

Article

Genetic and biomechanics insights into cathepsins and non-cancerous digestive diseases: A bidirectional two-sample Mendelian randomization study

Ruiming Ou, Shengping Hu, Yu Liu*

Department of General Surgery, The First Affiliated Hospital of Harbin Medical University, No.23 6 Postal Road, Nangang District, Harbin 150000, China

* Corresponding author: Yu Liu, 974878885@qq.com

CITATION

Ou R, Hu S, Liu Y. Genetic and biomechanics insights into cathepsins and non-cancerous digestive diseases: A bidirectional two-sample Mendelian randomization study. Molecular & Cellular Biomechanics. 2025; 22(5): 1631. https://doi.org/10.62617/mcb1631

ARTICLE INFO

Received: 19 February 2025 Accepted: 24 March 2025 Available online: 10 June 2025

COPYRIGHT



Copyright © 2025 by author(s). *Molecular & Cellular Biomechanics* is published by Sin-Chn Scientific Press Pte. Ltd. This work is licensed under the Creative Commons Attribution (CC BY) license. https://creativecommons.org/licenses/ by/4.0/

Abstract: Background: Digestive diseases have high incidence and mortality rates, posing a significant threat to global health. However, research on these disorders is uneven, while digestive cancers are well-studied and non-cancerous digestive diseases, despite their considerable health impact, have received less attention. Although cathepsins (CTSs), proteases that regulate extracellular matrix (ECM) turnover and cellular stiffness, have been implicated in digestive disorders, their role in disease-specific mechanical perturbations remains unclear. This study bridges this gap by integrating genetic causation with organ-level biomechanics. Methods: To overcome the constraints of conventional epidemiological methods, we employed a dual-sample bidirectional Mendelian randomization (MR) analysis leveraging genome-wide association study (GWAS) data to explore the causal relationships among 9 cathepsins and 23 non-cancerous digestive diseases. We conducted inverse variance weighted (IVW), weighted median (WM), MR-Egger, MR-PRESSO, Cochran's Q, and sensitivity analyses for thorough evaluation. We also performed correlation analyses to link the biomechanical data with the genetic and disease outcomes, aiming to identify the relationships between mechanical factors, CTSs, and non-cancerous digestive diseases. Results: Forward MR analysis indicated that CTSB promotes both cholecystitis and cholelithiasis and CTSZ promotes chronic gastritis and diverticulosis. Higher CTSL2 levels promote non-alcoholic fatty liver disease (NAFLD) and liver cirrhosis, whereas upregulated CTSG reduces NAFLD risk. Reverse MR analyses indicated that gastroesophageal reflux, gastric ulcer, NAFLD, and cholecystitis elevated CTSE, G, Z, and B levels, respectively; nonalcoholic steatohepatitis elevates CTSB and H levels. Liver cirrhosis increases CTSB, S, and Z; Barrett's esophagus, celiac disease, and diverticulosis downregulate CTSO, F, and H respectively; chronic pancreatitis lowers CTSE, F, and L2. Multivariable MR analyses revealed the independent effects of individual CTSs on specific diseases: CTSZ as a promoter for diverticulosis, CTSG as a protective factor for NAFLD, and CTSB as a promoter for cholecystitis and cholelithiasis. Conclusion: This study confirmed the causal relationships between cathepsins, mechanical factors in the digestive system, and non-cancerous digestive diseases. By integrating genetic and biomechanical analyses, we have provided a more in-depth understanding of how mechanical forces interact with biological molecules during the development of non-cancerous digestive diseases. Moreover, they may lead to the establishment of novel clinical practice approaches that take into account both the mechanical and biological aspects of digestive diseases, ultimately improving the diagnosis, treatment, and management of these conditions.

Keywords: Mendelian randomization; biomechanics; genome-wide association; single nucleotide polymorphisms; cathepsins; non-cancerous digestive diseases; causal relationship

1. Introduction

Non-cancerous digestive system diseases include a wide array of conditions that affect the digestive tract and accessory organs, such as the liver, gallbladder, and pancreas. The high prevalence of non-cancerous digestive diseases such as gastritis, peptic ulcers, enteritis, and liver diseases significantly affects public health worldwide [1,2]. These conditions have led to a decrease in quality of life and substantial healthcare burdens [1,3]. According to a study focusing on the global burden of digestive diseases, they accounted for over 7.3 billion cases in 2019 alone. The global age-standardized incidence and mortality rates of these conditions have not shown a downward trend from 1990 to 2019, with digestive disorders constituting more than one-third of the overall disease burden [4,5]. This situation highlights the pervasive impact of digestive disorders on well-being worldwide and the critical need for a wider understanding of the factors contributing to the development of these diseases [4].

Cathepsins are a critical subset of the protease enzyme family [6], mainly including B(CTSB), E(CTSE), F(CTSF), G(CTSG), H(CTSH), L2(CTSL2), O(CTSO), S(CTSS), and Z(CTSZ) [7], among others, serve as vital components in maintaining cellular homeostasis through their roles in lysosomal degradation, immune regulation, autophagy, protein metabolism, and extracellular matrix remodeling [8-11]. These functions form the basis of mechanisms implicated in various digestive diseases, with each cathepsin contributing distinctly to the progression of various disorders [11,12]. For instance, elevated levels of CTSB [13,14], CTSS [13], and CTSZ [15,16] in Helicobacter pylori-infected gastritis tissues drive local immune and inflammatory responses, thereby influencing the development of peptic ulcers. In pancreatitis, heightened CTSB and CTSS activity is observed in macrophages and pancreatic acinar cells, emphasizing their roles in the inflammatory response [17]. CTSD, expressed in pancreatic acinar and inflammatory cells, intensifies acute pancreatitis by amplifying CTSB, which subsequently triggers intracellular trypsinogen activation, further aggravating the condition [18,19]. On the other hand, CTSE is broadly expressed in immune cells, underscoring its diverse roles in immune regulation [10,20]. The relationship between CTSG and inflammatory bowel diseases such as ulcerative colitis (UC) remains complex. While a 1992 study by Mayet et al. detected no antibodies against CTSG in patients with UC [21], a later 2000 study by Kuwana et al. reported significantly higher CTSG antibody levels in patients with active UC than in healthy individuals [22], suggesting a potential but unresolved connection between CTSG and inflammatory bowel disease. CTSH contributes to fibrotic progression in cirrhotic livers by modulating mesenchymal cell plasticity and extracellular matrix responsiveness, although its effects diminish in advanced fibrosis due to its downregulation [23]. In pancreatic β -cells, high CTSH levels protect against cytokineinduced apoptosis, helping to preserve cellular function amidst immune-mediated damage [24]. CTSS activity, which can be upregulated by pathobionts, drives T cellmediated colonic inflammation, whereas cystatin C-dependent regulation of CTSS activity contributes to the generation of tolerant intestinal dendritic cells, thereby reducing inflammatory responses [25]. Additional studies are required to clarify the distinct roles of CTSF, CTSL2, and CTSO in noncancerous digestive disorders.

Despite these findings, the causal relationships between specific cathepsins and non-cancerous digestive diseases remain unclear. Conventional observational studies frequently encounter challenges arising from confounding variables and the possibility of reverse causation [26], making it challenging to establish definitive causal links. To address these limitations, our research employs Mendelian randomization (MR), which employs genetic variants as instrumental variables (IVs) to determine causality between exposures (nine cathepsins) and outcomes (23 non-cancerous digestive diseases) [27,28]. Because genetic variants are essentially randomized at the time of conception, this approach reduces bias from acquired factors, providing a more robust framework for causal inference. Furthermore, MR extends causal inference to domains where traditional randomized controlled trials (RCTs) are unlikely to be conducted, such as studying the effects of long-term exposure or those involving ethical risks. Using existing genetic data, MR avoids the high costs and complications of primary data collection in large-scale RCTs, making MR a cost-effective solution for evidence generation in modern epidemiological research [29]. Compared with previous MR analyses, guided by the concept of benefiting a wider range of people, we expanded the types and scope of digestive diseases, covering as many common non-cancerous digestive diseases as possible, such as gastroesophageal reflux disease, gastritis, peptic ulcer, inflammatory bowel disease, liver cirrhosis and other chronic liver diseases, gallbladder diseases, pancreatic diseases, etc. [4].

Based on this situation, we aimed to employ MR to investigate the potential causal links between more non-cancerous digestive diseases and nine cathepsins (B, E, F, G, H, O, S, L2, Z) to provide a focused and comprehensive analysis of their potential causal impacts on digestive health. The insights gained from this research could revolutionize our understanding and management of these conditions, ultimately leading to better patient outcomes and advancements in digestive health care.

2. Materials and methods

2.1. Study design and selection of IVs

To ensure that the selected IVs are valid for MR analysis, they must fulfill three core assumptions: exclusion restriction, relevance, and independence [30] (**Figure 1**). (1) The relevance assumption: The selected IVs should have a strong correlation with the exposure variable; (2) the exclusion restriction assumption: The IVs must be unrelated to any potential confounding factors, meaning that they must not be linked to any confounding factors that could affect the outcome; (3) the independence assumption: There is no direct association between the IVs and the outcome variable; the IVs can modulate the outcome variable exclusively by exposure.

Therefore, strict criteria were applied for the genome-wide association study (GWAS) data selection of cathepsin-related IVs. We ensured low LD with $r^2 < 0.001$ within a 10,000 kb interval and a significant correlation with each phenotype with *p*-values $< 5 \times 10^{-6}$. All single-nucleotide polymorphisms (SNPs) of cathepsins selected had an *F*-value > 10 to maintain the validity and effectiveness of the IVs (Supplementary **Table S1**). The same rigorous standards were applied to non-cancerous digestive disease analyses. We confirmed that the selected SNPs had no

association with confounding factors that could interfere with the relationship between exposure and outcome. This process involved excluding SNPs linked to the outcome, managing LD, correcting for pleiotropy using the MR pleiotropy residual sum and outlier (MR-PRESSO), and filtering IVs according to strength and association, thereby guaranteeing the reliability of causal inference in our MR study (**Figure 1**).



Figure 1. Study design and workflow.

2.2. Data source of cathepsins

Genetic summary statistics of nine cathepsins (B, E, F, G, H, L2, O, S, and Z) were sourced from the INTERVAL study, a comprehensive genetic research program that recruited 3301 European participants (**Table 1**). The IEU OpenGWAS project website for accessing the data is https://www.ebi.ac.uk. Notably, the study was conducted under ethical standards and each participant provided informed consent to ensure voluntary and informed participation [31].

GWAS ID	Year	Trait	Sample size	Number of SNPs
prot-a-718	2018	Cathepsin B	3301	10,265,264
prot-a-720	2018	Cathepsin E	3301	10,265,264
prot-a-722	2018	Cathepsin F	3301	10,265,264
prot-a-723	2018	Cathepsin G	3301	10,265,264
prot-a-725	2018	Cathepsin H	3301	10,265,264
prot-a-728	2018	Cathepsin L2	3301	10,265,264
prot-a-726	2018	Cathepsin O	3301	10,265,264
prot-a-727	2018	Cathepsin S	3301	10,265,264
prot-a-729	2018	Cathepsin Z	3301	10,265,264
prot-a-728 prot-a-726 prot-a-727 prot-a-729	2018 2018 2018 2018 2018	Cathepsin L2 Cathepsin O Cathepsin S Cathepsin Z	3301 3301 3301 3301 3301	10,265,264 10,265,264 10,265,264 10,265,264

Table 1. GWAS data of 9 cathepsins.

2.3. Genetic association of SNPs with non-cancerous digestive diseases

Statistics on 23 non-cancerous digestive diseases were obtained from the FinnGen database following the coding of the International Classification of Diseases Tenth Revision (ICD-10). The latest summary statistics of 23 non-cancerous digestive diseases were obtained from the FinnGen Consortium (data freeze 11), including gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), gastric ulcer, duodenal ulcer (DU), acute gastritis (AG), chronic gastritis (CG), irritable bowel syndrome, celiac disease (CD), diverticulosis, Crohn's disease of the small intestine, Crohn's disease of the large intestine, ulcerative colitis (UC), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcoholic liver disease, liver cirrhosis, cholangitis, cholecystitis, cholelithiasis, acute pancreatitis, chronic pancreatitis (CP), acute appendicitis, and other appendicitis. Importantly, informed written consent was obtained from each participant, and relevant institutional ethical approval or license was necessary for this MR study. **Table 2** provides details on the sample size, number of cases, and control groups.

Table 2. (GWAS	data	of 23	non-cancerous	digestive	diseases.
------------	------	------	-------	---------------	-----------	-----------

Phenotype	Number of SNPs	Cases	Controls	Sample size	Ancestry	Year	Website
Gastroesophageal reflux disease (GERD)	21,306,231	32,232	385,082	417,314	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_REFLUX.gz
Barrett's esophagus (BE)	21,305,544	1486	385,082	386,568	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_BARRET.gz
Gastric ulcer	21,305,720	7012	385,082	392,094	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_GULC.gz
Duodenal ulcer (DU)	21,305,607	4115	385,082	389,197	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_DULC.gz
Acute gastritis (AG)	21,305,600	2770	385,082	387,852	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_ACUTGASTR.gz
Chronic gastritis (CG)	21,305,758	11,226	385,082	396,308	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_CHRONGASTR.gz
Irritable bowel syndrome	21,305,149	11,742	360,393	372,135	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_IBS.gz
Celiac disease (CD)	21,306,572	4568	433,899	438,467	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_COELIAC.gz
Diverticular disease	21,305,864	37,886	360,393	398,279	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_DIVERTIC.gz
Crohn's disease of small intestine	21,306,471	2457	432,380	434,837	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_CHRONSMALL.gz
Crohn's disease of large intestine	21,306,421	1870	432,380	434,250	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_CHRONLARGE.gz
Ulcerative colitis (UC)	21,306,469	6435	446,419	452,854	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_UC_STRICT2.gz
Non-alcoholic fatty liver disease (NAFLD)	21,306,794	3006	450,727	453,733	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_NAFLD.gz

Phenotype	Number of SNPs	Cases	Controls	Sample size	Ancestry	Year	Website
Nonalcoholic steatohepatitis (NASH)	21,306,788	218	453,515	453,733	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_NASH.gz
Alcoholic liver disease	21,306,599	3330	440,301	443,631	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_ALCOLIV.gz
Cirrhosis of liver	21,306,741	1470	448,864	450,334	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_CHIRHEP_NAS.gz
Cholangitis	21,305,815	2068	397,583	399,651	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_CHOLANGI.gz
Cholecystitis	21,305,892	5237	397,583	402,820	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_CHOLECYST.gz
Cholelithiasis	21,306,603	44,582	397,583	442,165	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_CHOLELITH.gz
Acute pancreatitis	21,305,964	7562	397,583	405,145	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_ACUTPANC.gz
Chronic pancreatitis (CP)	21,305,846	4222	397,583	401,805	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_CHRONPANC.gz
Acute appendicitis	21,306,750	35,180	415,845	451,025	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_APPENDACUT.gz
Other appendicitis	21,306,290	3101	415,845	418,946	European	2024	https://storage.googleapis.com/finngen-public-data- r11/summary_stats/finngen_R11_K11_APPENDOTH.gz

Table 2. (Continued).

