-b-7953) were derived from the summary GWAS results in the UK Biobank (https://gwas.mrcieu.ac.uk/), with 421,986 subjects of European ancestry. UK Biobank is one of the most accessible, largest, and in-depth cohort studies in the world, collecting data from over half a million people (aged 40 to 69) at recruitment in the UK between 2006 and 2010. Trained healthcare staff performed pre-bronchiectasis

132 pulmonary function tests using a Vitalograph Pneumtrac 6800 spirometer (Maids

133 Moreton, UK). Genotyping was performed using Affymetrix UK BiLEVE Axiom array

134 (~50000 participants) and Affymetrix UK Biobank Axiom array (~450000 participants).

135 Statistical summaries were produced using linear mixed models to account for

136 relatability and population stratification, and then adjusted for sex and genotyping array.

SNP-NAFLD association estimates

138 Summary-level statistical data for NAFLD were sourced from FinnGen

139 consortium. We limited the NAFLD data to samples of European ancestry to refrain

140 from potential bias caused by population stratification. The dataset consists of 894 cases

141 and 217,898 controls. FinnGen project is a public-private partnership, which integrates

142 disorder endpoint genetic data from the Finnish biobanks and the Finnish health

143 registries. NAFLD cases was defined by using the EHR codes International

144 Classification of Diseases (ICD)10: K76.0, and determined by electronic medical

145 records.   Detailed   documentations   can   be   found   at   FinnGen   website

146 (https://finngen.gitbook.io/documentation/v/r5/).

Instrumental SNPs selection

148 Strict inclusion criteria were developed to screen qualified IVs. We selected the

149 SNPs for exposure with a genome-wide significant association (For lung function: $P <$

150 5E-08; For NAFLD: $P <$ 1E-05), and without linkage disequilibrium (LD) ($r^2 < 0.001$,

151 clumping distance = 10,000kb). We searched for the exposure-related SNPs in the

152 outcome dataset. When some genetic instruments were not provided in the outcome

153 dataset, we chosen proxy SNPs that were in significant correlation with these SNPs ($r^2 >$

154 0.8). To assure that the impact of SNPs on the studied exposure corresponds to the same

155 allele as the effect on the studied outcome, palindromic SNPs with intermediate allele

156 frequencies were eliminated from the IVs. SNPs which had the minor allele frequency

157 (MAF) less than 0.01 were also not included. Eligible SNPs were finally identified as

158 IVs for exposure to conduct the MR analysis.

159 **Statistical analysis**

160 The inverse-variance weighted (IVW) method was recognized as the main

161 statistical model to explore the potential causal relationship between lung function and

162 NAFLD [26]. The IVW method is a weighted regression of the effect of SNP exposure on

163 SNP outcome, with a fixed y-intercept of zero [27]. Given that the presence of horizontal

164 pleiotropy in IVW estimates can affect the results, three complementary methods were

165 adopted to test the robustness of the conclusions, including the MR-Egger regression

166 method, the weighted median method, and the weighted mode method. The MR-Egger

167 regression method relies on the assumption that the polymorphism of the genetic

168 instrument is independent of the instrument strength and can offer an effective estimate

169 of the causal effect [28]. The weighted median method can permit a high pleiotropy and

170 generate robust estimates under the circumstance of at least 50% of the information

171 contributing to the analysis comes from valid SNPs [29].The weighted mode method can

172 produce consistent outcomes, even though most IVs are noneffective [30]. These three

173 complementary methods provided relatively robust outcomes at the expense of

174 statistical power.

175 **Sensitivity analysis**

176   To guarantee the reliability of our conclusion, six sensitivity analyses were

177   performed to check and adjust the presence of pleiotropy in the causal estimates. Firstly,

178   the Cochran's Q statistic was used to quantify the heterogeneity among the chosen

179   genetic instruments. When heterogeneity was suspected ($P < 0.05$), random-effects

180   model of IVW was performed to calculate the effect size; otherwise, a fixed-effects

181   model was applied. Secondly, previous studies have demonstrated that height is an

182   established cause of pulmonary function and might bias the Mendelian randomization

183   estimates for FEV1 and FVC [31], so multivariable MR (MVMR) was conducted to

184   additionally adjust for height. Thirdly, the MR-Egger regression was used to test and

185   correct the potential horizontal pleiotropy among the selected IVs by evaluating the

186   intercept. Fourthly, MR pleiotropy residual sum and outlier (MR-PRESSO) test was

187   implemented to identify underlying outliers, and MR-PRESSO global test of

188   heterogeneity was used to detect potential horizontal pleiotropy. Fifthly, the asymmetry

189   of funnel plot was also used as an index to judge horizontal pleiotropy. Finally, the

190   leave-one-out sensitivity analysis was conducted by sequentially deleting each SNP to

191   determine whether MR estimates were driven by certain strong SNPs.

