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·临床研究·

牙周炎与肝胆疾病的因果关联：一项双向孟德尔随机化分析

赵莉[1]，　陈少鹏[2]，　陈圳[2]，　陈跃圻[2]，　余挺[2]
1.中山大学附属口腔医院，广东省口腔医学重点实验室，广东 广州（510055）；　2.广州医科大学口腔医学院·附属口腔医院牙周病科·广东省口腔组织修复与重建工程技术研究中心·广州市口腔再生医学基础与应用研究重点实验室，广东 广州（510182）

【摘要】　目的　利用孟德尔随机化（Mendelian randomization，MR）方法探究牙周炎与肝胆疾病的双向因果关联，为牙周炎与肝胆疾病共发患者的联合防治以及临床诊治决策提供证据。**方法**　从欧洲人群牙周炎的全基因组关联分析（genome-wide association study，GWAS）数据集中（17 353例牙周炎，28 210例对照）提取与暴露强相关的单核苷酸多态性（single nucleotide polymorphisms，SNPs）作为工具变量（instrumental variants，IVs），通过计算SNP的*F*值验证SNP与暴露的关联程度，并使用在线平台PhenoScanner剔除与结局强相关的IVs。使用逆方差加权（inverse variance weighted，IVW）、MR-Egger回归及加权中位数等方法估计牙周炎和肝胆疾病的双向因果关联。进行稳健性分析：Cochran's Q statistic（IVW）和Rucker's Q statistic（MR-Egger）用于评估异质性；MR-PRESSO用于评估水平多效性；使用Leave-one-out方法逐个剔除IVs后重新进行MR分析，以检验是否存在IV显著影响结果；并使用肝胆疾病相关的独立GWAS验证结果的稳健性。**结果**　IVW法提示牙周炎可能增加27.7%无胆石性胆囊炎风险（*OR* = 1.277，95% *CI*：1.097~1.485，*P*=0.002），MR-Egger回归及加权中位数法未观察到牙周炎对无胆石性胆囊炎的显著因果效应。然而，使用IVW法、MR-Egger回归及加权中位数法未观察到其他肝胆疾病与牙周炎的双向因果关联。稳健性分析显示，双向因果效应分析结果均未观察到异质性存在，不受水平多效性影响，Leave-one-out分析也提示分析结果稳健。使用独立数据集开展的MR分析与主分析结果无明显差异。**结论**　MR分析提示牙周炎可能是无胆石性胆囊炎的风险因素，该结果亟待包括临床研究及机制探究的进一步验证。此外，MR分析不支持牙周炎与非酒精性肝病、肝硬化或肝癌的双向因果关联。

【关键词】　牙周病；　肝胆疾病；　非酒精性脂肪肝；　肝硬化；　肝癌；　胆石病；　无胆石性胆囊炎；　孟德尔随机化
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Causal association between periodontitis and hepatobiliary diseases: genetic insights from Mendelian randomization

ZHAO Li[1], CHEN Shaopeng[2], CHEN Zhen[2], CHEN Yueqi[2], YU Ting[2]. 1. Hospital of Stomatology, Sun Yat-sen University & Guangdong Provincial Key Laboratory of Stomatology, Guangzhou 510055, China　2. Department of Periodontology, School and Hospital of Stomatology, Guangdong Engineering Research Center of Oral Restoration and Reconstruction & Guangzhou Key Laboratory of Basic and Applied Research of Oral Regenerative Medicine, Guangzhou Medical University, Guangzhou 510182, China
Corresponding author: YU Ting, Email: dent_yu@163.com;CHEN Yueqi, Email: jackiethedentist@163.com

【Abstract】　Objective　To investigate the reciprocal causal relationships between periodontitis and hepatobiliary

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【作者简介】赵莉，主治医师，博士，Email: xiaoyi502157882@163.com
【通信作者】余挺，副教授，博士，Email: dent_yu@163.com；陈跃圻，博士研究生在读，Email: jackiethedentist@163.com

diseases through Mendelian randomization (MR) analyses, to provide evidence for joint prevention and clinical decision-making in patients with concurrent periodontitis and hepatobiliary diseases. **Methods** Single nucleotide polymorphisms (SNPs) were extracted from the largest genome-wide association study on periodontitis (17 353 cases, 28 210 controls) and hepatobiliary diseases within the European ancestry and used as instrumental variables (IVs). The strength of the associations was examined by calculating the *F*-statistic. The SNPs significantly associated with the outcome were removed by scanning on Phenoscanner platform. Bidirectional causal associations between periodontitis and hepatobiliary diseases were estimated using inverse variance weighted (IVW), MR-Egger, and Weighted Median methods. The robustness of the findings was further verified through additional sensitive MR approaches, including Cochran's Q statistic (IVW), Rucker's Q statistic (MR-Egger), MR-PRESSO and Leave-one-out analysis. Further MR analyses, utilizing other available genome-wide association studies (GWAS) on hepatobiliary diseases, were conducted to validate the results. **Results** The IVW method found that periodontitis had a causal impact on acalculous cholecystitis (odds ratio = 1.277, 95% *CI* 1.097-1.485, *P*=0.002), implying an increased risk of acalculous cholecystitis associated with periodontitis, while the MR-Egger regression and Weighted Median failed to observe significant causal effects of periodontitis on acalculous cholecystitis. However, no bidirectional causal associations between periodontitis and nonalcoholic fatty liver disease, cirrhosis or liver cancer were observed using IVW, MR-Egger regression and Weighted Median. The bidirectional causal relationships were deemed unlikely to be influenced by horizontal pleiotropy. Further, the validation analysis based on alternative GWAS data suggested parallel results. **Conclusions** The MR analyses suggest that periodontitis may elevate the risk of acalculous cholecystitis. Further investigations, including clinical studies and mechanistic explorations, are warranted to validate these findings. However, the MR analyses do not support bidirectional causal associations between periodontitis and nonalcoholic fatty liver disease, cirrhosis or liver cancer.