2.4. MR analysis

We aimed to understand the potential forward and reverse causations between a single cathepsin and single diseases, as well as the effects of multiple cathepsins in a more physiological state on specific diseases. To this end, we performed forward MR, reverse MR, and multivariate MR analyses. First, we performed forward MR analysis. Three analysis methods were employed: inverse variance weighting (IVW), MR-Egger, and weighted median (WM). In our study, IVW was used as the main method for estimating the bidirectional effect, owing to its stability. Widely considered the primary approach in MR studies for assessing causality, IVW uses the Wald ratio to weigh the impact of each variant on disease risk relative to its effect on exposure [29]. It provides a total estimated effect of exposure on outcome, accounting for both fixed and random influences, enabling subsequent adjustments to achieve more reliable outcomes in the face of result heterogeneity [32]. Additionally, MR-Egger and weighted median analyses were performed as complementary approaches to validate the robustness of our MR analysis.

To further explore bidirectional causality by assessing reverse causality, we performed reverse MR analyses using the same GWAS dataset, data selection threshold, and analytical methods described above, with non-cancer digestive diseases as the exposure and cathepsins as the outcome.

Multivariable MR (MVMR) was designed to analyze the causal impact of multiple exposure factors on every outcome, making it particularly suitable for exploring the interactions between the nine cathepsins and their independent and comprehensive effects on a single non-cancerous digestive disease. When investigating the effect of a specific cathepsin, we controlled for the other eight cathepsins within the same disease context. Consequently, we can confidently conclude that our results reveal that the effect of several cathepsins on digestive diseases is independent and not mediated by other factors.

Causality was assessed using odds ratios (OR) and 95% confidence intervals (CIs). An OR < 1 indicates a protective effect, whereas an OR > 1 suggests a risk factor.

2.5. Sensitivity analyses

We utilized multiple sensitivity analyses to bolster our study's rigor and reliability. The Cochrane Q Test identified heterogeneity among SNPs at a significance level of Q-p-value < 0.05. In the presence of heterogeneity, we switched to inverse variance weighting with a random-effects model for a more robust analysis, replacing the initial findings.

To examine the potential for pleiotropy in MR results, which could arise if IVs directly affect the outcome via pathways other than the exposure of interest, we utilized MR-PRESSO (including globe and outlier tests) and the MR-Egger intercept test [29,33]. Outliers were detected by the MR-PRESSO global test, where a *p*-value threshold of less than 0.05 was set to indicate significant pleiotropy, and the specific SNPs were pinpointed for removal using the MR-PRESSO outlier test [34]. By excluding outliers and reconducting the MR analysis, we were able to significantly minimize pleiotropy. Subsequently, the MR-Egger intercept test was employed to detect any remaining horizontal pleiotropy [29]; a *p*-value greater than 0.05 suggested the absence of significant pleiotropy.

By individually removing each IV and analyzing the effects of the remaining variables, the leave-one-out approach can evaluate the impact of each IV on the overall estimation. If there is little change in the results after removing a certain variable, it indicates that the model's results are robust.

Implementing these stringent methods and conducting sensitivity analyses helped bolster our research's scrutiny and stability, safeguarding against potential biases, and affirming the reliability of our findings.

2.6. Software and packages

All analyses were conducted using R version 4.4.1. The following R packages were utilized: Two-sample MR version 0.6.8 [35] for the IVW, MR-Egger, and weighted median methods; MR-PRESSO version 1.0 [36] for the MR-PRESSO global test and outlier removal, and Mendelian randomization version 0.10.0 [32] for Multivariable MR.

It is important to add that this study adheres to the STROBE-MR checklist for reporting Mendelian randomization research [37], and to enhance the reproducibility of our work.

3. Results

3.1. IV selection

In our study, we sourced genetic variants for analyzing nine cathepsins from the INTERVAL study, whereas data on 23 digestive diseases were gathered from the FinnGen GWAS databases. We identified multiple SNPs as IVs for each cathepsin and disease in the bidirectional MR analysis by applying a genome-wide significance threshold of $P < 5 \times 10^{-6}$. Each IV demonstrated an *F*-statistic > 10, indicating no weak bias. Detailed information on the SNPs is provided in Supplementary **Table S1**.

3.2. MR analysis results

3.2.1. Forward MR analysis

First, we performed 2-sample MR analysis. Causal relationships indicating potential horizontal pleiotropy, as identified by the MR-Egger intercept, were excluded to ensure the robustness of the findings. The following findings (Figures 2-4) after this exclusion revealed that elevated CTSB levels were linked to a higher risk of developing cholecystitis (OR = 1.074, 95% CI: 1.007-1.145, IVW: P = 0.029) and cholelithiasis (OR = 1.047, 95% CI: 1.017-1.078, IVW: P = 0.002). Furthermore, a significant positive association was found, indicating that higher CTSL2 levels were associated with an increased risk of liver cirrhosis (OR = 1.221, 95% CI, 1.011–1.473; IVW: P = 0.038). Similarly, CTSL2 levels predict an increased risk of NAFLD, highlighting its role as a potential predictor (OR = 1.147; 95% CI, 1.006–1.308; IVW: P = 0.041). A positive correlation was observed between CTSZ levels and the incidence of CG (OR = 1.063, 95% CI: 1.004–1.125, IVW: P = 0.036), and heightened CTSZ levels were associated with a greater risk of diverticulosis (OR = 1.040; 95%) CI, 1.006–1.075; IVW: P = 0.021). In addition, heterogeneity in the results between CTSZ and diverticulosis was detected after the removal of outliers, indicating that the findings are not robust and may require further experimental validation. Conversely, a notable decrease in the risk of NAFLD was associated with higher CTSG levels (OR = 0.897, 95% CI, 0.806–0.999; P = 0.047). These findings emphasize the crucial role of cathepsin level in disease risk assessment and may have implications for future diagnostic and therapeutic strategies. The full forward MR results, including detailed statistics and methods, are available in Supplementary Table S2.

	Cholecystitis					Cholelithiasis			
Exposure	Method	pval		OR(95% CI)	Exposure	Method	pval		OR(95% CI)
cathepisn B			1		cathepisn B			1	
	MR-Egger	0.015		1.230 (1.057 to 1.431)		MR-Egger	0.046		1.077 (1.007 to 1.153)
	Weighted median	0.051	-	1.102 (1.000 to 1.215)		Weighted median	0.005	-	1.054 (1.016 to 1.093)
	Inverse variance weighted	10.029	•	1.074 (1.007 to 1.145)		Inverse variance weighted	10.002	-	1.047 (1.017 to 1.078)
cathepisn E					cathepisn E				
	MR-Egger	0.268		0.924 (0.813 to 1.051)		MR-Egger	0.911	+	1.003 (0.956 to 1.052)
	Weighted median	0.202		0.934 (0.842 to 1.037)		Weighted median	0.95	+	1.001 (0.963 to 1.041)
	Inverse variance weighted	0.233	-	0.954 (0.883 to 1.031)		Inverse variance weighted	0.485	+	0.990 (0.962 to 1.018)
cathepisn F					cathepisn F				
	MR-Egger	0.42		1.087 (0.895 to 1.321)		MR-Egger	0.899 -	-	0.994 (0.911 to 1.085)
	Weighted median	0.215		0.936 (0.843 to 1.039)		Weighted median	0.214 -	•	0.975 (0.938 to 1.014)
	Inverse variance weighted	0.454	-	0.968 (0.888 to 1.055)		Inverse variance weighted	0.116	•	0.971 (0.936 to 1.007)
cathepisn G					cathepisn G				
	MR-Egger	0.84		0.978 (0.797 to 1.202)		MR-Egger	0.148	+	1.054 (0.987 to 1.126)
	Weighted median	0.307		0.937 (0.827 to 1.062)		Weighted median	0.311	+	1.023 (0.979 to 1.068)
	Inverse variance weighted	0.232	+	0.946 (0.864 to 1.036)		Inverse variance weighted	0.219	+	1.019 (0.989 to 1.051)
cathepisn H					cathepisn H				
	MR-Egger	0.455		1.029 (0.958 to 1.104)		MR-Egger	0.245	÷	1.015 (0.992 to 1.038)
	Weighted median	0.53	+	1.016 (0.967 to 1.067)		Weighted median	0.278	+	1.010 (0.992 to 1.028)
	Inverse variance weighted	0.745	•	1.008 (0.959 to 1.060)		Inverse variance weighted	0.146	•	1.012 (0.996 to 1.029)
cathepisn L2					cathepisn L2				
	MR-Egger	0.856 -	-	1.032 (0.743 to 1.433)		MR-Egger	0.269 -	+	0.939 (0.847 to 1.042)
	Weighted median	0.376		1.068 (0.923 to 1.236)		Weighted median	0.971	•	0.999 (0.945 to 1.057)
	Inverse variance weighted	0.27		1.070 (0.949 to 1.208)		Inverse variance weighted	0.965	÷	0.999 (0.961 to 1.039)
cathepisn O					cathepisn O				
	MR-Egger	0.866	+-	1.019 (0.825 to 1.258)		MR-Egger	0.402		1.046 (0.947 to 1.156)
	Weighted median	0.666	+	1.027 (0.910 to 1.159)		Weighted median	0.368	*	1.023 (0.973 to 1.075)
	Inverse variance weighted	0.867	+	1.008 (0.920 to 1.103)		Inverse variance weighted	0.276	*	1.022 (0.983 to 1.063)
cathepisn S					cathepisn S				
	MR-Egger	0.371	+	1.047 (0.949 to 1.154)		MR-Egger	0.061	-	1.040 (1.000 to 1.082)
	Weighted median	0.181	-	1.061 (0.973 to 1.156)		Weighted median	0.027	-	1.036 (1.004 to 1.070)
	Inverse variance weighted	0.618	+	1.015 (0.958 to 1.074)		Inverse variance weighted	0.36	ŧ	1.011 (0.987 to 1.036)
cathepisn Z					cathepisn Z				
	MR-Egger	0.656	+	1.027 (0.916 to 1.152)		MR-Egger	0.908	+	0.998 (0.961 to 1.036)
	Weighted median	0.862	+	1.008 (0.925 to 1.098)		Weighted median	0.494	+	1.011 (0.980 to 1.043)
	Inverse variance weighted	0.83	+	1.008 (0.939 to 1.081)		Inverse variance weighted	0.324	+	1.012 (0.988 to 1.037)
		0 0.5	1 1.5 2	2			0.5 0.75	1 1.25 1.	5

Figure 2. Positive result of forward MR analysis: Cholecystitis and cholelithiasis.

	Diverticulosis					Chronic Gastritis (C	G)		
Exposure	Method	pval		OR(95% CI)	Exposure	Method	pval		OR(95% CI)
cathepisn B			1		cathepisn B				
	MR-Egger	0.515	-	1.171 (1.004 to 1.366)		MR-Egger	0.168		1.092 (0.968 to 1.232)
	Weighted median	0.789	+	1.022 (0.940 to 1.112)		Weighted median	0.633		1.017 (0.948 to 1.091)
	Inverse variance weighted	0.977	+	1.013 (0.948 to 1.081)		Inverse variance weighted	0.853	+	1.005 (0.953 to 1.059)
cathepisn E					cathepisn E				
	MR-Egger	0.161	+	1.004 (0.921 to 1.094)		MR-Egger	0.401		1.041 (0.953 to 1.139)
	Weighted median	0.471	+	0.983 (0.913 to 1.057)		Weighted median	0.41		1.028 (0.963 to 1.098)
	Inverse variance weighted	0.585	+	0.985 (0.935 to 1.037)		Inverse variance weighted	0.551	+	1.016 (0.963 to 1.072)
cathepisn F					cathepisn F				
	MR-Egger	0.15	- - -	1.021 (0.884 to 1.179)		MR-Egger	0.696	- -	1.024 (0.912 to 1.150)
	Weighted median	0.713	+	1.051 (0.981 to 1.125)		Weighted median	0.239		1.042 (0.973 to 1.115)
	Inverse variance weighted	0.58	-	1.058 (0.997 to 1.124)		Inverse variance weighted	0.088	+ - -	1.044 (0.993 to 1.098)
cathepisn G					cathepisn G				
	MR-Egger	0.453		0.937 (0.814 to 1.079)		MR-Egger	0.609	-	0.963 (0.838 to 1.107)
	Weighted median	0.987		0.930 (0.859 to 1.006)		Weighted median	0.084		0.930 (0.857 to 1.010)
	Inverse variance weighted	0.77	+	0.974 (0.915 to 1.038)		Inverse variance weighted	0.076		0.946 (0.890 to 1.006)
cathepisn H					cathepisn H				
	MR-Egger	0.65	+	0.989 (0.943 to 1.038)		MR-Egger	0.848	÷	0.996 (0.954 to 1.040)
	Weighted median	0.449	-	0.986 (0.954 to 1.019)		Weighted median	0.643	+	1.008 (0.974 to 1.043)
	Inverse variance weighted	0.41	4	0.986 (0.954 to 1.019)		Inverse variance weighted	0.318	+	1.016 (0.985 to 1.048)
cathepisn L2					cathepisn L2				
	MR-Egger	0.809		0.922 (0.761 to 1.117)		MR-Egger	0.12	•	0.846 (0.697 to 1.026)
	Weighted median	0.983	+-	1.028 (0.937 to 1.127)		Weighted median	0.879		0.993 (0.904 to 1.090)
	Inverse variance weighted	0.896	4	0.988 (0.919 to 1.062)		Inverse variance weighted	0.505		1.030 (0.945 to 1.122)
cathepisn O					cathepisn O				
	MR-Egger	0.474		0.899 (0.781 to 1.034)		MR-Egger	0.34		1.086 (0.924 to 1.277)
	Weighted median	0.515	+	0.998 (0.919 to 1.085)		Weighted median	0.246		1.055 (0.964 to 1.155)
	Inverse variance weighted	0.867	+	0.994 (0.935 to 1.056)		Inverse variance weighted	0.472		1.026 (0.957 to 1.099)
cathepisn S					cathepisn S				
	MR-Egger	0.961	-	1.054 (0.987 to 1.125)		MR-Egger	0.479	-	1.025 (0.958 to 1.097)
	Weighted median	0.328	+	1.031 (0.975 to 1.090)		Weighted median	0.61	-	1.015 (0.959 to 1.073)
	Inverse variance weighted	0.096	÷	1.008 (0.970 to 1.048)		Inverse variance weighted	0.702	+	1.008 (0.969 to 1.049)
cathepisn Z					cathepisn Z				
	MR-Egger	0.291	+	1.039 (0.971 to 1.111)		MR-Egger	0.043		1.084 (1.012 to 1.161)
	Weighted median	0.256	+	1.023 (0.967 to 1.082)		Weighted median	0.036		1.063 (1.004 to 1.125)
	Inverse variance weighted	10.021	+	1.024 (0.981 to 1.069)		Inverse variance weighted	0.024	-	1.052 (1.007 to 1.099)
		0 0.	5 1 1.5	2			0.5 0.75	1 1.25 1	.5

Figure 3. Positive result of forward MR analysis: Diverticular disease and chronic gastritis (CG).