192   Data analyses in the current study were completed using the "TwoSampleMR" and

193   "MRPRESSO" packages of the R 4.0.3 software (www.r-project.org). Multiple

194   comparisons were corrected by the Bonferroni approach, with a *P* value of less than

195   0.025 (0.05/2) indicating convincing evidence of causality.

196   **Results**

197   **Instrumental variables selection**

198   For FEV1, 260 SNPs ($P < $5E-08, $r^2 < 0.001$) were totally identified, explaining up

199   to 2.24% of the FEV1 variance. The F statistic of these instruments ranged from 30 to

200   271 with the average value of 57, all exceeding the threshold value of 10. For FVC, 320

201   SNPs (all $P < $5E-08, $r^2 < 0.001$) were identified, explaining up to 2.76% of the FVC

202   variance. The F statistic of these instruments ranged from 30 to 457 with the average

203   value of 63, indicating low evidence for weak instrument bias. **Supplementary Table**

204   **S1 and S2** described the detailed information of the lung function instruments used in

205   the current study.

MR analysis and sensitivity analysis

207   Since heterogeneity was suspected in the analysis of FEV1 and FVC with NAFLD

208   (P for Cochran's Q = 0.026 and 0.016, respectively) (**Table 1**), the IVW approach with

209   multiplicative random effects model was applied. The primary results of IVW analysis

210   uncovered that genetically predicted each SD increase in FEV1 and FVC was in relation

211   to lower risk of NAFLD (FEV1: odds ratio [OR] = 0.434, 95% confidence interval [CI]

212   = 0.251-0.749, P = 0.003; FVC: OR = 0.363, 95%CI = 0.224-0.588, $P < 0.001$) (**Table**

213   **1, Figure 1**). Similar relations were obtained in the MR-Egger regression (FEV1: OR

214   = 0.145, 95%CI = 0.027-0.782, P = 0.026; FVC: OR = 0.260, 95%CI = 0.068-0.995, P

215   = 0.050), weighted median (FEV1: OR = 0.408, 95%CI = 0.193-0.866, P = 0.020; FVC:

216   OR = 0.448, 95%CI = 0.233-0.863, P = 0.016), and weighted mode methods (FEV1:

217   OR = 0.535, 95%CI = 0.090-3.177, P = 0.492; FVC: OR = 0.670, 95%CI = 0.107-4.205,

218   P = 0.669), at a cost of lower statistical power and broader confidence levels (**Table 1,**

219   **Figure 1, 2**).

220   For FEV1, results from sensitivity analysis demonstrated that the causal estimates

221   were unlikely to be biased by the outlier SNPs and pleiotropic effects (P for MR-Egger

222   intercept = 0.180, P for MR-PRESSO global test = 0.060). Funnel plot showed

223   symmetric result, implying that the directional and horizontal pleiotropy did not reach

224   significant levels (**supplementary Figure S1**). The leave-one-out and forest plots

225   yielded consistent results, and did not find noticeable effects of any single SNP that

226   could drive the results (**supplementary Figure S2 and S3**). For FVC, the MR-Egger

227   regression analyses suggested that there was relatively weak evidence of horizontal

228   pleiotropy, and one outlier SNP (rs11967850) was identified by MR-PRESSO

229   approaches (P for MR-Egger intercept = 0.602, P for MR-PRESSO global test = 0.043).

230   After excluding this outlier, FVC still showed a converse causal effect on the risk of

231   NAFLD (OR = 0.376, 95%CI = 0.234-0.604, $P < 0.001$). The funnel plot was visually

232   symmetric (**supplementary Figure S1**). The leave-one-out and forest plots further

233   verified the robustness of the MR results (**supplementary Figure S2 and S3**).

234   Furthermore, in the MVMR analysis adjusted for height, the converse causal

235   associations of FEV1 and FVC with NAFLD were consistent with the main analyses,

236   but estimates for FVC was attenuated (FEV1: OR = 0.648, 95%CI = 0.446-0.941, P =

237   0.023; FVC: OR = 0.774, 95%CI = 0.501-1.195, P = 0.248) (**Table 1, Figure 1**).

238   **Further analyses**

239   To deeply elucidate the causal effect of NAFLD on lung function, we further

240   perform a two-sample MR analysis by using NAFLD as exposure and lung function as

241   outcome. 19 significant and independent SNPs ($P < $1E-05, $r^2 < 0.001$) were identified,

242 explaining 69.33% of the variance for NAFLD. The *F* statistics of these SNPs ranged

243 from 20 to 102 with the average *F* value of 28 for NAFLD, showing the absence of

244 weak IV bias (**Supplementary Table S3**).

