Causal association between gut microbiota composition and the risk of atrial fibrillation

Jiangshan Tan#, Jingyang Wang#, Yanmin Yang, Jun Zhu, Lulu Wang, Wei Xu, Siqi Lyu, Shuang Wu, Juan Wang, Han Zhang

Emergency and Critical Care Center, Fuwai Hospital, National Center for Cardiovascular Diseases of China, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

ABSTRACT

Background and Objectives: Considerable evidence has shown that alterations in gut microbiota composition are associated with atrial fibrillation (AF). However, the causal associations remain largely unresolved. This study aims to reveal the causality between gut microbiota and AF. **Methods:** We incorporated data from the largest genome-wide association studies (GWASs) of gut microbiota composition (involving 18,304 individuals) and GWASs of AF (comprising 60,620 cases and 970,216 controls) in European individuals. A two-sample Mendelian randomization framework was designed to investigate the role of gut microbiota in the development of AF. The inverse variance weighted method was applied for the main causal estimate. Complementary sensitivity analyses were utilized to confirm the robustness of the results. Finally, gene ontology enrichment analyses and Kyoto Encyclopedia of genes and genomes pathway analysis are used to investigate the bio-function. **Results:** Among all gut microbiota, five microbial taxa, namely *Lachnospiraceae FCS020*, *Rikenellaceae RC9 gut group*, *Catenibacterium*, *Victivallis*, and *Erysipelatoclostridium* were identified to be causally associated with the higher risk of AF. Besides, genetically predicted eight microbial taxa, namely *Lachnospiraceae NK4A136 group*, *Howardella*, *Intestinibacter bartlettii*, *Alloprevotella*, *Anaerostipes*, *Odoribacter*, *Ruminococcus (gnavus group)*, and *Ruminiclostridium 5* can prevent AF. **Conclusion:** Our study provides evidence of the causal effect of the gut microbiota on AF, highlighting causal microbial taxa. Our results may offer novel insights into gut microbiota-mediated mechanisms and interventions of AF.

Key words: gut microbiota, atrial fibrillation, causal associations, Mendelian randomization

INTRODUCTION

Atrial fibrillation (AF), the most common cardiac arrhythmia, associated with increased risk for multiple diseases, is considered a significant cause of morbidity and mortality.[1] Accompanying the aging of populations, the incidence and prevalence of AF are increasing globally. However, the underlying etiology and pathogenesis of AF are uncertain and both environmental and genetic factors contribute to the occurrence and development of AF.

Gut microbiota is a complex assembly of thousands of microbial species that live in the human intestinal tract.[2] Gut microbiota having symbiotic relationships with their host, influences host physiology not only locally but beyond the gut.[3] Increasing evidence indicates that microbiomes and their metabolites play a role in cardiometabolic diseases including coronary artery disease,[4] heart failure,[5] hypertension,[6] diabetes,[7] and obesity.[8,9]

Previous studies have shown that gut dysbiosis could facilitate AF, either directly or indirectly. Early sequencing studies demonstrated compositional changes in gut microbial community of patients with AF.[10-14] Subsequently, observational and experimental studies reported inconclusive causal associations between the gut

#These authors contributed equally to this work.

Address for Correspondence:
Yanmin Yang, Emergency and Intensive Care Center, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, 167 Beilishilu, Xicheng District, Beijing 100037, China.
Email: yymfuwai@163.com

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microbiome (mainly gut microbial metabolites such as trimethylamine, lipopolysaccharide, and bile acids) and AF.[15] Zhang *et al.* established a rat model of fecal microbiota transplantation, providing significant causal evidence that age-associated microbial dysbiosis promoted AF susceptibility through enhanced activity of atrial NLRP3-inflammasome.[16] However, observational studies are prone to be biased by residual confounding effects and reverse causation. Besides, controlled experiments for specific causative bacterial species are costly and difficult to achieve translation in the human realm. Although the identification of specific gut microbial causing AF could deepen the understanding of microbiota-derived pathogenesis and thus develop microbiota-related therapies. However, to this day, no studies outlining the causal effects of the specific taxon of gut microbiota on AF have been systematically investigated.

In such instances, Mendelian randomization (MR), widely accepted to explore the potential causal effect of exposure on disease, provides an alternative method for identifying the causation between gut microbiota and non-communicable diseases. First, MR uses genetic variant as instrument variables (IVs) to determine the genetic association between exposures and outcomes. The randomness in allele assortment during meiosis implies that genetic variants will be unrelated to other factors which may affect the outcome, thus excluding potential confounders. Second, the MR design also avoids reverse causation bias because the disease cannot affect genotype. Third, the MR can capture lifetime exposure instead of weeks or months of exposure. Finally, international genome-wide association study (GWAS) consortia provide the summary statistics of genome-wide association meta-analyses on the gut microbiota and AF, thus enabling MR studies. Although previous MR studies have suggested a potential link between certain gut microbial metabolites and AF, the evidence remains inconsistent.[17,18] Moreover, there is a lack of a direct causal relationship between the intestinal metabolites and the gut microbiota. Therefore, future research should focus on investigating the impact of gut microbiota on AF, rather than just focusing on the metabolites, to better understand the underlying mechanisms and to develop novel preventive and therapeutic interventions.

Thus, we performed comprehensive two-sample MR analyses based on public data from GWAS to investigate the causal relationship between gut microbiota and AF at the genus level.

METHODS

Study design

The single nucleotide polymorphisms (SNPs) employed as instrumental variables must adhere to three key assumptions: firstly, the SNPs must be correlated with the exposures, specifically gut microbiota; secondly, the SNPs cannot be correlated with any confounding variables that might potentially affect the association between the exposures and AF; and thirdly, the SNPs must be exclusively correlated with AF through the exposures and not through any other pathways. Our investigation explored the causal relationship between gut microbiota and AF, as illustrated in Figure 1. Our MR study utilized genetic variants as IVs to assess the causality between an exposure and an outcome, rather than directly manipulating the exposure in human subjects. Additionally, the genetic variants used in the present MR study are typically already available from large-scale GWAS, minimizing necessity for human participation and informed consent.