【Key words】 Periodontal diseases; hepatobiliary diseases; nonalcoholic fatty liver disease; cirrhosis; liver cancer; cholelithiasis; acalculous cholecystitis; Mendelian randomization

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牙周炎是一种以牙周组织进行性破坏为特征的炎性感染性疾病，是宿主免疫失衡与口腔微生物失调互促的结果[1]。重度牙周炎最终可导致患者失牙，其全球患病率约为23.6%[2]。牙周炎与多种全身系统病相关，其感染可扩散至全身并引起系统性炎症。目前，超过28种疾病与牙周炎的关联得到充分证据支持[3]。此外，不断有新的临床证据提示牙周炎可能存在其他全身并发症，如新冠肺炎及银屑病[4-5]。肝胆疾病是消化系统疾病的重要组成，主要包括肝癌，肝硬化，非感染性疾病如非酒精性脂肪肝（non-alcoholic fatty liver disease，NAFLD）、肝炎、胆囊炎。全球约有2%人口患有慢性肝病，其中约60%为NAFLD。慢性肝病存在发展为肝癌及肝硬化等致死性疾病的风险[6]。越来越多的流行病学证据不断提示牙周炎和肝病之间的潜在关联，包括牙周炎对NAFLD、肝癌及肝硬化的潜在影响，以及肝硬化对牙周炎的负面效应。一篇前瞻性研究指出，在肝硬化患者中，患有重度牙周炎者相较轻中度牙周炎者，其在1年的随访期内死亡率高1.19倍[7]。另一篇系统性评价则指出，肝硬化患者发生牙周炎的风险相较于对照组高1.63倍[8]。

在已有证据提示牙周炎与NAFLD关联的情况下，开展相关前瞻性研究面临巨大的伦理风险。孟德尔随机化（Mendelian randomization，MR）的研究设计利用基因在遗传时随机分配的特点，在概念上实现了随机化。这一特点使得MR分析能够进行因果推断、规避伦理风险并降低人力财力消耗。目前，仅有1篇MR研究探索了牙周炎和NAFLD及肝硬化的双向因果关联，并发现NAFLD及肝硬化对牙周炎有显著因果效应，分别可能增加牙周炎9%（*OR* = 1.094，95% *CI* = 1.006~1.189，*P* = 0.036）及13.8%（*OR* =1.138，95%*CI*=1.001~1.294，*P*=0.048）的患病风险。然而，该研究所使用

了样本量相对较小的（cases = 1 908 vs. controls = 340 591）NAFLD的全基因组关联分析（genome-wide association study，GWAS）数据集，且未使用其他独立GWAS验证分析结果[9]。因此，尚不能明确牙周炎是否与NAFLD、肝硬化以及肝胆疾病存在双向因果关联。综上，本研究旨在使用MR分析评估牙周炎和肝胆疾病的双向因果关联，既是对当前观察性证据的有力补充及检验，也可快速推进对牙周炎与肝胆疾病关系的科学认识及探索过程，为牙周炎与肝胆疾病的共发患者的联合防治以及临床诊治决策提供证据。

1　资料和方法

1.1　数据集来源

1.1.1　牙周炎相关数据集来源　牙周炎GWAS数据集来自Gene-Life style Interactions in Dental Endpoints，该研究包含7篇原始GWAS[10]。该数据集涵盖17 353例牙周病患者及28 210例健康对照，参与者均来自欧洲人群。其中4篇根据Centers for Disease Control and Prevention/American Academy of Periodontology标准诊断牙周炎。余下3篇中，1篇将牙周炎定义为：≥2个牙面存在探诊深度≥5 mm或≥4个牙面存在探诊深度≥4 mm；1篇研究将牙周炎定义为≥2个牙面探诊深度≥5.5 mm；另外1篇研究根据患者自我报告诊断牙周炎。该GWAS数据集校正了来自年龄及性别的偏倚。

1.1.2　肝胆疾病相关数据集来源　本研究通过在3个GWAS数据库检索获取肝胆疾病GWAS，包括openGWAS、FinnGen及GWAScatalog。最终检索得3种肝脏疾病（NAFLD、肝硬化及肝癌）和三种胆囊疾病（胆囊炎、无胆石性胆囊炎和胆石病）的GWAS数据集。上述GWAS使用电子健康记录系统所记录的诊断信息（基于国际疾病诊断第10版，International Classification of Diseases-10）定义相关肝胆疾病，其参与者均来自欧洲人群且与牙周炎GWAS样本人群不存在重叠（表1）。

表1　本孟德尔随机化分析使用的全基因组关联研究详情

Table 1　Detailed information of GWAS used in the present Mendelian randomization analyses