245   According to the results of IVW, there was no evidence that genetic responsibility

246 for NAFLD had a causal effect on FEV1 (OR = 1.000, 95%CI = 0.996-1.004, *P* = 0.987)

247 and FVC (OR = 1.003, 95%CI = 0.999-1.006, *P* = 0.118). MR-Egger, weighted median,

248 and weighted mode approaches also obtained similar resluts (**Table 2, Figure 3, 4**).

249 Cochran's Q test showed that there was no significant heterogeneity across the

250 estimates of incorporated SNPs (*P* for FEV1 = 0.332; *P* for FVC = 0.363, **Table 2**).

251 Moreover, no significantly horizontal pleiotropy was detected by MR-Egger regression

252 (*P* for FEV1 = 0.054; *P* for FVC = 0.769, **Table 2**). The MR-PRESSO global test

253 observed no outlier SNPs or a horizontal pleiotropic effect of NAFLD on FEV1 and

254 FVC (*P* for FEV1 = 0.182; *P* for FVC = 0.511). The funnel plot also displayed a

255 symmetrical distribution (**supplementary Figure S4**). The leave-one-out as well as the

256 forest plots further verified the reliability of the MR results (**supplementary Figure S5**

257 **and S6**).

258 **Discussion**

259   To the best of our knowledge, the current MR study is the first to investigate the

260 bidirectional causal relationship between pulmonary function and NAFLD. Our

261 analyses revealed that each genetically predicted SD increase in FEV1 and FVC was

262 associated with a lower risk of NAFLD. Multivariate MR analysis indicated that after

263 adjusting for height, FEV1 and FVC maintained a reverse causal association with

264   NAFLD, but only FEV1 reached a significant level. FEV1 is considered an indicator of

265   the severity of airway obstruction, while FVC is a measure of overall lung capacity[32].

266   They reflect different aspects of pulmonary function, which might account for the

267   differences in their effects on NAFLD. Furthermore, in the reverse MR analysis, there

268   was no causal effect of NAFLD on FEV1 and FVC.

269   The association between lung function and NAFLD has been explored in several

270   epidemiological studies. Qin et al. found that damaged lung function as measured by

271   FEV1 and FVC was significantly negatively associated with the prevalence of NAFLD

272   in middle-aged and elderly people without chronic lung disorders[20]. Jung et al. found

273   that NAFLD was independently related to decreased lung function, and the severity of

274   NAFLD was negatively correlated with pulmonary function[21]. Based on the data of a

275   cross-sectional study, Peng et al. found that individuals with higher levels of hepatic

276   steatosis were at higher risk for poor lung function, and damaged pulmonary function

277   may be an extrahepatic complication of NAFLD[22]. However, these cross-sectional

278   studies failed to elucidate the sequence of the occurrence of impaired lung function and

279   NAFLD, making it unclear whether there was a causal association between them. In a

280   large cohort of middle-aged South Koreans with more than half a million person-years

281   of follow-up, Song et al. reported that the risk of NAFLD increased with the decrease

282   of FEV1 and FVC in a dose-response manner, and they concluded that decreased

283   pulmonary function was a risk factor for NAFLD[33]. Our findings were consistent with

284   those of Song et al. that lung function had a reverse causal association with NAFLD

285   risk. Additionally, we also found no causal effect of NAFLD on lung function.

286   The exact mechanisms by which reduced lung function contributes to the increased

287   risk of NAFLD remains unknown. However, low-grade chronic inflammation may

288   play a critical role as it is strongly linked with both damaged pulmonary function and

289   NAFLD. The lungs and the liver are known to be both highly vascular organs with a

290   dual blood supply[34]. Environmental exposures, for example, occupational dusts,

291   industrial chemicals, as well as particulate air pollution, are well-known adverse factors

292   for the decline in lung function[35]. Inhalation of these harmful particulates from

293   environmental pollution may promote airway inflammation and the production of pro-

294   inflammatory cytokines (such as interleukin-6) from alveolar macrophages, and finally

295   leading to airway damage and impaired lung function[36]. Additionally, these

296   inflammatory cytokines can also enter the circulation and induce low-grade

297   inflammation throughout the body, and then accelerating the occurrence and

298   development of NAFLD[37].

299   The current study has a relatively large sample size, and is known to be the first

300   MR study to investigate the bidirectional causal relationship between lung function and

301   NAFLD. The usage of MR approach freed this study from potential bias and reverse

302   causality. Moreover, the findings of this study suggest that it is necessary to improve

303   lung function in people at high risk of NAFLD. However, several limitations should

304   also be noticed. First, since all subjects included in this study were of European descent,

305   it should be cautious to extrapolate the results to other ethnicities. Second, the variables

306   were binary, so it is difficult to assess possible dose-response relationships. Third, the

307   dataset in this study was derived from large-scale GWAS studies, so individual-level

308 detailed information was lacking, which made it impossible to perform stratified

309 analysis.