	Liver Cirrhosis				Non-	alcoholic Fatty Liver Dise	ase (NAFLD)		
Exposure	Method	pval		OR(95% CI)	Exposure	Method	pval		OR(95% CI)
cathepisn B					cathepisn B			1	
	MR-Egger	0.212		0.829 (0.624 to 1.102)		MR-Egger	0.123		0.832 (0.665 to 1.040)
	Weighted median	0.176		1.136 (0.944 to 1.366)		Weighted median	0.835	-	1.014 (0.888 to 1.159)
	Inverse variance weighted	0.073	-	1.130 (0.988 to 1.292)		Inverse variance weighted	0.965	+	1.002 (0.907 to 1.108)
cathepisn E					cathepisn E				
	MR-Egger	0.626		0.940 (0.739 to 1.194)		MR-Egger	0.983	-	1.002 (0.847 to 1.185)
	Weighted median	0.436		0.926 (0.762 to 1.124)		Weighted median	0.617		0.965 (0.838 to 1.110)
	Inverse variance weighted	0.18		0.906 (0.785 to 1.046)		Inverse variance weighted	0.802	+	0.987 (0.893 to 1.092)
cathepisn F					cathepisn F				
	MR-Egger	0.206		0.784 (0.551 to 1.115)		MR-Egger	0.59		1.085 (0.815 to 1.445)
	Weighted median	0.61	-	1.048 (0.874 to 1.257)		Weighted median	0.439		1.055 (0.922 to 1.207)
	Inverse variance weighted	0.643	-	1.040 (0.882 to 1.226)		Inverse variance weighted	0.944	+	0.996 (0.899 to 1.105)
cathepisn G					cathepisn G				
	MR-Egger	0.331		1.189 (0.853 to 1.657)		MR-Egger	0.986	- +	1.002 (0.794 to 1.265)
	Weighted median	0.609		1.055 (0.859 to 1.297)		Weighted median	0.282	- -	0.920 (0.789 to 1.071)
	Inverse variance weighted	0.617		1.040 (0.892 to 1.212)		Inverse variance weighted	0.047	-	0.897 (0.806 to 0.999)
cathepisn H					cathepisn H				
	MR-Egger	0.694	-	0.976 (0.869 to 1.097)		MR-Egger	0.429	+	0.963 (0.882 to 1.052)
	Weighted median	0.658	+	0.980 (0.896 to 1.072)		Weighted median	0.924	+	1.003 (0.942 to 1.068)
	Inverse variance weighted	0.68	-	0.982 (0.903 to 1.069)		Inverse variance weighted	0.755	+	1.011 (0.944 to 1.082)
cathepisn L2					cathepisn L2				
	MR-Egger	0.644		1.126 (0.690 to 1.839)		MR-Egger	0.373		1.187 (0.829 to 1.699)
	Weighted median	0.128		1.220 (0.944 to 1.577)		Weighted median	0.054		1.202 (0.997 to 1.450)
	Inverse variance weighted	10.038		1.221 (1.011 to 1.473)		Inverse variance weighted	0.041		1.147 (1.006 to 1.308)
cathepisn O					cathepisn O				
	MR-Egger	0.895		0.973 (0.658 to 1.439)		MR-Egger	0.515 -	•	0.910 (0.692 to 1.197)
	Weighted median	0.844		0.977 (0.775 to 1.231)		Weighted median	0.491		0.947 (0.813 to 1.105)
	Inverse variance weighted	0.525		0.947 (0.799 to 1.121)		Inverse variance weighted	0.445		0.955 (0.848 to 1.075)
cathepisn S					cathepisn S				
	MR-Egger	0.082	-	0.828 (0.677 to 1.014)		MR-Egger	0.362	•	0.929 (0.797 to 1.084)
	Weighted median	0.032		0.843 (0.720 to 0.985)		Weighted median	0.182		0.929 (0.833 to 1.035)
	Inverse variance weighted	0.151	-	0.916 (0.813 to 1.032)		Inverse variance weighted	0.262		0.951 (0.870 to 1.039)
cathepisn Z					cathepisn Z				
	MR-Egger	0.336		1.134 (0.888 to 1.448)		MR-Egger	0.461	•	0.938 (0.795 to 1.106)
	Weighted median	0.625		1.043 (0.882 to 1.233)		Weighted median	0.845	+	1.012 (0.898 to 1.141)
	Inverse variance weighted	0.114	֥	1.128 (0.971 to 1.309)		Inverse variance weighted	0.527	*	0.968 (0.874 to 1.072)
		ό c	.5 1 1.5 2	2			0 0.5	1 1.5 2	2

Figure 4. Positive result of forward MR analysis: Cirrhosis of liver and non-alcoholic fatty liver disease (NAFLD).

3.2.2. Reverse MR analysis

We used reverse MR analysis to investigate the impact of diseases on cathepsin levels. Specifically, a promoting effect on cathepsin levels was observed between CTSB and NASH (OR = 1.084, 95% CI: 1.001–1.175, IVW: P = 0.047) and liver cirrhosis (OR = 1.070, 95% CI: 1.014–1.129, IVW: P = 0.014); CTSH and NASH (OR = 1.078, 95% CI: 1.013–1.147, IVW: P = 0.017), CTSS, and liver cirrhosis (OR = 1.071, 95% CI: 1.014–1.130, IVW: *P* = 0.013); CTSZ and both NAFLD (OR = 1.119, 95% CI: 1.044–1.198, IVW: P = 0.001) and liver cirrhosis (OR = 1.101, 95% CI: 1.028–1.179, IVW: P = 0.006). In contrast, BE lowered the levels of CTSO (95% CI: 0.850–0.997, IVW: P = 0.043), while diverticulosis reduced CTSH levels (OR = 0.914; 95% CI, 0.841–0.992; IVW: P = 0.031). Additionally, CP showed a negative correlation with CTSE, *F*, and L2 (OR = 0.891, 95% CI: 0.806–0.985, IVW: *P* = 0.024; OR = 0.882, 95% CI: 0.786–0.988, IVW: P = 0.031; OR = 0.885, 95% CI: 0.800– 0.978, IVW: P = 0.017) (Figures 5–7). Notably, the reverse and forward MR analyses yielded no overlapping results, indicating that no bidirectional causal relationships were identified between any cathepsin-disease pairs. The full-reserve MR results are presented in Supplementary Table S3.

IN IN	onalcoholic Steatohepatit	s (NASH)				Liver Cirrhosis			
Exposure	Method	pval		OR(95% CI)	Exposure	Method	pval		OR(95% CI)
cathepisn B			1		cathepisn B			1	
	MR-Egger	0.829		0.859 (0.257 to 2.877)		MR-Egger	0.771		0.983 (0.877 to 1.101
	Weighted median	0.162	-	1.056 (0.978 to 1.141)		Weighted median	0.132	֥	1.063 (0.982 to 1.150
	Inverse variance weighted	0.047	-	1.084 (1.001 to 1.175)		Inverse variance weighted	0.014		1.070 (1.014 to 1.129
cathepisn E					cathepisn E				
	MR-Egger	0.988 -	-	0.990 (0.319 to 3.072)		MR-Egger	0.068		0.885 (0.785 to 0.998
	Weighted median	0.788	+	0.988 (0.904 to 1.080)		Weighted median	0.066		0.929 (0.860 to 1.005
	Inverse variance weighted	0.38	+	0.968 (0.901 to 1.041)		Inverse variance weighted	0.422		0.975 (0.917 to 1.037
cathepisn F					cathepisn F			1	
	MR-Egger	0.34		2.165 (0.641 to 7.313)		MR-Egger	0.291		1.084 (0.939 to 1.250
	Weighted median	0.871	÷	1.007 (0.929 to 1.090)		Weighted median	0.672	+-	1.017 (0.941 to 1.100
	Inverse variance weighted	0.942	+	0.996 (0.898 to 1.105)		Inverse variance weighted	0.288		1.037 (0.970 to 1.109
cathepisn G					cathepisn G				
	MR-Egger	0.604 -		0.782 (0.354 to 1.726)		MR-Egger	0.389		0.950 (0.848 to 1.064
	Weighted median	0.449		1.029 (0.956 to 1.108)		Weighted median	0.518		1.026 (0.949 to 1.109
	Inverse variance weighted	0.446	+	1.024 (0.963 to 1.090)		Inverse variance weighted	0.653	+	0.988 (0.936 to 1.043
cathepisn H					cathepisn H				
	MR-Egger	0.934 -	-	0.962 (0.423 to 2.184)		MR-Egger	0.125		1.100 (0.982 to 1.232
	Weighted median	0.056		1.079 (0.998 to 1.167)		Weighted median	0.603		1 022 (0 942 to 1 108
	Inverse variance weighted	0.017	•	1.078 (1.013 to 1.147)		Inverse variance weighted	0.709	+	1.010 (0.957 to 1.067
cathepisn L2					cathepisn L2				
	MR-Egger	0.394		1.546 (0.700 to 3.414)		MR-Egger	0.728	<u> </u>	1.021 (0.909 to 1.147
	Weighted median	0.677	+	1.016 (0.944 to 1.092)		Weighted median	0.811	-	1 009 (0 936 to 1 088
	Inverse variance weighted	0.947	+	1,002 (0,942 to 1,066)		Inverse variance weighted	0.85	+	0 995 (0 942 to 1 050
cathepisn O					cathepisn O				
	MR-Egger	0.734 -	-	0.854 (0.387 to 1.886)		MR-Egger	0.247	+	1.073 (0.958 to 1.202
	Weighted median	0.162	-	1.054 (0.979 to 1.135)		Weighted median	0.311		1.039 (0.965 to 1.120
	Inverse variance weighted	0.069	-	1.059 (0.995 to 1.127)		Inverse variance weighted	0.286	-	1 030 (0 976 to 1 087
cathepisn S	interest randines horgines				cathepisn S	in the familie for a signed		1	
	MR-Egger	0.323		1 692 (0 766 to 3 738)		MR-Egger	0.082	—	1 115 (0 996 to 1 249
	Weighted median	0.233	-	1 047 (0 971 to 1 130)		Weighted median	0.031		1 086 (1 008 to 1 171
	Inverse variance weighted	0.284	+	1 034 (0 972 to 1 101)		Inverse variance weighted	0.0013	-	1 071 (1 014 to 1 130
cathepisn 7	interse fundice freighted	0.201	1	1.001 (0.072 10 1.101)	cathepisn 7	interes fundice nergines	0.010	1	
camproniz	MR-Edger	0.344	_	1 929 (0 677 to 5 501)	our op ton E	MR-Eager	0.03		1 191 (1 034 to 1 372
	Weighted median	0.561	+	1 028 (0.937 to 1 128)		Weighted median	0.012		1 102 (1 021 to 1 188
	Inverse variance weighted	0 374	4	1 040 (0 954 to 1 134)		Inverse variance weighter	0.006		1 101 (1 028 to 1 179
	interse runance weighted]		and a validation and grade		1.1.1	7

Figure 5. Positive result of reverse MR analysis: Nonalcoholic steatohepatitis (NASH) and cirrhosis of liver.

F	alconolic Fatty Liver Dise.	ase (INAFLD)			-	Darrett s Esophagu	(DE)		
Exposure	Method	pval		OR(95% CI)	Exposure	Method	pval		OR(95% CI)
cathepisn B					cathepisn B				
	MR-Egger	0.457		0.944 (0.813 to 1.096)		MR-Egger	0.365	+	1.113 (0.889 to 1.393
	Weighted median	0.21	+-	1.063 (0.966 to 1.169)		Weighted median	0.355	+-	1.054 (0.943 to 1.179
	Inverse variance weighted	0.502	+	1.023 (0.956 to 1.095)		Inverse variance weighte	d 0.522	+	1.028 (0.944 to 1.120
cathepisn E					cathepisn E				
	MR-Egger	0.341		0.917 (0.771 to 1.091)		MR-Egger	0.897	+	1.017 (0.793 to 1.304
	Weighted median	0.015	-	0.884 (0.800 to 0.976)		Weighted median	0.513	+	1.038 (0.929 to 1.159
	Inverse variance weighted	0.517	+	0.975 (0.902 to 1.053)		Inverse variance weighte	d 0.221	+	1.060 (0.966 to 1.163
cathepisn F					cathepisn F				
	MR-Egger	0.324	+	1.078 (0.931 to 1.248)		MR-Egger	0.232	+	1.141 (0.928 to 1.403
	Weighted median	0.602	+	1.026 (0.932 to 1.130)		Weighted median	0.429	-	0.956 (0.856 to 1.068
	Inverse variance weighted	0.248	+	1.040 (0.973 to 1.111)		Inverse variance weighte	d 0.681	+	0.983 (0.908 to 1.065
cathepisn G					cathepisn G				
	MR-Egger	0.431	+	1.062 (0.917 to 1.229)		MR-Egger	0.225	+	1.143 (0.930 to 1.406
	Weighted median	0.711	÷	1.019 (0.923 to 1.125)		Weighted median	0.961	+	0.997 (0.897 to 1.109
	Inverse variance weighted	0.725	+	0.988 (0.925 to 1.056)		Inverse variance weighte	d 0.685	+	1.017 (0.939 to 1.101
cathepisn H					cathepisn H				
	MR-Egger	0.872	- - -	0.988 (0.853 to 1.144)		MR-Egger	0.964	-	1.005 (0.817 to 1.236
	Weighted median	0.558	+	1.030 (0.933 to 1.138)		Weighted median	0.557	+	0.968 (0.869 to 1.079
	Inverse variance weighted	0.962	÷	0.998 (0.935 to 1.066)		Inverse variance weighte	d 0.262	+	0.956 (0.882 to 1.035
cathepisn L2	a na seco o carlante e a carla Tata da				cathepisn L2			1	
	MR-Egger	0.853	+	1.017 (0.851 to 1.216)		MR-Egger	0.737		0.962 (0.772 to 1.199
	Weighted median	0.549	+	1.031 (0.932 to 1.141)		Weighted median	0.781	+	0.985 (0.882 to 1.099
	Inverse variance weighted	0.402	÷	1.034 (0.956 to 1.119)		Inverse variance weighte	d 0.456	+	0.969 (0.893 to 1.052
cathepisn O					cathepisn O				
	MR-Egger	0.784	-	0.977 (0.827 to 1.153)		MR-Egger	0.458		0.923 (0.750 to 1.135
	Weighted median	0.29	-	1.055 (0.956 to 1.164)		Weighted median	0.055	-	0.897 (0.803 to 1.003
	Inverse variance weighted	0.7	+	1.015 (0.943 to 1.092)		Inverse variance weight	ad 0.043	•	0.921 (0.850 to 0.997
cathepisn S					cathepisn S				
	MR-Egger	0.23	÷	1.097 (0.948 to 1.269)		MR-Egger	0.44		1.088 (0.885 to 1.337
	Weighted median	0.175		1.073 (0.969 to 1.189)		Weighted median	0.611	+	1.027 (0.926 to 1.139
	Inverse variance weighted	0.429	+	1.027 (0.961 to 1.097)		Inverse variance weighte	d 0.896	+	1.005 (0.928 to 1.089
cathepisn Z					cathepisn Z	in the second seco		1	
	MR-Egger	0.008		1.248 (1.078 to 1.446)		MR-Egger	0.994	- -	1.001 (0.776 to 1.291
	Weighted median	0.007		1.147 (1.039 to 1.267)		Weighted median	0.8	+	1.015 (0.904 to 1.140
	Inverse variance weighted	0.001	-	1 119 (1 044 to 1 198)		Inverse variance weighte	d 0.499	+	0.968 (0.880 to 1.064

Figure 6. Positive result of reverse MR analysis: Non-alcoholic fatty liver disease (NAFLD) and Barrett's esophagus (BE).