Data source

The genetic variants for gut microbiota were obtained from the largest published GWAS to date, which focused on gut microbiota composition and was conducted by the MiBioGen consortium.[19,20] This study comprised 18,340 individuals from 24 cohorts, with a majority of European ancestry (n = 13,266). The study utilized variable regions V4, V3-V4, and V1-V2 of the 16S rRNA gene to profile the microbial composition, perform taxonomic classification, and conducted microbiota quantitative trait loci mapping analysis. During this analysis, host genetic variants were identified and linked to genetic loci associated with the abundance levels of bacterial taxa. In total, the study identified 131 genera, 12 of which were unknown, and had a mean abundance greater than 1% at the lowest taxonomic level of the genus.[19] Therefore, the present study analyzed 119 genus-level taxa. The AF data was extracted from the largest GWAS that tested the association between AF and 34,740,186 genetic variants (minor allele frequency > 2.5×10^{-5}).[21] This GWAS compared a total of 60,620 cases and 970,216 controls of European ancestry, sourced from six contributing studies, namely The Nord-Trøndelag Health Study (HUNT), deCODE, the Michigan Genomics Initiative (MGI), DiscovEHR, UK Biobank, and the AFGen Consortium. The detailed information is shown in Table 1.

Instrumental variables

To choose the IVs, the following selection criteria were employed: First, potential IVs were selected based on SNPs associated with each genus at the locus-wide significance threshold ($P < 1.0 \times 10^{-5}$). Second, the linkage disequilibrium between the SNPs was calculated using the reference panel from the 1000 Genomes project European samples data, and among those SNPs with $R^2 < 0.001$ (clumping window size = 10,000 kb), only the SNPs with the lowest P values were retained. Third, SNPs with minor

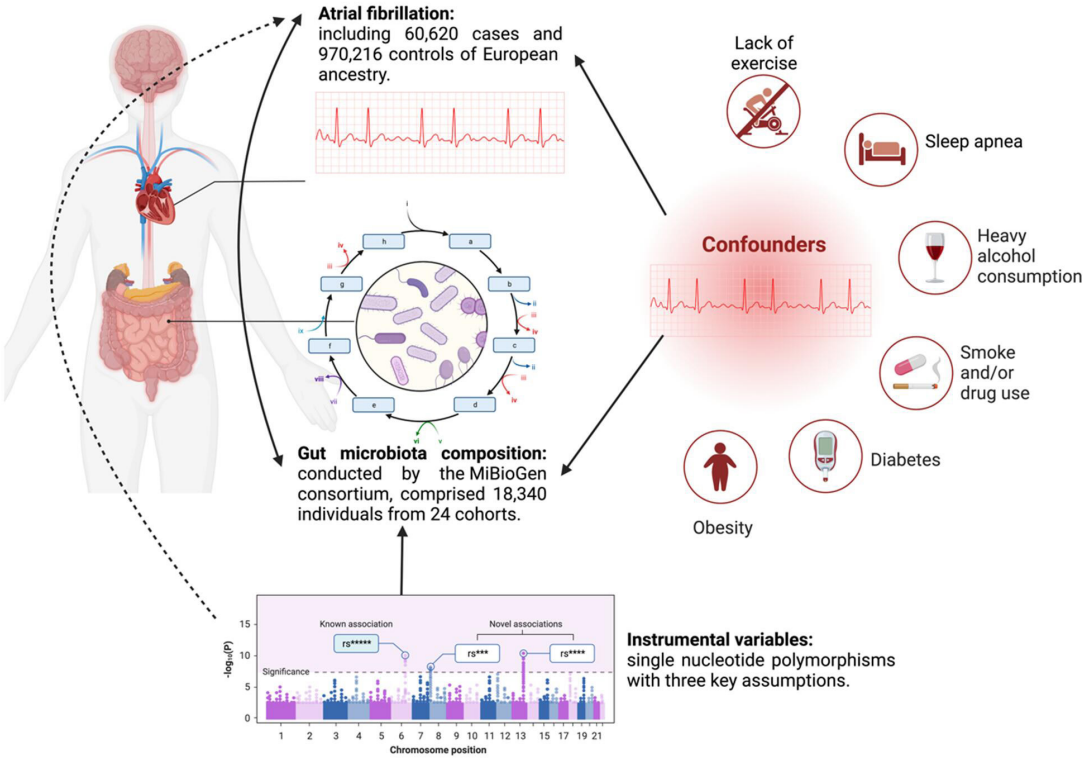

Figure 1: Schematic representation of the present Mendelian Randomization analysis.

allele frequency ≤ 0.01 were excluded. Finally, in cases where palindromic SNPs existed, the forward strand alleles were inferred using allele frequency information.

Gene ontology and pathway enrichment analysis

In our study, gene ontology (GO) analysis was employed to annotate gene products and elucidate their functional attributes, encompassing three primary domains: cellular component (CC), molecular function (MF), and biological process (BP). For pathway enrichment analysis, we utilized the Kyoto Encyclopedia of Genes and Genomes (KEGG). Enrichment of overlapping gene candidates was carried out using DAVID, accessible at https://david.ncifcrf.gov/. Results yielding a *P* value below 0.05 were deemed statistically significant.