310 In a word, using genetic data, we provide strong evidence that reduced lung

311 function, especially FEV1, is causally associated with NAFLD. Despite the exact

312 mechanism remains unclear, FEV1 could be considered when assessing NAFLD risk

313 and as a potential target for NAFLD prevention.

314 **References:**

315 1. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K et al. The
316 diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by
317 the American Association for the Study of Liver Diseases, American College of
318 Gastroenterology, and the American Gastroenterological Association. Hepatology
319 (Baltimore, Md) 2012;55(6):2005-23.
320 2. Manne V, Handa P, Kowdley KV. Pathophysiology of Nonalcoholic Fatty Liver
321 Disease/Nonalcoholic Steatohepatitis. Clinics in liver disease 2018;22(1):23-37.
322 3. Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical
323 epidemiology and disease burden of nonalcoholic fatty liver disease. World journal
324 of gastroenterology 2017;23(47):8263-76.
325 4. Alqahtani SA, Chan WK, Yu ML. Hepatic Outcomes of Nonalcoholic Fatty Liver
326 Disease Including Cirrhosis and Hepatocellular Carcinoma. Clinics in liver disease
327 2023;27(2):211-23.
328 5. Ziolkowska S, Binienda A, Jabłkowski M, Szemraj J, Czarny P. The Interplay between
329 Insulin Resistance, Inflammation, Oxidative Stress, Base Excision Repair and
330 Metabolic Syndrome in Nonalcoholic Fatty Liver Disease. International journal of
331 molecular sciences 2021;22(20).
332 6. Fiorentino TV, Miceli S, Succurro E, Sciacqua A, Andreozzi F, Sesti G.
333 Nonalcoholic fatty liver disease is associated with a decreased myocardial mechano-
334 energetic efficiency. Journal of internal medicine 2021;289(2):221-31.
335 7. Han E, Lee YH, Kim YD, Kim BK, Park JY, Kim DY et al. Nonalcoholic Fatty Liver
336 Disease and Sarcopenia Are Independently Associated With Cardiovascular Risk. The
337 American journal of gastroenterology 2020;115(4):584-95.
338 8. Peng TC, Wu LW, Chen WL, Liaw FY, Chang YW, Kao TW. Nonalcoholic fatty liver
339 disease and sarcopenia in a Western population (NHANES III): The importance of
340 sarcopenia definition. Clinical nutrition (Edinburgh, Scotland) 2019;38(1):422-8.
341 9. Mantovani A, Zaza G, Byrne CD, Lonardo A, Zoppini G, Bonora E et al. Nonalcoholic
342 fatty liver disease increases risk of incident chronic kidney disease: A systematic
343 review and meta-analysis. Metabolism: clinical and experimental 2018;79:64-76.
344 10. Yu Q, Lee YY, Xia ZY, Liong EC, Xiao J, Tipoe GL. S-allylmercaptocysteine improves

nonalcoholic steatohepatitis by enhancing AHR/NRF2-mediated drug metabolising enzymes and reducing NF-κB/IκBα and NLRP3/6-mediated inflammation. European journal of nutrition 2021;60(2):961-73.

11. Cantero I, Abete I, Bullón-Vela V, Crujeiras AB, Casanueva FF, Zulet MA et al. Fibroblast growth factor 21 levels and liver inflammatory biomarkers in obese subjects after weight loss. Archives of medical science : AMS 2022;18(1):36-44.

12. Małujło-Balcerska E, Sipowicz K, Pietras T. Comparing chronic obstructive pulmonary disease and depressive disorder in terms of inflammation-related biomarkers. Archives of medical science : AMS 2023;19(3):814-9.

13. Piccioni P, Tassinari R, Carosso A, Carena C, Bugiani M, Bono R. Lung function changes from childhood to adolescence: a seven-year follow-up study. BMC pulmonary medicine 2015;15:31.

14. Zappala CJ, Latsi PI, Nicholson AG, Colby TV, Cramer D, Renzoni EA et al. Marginal decline in forced vital capacity is associated with a poor outcome in idiopathic pulmonary fibrosis. The European respiratory journal 2010;35(4):830-6.

15. Soler Artigas M, Wain LV, Miller S, Kheirallah AK, Huffman JE, Ntalla I et al. Sixteen new lung function signals identified through 1000 Genomes Project reference panel imputation. Nature communications 2015;6:8658.

16. Ortega VE, Kumar R. The Effect of Ancestry and Genetic Variation on Lung Function Predictions: What Is "Normal" Lung Function in Diverse Human Populations? Current allergy and asthma reports 2015;15(4):16.