Variable	First author and publication year	Consortium	Sample size	GWAS Data source
Periodontitis	Shungin (2019)[10]	GLIDE	Cases=17 353 Controls=28 210	https://data.bris.ac.uk/data/dataset/2j2rqgzedxlq02oqbb4vmycnc2
NAFLD	Ghodsian (2021)[11]	Summary data	Cases=8 434 Controls=770 180	https://www.ebi.ac.uk/gwas/studies/GCST90091033
Cirrhosis	Kurki(2023)[12]	FinnGen	Cases = 3 970 Controls=373 307	https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_CIRRHOSIS_BROAD.gz
Liver cancer	Kurki(2023)[12]	FinnGen	Cases=1 081 Controls=287 137	https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_C3_BILIARY_GALLBLADDER_EXALLC.gz
Cholecystitis	Kurki(2023)[12]	FinnGen	Cases=4 299 Controls=330 903	https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_K11_CHOLECYST.gz
Cholelithiasis	Jiang (2021)[13]	UKB	Cases= 7 426 Controls=448 922	https://www.ebi.ac.uk/gwas/studies/GCST90044198
AC	Jiang (2021)[13]	UKB	Cases = 2 650 Controls=453 698	https://www.ebi.ac.uk/gwas/studies/GCST90044196

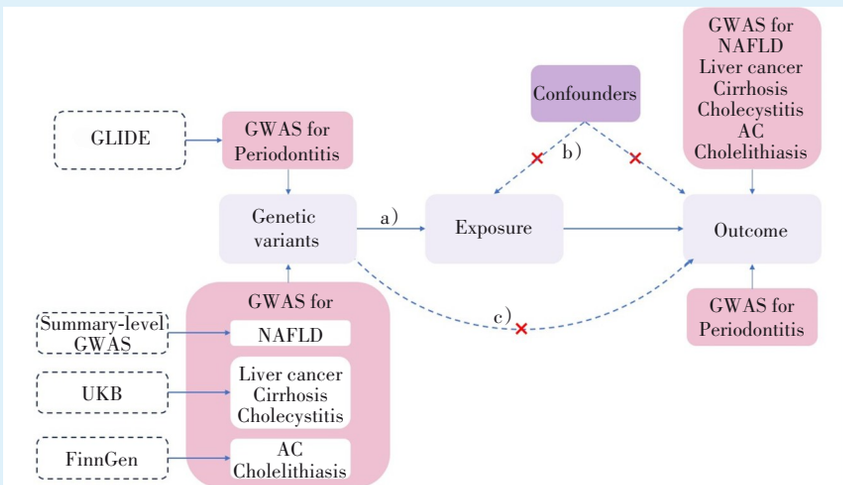
GWAS: genome-wide association study. GLIDE: gene-life style interactions in dental endpoints consortium; NAFLD: nonalcoholic fatty liver disease; AC: acalculous cholecystitis; UKB: UK biobank. Summary data from serial consortium, including Estonian Biobank, Electronic Medical Records and Genomics, and UK biobank; GWAS data of both periodontitis and hepatobiliary diseases were derived from European ancestry

1.2　研究设计

本研究使用双向MR分析探索牙周炎及肝胆疾病的双向因果关联，其研究设计如图1所示。本研究使用开放获取的GWAS数据集开展，并根据相关指南报告结果。研究过程符合伦理规范，不涉及人体及动物实验[14]。

1.3　工具变量筛选

筛选与暴露强相关的单核苷酸多态性（single nucleotide polymorphisms，SNPs）作为工具变量（instrumental variants，IVs）[15]。在筛选肝胆疾病的IVs



GLIDE: gene-lifestyle interactions in dental endpoints consortium; GWAS: genome-wide association study; AC: acalculous cholecystitis; NAFLD: nonalcoholic fatty liver disease; UKB: UK biobank. A genetic variant (i.e., a single nucleotide polymorphism) was considered an instrumental variable under the following three key assumptions: a) the genetic variant is strongly associated with the exposure of interest. This assumption was insured by setting significance thresholds for SNPs; b) the genetic variant is not associated with the outcome through a confounder-related pathway. This assumption was insured by sensitivity analyses and scoping IVs for underlying confounders on a phenotype scanning platform; c): the genetic variant can only influence the outcome through an exposure-related pathway, without directly affecting the outcome. This assumption was insured by an exclusion of any IVs with significant association ($P< 5\times10^{-08}$) with the outcome trait. IVs: instrumental variants. SNPs: single nucleotide polymorphisms.

Figure 1　Basic design of the Mendelian randomization analyses

图1　孟德尔随机化分析设计

时，除特殊说明，筛选阈值均设置为$P<5\times10^{-08}$。在筛选牙周炎IVs时，由于在$P<5\times10^{-08}$的阈值下无法筛得有效IVs，因此将阈值调整为$P<5\times10^{-06}$[9]，上述两个标准均被学界广泛使用。放宽阈值可能引入弱工具变量偏倚。本研究通过计算SNP的F值验证SNP与暴露的关联程度，以避免弱工具变量偏倚，$F>10$即认为该SNP与暴露强相关[16]。

此外，剔除存在连锁不平衡（$r^2>0.01$，筛选窗口 = 10 000 kb）及回文序列的IVs。最后，使用在线平台（PhenoScanner，http://www.phenoscanner.medschl.cam.ac.uk）剔除与结局强相关的IVs，以避免水平多效性偏倚。R^2和F值的计算公式如下。

$$R^2 = 2 \times MAF(1 - MAF) \times \frac{\beta^2}{SD^2}$$

MAF: minor allele frequency; β: effects size of minor allele; SD: standard error。

$$F = \frac{N - K - 1}{K} \times \frac{R^2}{1 - R^2}.$$ [17]