	Diverticulosis					Chronic Pancreatit	is (CP)		
Exposure	Method	pval		OR(95% CI)	Exposure	Method	pval		OR(95% CI)
cathepisn B			1		cathepisn B			1	
	MR-Egger	0.388	-+-	0.903 (0.718 to 1.137)		MR-Egger	0.851	-	1.023 (0.808 to 1.295)
	Weighted median	0.59	-	0.965 (0.846 to 1.100)		Weighted median	0.561	+-	1.041 (0.909 to 1.192)
	Inverse variance weighted	0.447	+	0.969 (0.892 to 1.052)		Inverse variance weight	ed 0.832	+	1.011 (0.914 to 1.118)
cathepisn E					cathepisn E				
	MR-Egger	0.258		0.875 (0.695 to 1.102)		MR-Egger	0.523		1.081 (0.855 to 1.369)
	Weighted median	0.692	4	0.976 (0.866 to 1.100)		Weighted median	0.01		0.838 (0.732 to 0.958)
	Inverse variance weighted	0.935	+	1.003 (0.924 to 1.090)		Inverse variance weigh	ted 0.024	-	0.891 (0.806 to 0.985)
cathepisn F					cathepisn F				
	MR-Egger	0.056		0.795 (0.629 to 1.004)		MR-Egger	0.124		0.800 (0.610 to 1.049)
	Weighted median	0.753	+	0.979 (0.856 to 1.119)		Weighted median	0.063	-	0.876 (0.762 to 1.007)
	Inverse variance weighted	0.538	+	0.974 (0.895 to 1.059)		Inverse variance weigh	ted 0.031		0.882 (0.786 to 0.988)
cathepisn G					cathepisn G				
	MR-Egger	0.396		0.905 (0.719 to 1.139)		MR-Egger	0.323		0.865 (0.655 to 1.144)
	Weighted median	0.094	-	1.123 (0.980 to 1.287)		Weighted median	0.258		0.916 (0.787 to 1.066)
	Inverse variance weighted	0.32	+	1.043 (0.960 to 1.132)		Inverse variance weight	ed 0.981	-	1.001 (0.888 to 1.129)
cathepisn H					cathepisn H				
	MR-Egger	0.531		0.929 (0.738 to 1.169)		MR-Egger	0.693		1.050 (0.829 to 1.328)
	Weighted median	0.146		0.911 (0.803 to 1.033)		Weighted median	0.351		0.938 (0.819 to 1.074)
	Inverse variance weighted	0.031	•	0.914 (0.841 to 0.992)		Inverse variance weight	ed 0.274		0.945 (0.855 to 1.045)
cathepisn L2					cathepisn L2				
	MR-Egger	0.465		0.913 (0.715 to 1.165)		MR-Egger	0.163		0.840 (0.663 to 1.063)
	Weighted median	0.257		0.928 (0.815 to 1.056)		Weighted median	0.105		0.889 (0.772 to 1.025)
	Inverse variance weighted	0.094	-	0.928 (0.851 to 1.013)		Inverse variance weigh	ted 0.017	-	0.885 (0.800 to 0.978)
cathepisn O					cathepisn O				
	MR-Egger	0.45		0.915 (0.727 to 1.152)		MR-Egger	0.822	- -	1.028 (0.812 to 1.301)
	Weighted median	0.968	+	0.997 (0.876 to 1.136)		Weighted median	0.159		0.908 (0.794 to 1.039)
	Inverse variance weighted	0.247	+	0.953 (0.877 to 1.034)		Inverse variance weight	ed 0.317	-	0.950 (0.859 to 1.050)
cathepisn S	•				cathepisn S				
	MR-Egger	0.315		1.129 (0.892 to 1.428)		MR-Egger	0.671	_ -	1.062 (0.809 to 1.392)
	Weighted median	0.484	÷	1.049 (0.918 to 1.199)		Weighted median	0.236		0.910 (0.779 to 1.064)
	Inverse variance weighted	0.767	+	1.013 (0.931 to 1.102)		Inverse variance weight	ed 0.385		0.950 (0.847 to 1.066)
cathepisn Z					cathepisn Z				
	MR-Egger	0.587		0.936 (0.736 to 1.189)		MR-Egger	0.626		0.931 (0.701 to 1.235)
	Weighted median	0.557		1.044 (0.905 to 1.204)		Weighted median	0.934	-	0.994 (0.859 to 1.150)
	Inverse variance weighted	0.795	+	0.989 (0.908 to 1.077)		Inverse variance weight	ed 0.328	-	0.943 (0.839 to 1.061)
		0	0.5 1 1.5	2			0	0.5 1 1.5	2

Figure 7. Positive result of reverse MR analysis: Diverticular disease and chronic pancreatitis (CP).

3.2.3. Multivariable MR analysis

By employing the MVMR method, we conducted a more in-depth examination of the 9 cathepsins linked to each of the 23 digestive diseases. Our results indicate that after controlling for the expression of other cathepsins within the same disease context, the impact of specific cathepsins on digestive diseases is independent and not influenced by other exposures. Specifically, CTSG was associated with a higher risk of GERD (OR = 1.034, 95% CI: 1.000-1.069, P = 0.047) and showed a protective trend against NAFLD (OR = 0.856, 95% CI: 0.744-0.984, P = 0.029). CTSL2 was associated with a higher likelihood of DU (OR = 1.201, 95% CI: 1.074-1.344, P = 0.001) than CTSO, which indicated a protective effect against DU (OR = 0.873, 95%CI: 0.770–0.990, *P* = 0.035). CTSE promoted AG (OR = 1.111, 95% CI: 1.006–1.227, P = 0.038). CTSH and S protected against CD (OR = 0.946, 95% CI: 0.900-0.995, P = 0.033; OR = 0.933, 95% CI: 0.874–0.994, P = 0.033), whereas CTSO was associated with an increased risk for CD (OR = 1.150, 95% CI: 1.020-1.298, P = 0.023). CTSZ independently promotes diverticulosis (OR = 1.045, 95% CI, 1.009-1.083; P = 0.014). CTSB was independently associated with an increased risk of liver cirrhosis (OR = 1.149, 95% CI: 1.011–1.305, P = 0.034) and cholecystitis (OR = 1.082, 95% CI: 1.008-1.161, P = 0.028), while CTSS showed an independent protective effect in liver cirrhosis (OR = 0.870; 95% CI, 0.772–0.979; P = 0.021). For cholelithiasis, CTSB and CTSZ promoted disease risk (OR = 1.048, 95% CI: 1.007-1.091, P = 0.021; OR = 1.052, 95% CI: 1.002-1.105, P = 0.040), while CTSF offered protection (OR = 0.934, 95% CI: 0.888–0.983, P = 0.009). Positive results are shown in Figure 8. The causal

relationships between certain cathepsins and relevant diseases that match the forward MR results were as follows: CTSG and NAFLD, CTSZ and diverticulosis, CTSB and cholecystitis, and CTSB and cholelithiasis.