Statistical analysis

Multiple methods, including the inverse variance weighted (IVW), MR-Egger regression, and weighted median, were employed in this study to investigate whether a causal association exists between gut microbiota and AF. The IVW method, utilizing a meta-analysis approach combined with Wald estimates for each SNP, produced an overall estimate of the effect of gut microbiota on AF. In the absence of horizontal pleiotropy, the IVW results would be unbiased. The MR-Egger regression, based on the assumption of instrument strength independent of direct effect, enables the evaluation of pleiotropy's existence with the intercept term. A zero intercept term indicates the absence of horizontal pleiotropy, and the MR-Egger regression result agrees with the IVW.[22] The weighted median method allows for the accurate estimation of causal association even if up to 50% of IVs are invalid.[23] Besides, the "simple mode", often termed the "Two-Stage Least Squares" or "2SLS", is an approach that operates in two primary steps: initially, genetic variants are regressed on the exposure, followed by the outcome being regressed on the predicted values from the initial step. This linear model serves as an efficacious tool, particularly when singular genetic variants are under consideration. The "weighted mode" and IVW method amalgamates data across a spectrum of genetic variants. By assigning greater weight to variants that elucidate a more substantial proportion of variance in the exposure, this method enables a nuanced, aggregate perspective. Especially advantageous when handling multiple genetic variants, the "weighted mode" boosts statistical power, allowing for more robust and comprehensive insights. Therefore, different MR analyses can ensure the consistency and reliability of our MR analysis.

To measure the heterogeneity of IVs, Cochran's IVW Q statistics were utilized. Moreover, the "leave-one-out" analysis was conducted by sequentially omitting each

Table 1: Study-specific basic information and numbers of genetic variants in international genome-wide association study of atrial fibrillation

Traits	Cases (*N*)	Controls (*N*)	Sample size (*N*)	Unique markers tested (*N*)
HUNT	6493	63,142	69,635	20,013,723
deCODE	13,471	358,161	371,632	18,134,320
MGI	1226	11,049	12,275	12,948,440
DiscoverEHR	6679	41,803	48,482	12,184,179
UKB	14,820	380,919	395,739	28,226,282
AFGen	17,931	115,142	133,073	11,792,062

instrumental SNP to identify potentially heterogeneous SNPs.[24] To evaluate the causal association between gut microbiota and AF, a reverse MR analysis was also carried out on the bacteria found to have a causal link with AF in the forward MR analysis. The methods and settings used were consistent with previous MR analyses.[22-24]

To assess the strength of IVs, the *F*-statistic was calculated. If the corresponding F-statistic was > 10, it was deemed that there was no significant weak instrumental bias.[25] The Bonferroni procedure was used to apply a false discovery rate correction to all indexes with < 0.05. Genera of gut microbiota and AF were considered to have a causal association when $P < 0.05$ and a suggestive association when $0.003\ (0.05/18) < P < 0.05$.

All statistical analyses were carried out using R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). The TwosampleMR (version 0.5.6) R packages were used to perform MR analyses.[26]

RESULTS

As per the IV selection criteria, a total of 1245 SNPs were employed as IVs for 119 bacterial genera. Additional information about the selected instrumental variables was showed in Supplementary Table S1.

Eighteen bacterial genera, namely *Alloprevotella*, *Anaerostipes*, *Anaerotruncus*, *Bacteroides*, *Catenibacterium*, *Erysipelatoclostridium*, *Eubacterium (nodatum group)*, *Howardella*, *Intestinibacter*, *Lachnospiraceae FCS020 group*, *Lachnospiraceae NK4A136 group*, *Lactobacillus*, *Odoribacter*, *Prevotella 9*, *Rikenellaceae RC9* gut group, *Ruminiclostridium 5*, *Ruminococcus (gnavus group)*, and *Victivallis* were found to be associated with AF in at least one MR method, as shown in Figure 2 and Supplementary Table S2.

IVW estimate suggests that *Alloprevotella* (OR = 0.942, 95% CI: 0.896–0.992, $P = 0.022$), *Anaerostipes* (OR = 0.922, 95% CI: 0.857–0.992, $P = 0.030$), *Howardella* (OR = 0.948, 95% CI: 0.910–0.989, $P = 0.012$), *Intestinibacter* (OR = 0.933, 95% CI: 0.879–0.991, $P = 0.024$), *Lachnospiraceae NK4A136 group* (OR = 0.918, 95% CI: 0.865–0.973, $P = 0.004$), *Odoribacter* (OR = 0.910, 95% CI: 0.831–0.996, $P = 0.041$), *Ruminococcus (gnavus group)*, OR = 0.952, 95% CI: 0.908–0.999, $P = 0.044$) had a suggestively protective effect on AF (all $0.003 < P < 0.050$) while *Catenibacterium* (OR = 1.060, 95% CI: 1.002–1.122, $P = 0.043$), *Lachnospiraceae FCS020 group* (OR = 1.077, 95% CI: 1.011–1.148, $P = 0.021$), *Rikenellaceae RC9 gut group* (OR = 1.047, 95% CI: 1.010–1.086, $P = 0.012$), and *Victivallis* (OR = 1.038, 95% CI: 1.001–1.077, $P = 0.044$) had a suggestively harmful effect on AF (all $0.003 < P < 0.050$, Figure 2 and Supplementary Table S2). At least three sensitivity analyses were consistent with the IVW primary outcome, indicating the reliability of our results (Table 2).

Among these eighteen causal associations, the *F*-statistics of the IVs ranged from 14.44 to 85.21, eliminating the bias of weak IVs. The results of Cochran's IVW *Q* test showed no significant heterogeneity of these IVs (Supplementary Table S3) in all the positive causal associations in IVW estimates (including the protective and harmful effects). In addition, there were no significant signs of directional horizontal pleiotropy observed in all the positive causal associations in IVW estimates, as indicated by the results of the MR-Egger regression intercept analysis (Figure 3 and Supplementary Table S4). Cochran's IVW *Q* test and MR-Egger regression intercept analysis suggested that the results of IVW may provide us with a more accurate and reliable estimation of these associations.

Notably, some other MR-Egger regression intercept analyses indicated significant directional horizontal pleiotropy in *Ruminiclostridium 5* and *Erysipelatoclostridium*, suggesting the results of the MR-Egger analysis were more accurate and reliable. Therefore, a suggestively protective effect and a suggestively harmful effect on AF were observed in the MR Egger analyses, respectively (Figure 2, Figure 3 and Supplementary Table S4).

