Letter to the Editor

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Assessing causal relationship between non-alcoholic fatty liver disease and risk of atrial fibrillation

To the Editor:

Non-alcoholic fatty liver disease (NAFLD) and atrial fibrillation (AF) have become the most prevalent diseases in the fields of chronic liver disease and sustained cardiac arrhythmia, respectively, which attract significant interest due to their affiliated morbidity and increased mortality.[1] Epidemiological evidence suggests that NAFLD may increase the risk of AF.[2] The findings, however, were not completely consistent. In a large prospective cohort, Van Kleef *et al.* discovered that fatty liver disease was not related to prevalent or incident AF.[3] Observational studies have methodological constraints and are inevitably subject to residual confounding factors. It is still uncertain if NAFLD is a causal factor in AF.

Mendelian randomisation (MR) analysis, which uses genetic variants as instrumental variables, is a powerful statistical tool for investigating causal relationships that have grown in popularity in epidemiology.[4] Genetic variants, which are unrelated to environmental factors, are randomly distributed at conception, which minimizes confounding and reverse causality. Hence, we used a two-sample MR analysis to assess the potential causal relationship between NAFLD and the risk of AF.

A genome-wide association study (GWAS) analysis in a histologically-characterized cohort of 1,483 biopsied NAFLD cases and 17,781 controls yielded 12 significant single nucleotide polymorphisms (SNPs) as genetically instrumental variables for NAFLD.[5] Because non-alcoholic steatohepatitis (NASH) and liver fibrosis are clinically important phenotypes in NAFLD and are more closely related to cardiovascular diseases, eight significant SNPs as genetic instrumental variables for NASH and three significant SNPs for advanced fibrosis (fibrosis stage F3 or F4) were extracted from this GWAS study to further investigate whether these severe NAFLD phenotypes lead to the development of AF. Furthermore, 55 significant SNPs (p <5×10^{-8}) were procured from another large-scale GWAS study as genetic instruments of NAFLD for validation analysis, in which NAFLD was proxied by chronic alanine aminotransferase (cALT) elevation.[6] The summary statistics for AF were obtained from a GWAS meta-analysis (60,620 cases and 970,216 controls).[7] AF was mainly diagnosed according to ICD-9 and ICD-10 codes. Details of instrumental variable selection and statistical analysis are included in the supplementary information.

To yield an overall estimate of the causal effect of NAFLD, NASH, and advanced fibrosis on AF, the inverse variance weighted method was used. To test the consistency of the results, three sensitivity analyses were performed: MR-Egger regression, weighted median, and MR-PRESSO analysis.

AF risk was not linked with NAFLD (odds ratio [OR] 0.99; 95% CI 0.97–1.01]; p = 0.15), nor NASH (OR 0.99; 95% CI 0.98–1.01; p = 0.43) or advanced fibrosis (OR 0.99; 95% CI 0.98–1.01; p = 0.25) (Fig. 1). If causality between NAFLD or severe NAFLD phenotypes and AF was prevalent at the strength denoted by observational epidemiology[2] (OR 1.27; 95% CI 1.18–1.37), our study had greater than 99% statistical power to detect a statistically significant effect. All sensitivity analyses produced generally consistent results. The Egger regression intercept analysis revealed no evidence of pleiotropy (intercept p value >0.05). Outliers were determined using the MR-PRESSO method, and their exclusion had no significant effect on the results. The results remained unchanged in the validation analysis (cALT, OR 1.00; 95% CI 0.96–1.04; p = 0.87) (Fig. 1).

There was no causal correlation in this MR study between genetically determined NAFLD or severe NAFLD phenotypes (NASH/advanced fibrosis) and the risk of AF. Although our study had enough statistical power to evaluate the causal relationships, the findings should be interpreted with prudence. The genetic instruments were chosen according to a relatively

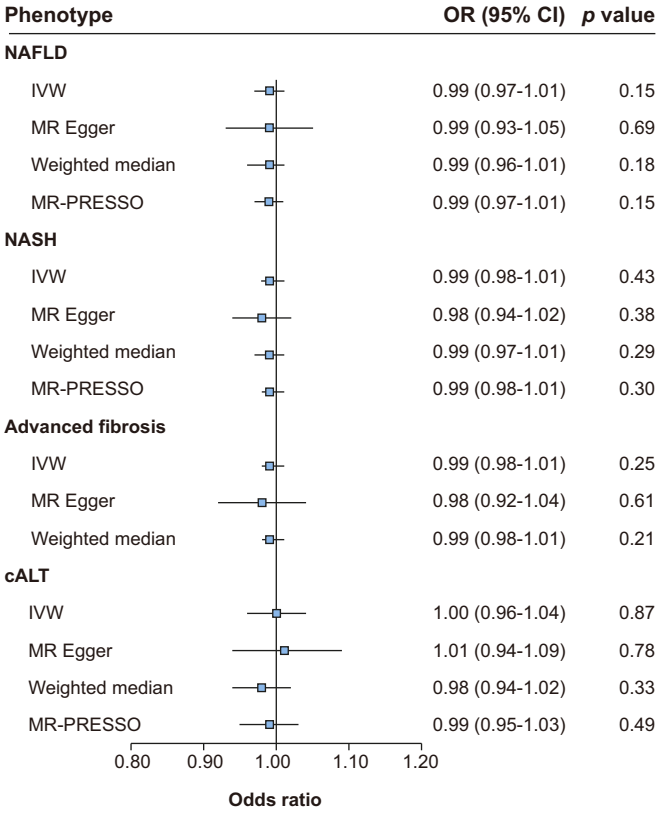

Fig. 1. Forest plot of MR estimates. Forest plot of MR results of NAFLD, NASH, advanced liver fibrosis, and cALT on AF. AF, atrial fibrillation; cALT, chronic alanine aminotransferase elevation; IVW, inverse variance weighted; MR, mendelian randomisation; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; SNP, single nucleotide polymorphism.

small-scale GWAS study (NAFLD: n = 1,434; NASH: n = 836; advanced fibrosis: n = 386). The genetic instruments utilized accounted for comparatively little genetic variance of phenotypes (NAFLD: 2.09%; NASH: 1.88%; advanced fibrosis: 1.27%), which could result in weak instrumental variables bias. Although we utilized another large-scale GWAS analysis with more individual SNPs for additional validation, NAFLD was defined in that study by cALT elevation rather than the presence of NAFLD *per se*. Besides, it did not provide stratification analyses for NASH or liver fibrosis. Given that there have only been a few GWAS studies of NAFLD based on liver biopsy, large-scale GWAS datasets and additional potentially related genetic variants are required for further validation. Furthermore, even though we used several sensitivity analyses to eliminate outlier variants and improve the robustness of the results, horizontal pleiotropy cannot be totally excluded.

In conclusion, our research did not show a causal relationship between genetically determined NAFLD or NAFLD subtypes and the risk of AF. MR analyses based on larger-scale GWAS summary data and more genetic instruments are needed to verify the results of our study.

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Conflict of interest

All authors declared that no potential conflicts of interest should be disclosed in this study.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Z.L and B.Z proposed the idea, performed the MR analyses, and drafted the manuscript. J.L and Z.T checked the integrity and plausibility of data analysis. Y.W revised the manuscript and was responsible for the integrity of data acquisition and statistical analyses.

Data availability statement

NAFLD GWAS summary statistics can be downloaded from the GWAS catalog (http://www.ebi.ac.uk/gwas). GWAS summary statistics for AF can be downloaded from open GWAS (https://gwas.mrcieu.ac.uk/).

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Supplementary data

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