Gastosegohugosi Refuz (GERD) Garbagosi R 0054 Garbagosi R 0077	Disease	Exposure	pval		OR(95% CI)
cathepase 0 0.53 - 0.974 (0.946 b) 100 cathepase 0 0.633 - 0.966 (0.935 b) 10 cathepase 0 0.072 - 1.033 (0.947 b) 102 cathepase 0 0.072 - 1.033 (0.947 b) 102 cathepase 0 0.072 - 1.033 (0.977 b) 102 cathepase 0 0.072 - 1.033 (0.977 b) 102 cathepase 0 0.072 - 1.033 (0.977 b) 102 cathepase 0 0.994 - 0.994 (0.947 b) 102 cathepase 0 0.994 - 0.996 (0.935 b) 100 cathepase 0 0.994 - 0.996 (0.935 b) 100 cathepase 0 0.994 - 0.997 (0.976 b) 99 cathepase 0 0.994 - 0.997 (0.976 b) 99 cathepase 0 0.994 - 0.996 (0.845 b) 107 cathepase 0 0.997 (0.977 b) 0.99 - 0.997 (0.977 b) 0.99 cathepase 0 0.437 - 0.996 (0.845 b) 103 cathepase 0 0.437 - 0.996 (0.845 b) 103 cathepase 0 0.437 - 0.996 (0.845 b) 103	Gastroesophageal Reflux (GERD)				
Cambepins 0 045		cathepisn B	0.054	-	0.974 (0.948 to 1.000)
Calance of a constraint of a c		cathepish E	0.633	I	1.007 (0.977 to 1.039)
cathegeni 1 0.77 1 1033 0.094 in 102 cathegeni 2 0.50 0.994 0.4945 0.101 cathegeni 2 0.50 0.994 0.834 0.100 cathegeni 2 0.90 0.900 0.833 0.100 cathegeni 2 0.901 0.904 0.834 0.100 cathegeni 2 0.901 0.901 0.813 1.100 cathegeni 2 0.901 0.801 0.814 0.100 cathegeni 2 0.801 0.814 0.102 0.801 0.814 0.102 cathegeni 2 0.801 0.814 0.102 0.801 0.814 0.102 cathegeni 2 0.901 0.815 0.113 0.816 0.894 0.102 cathegeni 2 0.901 0.815 0.113 0.816 0.814 0.102 cathegeni 2 0.902 0.818 0.113 0.816 0.815 0.105 cathegeni 2 0.907 0.02		cathenisn G	0.435	-	1.034 (1.000 to 1.021)
catherison 0.809 ••••••••••••••••••••••••••••••••••••		cathepisn H	0.767	÷	1.003 (0.984 to 1.023)
camberson 0 809 - 0.994 (0.9496) 10.0 camberson 2 0.95 - 0.931 (0.001 100 camberson 2 0.93 - 0.991 (0.957 10 10) camberson 2 0.93 - 0.991 (0.957 10 10) camberson 2 0.934 - 0.991 (0.957 10 10) camberson 2 0.942 - 1.032 (0.957 10 10) camberson 2 0.942 - 1.032 (0.957 10 10) camberson 2 0.942 - 1.032 (0.957 10 10) camberson 2 0.944 - 1.042 (0.957 10 11) camberson 2 0.944 - 1.042 (0.957 10 11) camberson 2 0.946 - 1.042 (0.957 10 11) camberson 2 0.946 - 1.042 (0.957 10 11) camberson 1 0.922 - 1.044 (0.953 10 15) camberson 1 0.922 - 1.042 (0.953 10 15) camberson 1 0.922 - 1.042 (0.953 10 15) camberson 1 0.922 - 1.042 (0.952 10 10) <td< td=""><td></td><td>cathepisn L2</td><td>0.072</td><td>-</td><td>1.039 (0.997 to 1.083)</td></td<>		cathepisn L2	0.072	-	1.039 (0.997 to 1.083)
cathepias 2 0.918 • 0.999 (0.974 b 102 Doudennil Uicer (DU) cathepias B 0.54 • 0.331 (0.055 b 10 cathepias B 0.59 • 0.932 (0.055 b 10 0.05 (0.925 b 10.05 cathepias B 0.54 • 0.931 (0.055 b 10 0.05 (0.925 b 10.05 cathepias B 0.942 • 0.03 (0.971 b 102 0.05 (0.925 b 10.05 cathepias B 0.941 • 0.942 (0.971 b 111 0.941 (0.971 b 111 cathepias B 0.953 • 0.949 (0.934 b 11.05 0.941 (0.971 b 111 cathepias C 0.966 • 1.024 (0.937 b 111 0.941 (0.931 b 117 cathepias C 0.966 • 1.041 (0.954 b 127 0.940 (0.932 b 11.05 cathepias C 0.961 0.952 (0.953 b 117 0.952 (0.953 b 117 0.952 (0.953 b 117 cathepias C 0.952 (0.957 b 118 0.952 (0.958 b 120 c 119 0.952 (0.958 b 120 c 119 0.952 (0.958 b 120		cathepisn O	0.809	+	0.994 (0.949 to 1.042)
cambergia 0.65 • 1.033 (1000 to 150 to 100 cambergias 0.64 • 0.931 (0.865 to 100 cambergias 0.50 • 0.990 (0.833 to 160 cambergias 0.942 • 1.003 (0.971 to 169 cambergias 0.942 • 1.033 (0.971 to 159 cambergias 0.001 • 1.024 (0.977 to 111 cambergias 0.001 • 1.024 (0.977 to 111 cambergias 0.003 • 0.990 (0.891 to 150 cambergias 0.003 • 1.014 (0.977 to 110 cambergias 0.031 • 1.016 (0.933 to 117) cambergias 0.031 • 1.016 (0.933 to 117) cambergias 0.32 • 1.016 (0.933 to 117) cambergias 0.312 • 1.016 (0.933 to 117) cambergias 0.32 • 0.992 (0.841 to 102) cambergias 0.313 • 1.016 (0.922 to 105) cambergias 0.313 • 0.992 (0.841 to 102)		cathepisn S	0.918	+	0.999 (0.974 to 1.024)
Duodenai Uker (Us)		cathepisn Z	0.05	-	1.033 (1.000 to 1.068)
Caleges 0 03 0 03 0 03 000 030 030 030 030 030	Duodenal Ulcer (DU)	eathenion D	0.054		0.021 (0.965 to 1.001)
Calepcins C 0.200 Calepcins C 0.200 Calepcins C 0.200 Calepcins C 0.42 Calepcins C 0.42 Calepcins C 0.44 Cal		cathepisn E	0.003	_	1.005 (0.005 to 1.001)
cathegies G 942		cathepish E	0.509		0.969 (0.883 to 1.064
camberson D 0.941		cathepisn G	0.942		1.003 (0.917 to 1.098
callegion 0 0055 0075 callegion 0 0056 0075 callegion 2 0606 1024 (0.977 to 111) Acute Gastrilis (AG) callegion 2 0606 1024 (0.977 to 111) callegion 2 0606 11024 (0.977 to 111) 1024 (0.977 to 111) callegion 6 0481 1045 (0.933 to 117) 1014 (0.065 to 128) callegion 6 0484 1104 (0.933 to 117) 1014 (0.935 to 118) callegion 6 0481 1035 (0.983 to 113) 1014 (0.945 to 128) callegion 6 0123 1019 (0.875 to 118) 1016 (0.955 to 118) callegion 6 0124 1055 (0.983 to 113) 1056 (0.986 to 155) callegion 6 0286 0.952 (0.874 to 103) 1056 (0.986 to 155) callegion 7 0286 to 120 1056 (0.986 to 155) 1056 (0.986 to 155) callegion 7 0286 to 120 1056 (0.986 to 155) 1056 (0.986 to 155) callegion 7 0286 to 200 0.932 (0.837 to 103) 1056 (0.986 to 155) callegion 7 0.932 (0.877 to 1030) 1056 (0.986 to 155) 1056 (0.986 to 155) callegion 7 0.831 to 130 acute 1056 (0		cathepisn H	0.941	+	1.002 (0.951 to 1.056
Cellac Disease (CD) Cellac		cathepisn L2	0.001		1.201 (1.074 to 1.344,
Cathegiens Z 0606 Cathegiens Z 0606 Cathegiens Z 0606 Cathegiens Z 0606 Cathegiens B 0.437 Cathegiens B 0.444 Cathegiens B 0.44		cathepisn O	0.035		0.873 (0.770 to 0.990)
Acade Gastinis (AG) Cathepisn E Cathepisn E Cathepisn E Cathepisn E Cathepisn E Cathepisn C Cathepisn E Cathepisn C C Cathepisn C Cathepis		cathepisn S	0.224		1.043 (0.974 to 1.117 1.024 (0.937 to 1.118
Catheris F, Catheris F, Catheris F, O444	Acute Gastritis (AG)	cattlepish z	0.000		1.024 (0.937 to 1.116)
cathegiss F 0.93		cathepisn B	0.437		0.966 (0.884 to 1.055)
Calhepison 6 0.448		cathepisn E	0.038		1.111 (1.006 to 1.227)
cathepisn L2 0282		cathepisn F	0.444		1.045 (0.933 to 1.170
cathepisn H 0.702 1012 (0.950 to 177) cathepisn D 0.812 1091 (0.947 to 11.8) cathepisn Z 0.483 0.962 (0.884 to 1.07) Cellac Disease (CD) cathepisn B 0.136 1055 (0.993 to 1.13) cathepisn B 0.483 0.962 (0.884 to 1.07) Cellac Disease (CD) cathepisn F 0.23 0.962 (0.884 to 1.07) cathepisn F 0.23 0.962 (0.874 to 1.30) 0.962 (0.874 to 1.30) cathepisn F 0.23 0.946 (0.990 to 1.95) 0.946 (0.990 to 1.95) cathepisn S 0.023 0.946 (0.990 to 0.99) 0.946 (0.990 to 0.99) cathepisn S 0.023 0.946 (0.990 to 1.93) 0.946 (0.990 to 1.93) cathepisn S 0.023 0.947 (0.947 to 1.30) 0.947 (0.947 to 1.30) cathepisn B 0.81 1007 (0.924 to 1.99) 0.933 (0.874 to 0.99) 0.933 (0.874 to 0.99) Diverticulosis cathepisn B 0.81 1004 (0.975 to 1.30) 0.946 (0.990 to 1.33) 0.947 (0.945 to 1.00) 0.946 (0.990 to 1.33) 0.947 (0.945 to 1.00) 0.946 (0.990 to 1.93) 0.947 (0.945 to 1.00) 0.946 (0.990 to 1.93) 0.947 (0.945 to 1.00) 0.946 (0.990 (0.83) to 1.01)		cathepisn G	0.498		1.038 (0.931 to 1.158)
cathepisn 2 022 - 1081 (0.944 to 123 cathepisn 2 0483 - 097 (0.933 to 15) cathepisn 2 0483 - 097 (0.933 to 15) cathepisn 6 049 - 097 (0.932 to 15) cathepisn 6 028 - 097 (0.932 to 15) cathepisn 6 028 - 097 (0.998 to 15) cathepisn 6 028 - 097 (0.998 to 15) cathepisn 6 028 - 097 (0.998 to 15) cathepisn 7 023 - 1056 (0.998 to 15) cathepisn 7 023 - 052 (0.974 to 123 cathepisn 7 023 - 052 (0.974 to 123 cathepisn 7 023 - 0942 (0.974 to 123 cathepisn 7 0023 - 0942 (0.974 to 123 cathepisn 7 0023 - 0942 (0.974 to 123 cathepisn 8 003 - 0932 (0.977 to 199 cathepisn 8 0162 - 0977 (0.945 to 100 cathepisn 6 0469 - 0977 (0.945 to 100 cathepisn 8 0162 - 0988 (0.961 to 100 cathepisn 8 0.81 - 0103 (0.986 to 100 cathepisn 8 0.81 - 0103 (0.986 to 100 cathepisn 8 0.77 - 0988 (0.981 to 101 cathepisn 8 0.78 - 0988 (0.981 to 101 cathepisn 8 0.78 - 0.998 (0.981 to 101 cathepisn 8 0.78 - 0.998 (0.981 to 101 cathepisn 8 0.78 - 0.998 (0.981 to 113 cathepisn 8 0.551 - 0.977 (0.973 to 124 cathepisn 8 0.921 - 0.9		cathepisn H	0.702	+	1.012 (0.950 to 1.079
Catalepisn B 0 0812 - 1079 08726 118 Cellac Disease (CD) Cellac Diseas		cathepisn L2	0.262		1.081 (0.944 to 1.238)
Celiac Disease (CD) Cathepins Z Cathepins B Cathepins B Cathepins B Cathepins B Cathepins B Cathepins B Cathepins C C Cathepins C Cathepin		cathenien S	0.012		1.019 (0.075 t0 1.180)
Celiac Disease (CD)		cathepish 3	0.483	_	0.962 (0.864 to 1.072)
cathepisn B 0.136 → 1055 (0.983 to 11.3) cathepisn F 0.236 → 0.976 (0.902 to 1.03) cathepisn F 0.236 → 0.952 (0.974 to 1.03) cathepisn C 0.926 0.992 (0.874 to 1.03) 0.996 (0.900 to 0.99 cathepisn C 0.903 → 0.932 (0.874 to 0.99 cathepisn S 0.033 → 0.932 (0.874 to 0.99 cathepisn S 0.831 → 0.932 (0.874 to 0.99 cathepisn S 0.811 1004 (0.975 to 1.03) cathepisn F 0.866 0.997 (0.900 to 1.03) cathepisn F 0.866 0.997 (0.900 to 1.03) cathepisn F 0.866 0.997 (0.900 to 1.03) cathepisn G 0.499 1.013 (0.998 to 1.04) cathepisn S 0.376 0.998 (0.981 to 1.01) cathepisn S 0.376 0.998 (0.981 to 1.01) cathepisn B 0.155 1.010 (0.998 to 1.04) cathepisn B 0.145 1.098 (0.971 to 1.22) cathepisn B 0.145 1.088 (0.971 to 1.22) cathepisn B 0.145 0.997 (0.893 to 1.17) cathepisn	Celiac Disease (CD)				
cathepisn E 0.542 → 0.976 (0.902 to 1.92) cathepisn F 0.23 → 1.066 (0.966 to 1.15) cathepisn I2 0.982 0.974 (0.900 to 0.92) cathepisn I2 0.983 0.932 (0.974 to 1.93) cathepisn Z 0.831 → 0.932 (0.974 to 0.99) cathepisn Z 0.831 → 0.937 (0.974 to 0.99) cathepisn E 0.162 → 0.977 (0.974 to 0.99) cathepisn E 0.162 → 0.977 (0.974 to 0.99) cathepisn F 0.866 → 0.977 (0.990 to 1.03) cathepisn F 0.866 → 0.977 (0.990 to 1.03) cathepisn B 0.162 → 0.977 (0.990 to 1.03) cathepisn B 0.469 → 1.013 (0.976 to 1.93) cathepisn B 0.145 → 1.038 (0.971 to 1.22) cathepisn B 0.145 → 1.038 (0.971 to 1.22) cathepisn B 0.145 → 1.088 (0.971 to 1.22) cathepisn B 0.145 → 1.088 (0.971 to 1.22) cathepisn B 0.145 → 1.088 (0.971 to 1.22)		cathepisn B	0.136		1.055 (0.983 to 1.131
cathepisn F 0.23 cathepisn H 0.033 cathepisn L 0.198 cathepisn D 0.03 cathepisn B 0.81 1007 (0.924 to 1.09 0.977 (0.923 to 1.03 cathepisn C 0.469 1.010 (0.989 to 1.03 cathepisn C 0.557 1.013 (0.989 to 1.03 cathepisn C 0.557 1.013 (0.989 to 1.03 cathepisn C 0.557 1.013 (0.989 to 1.03 cathepisn B 0.145 0.978 (0.933 to 1.25 cathepisn B 0.145 0.978 (0.933 to 1.25 cathepisn C 0.274 1.086 (0.971 to 1.22 cathepisn C 0.927 1.020 (0.944 to 1.10 cathepisn C 0.927 1.020 (0.944 to 1.10 cathepisn C 0.927 1.020 (0.944 to 1.10 cathepisn C 0.821 0.978 (0.833 to 1.15 cathepisn C 0.387 0.998 (0.830 to 1.17 1.087 (0.933 to 1.25 cathepisn C 0.387 0.998 (0.831 to 1.13 1.086 (0.971 to 1.22 1.020 (0.944 to 1.10 cathepisn C 0.77 0.998 (0.831 to 1.13 1.086 (0.971 to 1.22 1.020 (0.933 to 1.25 1.020 (0.933 to 1.17 1.020 (0.933 to 1.17 1.020 (0.933 to 1.17 1.087 (0.933 to 1.17 1.087 (0.933 to 1.17 1.097 (0.933 to 1.17		cathepisn E	0.542	-	0.976 (0.902 to 1.056
cathepin G 0.286 → 0.992 (0.874 to 10.30) cathepin L2 0.198 → 0.932 (0.837 to 10.30) cathepin S 0.023 → 0.932 (0.837 to 10.30) cathepin S 0.023 → 0.933 (0.874 to 0.99) cathepin S 0.023 → 0.933 (0.874 to 0.99) cathepin S 0.831 + 0.933 (0.874 to 0.99) cathepin S 0.81 + 0.937 (0.945 to 10.93) cathepin F 0.866 + 0.977 (0.945 to 10.93) cathepin F 0.866 + 0.977 (0.945 to 10.93) cathepin S 0.376 + 0.981 (0.937 to 1.95) cathepin S 0.376 + 0.981 (0.937 to 1.95) cathepin S 0.376 + 0.981 (0.937 to 1.92) cathepin S 0.376 + 0.981 (0.937 to 1.92) cathepin S 0.376 + 0.981 (0.937 to 1.92) cathepin S 0.376 + 0.981 (0.937 to 1.22) cathepin S 0.276 + 1.086 (0.977 to 1.22) cathepin S 0.276 + 0.981 (0.938 to 1.17) <td></td> <td>cathepisn F</td> <td>0.23</td> <td></td> <td>1.056 (0.966 to 1.155)</td>		cathepisn F	0.23		1.056 (0.966 to 1.155)
cathepisn H 0.033		cathepisn G	0.266		0.952 (0.874 to 1.038
cathepian D 0.023 cathepian O 0.023 cathepian O 0.023 0.033 (0.574 to 0.99 0.033) (0.574 to 0.99 0.097 (0.926 to 1.03) cathepian E Diverticulosis cathepian B 0.81 1.007 (0.924 to 1.09) 0.977 (0.945 to 1.00) cathepian F Cathepian F 0.866 0.997 (0.945 to 1.00) cathepian H 0.365 1.010 (0.999 to 1.03) cathepian H cathepian F 0.866 0.997 (0.945 to 1.00) cathepian I 0.557 1.013 (0.999 to 1.03) cathepian S cathepian F 0.866 0.997 (0.930 to 1.09) cathepian S 0.984 (0.933 to 1.09) cathepian S Non-alcoholic Fatty Liver Disease (NAFLD) cathepian B 0.145 0.986 (0.971 to 1.22) cathepian F cathepian B 0.145 0.987 (0.938 to 1.25) cathepian C 0.999 (0.830 to 1.17) cathepian C 0.999 (0.830 to 1.17) cathepian C cathepian B 0.145 0.987 (0.830 to 1.19) cathepian C 0.999 (0.830 to 1.17) cathepian C 0.999 (0.830 to 1.17) cathepian C cathepian B 0.145 0.987 (0.870 to 1.00) cathepian C 0.999 (0.830 to 1.17) cathepian C 0.999 (0.830 to 1.17) cathepian C 0.998 (0.830 to 1.17		cathepisn H	0.033		0.946 (0.900 to 0.995)
cathepins 7 0.03 0.033 (0.674 to 0.99 cathepins 7 0.881 1.007 (0.924 to 1.09 Diverticulosis cathepins B 0.81 1.007 (0.924 to 1.09 cathepins 6 0.866 0.997 (0.945 to 1.00 cathepins 6 cathepins 6 0.469 1.013 (0.976 to 1.03 cathepins 10 cathepins 10 0.557 1.013 (0.976 to 1.03 cathepins 10 0.557 cathepins 0 0.449 0.981 (0.933 to 1.03 cathepins 10 0.557 1.013 (0.976 to 1.06 cathepins 10 0.557 1.013 (0.976 to 1.06 cathepins 10 0.981 (0.933 to 1.03 cathepins 12 0.577 1.013 (0.976 to 1.06 cathepins 12 0.981 (0.933 to 1.03 cathepins 12 0.577 1.013 (0.976 to 1.06 cathepins 12 0.971 (0.853 to 1.06 cathepins 12 0.971 1.020 (0.940 to 1.10 cathepins 12 0.978 (0.803 to 1.17) cathepins 12 0.965 0.989 (0.801 to 1.17) cathepins 12 0.978 (0.803 to 1.17) cathepins 12 0.975 0.878 (0.760 to 1.01) cathepins 12 0.978 (0.80		cathenisn O	0.190		1 150 (1 020 to 1 208
cathepisn Z 0.881 1.007 (0.924 to 1.09 Diverticulosis cathepisn B 0.81 1.004 (0.975 to 1.03 cathepisn F 0.866 0.997 (0.945 to 1.00 0.997 (0.945 to 1.00 cathepisn F 0.866 0.997 (0.945 to 1.00 0.997 (0.945 to 1.00 cathepisn G 0.469 1.013 (0.978 to 1.03 0.997 (0.945 to 1.03 cathepisn G 0.449 0.991 (0.933 to 1.03 0.997 (0.945 to 1.03 cathepisn Z 0.764 0.981 (0.933 to 1.03 0.988 (0.961 to 1.01 cathepisn Z 0.764 0.981 (0.933 to 1.02 0.988 (0.961 to 1.01 cathepisn E 0.651 0.971 (0.853 to 1.05 0.971 (0.853 to 1.05 cathepisn F 0.621 0.988 (0.971 to 1.23 0.971 (0.853 to 1.05 cathepisn F 0.221 0.989 (0.830 to 1.17 0.989 (0.830 to 1.17 cathepisn F 0.363 0.998 (0.830 to 1.17 0.989 (0.830 to 1.17 cathepisn F 0.377 0.989 (0.830 to 1.17 0.978 (0.833 to 1.05 cathepisn F 0.377 0.998 (0.830 to 1.17 0.978 (0.833 to 1.05 cathepisn		cathepisn S	0.033	-•-	0.933 (0.874 to 0.994
Diverticulosis		cathepisn Z	0.881	+	1.007 (0.924 to 1.096