To further explore the potential mechanism of this causation, we focused on the most significant KEGG

Table 2: The results of sensitivity analysis of all positive associations

Method	Nsnp	pval	OR	OR_lci95	OR_uci95
Alloprevotella					
MR Egger	5	0.976	1.007	0.648	8.488
Weighted median	5	0.156	0.951	0.886	1.407
Inverse variance weighted	5	0.022	0.942	0.896	1.072
Simple mode	5	0.456	0.963	0.880	2.561
Weighted mode	5	0.489	0.965	0.882	2.733
Anaerostipes					
MR Egger	13	0.864	0.976	0.748	6.233
Weighted median	13	0.222	0.940	0.850	1.625
Inverse variance weighted	13	0.030	0.922	0.857	1.102
Simple mode	13	0.066	0.808	0.657	1.266
Weighted mode	13	0.756	1.032	0.851	4.859
Anaerotruncus					
MR Egger	12	0.042	0.732	0.563	1.241
Weighted median	12	0.283	0.945	0.853	1.833
Inverse variance weighted	12	0.095	0.939	0.872	1.251
Simple mode	12	0.520	0.941	0.785	3.036
Weighted mode	12	0.588	0.947	0.783	3.491
Bacteroides					
MR Egger	12	0.042	0.732	0.563	1.241
Weighted median	12	0.282	0.945	0.853	1.831
Inverse variance weighted	12	0.095	0.939	0.872	1.251
Simple mode	12	0.539	0.941	0.778	3.166
Weighted mode	12	0.569	0.947	0.791	3.348
Catenibacterium					
MR Egger	4	0.906	0.953	0.471	8.460
Weighted median	4	0.069	1.062	0.995	1.183
Inverse variance weighted	4	0.043	1.060	1.002	1.119
Simple mode	4	0.189	1.076	0.988	1.514
Weighted mode	4	0.216	1.076	0.981	1.601
Erysipelatoclostridium					
MR Egger	15	0.014	1.344	1.095	1.141
Weighted median	15	0.533	1.023	0.953	2.944
Inverse variance weighted	15	0.471	1.023	0.961	2.598
Simple mode	15	0.712	1.024	0.904	4.303
Weighted mode	15	0.625	1.030	0.916	3.618
Eubacterium (nodatum group)					
MR Egger	11	0.043	0.814	0.686	1.187
Weighted median	11	0.556	1.015	0.966	3.049
Inverse variance weighted	11	0.685	0.990	0.945	3.922
Simple mode	11	0.685	1.019	0.935	3.998
Weighted mode	11	0.661	1.019	0.938	3.810
Howardella					
MR Egger	9	0.670	0.961	0.806	4.066
Weighted median	9	0.041	0.944	0.893	1.115
Inverse variance weighted	9	0.012	0.948	0.910	1.046
Simple mode	9	0.197	0.939	0.860	1.539
Weighted mode	9	0.189	0.940	0.864	1.513
Intestinibacter					
MR Egger	16	0.061	0.816	0.671	1.245
Weighted median	16	0.052	0.921	0.848	1.155

To be continued

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Inverse variance weighted	16	0.024	0.933	0.879	1.081
Simple mode	16	0.236	0.918	0.802	1.701
Weighted mode	16	0.321	0.934	0.820	2.007
Lachnospiraceae FCS020 group					
MR Egger	12	0.951	0.995	0.852	6.979
Weighted median	12	0.218	1.057	0.968	1.603
Inverse variance weighted	12	0.021	1.077	1.011	1.076
Simple mode	12	0.496	1.050	0.917	2.835
Weighted mode	12	0.404	1.053	0.937	2.345
Lachnospiraceae NK4A136 group					
MR Egger	15	0.212	0.919	0.810	1.617
Weighted median	15	0.020	0.906	0.833	1.086
Inverse variance weighted	15	0.004	0.918	0.865	1.039
Simple mode	15	0.129	0.907	0.806	1.368
Weighted mode	15	0.100	0.913	0.824	1.280
Lactobacillus					
MR Egger	8	0.166	0.856	0.705	1.529
Weighted median	8	0.025	0.919	0.853	1.092
Inverse variance weighted	8	0.292	0.962	0.896	1.837
Simple mode	8	0.138	0.912	0.819	1.385
Weighted mode	8	0.143	0.913	0.820	1.397
Odoribacter					
MR Egger	7	0.173	0.787	0.586	1.631
Weighted median	7	0.031	0.872	0.771	1.131
Inverse variance weighted	7	0.041	0.910	0.831	1.134
Simple mode	7	0.134	0.840	0.690	1.438
Weighted mode	7	0.144	0.840	0.686	1.470
Prevotella 9					
MR Egger	16	0.473	0.933	0.774	2.780
Weighted median	16	0.035	0.924	0.858	1.111
Inverse variance weighted	16	0.173	0.960	0.906	1.445
Simple mode	16	0.074	0.895	0.799	1.225
Weighted mode	16	0.087	0.899	0.802	1.257
Rikenellaceae RC9gut group					
MR Egger	11	0.788	1.032	0.826	5.254
Weighted median	11	0.022	1.057	1.008	1.069
Inverse variance weighted	11	0.012	1.047	1.010	1.044
Simple mode	11	0.101	1.077	0.994	1.270
Weighted mode	11	0.120	1.074	0.989	1.319
Ruminiclostridium 5					
MR Egger	12	0.046	0.678	0.486	1.296
Weighted median	12	0.805	1.015	0.904	5.141
Inverse variance weighted	12	0.692	1.016	0.937	4.046
Simple mode	12	0.378	1.080	0.916	2.280
Weighted mode	12	0.934	0.993	0.842	6.789
Ruminococcus (gnavus group)					
MR Egger	11	0.045	0.770	0.617	1.223
Weighted median	11	0.254	0.964	0.905	1.700
Inverse variance weighted	11	0.044	0.952	0.908	1.116
Simple mode	11	0.850	0.991	0.903	5.550
Weighted mode	11	0.825	0.989	0.899	5.285
Victivallis					
MR Egger	10	0.452	0.896	0.682	2.787

To be continued

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Weighted median	10	0.668	1.011	0.961	3.801
Inverse variance weighted	10	0.044	1.038	1.001	1.110
Simple mode	10	0.989	1.001	0.925	7.233
Weighted mode	10	0.991	1.000	0.928	7.241

OR: odds ratio; OR_lci95: odds ratio lower confidence interval at 95%; OR_uci95: odds ratio upper confidence interval at 95%; Nsnp: number of single nucleotide polymorphisms.