N: sample size of exposure; K: number of instrumental variables。

1.4　牙周炎与肝胆疾病双向因果效应分析、结果稳健性分析

1.4.1　牙周炎与肝胆疾病双向因果效应分析　本研究使用逆方差加权（inverse variance weighted，IVW）法为最主要MR分析方法，并使用MR-Egger回归和加权中位数（weighted median，WM）法进行补充验证。SNP的Wald效应值由SNP与暴露的关联强度除以该SNP与结局的关联强度得出。IVW法假设所有SNP均有效，对SNP进行逐个Wald效应分析，并使用荟萃分析合并所有SNP的Wald效应值[18]。MR-Egger回归使用SNPs与暴露的关联强度作为权重，对SNP与暴露和结局关联强度进行加权线性回归。该方法假设SNP与暴露的关联与水平效应无关，因此可在无有效SNP的情况下提供有效因果估计。MR-Egger回归截距的显著性可反映水平多效性是否存在[19]，加权中位数法（WM法）可在少于50% IVs有效的情况下，提供有效因果估计。其结果可在异常值存在的情况下保持稳健[16]。本研究使用Bonferroni方法对检验水平进行校正，即$P< 0.01$时认为结果显著。

1.4.2　稳健性分析　通过Cochran's Q statistic（IVW），Rucker's Q statistic（MR-Egger）及MR-PRESSO排除异质性及水平多效性的存在以保证MR分析的稳健性。Cochran's Q statistic（IVW）和Rucker's Q statistic（MR-Egger）用于评估异质性[18-19]。MR-PRESSO用于评估水平多效性及IVs中异常值的存在[20]。

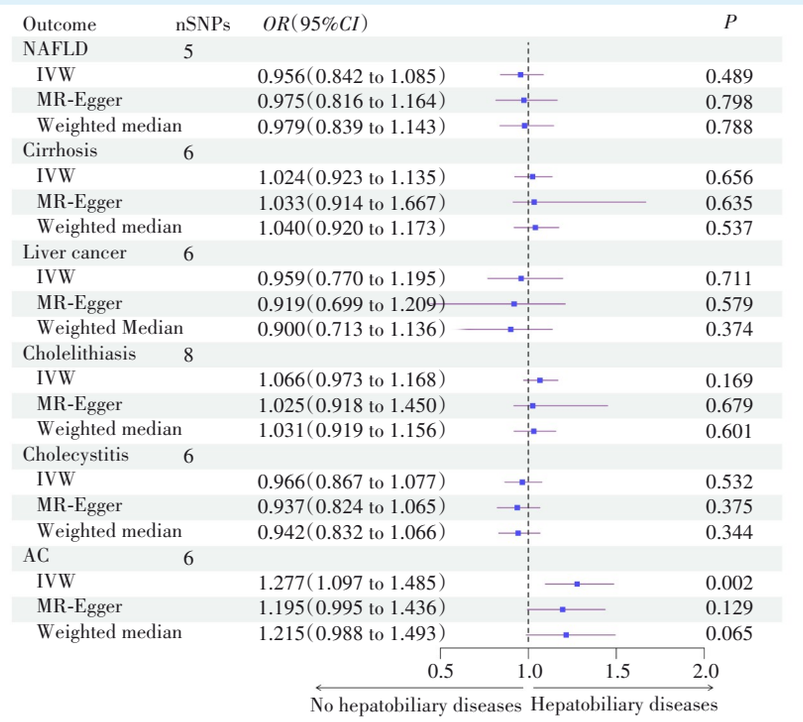
此外，使用Leave-one-out方法逐个剔除IVs后重新进行MR分析，以检验是否存在IV显著影响结果。本MR分析使用R4.3.0进行[21-22]。

最后，使用牙周炎与肝胆疾病相关的独立GWAS数据集开展MR分析，以验证主要结果的稳健性。

2　结　果

2.1　牙周炎对肝胆疾病的因果效应

IVW法显示，牙周炎对无胆石性胆囊炎有显著的因果效应，可使无胆石性胆囊炎的患病风险增加27.7%（*OR* = 1.277，95% *CI*：1.097~1.485，*P*=0.002）。基于MR-Egger（*OR* = 1.195，95% *CI*：0.995~1.436，*P*=0.128）及WM法（*OR* = 1.215，95% *CI*：0.988~1.493，*P*=0.065）的分析结果与IVW法效应方向一致，不同MR方法结果间的差异可归因于统计效能不同。三种MR方法均未观察到牙周炎对其他肝胆疾病的因果效应（图2）。



nSNPs: number of single nucleotide polymorphisms; *OR*: odds ratio; *CI*: confidence interval; IVW: inverse variance weighted; NAFLD: non-alcoholic fatty liver disease; AC: acalculous cholecystitis

Figure 2　Mendelian randomization estimates of periodontitis on the risk for hepatobiliary diseases

图2　牙周炎对肝胆疾病因果效应的孟德尔随机化估计

2.2　肝胆疾病对牙周炎的因果效应

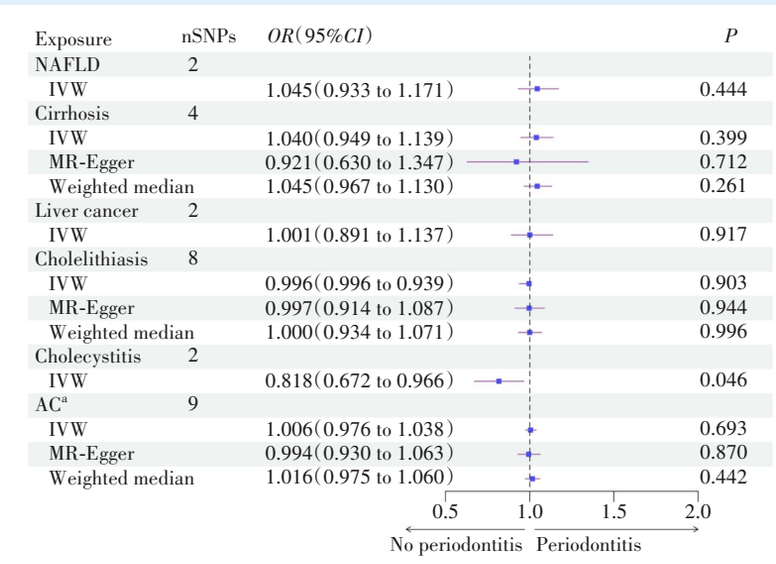
本MR分析使用IVW法、MR-Egger回归及WM法对肝胆疾病对牙周炎的因果效应进行了检验，然而并未发现肝胆疾病对牙周炎具有显著因果效应（图3）。对于NAFLD、肝癌、胆囊炎对牙周炎的因果效应估计，由于有效IVs不满足MR-Egger及加权中位数法开展的最低要求（nSNPs ≥ 3），因此仅使用IVW法进行MR分析。