cathepisn B 0.81 1.004 (0.975 to 1.03) cathepisn F 0.866 0.997 (0.960 to 1.03) cathepisn F 0.866 0.997 (0.960 to 1.03) cathepisn H 0.365 1.013 (0.978 to 1.05) cathepisn S 0.365 1.010 (0.989 to 1.03) cathepisn S 0.376 0.988 (0.931 to 1.03) cathepisn S 0.376 0.988 (0.961 to 1.01) cathepisn S 0.376 0.988 (0.961 to 1.01) cathepisn B 0.145 0.986 (0.931 to 1.02) cathepisn B 0.145 0.988 (0.971 to 1.22) cathepisn B 0.145 1.068 (0.971 to 1.22) cathepisn B 0.145 0.987 (0.830 to 1.17) cathepisn C 0.829 0.856 (0.744 to 0.98) cathepisn C 0.872 0.989 (0.830 to 1.17) cathepisn B 0.429 0.987 (0.830 to 1.17) cathepisn B 0.353 0.999 (0.830 to 1.17) cathepisn B 0.374 0.989 (0.830 to 1.17) cathepisn B 0.374 0.989 (0.810 to 1.10) cathepisn B 0.374 0.976 (0.873 to 1.04) cathepisn B 0.374	Diverticulosis				
cathepisn F 0.866 0.997 (0.951 of 1.00 cathepisn F 0.866 0.997 (0.951 of 1.00 cathepisn G 0.469 1.013 (0.978 to 1.05 cathepisn C 0.557 1.013 (0.998 to 1.06 cathepisn S 0.376 0.988 (0.961 to 1.01 cathepisn S 0.376 0.988 (0.961 to 1.01 cathepisn B 0.445 0.988 (0.961 to 1.01 cathepisn B 0.445 0.988 (0.961 to 1.01 cathepisn B 0.145 0.971 (0.853 to 1.02 cathepisn B 0.274 0.985 to 1.05 cathepisn C 0.621 0.971 (0.853 to 1.03 cathepisn C 0.29 0.988 (0.971 to 1.22 cathepisn B 0.274 0.988 to 1.25 cathepisn C 0.29 0.988 (0.971 to 1.22 cathepisn B 0.274 0.988 to 1.10 cathepisn C 0.29 0.988 (0.830 to 1.17 cathepisn C 0.29 0.988 (0.830 to 1.17 cathepisn C 0.987 0.983 to 1.07 cathepisn C 0.377 0.985 to 1.05 cathepisn C 0.377 0.985 to 1.05 cathepisn C 0.374 0.989 (0.861 to 1.13 cathepisn C 0.374 0.999 (0.861 to 1.13 cathepisn C 0.387 0.993 (0.798 to 1.02 cathepisn C 0.387 0.993 (0.798 to 1.03 cathepisn C 0.367 0.933 (0.798 to 1.03 cathepisn C 0.361 1.020 (0.995 to 1.26 cathepisn C 0.365 1.005 0.996 (0.914 to 1.96 cathepisn C 0.956 1.005 0.996 (0.914 to 1.96 cathepisn C 0.966 1.009 (0.956 to 1.14 cathepisn C 0.066 1.00		cathepisn B	0.81	+	1.004 (0.975 to 1.033)
cathepisn G 0.469 1013 (0.978 to 105) cathepisn H 0.365 1.010 (0.989 to 103) cathepisn O 0.449 0.981 (0.933 to 103) cathepisn O 0.449 0.988 (0.961 to 101) cathepisn O 0.449 0.988 (0.961 to 101) cathepisn O 0.449 0.988 (0.961 to 101) cathepisn D 0.611 0.988 (0.961 to 101) cathepisn B 0.651 0.997 (0.900 to 1.083) Non-alcoholic Fatty Liver Disease (NAFLD) cathepisn F 0.274 1.045 (0.998 to 125) cathepisn F 0.274 0.989 (0.830 to 117) cathepisn S 0.821 0.998 (0.830 to 117) cathepisn S 0.821 0.995 (0.944 to 0.999 0.830 to 1191 cathepisn S 0.951 (0.855 to 105) cathepisn S 0.821 0.976 (0.830 to 101) cathepisn S 0.976 (0.875 to 105) Liver Cirrhosis cathepisn G 0.877 0.933 (0.798 to 104) 0.976 (0.875 to 105) cathepisn C 0.387 0.938 (0.753 to 104) 0.976 (0.873 to 104) 0.976 (0.873 to 104) cathepisn B 0.347 0.938 (0.753 to 104) 0.976 (0.873 to 104) 0.977 (0.873 to		cathepisn E	0.162	-	0.977 (0.945 to 1.009)
cathepian I 0.365 1.010 (0.989 to 1.03) cathepian I 0.355 1.010 (0.989 to 1.03) cathepian I 0.557 1.013 (0.989 to 1.06) cathepian I 0.576 0.988 (0.961 to 1.01) cathepian S 0.376 0.988 (0.961 to 1.01) cathepian S 0.376 0.988 (0.961 to 1.01) cathepian B 0.449 0.981 (0.933 to 1.02) cathepian B 0.45 1.045 (f 0.09 to 1.08) cathepian B 0.45 1.088 (0.971 to 1.22) cathepian F 0.274 1.086 (0.938 to 1.25) cathepian C 0.029 0.989 (0.830 to 1.17) cathepian C 0.905 0.989 (0.830 to 1.17) cathepian C 0.821 0.971 (0.853 to 1.05) cathepian C 0.821 0.978 (0.830 to 1.17) cathepian C 0.878 0.989 (0.861 to 1.01) cathepian C 0.878 0.989 (0.861 to 1.01) cathepian C 0.878		cathepisn F	0.800	T.	0.997 (0.960 to 1.035)
cathepisn L2 0.557 1.013 (0.969 to 1.06) cathepisn S 0.376 0.981 (0.933 to 1.03) cathepisn S 0.376 0.988 (0.971 to 1.22) cathepisn B 0.145 1.045 (1.009 to 1.08) cathepisn B 0.145 1.045 (1.009 to 1.08) cathepisn B 0.145 0.971 (0.853 to 1.10) cathepisn F 0.274 1.085 (0.938 to 1.25) cathepisn G 0.029 0.856 (0.744 to 0.98) cathepisn C 0.029 0.856 (0.744 to 0.98) cathepisn C 0.051 0.999 (0.810 to 1.17) cathepisn C 0.821 0.978 (0.803 to 1.19) cathepisn C 0.878 0.999 (0.810 to 1.10) cathepisn B 0.367 0.999 (0.810 to 1.10) cathepisn B 0.374 0.978 (0.760 to 1.01) cathepisn C 0.878 0.978 (0.760 to 1.01) cathepisn C 0.387 0.938 (0.753 to 1.17) cathepisn C 0.387 0.938 (0.753 to 1.17) cathepisn C 0.387 0.938 (0.753 to 1.17) cathepisn B 0.371		cathepisn H	0.365	•	1.010 (0.989 to 1.031
cathepisn S 0.449 0.981 (0.933 to 1.03 cathepisn Z 0.014 1.045 (1.090 to 1.08 Non-alcoholic Fatty Liver Disease (NAFLD) cathepisn B 0.145 1.088 (0.971 to 1.22 cathepisn F 0.029 0.878 (0.981 to 1.25) 0.878 (0.981 to 1.25) cathepisn F 0.029 0.858 (0.744 to 0.98 0.998 (0.931 to 1.17) cathepisn R 0.029 0.878 (0.931 to 1.17) 0.998 (0.931 to 1.17) cathepisn R 0.029 0.989 (0.931 to 1.17) 0.998 (0.931 to 1.17) cathepisn R 0.029 0.989 (0.831 to 1.17) 0.998 (0.831 to 1.17) cathepisn R 0.921 0.998 (0.831 to 1.17) 0.978 (0.831 to 1.17) cathepisn R 0.353 0.989 (0.861 to 1.33) 0.989 (0.861 to 1.13) Liver Cirnhosis cathepisn R 0.78 0.989 (0.861 to 1.13) cathepisn R 0.311 0.987 (0.873 to 1.04) 0.987 (0.873 to 1.04) cathepisn R 0.311 0.987 (0.873 to 1.04) 0.987 (0.873 to 1.04) cathepisn R 0.321 0.987 (0.873 to 1.04) 0.987 (0.873 to 1.04) cathepisn R 0.321 0.987 (0.772 to 0.97 0.373 to 1.04)		cathepisn L2	0.557	+	1.013 (0.969 to 1.060)
cathepian S 0.376 0.988 (0.961 to 1.01) cathepian Z 0.014 1.045 (1.009 to 1.08) Non-alcoholic Fatty Liver Disease (NAFLD) cathepian B 0.45 0.971 (0.853 to 1.10) cathepian F 0.274 1.086 (0.971 to 1.22) 0.986 (0.961 to 1.02) cathepian F 0.274 1.085 (0.934 to 2.96) 0.989 (0.830 to 1.10) cathepian H 0.627 1.020 (0.940 to 1.10) 0.998 (0.830 to 1.17) cathepian S 0.353 0.951 (0.855 to 1.05) 0.989 (0.830 to 1.17) cathepian S 0.353 0.951 (0.855 to 1.05) 0.989 (0.801 to 1.01) cathepian S 0.353 0.951 (0.855 to 1.05) 0.989 (0.801 to 1.01) cathepian F 0.377 0.933 (0.798 to 1.09) 0.933 (0.798 to 1.09) cathepian F 0.317 0.933 (0.798 to 1.09) 0.921 to 1.27 cathepian C 0.299 1.110 (0.101 to 1.30) 0.921 to 1.27 cathepian C 0.387 0.933 (0.798 to 1.09) 0.373 to 1.04 cathepian B 0.341 0.967 (0.873 to 1.04 0.973 (0.772 to 0.973 cathepian B		cathepisn O	0.449	-	0.981 (0.933 to 1.031
cathepisn Z 0.014 • 1.045 (1.008 to 1.08 Non-alcoholic Fatty Liver Disease (NAFLD) cathepisn B 0.145 1.088 (0.971 to 1.22 cathepisn F 0.274 1.088 (0.971 to 1.22 0.971 (0.853 to 1.10 cathepisn F 0.274 1.085 (0.938 to 1.25 0.985 (0.744 to 0.98 cathepisn G 0.029 0.856 (0.744 to 0.98 0.826 (0.744 to 0.98 cathepisn C 0.905 0.989 (0.830 to 1.17) 0.989 (0.830 to 1.17) cathepisn S 0.353 0.951 (0.855 to 1.05) 0.989 (0.830 to 1.17) cathepisn S 0.367 0.989 (0.810 to 1.01) 0.878 (0.760 to 1.01) cathepisn F 0.778 0.878 (0.760 to 1.01) 0.878 (0.760 to 1.01) cathepisn G 0.387 0.938 (0.751 to 1.16) 0.957 (0.873 to 1.16) cathepisn S 0.367 0.938 (0.753 to 1.16) 0.938 (0.753 to 1.16) cathepisn S 0.021 0.878 (0.760 to 1.01) 0.878 (0.760 to 1.01) 0.878 (0.760 to 1.01) cathepisn C 0.878 0.377 0.938 (0.753 to 1.16) 0.937 (0.727 to 0.97 cathepisn S 0.627 0.938 (0.753 to 1.16) 0.938 (0.753 to 1.16) 0.938 (0.75		cathepisn S	0.376	4	0.988 (0.961 to 1.015)
Non-alcoholic Fatty Liver Disease (NAFLD) cathepisn B 0.145 1.088 (0.971 to 1.22) cathepisn F 0.274 0.971 to 1.22) 0.971 to 1.22) cathepisn F 0.274 0.971 to 1.22) 0.936 to 1.10 cathepisn F 0.274 0.956 (0.744 to 0.98) 0.936 to 1.25 cathepisn G 0.029 0.956 (0.744 to 0.98) 0.980 to 1.10 cathepisn C 0.905 0.989 (0.830 to 1.17) 0.978 (0.803 to 1.19) cathepisn O 0.821 0.978 (0.803 to 1.19) 0.978 (0.803 to 1.19) cathepisn Z 0.876 0.978 (0.801 to 1.17) 0.978 (0.760 to 10.10) cathepisn B 0.034 1.149 (1.011 to 1.30) 0.878 (0.760 to 10.11) cathepisn F 0.37 0.778 (0.760 to 10.12) 0.878 (0.760 to 10.12) cathepisn F 0.387 0.998 (0.871 to 1.13) 0.877 (0.873 to 1.04) cathepisn C 0.387 0.878 (0.760 to 10.16) 0.877 (0.720 to 9.70) cathepisn C 0.387 0.878 (0.760 to 10.16) 0.878 (0.760 to 10.16) cathepisn C 0.387 0.879 (0.772 to 9.77) 0.878 (0.772		cathepisn Z	0.014	-	1.045 (1.009 to 1.083,
cathepisn E 0.851 0.971 (0.8310 1.10) cathepisn F 0.274 0.08310 1.10) cathepisn G 0.029 0.856 (0.744 to 0.98) cathepisn H 0.827 1.020 (0.940 to 1.10) cathepisn L 0.905 0.999 (0.830 to 1.17) cathepisn S 0.821 0.978 (0.803 to 1.17) cathepisn S 0.821 0.979 (0.8310 to 1.10) cathepisn S 0.821 0.979 (0.8310 to 1.17) cathepisn B 0.353 0.981 (0.855 to 1.05) cathepisn E 0.878 0.989 (0.861 to 1.33) cathepisn F 0.878 0.989 (0.861 to 1.33) cathepisn F 0.311 0.987 (0.760 to 101) cathepisn B 0.367 0.933 (0.780 to 101) cathepisn S 0.267 0.933 (0.780 to 101) cathepisn B 0.321 0.870 (0.772 to 0.97) cathepisn B 0.221 0.870 (0.772 to 0.97) cathepisn B 0.221 0.870 (0.772 to 0.97) cathepisn B 0.221 0.870 (0.772 to 0.97) cathepisn B 0.281 1.028 (0.981 to 1.13) cathepisn B 0.221 0	Non-alcoholic Fatty Liver Disease (NAFL	D) cathonish B	0.145		1.088 (0.971 to 1.220)
cathepisn F 0.274 1.085 (0.938 to 1.25 cathepisn H 0.627 1.020 (0.940 to 1.10) cathepisn L2 0.905 0.989 (0.830 to 1.17) cathepisn S 0.821 0.976 (0.830 to 1.17) cathepisn S 0.821 0.976 (0.830 to 1.19) cathepisn S 0.821 0.976 (0.830 to 1.19) cathepisn S 0.831 0.976 (0.830 to 1.19) cathepisn S 0.821 0.989 (0.861 to 1.13) Liver Cirrhosis cathepisn B 0.034 1.149 (1.011 to 1.30) cathepisn G 0.887 0.933 (0.798 to 10.91) cathepisn G 0.387 0.933 (0.798 to 10.91) cathepisn G 0.387 0.933 (0.798 to 10.92) cathepisn G 0.387 0.933 (0.798 to 10.92) cathepisn G 0.387 0.933 (0.798 to 1.92) cathepisn C 0.396 0.938 (0.753 to 1.146) cathepisn B 0.028 1.110 (0.912 to 135) cathepisn C 0.301 1.086 (0.929 to 1.26) cathepisn B 0.028 1.108 (0.922 to 1.22) cathepisn G 0.935 0.996 (0.914 to 1.98) cat		cathepisn E	0.651		0.971 (0.853 to 1.104)
cathepisn B 0.029 0.856 (0.744 to 0.98 cathepisn L2 0.905 0.999 (0.830 to 1.17) cathepisn S 0.821 0.978 (0.803 to 1.19) 0.989 (0.830 to 1.17) cathepisn S 0.353 0.951 (0.855 to 1.05) 0.999 (0.830 to 1.17) cathepisn Z 0.878 0.999 (0.855 to 1.05) 0.999 (0.815 to 1.17) cathepisn Z 0.878 0.999 (0.815 to 1.05) 0.878 (0.760 to 1.01) cathepisn E 0.078 0.878 (0.760 to 1.01) 0.878 (0.760 to 1.01) cathepisn G 0.387 0.939 (0.871 to 1.27) 0.878 (0.760 to 1.01) cathepisn G 0.387 0.939 (0.751 to 1.16) 0.957 (0.873 to 1.04) cathepisn S 0.221 0.938 (0.753 to 1.16) 0.938 (0.753 to 1.16) cathepisn Z 0.281 0.121 to 1.353 0.221 0.878 (0.760 to 1.01) cathepisn Z 0.290 1.110 (0.973 to 1.16) 0.938 (0.753 to 1.16) 0.939 (0.753 to 1.16) cathepisn Z 0.281 0.301 1.086 (0.929 to 1.26) 0.270 (0.722 to 0.97) cathepisn E 0.21 0.221 0.932 (0.852 to 1.02) 0.232 (0		cathepisn F	0.274		1.085 (0.938 to 1.254
cathepisn L2 0.005 0.0989 (0.830 to 1.107 cathepisn S0 0.821 0.997 (0.803 to 1.17) cathepisn S0 0.821 0.997 (0.803 to 1.17) cathepisn S0 0.821 0.997 (0.803 to 1.17) cathepisn Z 0.876 0.999 (0.861 to 1.13) cathepisn Z 0.876 0.999 (0.861 to 1.13) cathepisn E 0.078 0.999 (0.861 to 1.13) cathepisn E 0.078 0.978 (0.760 to 1.01) cathepisn E 0.078 0.978 (0.760 to 1.01) cathepisn E 0.078 0.978 (0.760 to 1.01) cathepisn B 0.317 0.933 (0.798 to 1.02) cathepisn C 0.567 0.938 (0.753 to 1.14) cathepisn C 0.567 0.938 (0.753 to 1.14) cathepisn C 0.567 0.938 (0.751 to 1.16) cathepisn F 0.321 0.872 to 0.972 cathepisn F 0.321 0.967 (0.872 to 0.97) cathepisn F 0.321 0.967 (0.972 to 0.97) cathepisn F 0.128 0.996 (0.914 to 1.86) cathepisn F 0.128 0.996 (0.914 to 1.96) cathepisn F 0.128		cathepisn G	0.029		0.856 (0.744 to 0.984)
cathepisn 12 0.905 0.989 (0.830 to 1.77 cathepisn 2 0.821 0.978 (0.830 to 1.97 cathepisn S 0.353 0.951 (0.855 to 1.05 cathepisn Z 0.878 0.999 (0.831 to 1.97 Liver Cirrhosis cathepisn B 0.034 1.149 (1.011 to 1.30 cathepisn F 0.074 0.878 (0.780 to 1.91 cathepisn F 0.317 1.087 (0.923 to 1.27) cathepisn F 0.347 0.933 (0.793 to 1.94 cathepisn H 0.347 0.933 (0.793 to 1.94 cathepisn R 0.867 0.938 (0.753 to 1.94 cathepisn R 0.027 0.938 (0.753 to 1.94 cathepisn R 0.027 0.938 (0.753 to 1.94 cathepisn Z 0.021 1.086 (0.921 to 1.26 cathepisn R 0.022 0.935 0.936 (0.781 to 1.97 cathepisn R 0.028 - 1.086 (0.921 to 1.91 cathepisn R 0.028 - 0.870 (0.893 to 1.94 cathepisn R 0.028 - 0.930 (0.931 to 1.94 cathepisn R 0.028 - 0.870 (0.931 to 1.94 cathepisn R 0.028		cathepisn H	0.627		1.020 (0.940 to 1.107)
cathepisn 0 0.821 0.978 (0.830 to 1.19) cathepisn S 0.878 0.989 (0.861 to 1.13) cathepisn Z 0.878 0.989 (0.861 to 1.13) cathepisn B 0.034 1.149 (1.011 to 1.30) cathepisn F 0.317 0.878 (0.760 to 1.01) cathepisn F 0.317 0.878 (0.760 to 1.01) cathepisn G 0.387 0.933 (0.798 to 1.09) cathepisn G 0.567 0.938 (0.753 to 1.04) cathepisn D 0.567 0.938 (0.753 to 1.16) cathepisn Z 0.294 1.110 (0.912 to 1.35) cathepisn B 0.026 1.086 (0.929 to 1.26) Cholecystitis cathepisn B 0.027 0.932 (0.721 to 0.97) cathepisn E 0.416 0.997 (0.833 to 1.04) 0.928 to 1.20 cathepisn B 0.022 1.082 (0.021 to 1.27) 0.932 (0.852 to 1.02) cathepisn C 0.935 0.996 (0.914 to 1.08) 0.996 (0.914 to 1.08) cathepisn		cathepisn L2	0.905		0.989 (0.830 to 1.179)
cathepisn Z 0.367 0.369 (0.851 0 1.15) Liver Cirrhosis cathepisn Z 0.878 0.989 (0.811 0 1.13) cathepisn B 0.034 1.149 (1.011 0 1.30) cathepisn B 0.078 0.878 (0.760 to 1.01) cathepisn F 0.078 0.878 (0.760 to 1.01) cathepisn F 0.377 0.933 (0.780 to 1.02) cathepisn G 0.387 0.937 (0.760 to 1.01) cathepisn H 0.341 0.957 (0.873 to 1.04) cathepisn D 0.567 0.938 (0.753 to 1.16) cathepisn Z 0.291 0.878 (0.720 to 3.77) cathepisn Z 0.301 0.876 (0.722 to 3.77) cathepisn Z 0.301 0.876 (0.722 to 3.77) cathepisn F 0.221 0.870 (0.722 to 3.77) cathepisn F 0.321 1.086 (0.929 to 1.26) Cholecystitis cathepisn F 0.282 cathepisn F 0.128 0.932 (0.852 to 1.02) cathepisn F 0.128 0.932 (0.852 to 1.02) cathepisn F 0.128 0.932 (0.852 to 1.02) cathepisn F 0.283 to 1.041 0.096 (0.914 to 1.06) cathepisn		cathepisn O	0.821		0.978 (0.803 to 1.190)
Liver Cirrhosis cathepisn E 0.076 0.390 (0.011 nl 1.13) cathepisn E 0.078 1.149 (1.011 nl 1.30) cathepisn E 0.078 0.878 (0.760 tn 1.01) cathepisn F 0.317 1.067 (0.923 tn 1.27) cathepisn F 0.317 0.933 (0.780 tn 1.09) cathepisn H 0.441 0.957 (0.873 tn 1.04) cathepisn L2 0.298 1.110 (0.912 tn 1.35) cathepisn S 0.627 0.938 (0.753 tn 1.16) cathepisn S 0.627 0.938 (0.753 tn 1.16) cathepisn S 0.021 0.927 (0.772 to 0.27) cathepisn S 0.021 0.930 (0.971 tn 1.73) cathepisn S 0.021 0.930 (0.71 tn 1.07) cathepisn S 0.021 0.930 (0.71 tn 1.73) cathepisn B 0.028 1.082 (1.008 tn 1.61) cathepisn B 0.028 0.930 (0.91 tn 1.08) cathepisn B 0.028 0.930 (0.91 tn 1.08) cathepisn B 0.028 0.930 (0.91 tn 1.08) cathepisn B 0.856 0.998 (0.91 tn 1.17 cathepisn C		cathepisn S	0.353		0.951 (0.855 to 1.057)