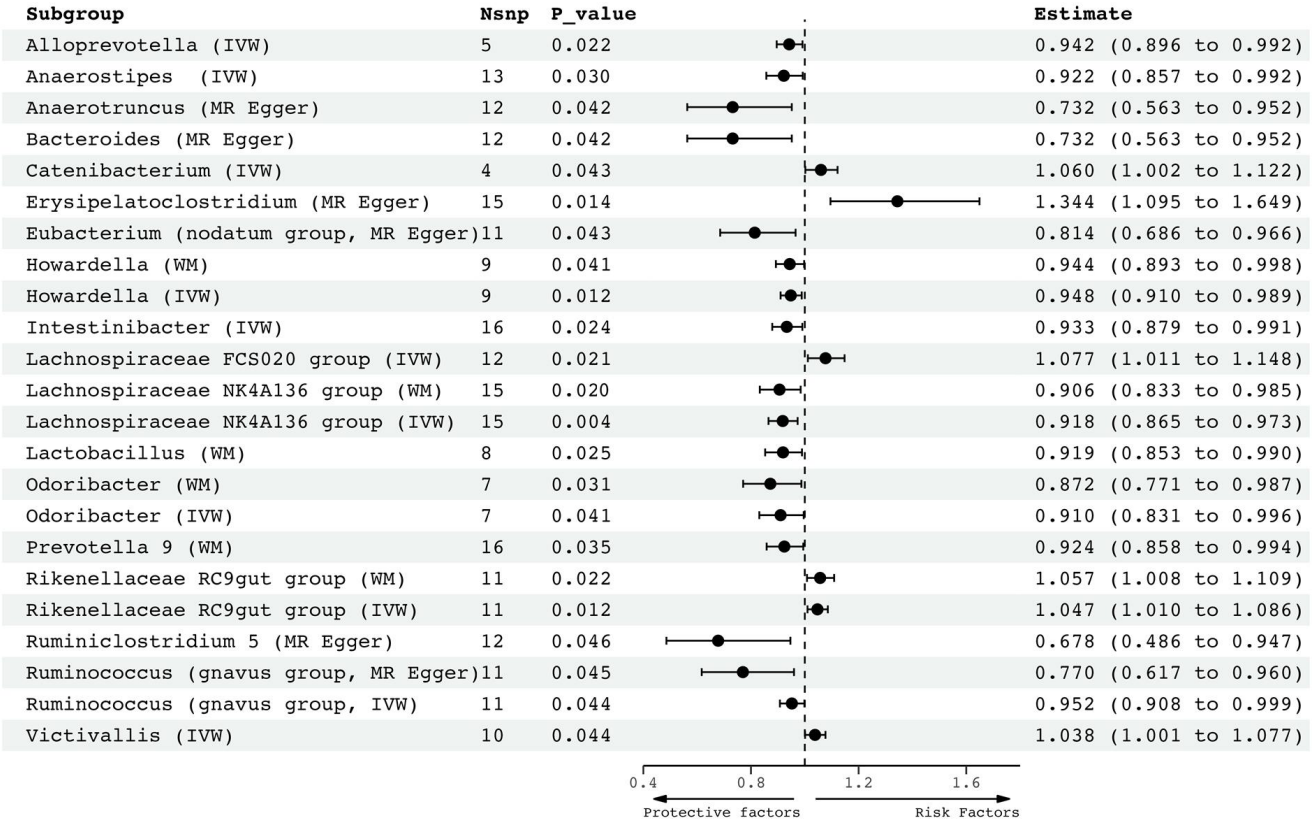

Figure 2: Mendelian Randomization estimates for the association between gut microbiota and atrial fibrillation. IVW: inverse variance weighted; WM: weighted median, Nsnp: number of single nucleotide polymorphisms.

signal pathways and the top 6 enrichment terms in BP, CC, and MF in the genetic variants and their associated genes, as illustrated in Figure 4 and Figure 5. For BP, the primary enrichments were: neuron development, cell and biological adhesion, nervous system evolution, and cellular morphogenesis, particularly in differentiation. Within CC, the genes were predominantly associated with postsynapse, synapse, basal cell structures, neuronal components, postsynaptic density, and asymmetric synapse. For MF, the genes largely showcased activities like protein serine/threonine kinase, combined serine/threonine/tyrosine kinase, general kinase, phosphotransferase with alcohol groups as acceptors, and binding to cell adhesion molecules. Regarding signal pathway enrichment, the genes prominently participated in circadian entrainment and the relaxin signaling pathway.