17. Cheng Y, Chen Q, Huang R, Lao C, Fu W. Moxibustion treatment increases the survival rate of lung infection of patients bed-ridden due to osteoporotic fracture of the spine via regulation of the inflammatory responses. Archives of medical science : AMS 2023;19(1):258-63.

18. Lee YY, Tsao YC, Yang CK, Chuang CH, Yu W, Chen JC et al. Association between risk factors of metabolic syndrome with lung function. European journal of clinical nutrition 2020;74(5):811-7.

19. Nerpin E, Jacinto T, Fonseca JA, Alving K, Janson C, Malinovschi A. Systemic inflammatory markers in relation to lung function in NHANES. 2007-2010. Respiratory medicine 2018;142:94-100.

20. Qin L, Zhang W, Yang Z, Niu Y, Li X, Lu S et al. Impaired lung function is associated with non-alcoholic fatty liver disease independently of metabolic syndrome features in middle-aged and elderly Chinese. BMC endocrine disorders 2017;17(1):18.

21. Jung DH, Shim JY, Lee HR, Moon BS, Park BJ, Lee YJ. Relationship between non-alcoholic fatty liver disease and pulmonary function. Internal medicine journal 2012;42(5):541-6.

22. Peng TC, Kao TW, Wu LW, Chen YJ, Chang YW, Wang CC et al. Association Between Pulmonary Function and Nonalcoholic Fatty Liver Disease in the NHANES III Study. Medicine 2015;94(21):e907.

23. Zhang Y, Liu Z, Choudhury T, Cornelis MC, Liu W. Habitual coffee intake and risk for nonalcoholic fatty liver disease: a two-sample Mendelian randomization study. European journal of nutrition 2021;60(4):1761-7.

24. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG. Using published

389 data in Mendelian randomization: a blueprint for efficient identification of causal
390 risk factors. European journal of epidemiology 2015;30(7):543-52.

391 25. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian
392 randomization: using genes as instruments for making causal inferences in
393 epidemiology. Statistics in medicine 2008;27(8):1133-63.

394 26. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with
395 multiple genetic variants using summarized data. Genetic epidemiology 2013;37(7):658-
396 65.

397 27. Chen Y, Shen J, Wu Y, Ni M, Deng Y, Sun X et al. Tea consumption and risk of
398 lower respiratory tract infections: a two-sample mendelian randomization study.
399 European journal of nutrition 2022:1-9.

400 28. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid
401 instruments: effect estimation and bias detection through Egger regression.
402 International journal of epidemiology 2015;44(2):512-25.

403 29. Burgess S, Bowden J, Fall T, Ingelsson E, Thompson SG. Sensitivity Analyses for
404 Robust Causal Inference from Mendelian Randomization Analyses with Multiple Genetic
405 Variants. Epidemiology (Cambridge, Mass) 2017;28(1):30-42.

406 30. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian
407 randomization via the zero modal pleiotropy assumption. International journal of
408 epidemiology 2017;46(6):1985-98.

409 31. Au Yeung SL, Borges MC, Lawlor DA, Schooling CM. Impact of lung function on
410 cardiovascular diseases and cardiovascular risk factors: a two sample bidirectional
411 Mendelian randomisation study. Thorax 2022;77(2):164-71.

412 32. VanDevanter DR, Pasta DJ. Evidence of diminished FEV1 and FVC in 6-year-olds
413 followed in the European Cystic Fibrosis Patient Registry, 2007-2009. Journal of
414 cystic fibrosis : official journal of the European Cystic Fibrosis Society
415 2013;12(6):786-9.

416 33. Song JU, Jang Y, Lim SY, Ryu S, Song WJ, Byrne CD et al. Decreased lung function
417 is associated with risk of developing non-alcoholic fatty liver disease: A
418 longitudinal cohort study. PloS one 2019;14(1):e0208736.

419 34. Miao L, Yang L, Guo LS, Shi QQ, Zhou TF, Chen Y et al. Metabolic Dysfunction-
420 associated Fatty Liver Disease is Associated with Greater Impairment of Lung Function
421 than Nonalcoholic Fatty Liver Disease. Journal of clinical and translational
422 hepatology 2022;10(2):230-7.

423 35. Miele CH, Grigsby MR, Siddharthan T, Gilman RH, Miranda JJ, Bernabe-Ortiz A et
424 al. Environmental exposures and systemic hypertension are risk factors for decline
425 in lung function. Thorax 2018;73(12):1120-7.

426 36. van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, Fujii T et al. Cytokines
427 involved in the systemic inflammatory response induced by exposure to particulate
428 matter air pollutants (PM(10)). American journal of respiratory and critical care
429 medicine 2001;164(5):826-30.

430 37. Luci C, Bourinet M, Leclère PS, Anty R, Gual P. Chronic Inflammation in Non-
431 Alcoholic Steatohepatitis: Molecular Mechanisms and Therapeutic Strategies.
432 Frontiers in endocrinology 2020;11:597648.