2.3　稳健性分析

所有MR分析均满足*F*>10（2 797.559~10 025.091），表明MR分析结果不太可能受弱工具变量偏倚影响。用于预测牙周炎的IVs（即遗传变异）对牙周炎的解释度为11.6%，肝胆疾病关联的IVs对相关疾病的解释度为1.6% ~19.2%（表2）。本研究所有MR分析中，均未观察到异质性存在，且MR-PRESSO未发现IVs中存在异常值。MR-Egger回归截距值与0无显著差异，提示分析不受水平多效性干扰（表3、表4）。另外，Leave-one-out分析也提示分析结果稳健（图4）。使用独立GWAS数据集开展的MR分析结果与主要结果趋势相同。

3　讨　论

既往临床研究提示牙周炎与多种肝病存在关联，包括NAFLD、肝癌及肝硬化。其中，支持牙周炎和NAFLD关联的证据最强，其次是肝癌和肝硬化。然而，少有研究探索过牙周炎与胆囊炎的相关性。本研究使用MR分析探索了牙周炎与多种肝胆疾病的双向关联，包括3种肝病和3种胆囊疾病。本研究观察到牙周炎对无胆石性胆囊炎的显著因果效应，即相较于对照组，牙周炎患者发生无胆石性胆囊炎的风险高0.277倍（*OR*=1.277，



nSNPs: number of single nucleotide polymorphisms; *OR*: odds ratio; *CI*: confidence interval; IVW: inverse variance weighted; NAFLD: nonalcoholic fatty liver disease; AC: acalculous cholecystitis. [a]: the association threshold of instrumental variables was set at 5×10^{-06} (i.e., those with $P<5\times10^{-06}$ were included). The causal effects of NAFLD, liver cancer, cholelithiasis on periodontitis were only estimated by IVW method, since the valid instrumental variables related to the exposure did not satisfy the minimum requirements of weighted median and MR-Egger

Figure 3　Mendelian randomization estimates of hepatobiliary on the risk for periodontitis

图3　肝胆疾病对牙周炎因果效应的孟德尔随机化估计

表2　本孟德尔随机化分析中暴露与结局变量的R^2与F值

Table 2　R^2 and F-statistic of the variables in the present Mendelian randomization study

Variable	Variance (R^2)	F
Periodontitis	0.115 85	746.114
NAFLD	0.048 98	10 025.091
Cirrhosis	0.083 43	8 585.631
Liver cancer	0.087 27	9 185.666
Cholecystitis	0.016 41	2 797.559
Cholelithiasis	0.149 80	8 040.634
AC	0.191 67	8 323.308

NAFLD: nonalcoholic fatty liver disease; AC: acalculous cholecystitis; R^2: represents the proportion of phenotypic variations of the exposure explained by the single nucleotide polymorphisms; F: F-statistic was used to estimate the strength of the single nucleotide polymorphisms

95%*CI*：1.097~1.485，P = 0.002）。然而，本研究未观察到牙周炎对其他5种肝胆疾病的因果效应，反之亦然。稳健性分析及基于独立GWAS数据集的MR分析验证了上述结果的稳健性。

一篇纳入27 703名参与者的荟萃分析（共纳入1篇队列研究及4篇横断面研究）指出，牙周炎患者发生NAFLD的风险相较对照组升高（*OR*=1.48，95% *CI*：1.15~1.89）。然而，在校正代谢疾病的影响后，其*OR*值下降至1.13（95%*CI*：0.95~1.35）[23]。另一篇荟萃分析（纳入1篇病例对照研究、2篇前瞻性队列研究及四篇横断面研究；共纳入192 815名参与者）则显示，牙周炎与NAFLD患病风险不存在关联（*OR*=1.04，95%*CI*：0.97~1.12）[24]。相比之下，针对NAFLD对牙周炎的影响的研究设计主要

表3　牙周炎对肝胆疾病因果效应的稳健性分析

Table 3　Robustness analyses on periodontitis-effects on hepatobiliary diseases

Outcome	Heterogeneity				Pleiotropy			
	IVW		MR-Egger		MR-Egger		MR-PRESSO	
	P	Q	P	Q	P	intercept	P	Q
NAFLD	0.927	0.881	0.852	0.788	0.780	−0.005	0.934	1.457
Cirrhosis	0.519	4.216	0.387	4.141	0.802	−0.005	0.618	6.031
Liver cancer	0.218	5.762	0.280	6.270	0.582	0.024	0.492	8.755
Cholelithiasis	0.976	1.982	0.852	0.472	0.286	0.017	0.840	3.266
Cholecystitis	0.294	6.127	0.280	5.607	0.412	0.017	0.405	10.104
AC	0.623	3.500	0.746	1.944	0.280	0.028	0.696	5.026