DISCUSSION

This is the first comprehensive examination of the causal association between gut microbiota and AF. We conducted MR analyses on 119 bacterial genera, involving 18,304 individuals from 24 cohorts, to reveal the potential role of gut in the development of AF. We identify that *Lachnospiraceae FCS020 group* (OR: 1.077; 95% CI: 1.011–1.148; $P = 0.021$), *Rikenellaceae RC9 gut group* (OR: 1.047; 95% CI: 1.010–1.086; $P = 0.012$), *Catenibacterium* (OR: 1.060; 95% CI: 1.002–1.122; $P = 0.043$), *Victivallis* (OR: 1.038; 95% CI: 1.001–1.077; $P = 0.044$), and *Erysipelatoclostridium* (OR: 1.344; 95% CI:1.095–1.649; $P = 0.014$) causally associate with the higher risk of AF. Our findings also suggest that *Lachnospiraceae NK4A136 group* (OR: 0.918; 95% CI: 0.865–0.973; $P = 0.004$), *Howardella* (OR: 0.948;

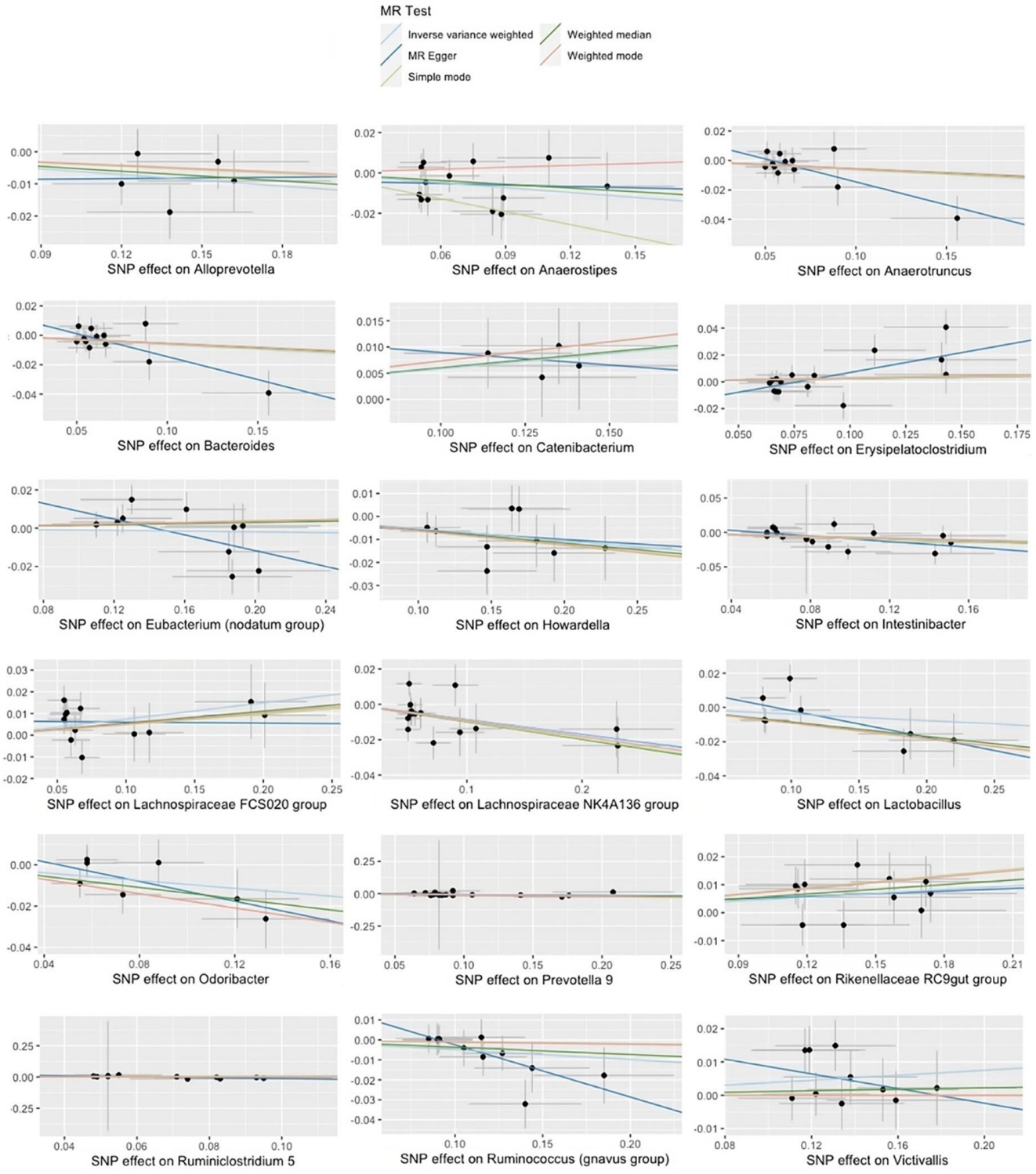

Figure 3: Scatter plots for the causal association between gut microbiota and atrial fibrillation. MR: Mendelian Randomization; SNP: single nucleotide polymorphism.

95% CI: 0.910– 0.989; $P = 0.012$), *Intestinibacter bartlettii* (OR: 0.933; 95% CI: 0.879–0.991; $P = 0.024$), *Alloprevotella* (OR: 0.942; 95% CI: 0.896–0.992; $P = 0.022$), *Anaerostipes* (OR: 0.922; 95% CI: 0.857–0.992; $P = 0.030$), *Odoribacter* (OR: 0.910; 95% CI: 0.831–0.996; $P = 0.041$), *Ruminococcus (gnavus group)* (OR: 0.952; 95% CI: 0.908–0.999; $P = 0.044$), and *Ruminiclostridium 5* (OR: 0.678; 95% CI: 0.486–0.947; $P = 0.046$), have a suggestively protective effect on AF.

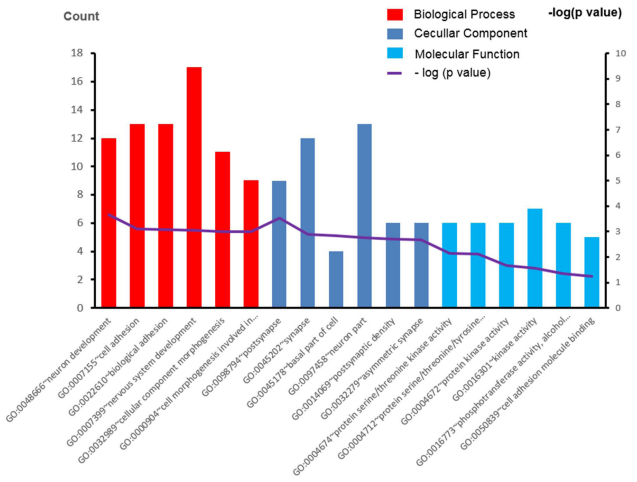

Figure 4: The results of GO enrichment analysis in all significant exposures associated genes. The top six significant enrichment terms of biological process, cellular component, and molecular function of overlapping genes are shown. GO: gene ontology.