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Figure legends

Figure 1. Mendelian randomization estimates of lung function with the risk of nonalcoholic fatty liver disease.

Figure 2. Scatterplot of the effect size for each SNP on lung function and the risk of nonalcoholic fatty liver disease.

Figure 3. Mendelian randomization estimates of nonalcoholic fatty liver disease on lung function.

Figure 4. Scatterplot of the effect size for each SNP on nonalcoholic fatty liver disease and lung function.

Sensitivity analysis:
Cochran's Q test; multivariable MR; MR-Egger;
MR-PRESSO; funnel plot; leave-one-out

Assumption 2:
IVs are independent of potential confounders that affect exposure and outcome

Confounders

Genetic instruments
SNPs: P < 5E-08/1E-05
r^2 < 0.001
kb = 10,000

Assumption 1:
the chosen IVs should be significantly
related to the exposure

Exposure

Methods for TSMR analysis:
Inverse variance weighted (IVW)
MR-Egger
Weighted median
Weighted mode

Outcome

Assumption 3: IVs affect the risk of the outcome merely via exposure
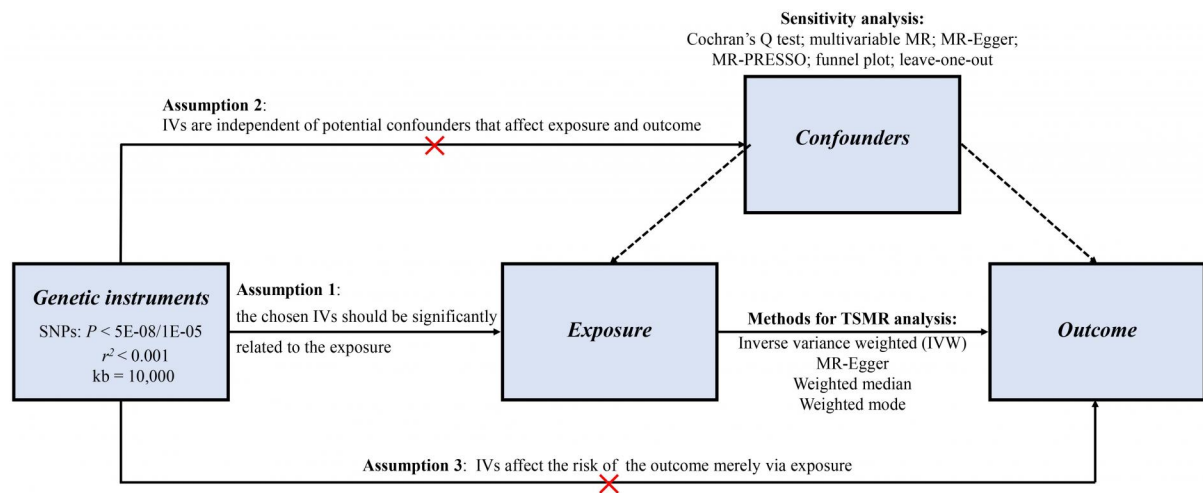
Preprint

Table 1. MR estimates from each method of assessing the causal effects of lung function on NAFLD risk.

Exposure	Outcome	Nsnp	Methods	Beta	SE	OR (95%CI)	P value	Horizontal pleiotropy			Heterogeneity	
								Egger intercept	SE	P value	Cochran's Q	P value
FEV1	NAFLD	218	IVW	-0.835	0.279	0.434 (0.251-0.749)	0.003	0.018	0.013	0.180	259.383	0.026
			MR Egger	-1.928	0.858	0.145 (0.027-0.782)	0.026					
			Weighted median	-0.895	0.383	0.408 (0.193-0.866)	0.020					
			Weighted mode	-0.626	0.909	0.535 (0.090-3.177)	0.492					
			MVMR	-0.434	0.191	0.648 (0.446-0.941)	0.023					
FVC	NAFLD	277	IVW	-1.013	0.246	0.363 (0.224-0.588)	<0.001	0.005	0.010	0.602	328.679	0.016
			MR egger	-1.346	0.684	0.260 (0.068-0.995)	0.050					
			Weighted median	-0.802	0.334	0.448 (0.233-0.863)	0.016					
			Weighted mode	-0.401	0.937	0.670 (0.107-4.205)	0.669					
			MVMR	-0.256	0.222	0.774 (0.501-1.195)	0.248					

CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; IVW, inverse variance weighted; MR, Mendelian randomization; MVMR, multivariable Mendelian randomization; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; SNP, single nucleotide polymorphism; SE, standard error.

$P < 0.025$ was considered statistically significant.

Table 2. MR estimates from each method of assessing the causal effects of NAFLD on lung function.