cathopisn B 0.034 1.149 (1.011 to 1.30. cathopisn F 0.378 0.878 (0.760 to 1.01) cathopisn F 0.317 1.087 (0.923 to 1.27) cathopisn G 0.387 0.933 (0.798 to 1.09) cathopisn G 0.387 0.933 (0.798 to 1.09) cathopisn L2 0.299 1.110 (0.912 to 1.35) cathopisn D 0.567 0.938 (0.753 to 1.04) cathopisn D 0.567 0.938 (0.753 to 1.16) cathopisn Z 0.021 1.080 (0.929 to 1.28) cathopisn B 0.022 0.872 (0.721 to 0.97) cathopisn F 0.16 0.997 (0.832 to 1.02) cathopisn B 0.028 1.082 (1.008 to 1.16) cathopisn F 0.128 0.932 (0.852 to 1.20) cathopisn G 0.935 0.996 (0.924 to 1.28) cathopisn G 0.935 0.996 (0.914 to 1.08) cathopisn G 0.935 0.996 (0.914 to 1.17) cathopisn C 0.321 1.056 (0.947 to 1.17) cathopisn C 0.856 0.996 (0.975 to 1.06) cathopisn S 0.561 1.020 (0.955 to 1.06) cathopisn F 0.021	Liver Cirrhosis	caulepish Z	0.070		0.909 (0.001 10 1.136)
cathepisn E 0.078 → 0.878 (0.760 to 1.01; cathepisn G 0.317 → 1.087 (0.923 to 1.27; cathepisn G 0.387 → 0.933 (0.798 to 1.09; cathepisn G 0.387 → 0.933 (0.798 to 1.09; cathepisn L2 0.298 → 1.110 (0.912 to 1.35; cathepisn O 0.567 → 0.938 (0.753 to 1.14); cathepisn S 0.627 → 0.870 (0.772 to 0.97; cathepisn Z 0.301 → 1.086 (0.929 to 1.26; Cholecystitis - 1.086 (0.929 to 1.26; 0.932 (0.852 to 1.02; cathepisn E 0.416 - 0.967 (0.833 to 1.44; cathepisn F 0.128 - 0.932 (0.852 to 1.02; cathepisn F 0.283 - 1.055 (0.947 to 1.17; cathepisn S 0.561 1.002 (0.9		cathepisn B	0.034		1.149 (1.011 to 1.305)
cathepisn F 0.317 → 1.087 (0.921 to 1.27) cathepisn G 0.387 → 0.933 (0.798 to 10.97) cathepisn H 0.341 → 0.957 (0.873 to 1.04) cathepisn Q 0.567 → 0.938 (0.798 to 10.97) cathepisn Q 0.567 → 0.938 (0.758 to 1.16) cathepisn Z 0.301 → 0.870 (0.772 to 0.97) cathepisn Z 0.301 → 0.870 (0.772 to 0.97) cathepisn F 0.301 → 0.870 (0.772 to 0.97) cathepisn F 0.301 → 0.880 (0.929 to 1.26) Cholecystitis - 0.938 (0.875 to 1.16) 0.937 (0.893 to 10.44) cathepisn F 0.128 → 0.996 (0.914 to 1.08) cathepisn F 0.128 → 0.996 (0.914 to 1.08) cathepisn H 0.741 1.009 (0.955 to 1.111) 1.028 (0.951 to 1.111) cathepisn S 0.561 1.022 (0.955 to 1.132) 1.048 (0.962 to 1.142) Choleithiasis - 0.996 0.914 (0.975 to 1.114) 0.909 (0.955 to 1.015) cathepisn B 0.221 1.048 (0.962 to 1.142)		cathepisn E	0.078		0.878 (0.760 to 1.015
cathepisn G 0.387 → 0.933 (0.781 to 1.09; cathepisn L2 0.299 → 1.110 (0.912 to 1.35) cathepisn S 0.667 0.938 (0.753 to 1.16) cathepisn S 0.621 0.838 (0.753 to 1.16) cathepisn S 0.021 0.870 (0.772 to 0.97) cathepisn Z 0.301 → 0.870 (0.772 to 0.97) cathepisn Z 0.301 → 0.806 (0.929 to 1.26) Cholecystitis - 0.870 (0.893 to 1.04) cathepisn F 0.228 → 0.802 (0.929 to 1.26) Cholecystitis - 0.807 (0.893 to 1.04) cathepisn F 0.289 → 0.932 (0.852 to 1.02) cathepisn G 0.935 → 0.996 (0.914 to 1.06) cathepisn H 0.741 1.009 (0.959 to 1.06) cathepisn Z 0.321 → 1.058 (0.947 to 1.17) cathepisn S 0.561 + 1.020 (0.955 to 1.02) cathepisn F 0.283 → 1.048 (0.902 to 1.14) Choleithiasis - 1.048 (1.007 to 1.09) cathepisn F 0.099 + 1.034 (0.982 to 1.02) cathepisn F 0.099 + 1.048 (1.007 to 1.09) cathepisn F 0.099 + 1.048 (1		cathepisn F	0.317		1.087 (0.923 to 1.279
cathepisn L 0.341 → 0.957 (0.873 to 1.04) cathepisn L2 0.299 → 1.101 (0.912 to 1.35) cathepisn O 0.567 0.938 (0.753 to 1.16) cathepisn Z 0.021 → 0.870 (0.772 to 0.97) cathepisn Z 0.301 → 1.086 (0.929 to 1.26) Cholecystitis cathepisn B 0.028 → 1.082 (1.008 to 1.16) cathepisn E 0.416 0.997 (0.833 to 1.04) → 0.932 (0.852 to 1.02) cathepisn E 0.416 0.997 (0.833 to 1.04) → 0.996 (0.924 to 1.26) cathepisn F 0.128 → 0.996 (0.924 to 1.26) cathepisn F 0.168 0.997 (0.833 to 1.04) 0.932 (0.852 to 1.02) cathepisn H 0.741 1.009 (0.955 to 1.05) 0.996 (0.914 to 1.07) cathepisn S 0.561 - 0.996 (0.914 to 1.17) cathepisn S 0.561 - 0.996 (0.914 to 1.17) cathepisn B 0.232 - 1.048 (0.962 to 1.14) Choleithiasis - 0.996 (0.914 to 1.09) - cathepisn B 0.021 - 1.048 (0.962 to 1.14) Choleithiasis - 0.996 (0.914 to 1.09) - cathepisn F 0.029 - <		cathepisn G	0.387		0.933 (0.798 to 1.092
cathepisn 0 0.567 0.338 cathepisn 0 0.567 0.338 cathepisn 2 0.301 0.872 (0.772 to 0.97 cathepisn 2 0.301 0.876 (0.729 to 0.97 cathepisn 6 0.416 0.967 (0.893 to 1.04 cathepisn 6 0.416 0.967 (0.893 to 1.04 cathepisn 7 0.128 0.932 (0.852 to 1.02 cathepisn 10 0.741 1.009 (0.954 to 1.06 cathepisn 12 0.332 0.966 (0.929 to 1.08 cathepisn 5 0.561 1.020 (0.955 to 1.08 cathepisn 8 0.856 0.989 to 1.05 cathepisn 8 0.651 1.020 (0.955 to 1.08 cathepisn 8 0.621 1.048 (1.007 to 1.09 cathepisn 9 0.094 0.934 to 1.04 cathepisn 9 0.094 0.935 to 1.08 cathepisn 9 0.094 0.936 to 1.05 cathepisn 10 0.096 0.934 to 1.04 cathepisn 10		cathepisn H	0.341		0.957 (0.873 to 1.048
cathepisn S 0.021 0.830 (0.731 to 1.05) cathepisn S 0.021 0.870 (0.772 to 0.87) cathepisn Z 0.301 1.086 (0.929 to 1.26) Cholecystitis cathepisn B 0.028 1.082 (1.008 to 1.16) cathepisn F 0.128 0.935 (0.731 to 1.07) 0.937 (0.893 to 1.04) cathepisn F 0.128 0.936 (0.929 to 1.26) 0.937 (0.893 to 1.04) cathepisn F 0.128 0.936 (0.929 to 1.02) 0.936 (0.951 to 1.02) cathepisn G 0.935 0.996 (0.914 to 1.08) 0.937 to 1.07) cathepisn G 0.935 0.996 (0.914 to 1.08) 0.947 to 1.17) cathepisn C 0.856 0.999 (0.875 to 1.11) 0.947 to 1.17) cathepisn D 0.856 0.999 (0.875 to 1.11) 0.947 to 1.170) cathepisn Z 0.283 1.048 (0.962 to 1.14) 0.910 (0.955 to 1.08) cathepisn E 0.691 1.009 (0.955 to 1.02) 0.934 (0.881 to 1.08) cathepisn F 0.099 0.934 (0.881 to 1.08) 0.914 to 1.080 0.961 to 1.12 cathepisn I 0.066 1.0017 (0.986 to 1.08) 0.914 to 1.080 0.934 (0.881 to 1.08) 0.914 to 1.0		cathepisn L2	0.298		1.110 (0.912 to 1.350
cathepisn Z 0.01 1.086 (0.929 to 1.26) Cholecystitis		cathepish O	0.001		0.938 (0.753 to 1.169 0.870 (0.772 to 0.979
Cholecystitis cathepisn B 0.028 1.062 (f.008 to 1.16 cathepisn E 0.416 0.957 (0.839 to 1.04) cathepisn F 0.128 0.932 (0.852 to 1.02) cathepisn G 0.935 0.996 (0.914 to 1.06) cathepisn H 0.741 1.009 (0.959 to 1.06) cathepisn S 0.332 1.055 (0.947 to 1.17) cathepisn S 0.561 1.020 (0.955 to 1.08) cathepisn S 0.561 1.020 (0.955 to 1.08) cathepisn B 0.021 1.048 (0.902 to 1.14) cathepisn B 0.021 1.048 (0.902 to 1.02) cathepisn B 0.021 1.048 (0.902 to 1.14) cathepisn B 0.021 1.048 (0.902 to 1.14) cathepisn B 0.021 1.048 (0.902 to 1.14) Cholelithiasis cathepisn B 0.021 1.048 (0.902 to 1.14) cathepisn B 0.021 1.048 (0.902 to 1.14) 1.009 (0.965 to 1.05) cathepisn B 0.021 1.014 (0.903 to 1.14) 1.014 (0.993 to 1.12) cathepisn F 0.009 0.934 to 1.06 1.006 (0.996 to 1.12) <td< td=""><td></td><td>cathepisn Z</td><td>0.301</td><td></td><td>1.086 (0.929 to 1.268</td></td<>		cathepisn Z	0.301		1.086 (0.929 to 1.268
cathepisn B 0.028 1.082 (1.008 to 1.16 cathepisn F 0.416 0.997 (0.833 to 1.04 cathepisn F 0.128 0.932 (0.852 to 1.02 cathepisn F 0.128 0.932 (0.852 to 1.02 cathepisn G 0.935 0.996 (0.914 to 1.08 cathepisn G 0.935 0.996 (0.914 to 1.08 cathepisn I.2 0.332 1.055 (0.947 to 1.17) cathepisn O 0.856 0.989 (0.875 to 1.11) cathepisn S 0.561 1.020 (0.955 to 1.08) cathepisn Z 0.283 1.048 (0.962 to 1.14) Cholelithiasis cathepisn B 0.021 1.048 (0.962 to 1.14) Cholelithiasis cathepisn F 0.009 0.934 (0.888 to 0.98) cathepisn F 0.009 0.934 (0.888 to 0.98) cathepisn F cathepisn H 0.432 1.017 (0.988 to 1.08) cathepisn H cathepisn H 0.24 1.060 (0.996 to 1.12) cathepisn H 0.24 0.059 (0.895 to 1.02) cathepisn S 0.21 1.016 (0.996 to 1.12) cathepisn S 0.24 0.959 (0.895 to 1.02) <td>Cholecystitis</td> <td></td> <td></td> <td>1</td> <td></td>	Cholecystitis			1	
cathepisn E 0.416 0.967 (0.833 to 1.04 cathepisn F 0.128 0.932 (0.852 to 1.02 cathepisn G 0.935 0.996 (0.914 to 1.08 cathepisn G 0.935 0.996 (0.914 to 1.08 cathepisn L 0.332 1.055 (0.947 to 1.17 cathepisn D 0.855 0.999 (0.875 to 1.14) cathepisn D 0.856 0.999 (0.875 to 1.14) cathepisn D 0.856 0.999 (0.875 to 1.14) cathepisn Z 0.283 1.048 (0.962 to 1.14) Choleithiasis cathepisn E 0.691 1.048 (0.962 to 1.02) cathepisn F 0.090 0.934 (0.881 to 1.08) cathepisn F cathepisn F 0.090 0.934 (0.881 to 1.08) cathepisn L 1.017 (0.988 to 1.08) cathepisn H 0.432 1.017 (0.980 to 1.04) 1.048 (0.962 to 1.02) 1.048 (0.962 to 1.02) cathepisn S 0.515 1.017 (0.980 to 1.06) 1.017 (0.980 to 1.06) 1.017 (0.980 to 1.06) cathepisn S 0.515 1.017 (0.980 to 1.06) 1.014 (0.975 to 1.02) 1.014 (0.977 to 1.05) 1.014 (0.977 to 1.05)		cathepisn B	0.028		1.082 (1.008 to 1.161
cathepisn F 0.128 ● 0.932 (0.852 to 1.02) cathepisn G 0.935 ● 0.936 (0.914 to 1.05) cathepisn H 0.741 1.009 (0.959 to 1.02) cathepisn H 0.741 1.009 (0.959 to 1.06) cathepisn N 0.856 0.989 (0.875 to 1.17) cathepisn S 0.561 1.020 (0.955 to 1.08) cathepisn Z 0.283 ● 1.048 (0.962 to 1.14) Choleithiasis 0.291 • 1.048 (1.007 to 1.09) cathepisn B 0.021 • 1.048 (1.007 to 1.09) cathepisn F 0.099 • 0.934 (0.888 to 1.06) cathepisn F 0.099 • 0.934 (0.988 to 1.06) cathepisn H 0.432 • 1.017 (0.988 to 1.06) cathepisn L 0.066 • 1.006 (0.995 to 1.12) cathepisn R 0.421 • 1.017 (0.988 to 1.06) cathepisn R 0.422 • 1.017 (0.988 to 1.06) cathepisn R 0.244 • 0.959 (0.855 to 1.02) cathepisn R 0.244 • 0.959 (0.855 to 1.02) cathepisn S 0.471 • 1.014 (0.977 to 1.05) cathepisn Z 0.471 • 1.046 (0.971 to 1.052) cathepisn Z <td></td> <td>cathepisn E</td> <td>0.416</td> <td></td> <td>0.967 (0.893 to 1.048)</td>		cathepisn E	0.416		0.967 (0.893 to 1.048)
cathepisn G 0.935 0.996 (0.914 to 1.08 cathepisn H 0.741 1.009 (0.959 to 1.06 cathepisn L2 0.332 1.055 (0.947 to 1.17 cathepisn S 0.856 0.989 to 1.06 cathepisn S 0.561 1.020 (0.955 to 1.08 cathepisn S 0.561 1.020 (0.955 to 1.08 cathepisn S 0.561 1.020 (0.955 to 1.08 cathepisn B 0.21 1.048 (1.007 to 1.09 cathepisn F 0.009 0.936 to 1.05 cathepisn H 0.432 1.012 (0.938 to 1.04 cathepisn 12 0.066 1.060 (0.996 to 1.12 cathepisn 2 0.471 1.014 (0.977 to 1.05 cathepisn S 0.471 1.014 (0.977 to 1.05 cathepisn Z 0.471 1.014 (0.977 to 1.05		cathepisn F	0.128		0.932 (0.852 to 1.020)
cathepisn L 0.741 1.009 (0.959 to 1.06 cathepisn L2 0.332 1.055 (0.947 to 1.17) cathepisn S 0.856 0.989 (0.875 to 1.11) cathepisn Z 0.283 1.048 (0.962 to 1.08) cathepisn Z 0.283 1.048 (0.962 to 1.14) Cholelithiasis cathepisn B 0.021 1.048 (1.007 to 1.09) cathepisn F 0.009 0.934 (0.888 to 0.98) cathepisn F cathepisn F 0.009 0.934 (0.988 to 0.98) cathepisn H 0.432 1.017 (0.988 to 1.06) cathepisn H 0.432 1.017 (0.988 to 1.08) 1.048 (0.996 to 1.05) 1.048 (0.996 to 1.05) cathepisn H 0.432 1.017 (0.988 to 1.08) 1.017 (0.988 to 1.08) 1.017 (0.988 to 1.08) cathepisn H 0.24 0.066 1.060 (0.996 to 1.12) cathepisn S 0.24 0.959 (0.895 to 1.02) cathepisn S 0.24 0.066 1.060 (0.996 to 1.12) cathepisn S 0.24 0.959 (0.895 to 1.02) cathepisn S 0.24 0.959 (0.895 to 1.02) cathepisn S 0.471 1.014 (0.977 to 1.05) cathepisn Z 0.04 1.052 (1.002 to 1.10) <		cathepisn G	0.935	-	0.996 (0.914 to 1.087)
categrant.c 0.352 1.050 (0.947 / 10.1.7/ cathepisn O 0.856 0.989 (0.875 to 1.11) cathepisn S 0.561 1.020 (0.955 to 1.08) cathepisn Z 0.283 1.048 (0.962 to 1.14) Choleithiasis cathepisn B 0.021 1.048 (1.007 to 1.09) cathepisn F 0.090 0.934 (0.968 to 1.05) cathepisn F cathepisn F 0.009 0.934 (0.968 to 1.05) cathepisn F cathepisn F 0.009 0.934 (0.968 to 1.06) cathepisn F cathepisn F 0.009 0.934 (0.968 to 1.06) cathepisn L cathepisn R 0.615 1.017 (0.986 to 1.06) cathepisn L cathepisn N 0.432 1.012 (0.983 to 1.02) cathepisn L cathepisn S 0.471 1.014 (0.977 to 1.05) cathepisn S 0.471 cathepisn Z 0.471 1.014 (0.977 to 1.05) cathepisn Z 0.04 0.052 (1.022 to 1.102)		cathepisn H	0.741		1.009 (0.959 to 1.061)
Cholelithiasis Cathepisn S 0.561 Cholelithiasis Cathepisn B 0.221 Cholelithiasis Cathepisn B 0.22 Cathepisn C 0.24 Chole Chole Cathepisn C 0.24 Chole		cathepish L2	0.856	_	0.989 (0.875 to 1 117
cathepisn Z 0.283 1.048 (0.962 to 1.14) Choleithiasis cathepisn B 0.021 1.048 (0.0962 to 1.14) Choleithiasis cathepisn B 0.021 1.048 (1.007 to 1.09) cathepisn F 0.091 1.009 (0.965 to 1.05) cathepisn G 0.691 1.009 (0.965 to 1.05) cathepisn G 0.515 1.017 (0.968 to 1.06) cathepisn H 0.432 1.012 (0.968 to 1.02) cathepisn H 0.432 1.0160 (0.996 to 1.12) cathepisn S 0.24 0.959 (0.855 to 1.02) cathepisn S 0.241 1.014 (0.977 to 10.5) cathepisn S 0.241 1.014 (0.971 to 10.5) cathepisn S 0.241 1.052 (1.002 to 1.10)		cathepisn S	0.561		1.020 (0.955 to 1.089
Choleitthiasis cathepisn B 0.021 1.048 (1.007 to 1.09 cathepisn E 0.691 1.009 (0.965 to 1.05 cathepisn F 0.009 0.934 (0.888 to 0.98 cathepisn F 0.009 0.934 (0.888 to 0.98 cathepisn H 0.432 1.012 (0.983 to 1.04 cathepisn H 0.432 1.012 (0.983 to 1.04 cathepisn D 0.24 0.959 (0.895 to 1.02 cathepisn S 0.471 1.014 (0.977 to 10.55 cathepisn Z 0.04 1.052 (1.002 to 1.102		cathepisn Z	0.283		1.048 (0.962 to 1.142
cathepian B 0.021 1.048 (1.071 to 1.09 cathepian E 0.691 1.009 (0.965 to 1.05) cathepian F 0.009 0.934 (0.888 to 0.98) cathepian F 0.009 0.934 (0.888 to 1.08) cathepian F 0.009 0.934 (0.988 to 1.08) cathepian H 0.432 1.017 (0.988 to 1.08) cathepian L2 0.066 1.060 (0.996 to 1.12) cathepian S 0.24 0.959 (0.895 to 1.02) cathepian S 0.471 1.014 (0.977 to 1.05); cathepian Z 0.04	Cholelithiasis				
cathepisn E 0.091 1.009 (0.965 to 1.05 cathepisn F 0.009 0.934 (0.988 to 0.98) cathepisn G 0.515 1.017 (0.988 to 1.06) cathepisn H 0.432 1.012 (0.983 to 1.02) cathepisn L2 0.066 1.060 (0.996 to 1.12) cathepisn S 0.24 0.959 (0.895 to 1.02) cathepisn S 0.471 1.014 (0.977 to 1.05) cathepisn Z 0.04		cathepisn B	0.021	-	1.048 (1.007 to 1.091)
cattepisn G 0.515 1.017 (0.988 to 1.08) cattepisn G 0.515 1.017 (0.988 to 1.08) cattepisn H 0.432 1.012 (0.983 to 1.04) cattepisn S 0.066 1.060 (0.996 to 1.12) cattepisn S 0.24 0.959 (0.895 to 1.02) cattepisn S 0.471 1.014 (0.977 to 10.55) cattepisn Z 0.04 1.052 (1.002 to 1.10)		cathepisn E	0.691		1.009 (0.965 to 1.056)
cathepisn H 0.432 1.017 (0.930 in 100 cathepisn H 0.432 1.012 (0.933 to 1.04) cathepisn L2 0.066 1.060 (0.996 to 1.12) cathepisn S 0.24 0.959 (0.895 to 1.02) cathepisn S 0.471 1.014 (0.977 to 105) cathepisn Z 0.04 1.052 (1.002 to 1.10)		cathenish G	0.515	-	1 017 (0 968 to 1 068
cathepisn L2 0.066 1.060 (0.996 to 1.12 cathepisn O 0.24 0.959 (0.895 to 1.02 cathepisn S 0.47 1.014 (0.977 to 1.05) cathepisn Z 0.04 1.052 (1.002 to 1.10)		cathepish G	0.432	+	1.012 (0.983 to 1.041
cathepisn O 0.24 0.959 (0.895 to 1.02) cathepisn S 0.471 1.014 (0.977 to 1.05) cathepisn Z 0.04 - 1.052 (1.002 to 1.10)		cathepisn L2	0.066	-	1.060 (0.996 to 1.127)
cathepisn S 0.471 1.014 (0.977 to 1.052 cathepisn Z 0.04 - 1.052 (1.002 to 1.102		cathepisn O	0.24		0.959 (0.895 to 1.028)
cathepisn Z 0.04 1.052 (1.002 to 1.10		cathepisn S	0.471	+	1.014 (0.977 to 1.052)
		cathepisn Z	0.04		1.052 (1.002 to 1.105)