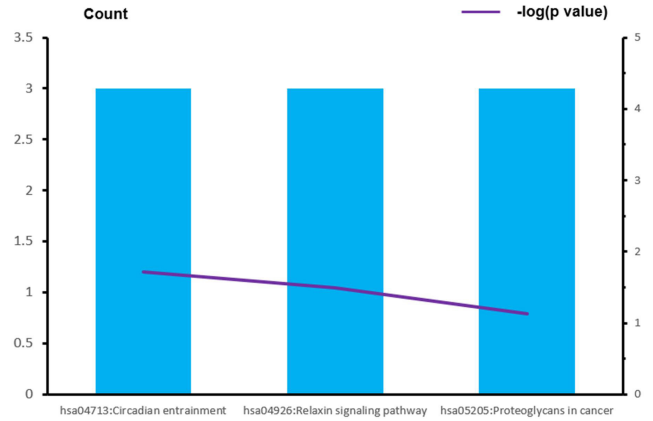

Figure 5: The results of KEGG enrichment analysis in all significant exposures associated genes. A total of 3 KEGG signaling pathways of overlapping genes are shown. KEGG: kyoto encyclopedia of genes and genomes.

Previous studies have explored potential associations between the gut microbiome and AF. Luo *et al.*[17] performed MR analyses to explore the effects of 3 bacterial genera (Shigella, campylobacter, and candida) and 12 metabolites (beta-hydroxybutyric acid, betaine, trimethylamine N-oxide (TAMO), carnitine, choline, glutamate, kynurenine, phenylalanine, propionic acid, serotonin, tryptophan, tyrosine) on AF and no causal relationship was found. Jia *et al.*[18] conducted similar MR studies with comparable results. However, the relationship between specific gut microbial metabolites and AF is not yet clear. While some studies have found that certain metabolites produced by the gut microbiota may promote inflammation and oxidative stress, which are known to be involved in the development of AF, other studies have failed to establish a direct causal link.[18] Therefore, it is necessary to conduct further research to investigate the causal relationship between gut microbiota and AF. Our study differs from these studies in the following two aspects: First, our study is more comprehensive in its investigation of causality between gut microbial and AF. Unlike the above two studies that analyzed 3 bacterial genera and several gut microbial metabolites, we comprehensively analyzed 119 bacterial genera. This allows us to assess the causal relationship between almost all available gut microbiota and AF. Second, the quality control procedure for selecting IVs is stricter in our study. We not only select independently significant GWAS SNPs ($P < 1 \times 10^{-5}$) as IVs but also conduct pleiotropy and heterogeneity analysis to maximally fulfill core MR assumptions. In contrast, the above two studies used a fairly loose P value threshold ($P < 5 \times 10^{-5}$) to select eligible IVs, which may introduce a weak instrument bias.

Our MR analyses could inspire more mechanistic and interventional studies of gut microbiome and AF. Recent research findings indicated a key role for inflammasomes as a root cause of AF. As exemplified in our study, *Lachnospiraceae NK4A136 group*, *Ruminococcus (gnavus group)*, *Ruminiclostridium 5*, *Anaerostipes*, *Alloprevotella*, and *Howardella* show protective effects for AF, which may attribute to anti-inflammatory effects of short-chain-fatty-acids (SCFAs). These genera belong to families that comprise well-known SCFAs producing bacteria, such as *Ruminococcaceae*, *Prevotellaceae*, and *Lachnospiraceae*.[27] SCFAs, the major products of dietary fibers from the microbial fermentative activity in the gut, have wide-ranging impacts on various aspects of host physiology, such as host metabolism, immune system, and cell proliferation.[28] Among the SCFAs, butyrate has been investigated extensively. Butyrate has multitude of anti-inflammatory physiological functions, both within and outside the intestine.[29] Butyrate provided energy to colonocytes and enhanced the gut barrier function, which reduces LPS intestinal leakage into the systemic circulation and further decreases inflammation.[30-32] Butyrate also ameliorated the overproduction of adhesion molecules (VCAM-1 and E-selectin), reduced oxidative stress (ROS and 4-HNE), and suppressed inflammation (MCP-1 and IL-8).[33] However, *Lachnospiraceae_FCS020_group*, as an IL-6 positive-related genus, also belongs to family Lachnospiraceae, inducing an inflammatory response in the body.[34] Besides, an increased in *Lachnospiraceae_FCS020_group* showed a positive correlation with LPS production and a negative correlation with acetate, propionate, and butyrate.[35] Our results demonstrated that bacterial species within the same genus can have different physiological effects, underscoring the importance of studying the pathogenesis of the intestinal flora at a more specialized level.

Gut microbial may impact on AF through their multiple effects on AF risk factors, such as diabetes mellitus, obesity,