Exposure	Outcome	Nsnp	Methods	Beta	SE	OR (95%CI)	*P* value	Horizontal pleiotropy			Heterogeneity	
								Egger intercept	SE	*P* value	Cochran's Q	*P* value
NAFLD	FEV1	15	IVW	0.000	0.002	1.000 (0.996-1.004)	0.987	-0.004	0.002	0.054	14.616	0.332
			MR Egger	0.007	0.004	1.007 (1.000-1.015)	0.080					
			Weighted median	0.001	0.003	1.001 (0.996-1.006)	0.578					
			Weighted mode	0.002	0.003	1.002 (0.996-1.008)	0.489					
NAFLD	FVC	14	IVW	0.003	0.002	1.003 (0.999-1.006)	0.118	-0.001	0.002	0.769	13.088	0.363
			MR egger	0.004	0.004	1.004 (0.995-1.012)	0.396					
			Weighted median	0.002	0.002	1.002 (0.997-1.006)	0.485					
			Weighted mode	0.002	0.003	1.002 (0.997-1.008)	0.495					

CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; IVW, inverse variance weighted; MR, Mendelian randomization; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; SNP, single nucleotide polymorphism; SE, standard error.
P < 0.025 was considered statistically significant.

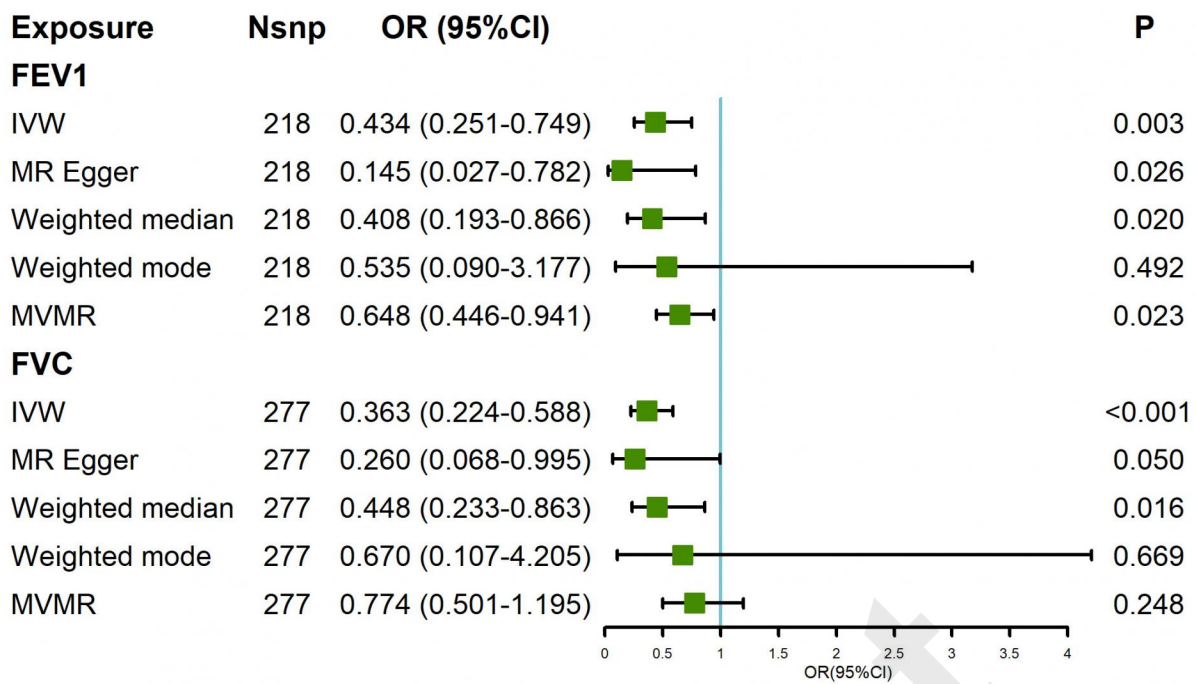| Exposure	Nsnp	OR (95%CI)		P
FEV1				
IVW	218	0.434 (0.251-0.749)		0.003
MR Egger	218	0.145 (0.027-0.782)		0.026
Weighted median	218	0.408 (0.193-0.866)		0.020
Weighted mode	218	0.535 (0.090-3.177)		0.492
MVMR	218	0.648 (0.446-0.941)		0.023
FVC				
IVW	277	0.363 (0.224-0.588)		<0.001
MR Egger	277	0.260 (0.068-0.995)		0.050
Weighted median	277	0.448 (0.233-0.863)		0.016
Weighted mode	277	0.670 (0.107-4.205)		0.669
MVMR	277	0.774 (0.501-1.195)		0.248