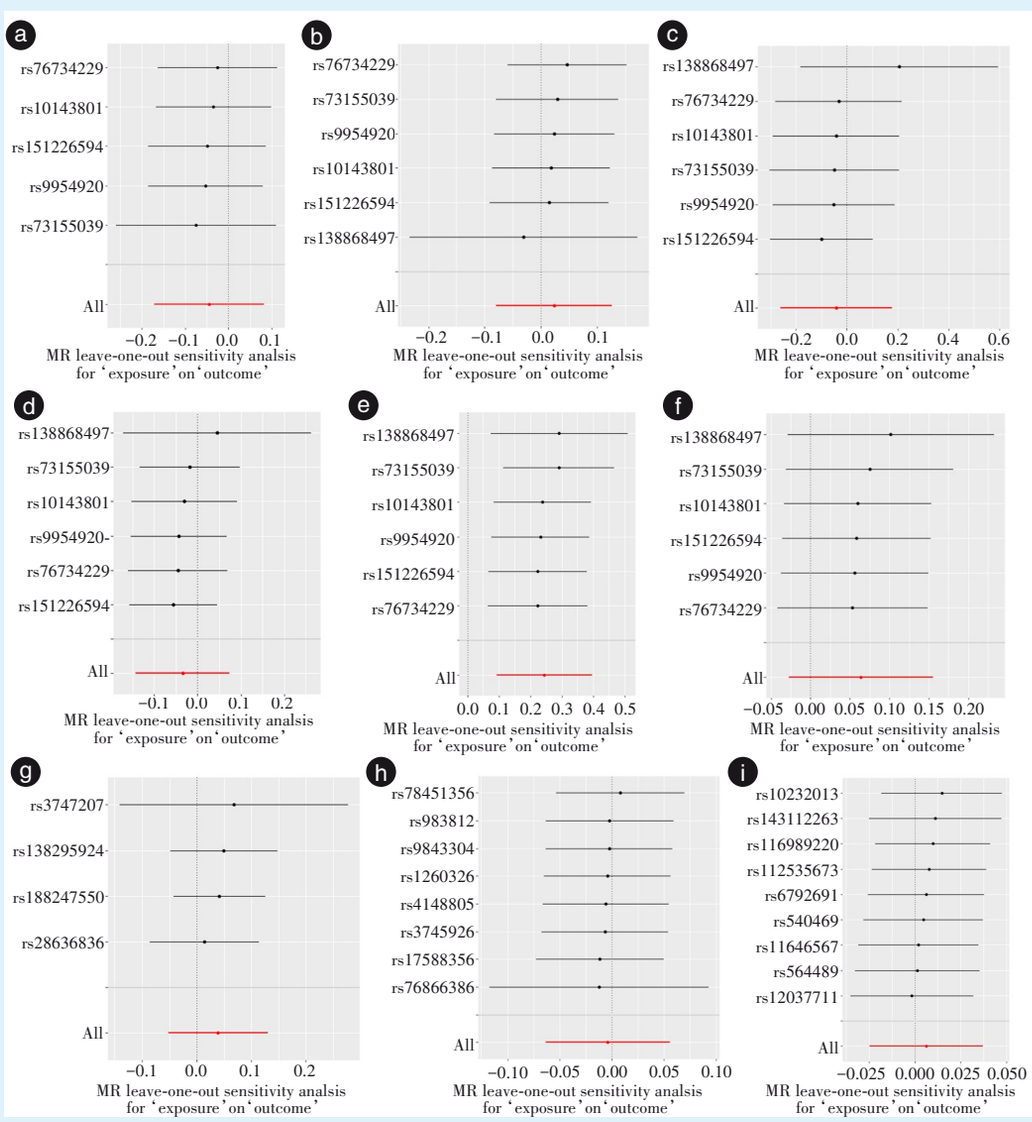
NAFLD: nonalcoholic fatty liver disease; AC: acalculous cholecystitis; IVW: inverse variance weighted

表4　肝胆疾病对牙周炎因果效应的稳健性分析

Table 4　Robustness analyses on hepatobiliary diseases-effects on periodontitis

Exposure	Heterogeneity				Pleiotropy			
	IVW		MR-Egger		MR-Egger		MR-PRESSO	
	P	*Q*	*P*	*Q*	*P*	intercept	*P*	*Q*
NAFLD	0.806	0.061	NA	NA	NA	NA	NA	NA
Cirrhosis	0.220	4.416	0.162	3.640	0.582	0.040	0.486	6.077
Liver cancer	0.220	1.504	NA	NA	NA	NA	NA	NA
Cholelithiasis	0.835	3.504	0.743	3.504	0.988	~0	0.884	4.060
Cholecystitis	0.304	1.055	NA	NA	NA	NA	NA	NA
AC	0.462	9.758	0.371	9.756	0.966	0.001	0.526	10.952

NAFLD: nonalcoholic fatty liver disease; AC: acalculous cholecystitis; IVW: inverse variance weighted; NA: not available. Because no enough single nucleotide polymorphisms could be used to perform MR-Egger and MR-PRESSO. However, the non-significant results remained robust as presence of pleiotropy, if any, would bias the results to significant associations



a: periodontitis as exposure, and nonalcoholic fatty liver disease as outcome; b: periodontitis as exposure, and cirrhosis as outcome. c: periodontitis as exposure, and liver cancer as outcome. d: periodontitis as exposure, and cholecystitis as outcome. e: periodontitis as exposure, and cholelithiasis as outcome; f: periodontitis as exposure, and acalculous cholecystitis as outcome. g: cirrhosis as exposure, and periodontitis as outcome. h: cholelithiasis as exposure, and periodontitis as outcome. i: acalculous cholecystitis as exposure, and periodontitis as outcome. The y-axis indicates the removed individual instrumental variable, while the x-axis stands for the causal effects and 95% confidence interval after removing the corresponding instrumental variable