coronary artery disease, heart failure, and so on. Because these risk factors are individually related to AF progression and are also associated with gut dysbiosis[15] On the one hand, gut microbial may aggravate inflammation and insulin resistance, leading to development of AF-risk factors and enhancing AF progression. Several suggestive gut microbial taxa were detected in our MR analysis, some of which have been proved in previous observational studies. Sun *et al.* reported that the significant increase of *Rikenellaceae RC9 gut group*, impairing intestinal barrier and stimulating gut inflammation, may contribute to the pathogenesis of acute myocardial infarction.[19,20] Gallardo-Becerra *et al.* suggested that *Catenibacterium* positively correlated with clinical and anthropometric parameters of obesity and metabolic syndrome and can be considered as new biomarkers.[36] He, *et al.* performed Spearman correlation analysis and identified that *Erysipelatoclostridium* were significantly associated with an increase in weight of mice in the high-fat diet-induced obese mice.[37] Similarly, Rodriguez J, *et al.* found that *Victivallis* positively correlated with hepatic lipid accumulation and myosteatosis in humanized obese mice. Moreover, *Victivallis* decreased after treatment with inulin in specific humanized obese mice.[38] On the other hand, gut microbial may reduce inflammation and improve metabolism, exerting protective effects on AF-related risk factors and inhibiting the progression of AF. For example, Zhang *et al.* report that in type 2 diabetes mellitus and diabetic nephropathy patients, the relative abundances of butyrate-producing bacteria and potential probiotics (*Lachnospira* and *Intestinibacter*) were significantly reduced, which may play a role in lipid metabolism and glucose metabolism.[39] Multiple studies have observed a decrease in the abundance of *Intestinibacter bartlettii* after intake of metformin, which may lead to the gastrointestinal side effects of metformin.[40-43] Thus, concurrent use of *Intestinibacter bartlettii* with metformin might result in an improvement in treatments.[43] Nevertheless, there were also some observational studies that showed inconsistent outcomes. For example, Li *et al.* found that *Victivallis* were reduced by diabetes. And tea polysaccharides could restore the relative abundance of these bacterial genera.[44] These inconsistencies may be due to the dynamic changes in gut microbiota caused by varies factors such as diet and health status. The potential for reverse causation in observational studies and the limitations of sequencing techniques hamper inferences about causality between gut microbiota and AF. The results of our MR study suggest some indication for future research of gut microbiota. Certainly, more animals and human studies, which fulfils an essential element of the "Koch's postulate" and proving causation between the gut microbiome and AF, needs to be completed.[45]

We delved into the causation mechanism by focusing on significant KEGG pathways and top 6 enrichment terms (BP, CC, MF) related to genetic variants and their associated genes. Notable enrichments included neuron development, cell adhesion, nervous system evolution, and protein kinase activities. Additionally, genes were prominent in pathways like circadian entrainment and relaxin signaling. The findings of the present study were partly in agreement with previous researches which highlighted a range of possible mechanisms, particularly cardiac autonomic nervous system activation and inflammation, that link TMAO with AF. Yu *et al.* injected TMAO into four major anterior right ganglionated plexi of canine model, resulting in increased autonomic activity and AF induction compared with saline controls. Cardiac autonomic nervous system activation processes were accompanied by the addition of inflammatory cytokines and the enhancement of inflammatory levels.[46]

We suggest several possible future gut microbiome-related clinical translations. First, oral consumption of prebiotics, such as galactooligosaccharides which increase SCFAs and decrease LPS, or probiotics (mainly SCFAs-producing bacteria). Second, faecal microbiota transplantation can introduce beneficial microbes from a healthy donor to a recipient which showed promise in health researches. Third, lifestyle factors, including sleep, stress, dietary habits, and exercise, impact gut microbiota, and their modification can induce favorable microbial shifts. Fourth, bacteriophages may offer targeted bacterial elimination. Fifth, gene editing techniques, such as clustered regularly interspaced short palindromic repeats, hold potential for modifying gut microbes.[47,48] While these potential clinical application pathways exist, it is imperative to first comprehend the causal relationship between these microorganisms and AF before embarking on the development of clinical products. Only through an in-depth investigation of this causality can we ensure a smoother clinical transition and identify products that possess authentic clinical significance.

However, our study has a few limitations. Firstly, although the GAWS associated with gut microbial that we included in this study had the largest known sample size, a small number of which were taken from sets consisting of other races, which may partially affect our estimates. Secondly, we conducted the MR analysis only at the genus level, which means we couldn't reveal causation between gut microbiota and AF at a more specialized level, such as the species or strain levels. With the rapid development of genome-sequencing technology, we believe that more SNPs will have been identified, leading to more specific and accurate results. Thirdly, AF is more prevalent in men than in women at all ages.[49,50] we just used the summary-level statistics in our study rather than individual-level data. Therefore, we can't further explore the causal association between subgroups (women vs. men), which may influence our results. Fourthly, our results may

not be universally applicable due to population-specific genetic variations. We tried to replicate our study in other populations. However, after a comprehensive search, no GWAS on gut microbiota had been performed on other populations. To further refine the causality between gut microbiota and AF, we conducted GO enrichment analyses and KEGG pathway analysis to investigate the bio-function.

In summary, our study provides an unprecedented and comprehensive profile of gut microbes associated with AF, supporting the potentially causal effects of the various gut microbiota on AF. Our results highlight the protective role of SCFAs producing bacteria (*Lachnospiraceae NK4A136 group*, *Ruminococcus (gnavus group)*, *Anaerostipes*, *Alloprevotella*, and *Howardella*) in the development of AF, providing valuable guidance for the prevention and treatment of AF in clinical practice. Our study also emphasizes that different bacteria species of same genus played distinct physiological effects on AF. Thus, the potential mechanisms of specific species and strains of AF-related gut microbiota need to be further investigated.

Supplementary Information

Supplementary materials are only available at the official site of the journal (www.intern-med.com).

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Author Contributions

Conceptualization: Jiangshan Tan and Jingyang Wang. Methodology: Jiangshan Tan, Jingyang Wang, Yanmin Yang and Jun Zhu. Resources and data curation: Jiangshan Tan, Jingyang Wang and Lulu Wang. Writing—Original Draft: Jiangshan Tan, Jingyang Wang, Yanmin Yang and Wei Xu. Writing—Review and Editing: Jiangshan Tan, Jingyang Wang, Juan Wang, Han Zhang, Siqi LYU, and Shuang Wu. Jiangshan Tan and Jingyang Wang contributed equally to this work.

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Ethical Approval

Not applicable.

Informed Consent

Not applicable.

Conflict of Interest

There is no conflict of interest in this study.

Use of Large Language Models, AI and Machine Learning Tools

None declared.

Data Availability Statement

All data and materials used in this research are publicly available.

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