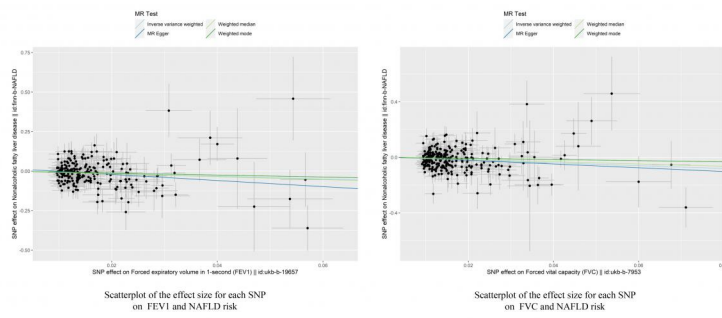Figure 1. Mendelian randomization estimates of lung function with the risk of nonalcoholic fatty liver disease.

Figure 2. Scatterplot of the effect size for each SNP on lung function and the risk of nonalcoholic fatty liver disease.

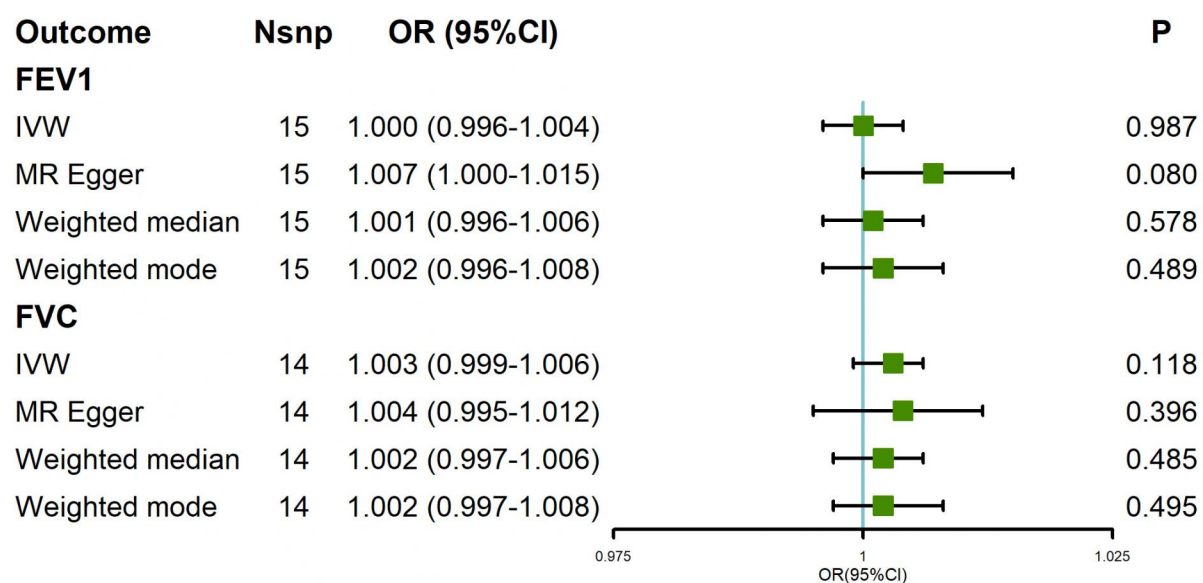| Outcome	Nsnp	OR (95%CI)		P
FEV1				
IVW	15	1.000 (0.996-1.004)		0.987
MR Egger	15	1.007 (1.000-1.015)		0.080
Weighted median	15	1.001 (0.996-1.006)		0.578
Weighted mode	15	1.002 (0.996-1.008)		0.489
FVC				
IVW	14	1.003 (0.999-1.006)		0.118
MR Egger	14	1.004 (0.995-1.012)		0.396
Weighted median	14	1.002 (0.997-1.006)		0.485
Weighted mode	14	1.002 (0.997-1.008)		0.495

OR(95%CI)

Figure 3. Mendelian randomization estimates of nonalcoholic fatty liver disease on lung function.
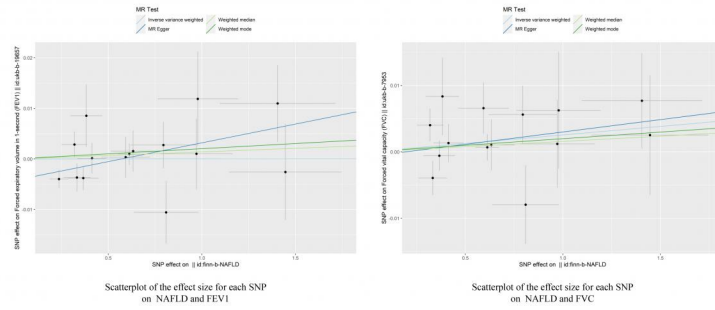
Figure 4. Scatterplot of the effect size for each SNP on nonalcoholic fatty liver disease and lung function.