Figure 4　Leave-one-out analysis for the validation of the robustness of Mendelian randomization analysis

图4　Leave-one-out法验证孟德尔随机化分析结果稳健性

为横断面及病例-对照。因此，牙周炎和NAFLD是否存在关联仍未有定论[25]。

NAFLD和牙周炎的严重程度均可能是潜在的偏倚来源。NAFLD包含两个阶段，即早期的非酒精性脂肪肝（non-alcoholic fatty liver，NAFL）和晚期的非酒精性肝炎（non-alcoholic steatohepatitis，NASH）[6]。多数临床研究通过检测血清肝酶水平识别NAFLD患者，而患者在NAFL阶段通常不会出现这一指标的异常，即相关研究纳入的患者多处于NASH阶段。因此，这些研究中的对照组中可能混有NAFL患者。而本研究使用的GWAS数据集中NAFL和NASH患者比例并不清楚，NAFL或NASH占比过重，可能导致低估或高估牙周炎和NAFLD的相关性[26]。

牙周炎的严重程度也可能影响分析结果。根据流行病学资料，绝大多数牙周炎患者为轻中度牙周炎。本研究使用的GWAS数据集纳入的7篇研究中参与者也符合这一特点，即牙周炎被定义为探诊深度≥4 mm或5 mm。一篇纳入了6 165名参与者的研究（健康对照，n=1 900；轻中度牙周炎，n=1 610；重度牙周炎，n=1 723；无牙颌，n=932）发现，相较健康对照组，重度牙周炎患者在12年的随访期内发生NAFLD的风险上升2.69倍（HR=3.69，95% CI：1.79~7.60），而轻中度牙周炎患者相对健康对照无显著差异[27]。由于重度牙周炎受遗传因素影响更大，因此不能排除牙周炎和NASH的潜在因果关联。

不断有新证据提示牙周炎与肝癌及肝硬化的潜在关联。一篇旨在评估失牙与肝癌的关联荟萃分析纳入了5篇队列研究及1篇横断面研究。其中3篇队列研究支持牙周炎可能增加肝癌风险，而另外3篇（即2篇队列及1篇横断面研究）不支持该观点。因此，现阶段证据并不足以定论二者的关联。此外，受研究使用的牙周指标（即牙周炎或牙齿丧失）及牙周炎（即自我报告或临床诊断）诊断标准差异及未校正混杂效应的影响，该系统评价纳入的研究间存在较高异质性[28]。针对肝癌对牙周炎的影响，只有少量研究探究过这一问题。尽管有回顾性研究初步提示牙周炎与肝癌之间存在关联，但二者的因果关联并未得到验证。从遗传角度而言，本MR分析不支持牙周炎与肝癌的双向因果关联，与临床研究结论一致。

针对于肝硬化与牙周炎的关联，一篇荟萃分析（纳入3篇病例-对照及9篇横断面研究，包括604例肝硬化患者及842例健康对照）提示，相较于对照组，肝硬化患者患牙周炎的风险高1.63倍（OR = 2.63，95%CI：1.531~4.520）。然而，该研究并未纳入前瞻性研究[8]。一篇纳入184例肝硬化患者的前瞻性研究发现，相较轻中度牙周炎患者，重度牙周炎患者在1年随访期内的死亡率增加119%（HR = 2.19，95% CI：1.07~4.50）。然而，该研究随访期相对较短，且未观察到牙周炎与肝功能（以Child-Pugh指数评估）间的显著关联[7]。关于牙周炎对肝硬化影响的证据有限，二者的双向因果关联仍未有定论。本MR分析与先前相关临床研究结论一致。需要指出的是，肝癌与肝硬化是需要及时干预的致死性疾病，其干预措施（如放化疗及肝移植）本身十分复杂，可能干扰临床研究的开展及牙周结局的评估。此外，通过队列研究探索牙周炎对这两种疾病的影响存在重大伦理风险，反之亦然。这可能是现有相关证据级别较低的原因。相比之下，MR分析受临床干预的影响更少。

现阶段，尚未有研究探索牙周炎与胆囊炎的关联。胆囊炎可分为非胆石性胆囊炎及胆石性胆囊炎[29]。本MR分析揭示了牙周炎对无胆石性胆囊炎的显著正向因果关联，即牙周炎可增加无胆石性胆囊炎27.7%的患病风险。无胆石性胆囊炎的确切病因尚不明了，主要与重病、严重创伤、烧伤以及心脏手术相关[30]。这一类患者的预后本身更容易受到并发症的影响[31]。本MR分析初步提示，牙周炎可能是无胆石性胆囊炎的预测因素。然而，牙周治疗是否能有效降低无胆石性胆囊炎的患病风险、改善相关疾病预后，则有待前瞻性队列研究验证。如果该关联真实存在，则潜在机制可能如下。首先，口腔细菌可能通过牙周袋、肠道黏膜及肝肠循环向胆囊远处转移。以胆汁淤积为特征的无胆石性胆囊炎可能增加胆囊对细菌感染的易感性，从而加剧胆汁淤积。Dyrhovden等[32]通过16SrRNA测序及微生物培养，检测了36例胆囊炎患者的胆汁样本，发现口腔菌群的存在（表5：胆囊炎患者胆汁样本与口腔微生态中共有菌群）。其次，无胆石性胆囊炎以胆囊黏膜上皮缺血性坏死、坏死组织中性粒细胞浸润及循环免疫反应水平上升为特征。基于牙周炎和胆囊炎均可诱发系统性炎症，牙周炎可能通过过度激活炎症反应而促进胆囊炎[1]。第三，牙周炎患者的牙龈组织中总胆汁酸及结合胆汁酸含量增高，其受体——鞘氨

醇-1-磷酸受体2(sphingosine-1-phosphate receptor 2,S1PR2)亦增多,提示牙周炎与胆汁酸代谢异常有关[33]。不排除牙周致病菌或引发的系统性炎症可通过影响系统的胆汁酸代谢,增加胆汁淤积及胆囊炎的患病风险[30]。

表5　胆囊炎患者胆汁样本与口腔微生态中共有菌群[32]

Table 5　Bacterial species shared by bile samples from patients with cholecystitis and the oral cavity[32]

Species	Strain	Positive frequency by sequencing	Identified in more than one article?	The highest proportion in individuals (%)	Number of samples containing the bacterium [n(%)]
Actinomyces			Yes	5.1	3 (10.0)
	*naeuslundii**	1	Yes		
	gerencseriae	1	No		
Bifidobacterium			No	0.6	3 (10.0)
	animalis	2	No		
	dentium	1	No		
	longum	1	No		
Dialister	*invisius*	1	No	0.1	1 (3.0)
Enterococcus			Yes	88.3	7 (24.0)
	faecalis	4	No		
	faecium	4	Yes		
Fusobacterium	*nucleatum**	5	Yes	47.4	5 (17.0)
Haemophilus	*parainfluenzae*	3	Yes	100.0	3 (10.0)
Klebsiella			Yes	100.0	11 (38.0)
	pneumoniae	3	Yes		
	michiganensis	3	No		
	Oxytoca	3	No		
	Variicola	2	No		
Streptococcus			Yes	100.0	13 (45.0)
	anginosus	3	No		
	salivarius	3	No		
	*Sanguinis**	2	No		
	gordonii	1	No		
	massiliensis	1	No		
	mitis	1	No		
	mutans	1	No		
	oraits	1	No		
	parasanguinis	1	No		

This table was organized based on prior investigations that analyzed the bacteria in bile samples from individuals with cholecystitis, employing 16S ribosomal RNA sequencing; *: periodontitis-related bacteria

本MR分析探索了牙周炎胆囊炎(不区分亚型)的因果关联,然而并未观察到二者的显著因果关联。本研究估计了5种肝胆疾病对牙周炎的因果效应,并使用$P<0.01$(即0.05/5)的检验水平以降低I类错误风险。此外,使用Benjamini-Hochberg方法校正后的分析结果(adjusted $P=0.276$)也不支持胆囊炎对牙周炎的因果效应。因此,无法推断胆囊炎是否对牙周炎有因果或保护效应。此外,尚未有证据支持胆囊炎是牙周炎(抑或是其他炎性疾病)的保护因素。

本研究存在一定局限性。第一,本研究在筛选牙周炎IVs时,将关联阈值从$P<5\times10^{-08}$调整为$P<5\times10^{-06}$,两种阈值标准均被广泛采纳。理论上放宽阈值可能引入弱工具变量偏倚,然而稳健性分析表明这种可能性较低(所有SNP的F值>10)。反之,在分析肝胆疾病对牙周炎的因果效应时,使用$P<5\times10^{-08}$的标准导致的有效IVs数量减少(IVs数目<3)也可能影响分析结果。而使用不同来源

的GWAS的验证分析(肝胆疾病相关IVs ≥ 3)的结果可以证实主要MR分析结果的稳健性[21]。已有证据证实,牙周炎与多种疾病的关联并非线性关联。因此,如果牙周炎与肝胆疾病的关联过度偏离线性关系,则本研究使用的MR方法不能观察到二者的因果关联。第二,本研究纳入的肝胆疾病及牙周炎均可存在性别差异[6, 34-37],但本研究并未展开基于性别的分层分析。本研究使用的汇总GWAS数据集纳入的7篇原始研究中,有1篇研究通过自我报告诊断牙周炎,该诊断方法可能存在回忆偏倚。第三,由于缺乏独立GWAS数据集,牙周炎对胆囊炎的因果效应并未得到基于独立验证集的MR分析支持。最后,由于本研究使用的GWAS数据集来自于欧洲人群样本,因此应谨慎将本研究结论应用于其他人群。

总体上,本MR分析发现牙周炎可能是无胆石性胆囊炎的风险因素。然而,应注意本研究存在一定局限性,且该结论需要后续临床研究验证。过往观察性研究曾提示牙周炎与NAFLD、肝硬化或肝癌存在相关性。从临床的角度,NAFLD-肝硬化-肝癌具备一定的病情发展演化关系。本MR分析提示牙周炎与NAFLD、肝硬化、肝癌均没有因果关联,与这种演进关系内部一致性在逻辑上是协调的,也提示牙周炎与三类肝病的演化进程没有相互因果作用。本MR分析的结论具有一定临床价值,其阐释的牙周炎与无胆石性胆囊炎的因果关系,以及与NAFLD、肝硬化、肝癌的非因果关系,是当下观察性研究结论的重要补充,有待大规模前瞻性研究进一步检验,因果关系也有待动物及细胞实验给出机制解释。

【Author contributions】 Zhao L conceived the study, performed the statistical analysis and wrote the manuscript. Chen SP, Chen Z performed the statistical analysis. Chen YQ, Yu T conceived the study, critically revised the manuscript. All authors read and approved the final manuscript submitted.

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