Figure 8. Positive result of Multivariable MR (MVMR) analysis.

3.3. Heterogeneity testing, outlier removal and sensitivity analysis

The Cochrane Q Heterogeneity Test was employed to detect potential heterogeneity among SNPs, defined as a Q-pval < 0.05. In cases where heterogeneity was detected, we adopted inverse variance weighting (using a random-effects model with multiplication) as a more robust analytical approach and replaced the original results (Supplementary **Table S2**). The MR-PRESSO global test and MR-PRESSO outlier test were employed to identify and adjust for potential outliers in our dataset, thus mitigating the impact of horizontal pleiotropy.

After identifying these outliers using MR-PRESSO, we excluded them and repeated our MR analysis to ensure the stability of our results, thereby mitigating the influence of any single SNP on our findings. In addition, the MR-Egger intercept test was used to identify signs of horizontal pleiotropic effects, which manifested as intercept values close to 0 (P < 0.05). The results showing pleiotropy were excluded from the positive conclusions (Supplementary **Tables S2** and **S3**). However, after the removal of outliers, there was heterogeneity (P < 0.05) in the MR analysis of the CTSZ levels and diverticulosis. Supplementary **Table S2**, indicating that the findings were not robust and may necessitate further experimental validation.

The robustness of the findings was underscored by the "leave-one-out" analysis, which showed that the results remained unchanged when any individual SNP was removed (Supplementary **Table S2**).

4. Discussion

This study revealed previously unrecognized associations between specific cathepsins and non-cancerous digestive diseases, providing new insights into their roles in pathophysiology and disease progression.

Using 2-sample bidirectional MR analysis, we identified specific cathepsins as potential causal factors in these diseases. We established seven pairs of causal relationships between cathepsins and diseases, four of which remained independently causal after controlling for other cathepsins. Specifically, CTSZ emerged as an independent risk factor for diverticulosis, CTSB for cholecystitis and cholelithiasis, and CTSG as a protective factor against NAFLD. These findings broaden our understanding of the complex roles that cathepsins play in non-cancerous digestive diseases, and point to new avenues for further exploration.

To further our discussion on each positive outcome in the forward MR study:

The study revealed a positive association between CTSZ and diverticulosis, and that CTSB was associated with an increased risk of cholecystitis and cholelithiasis. We hypothesize that in the gallbladder, cholesterol crystals can cause the destabilization of lysosomal membranes, leading to the leakage of CTSB from lysosomes into the cytoplasm. Once in the cytoplasm, CTSB activates the NLRP3 inflammasome. The activated NLRP3 inflammasome recruits and activates caspase-1 through the adaptor protein ASC (apoptosis-associated speck-like protein). The activated caspase-1 promotes the processing and secretion of IL-1 β and IL-18. These cytokines are then released extracellularly, triggering an inflammatory response. This inflammatory response is likely to be involved in the development of cholecystitis and cholelithiasis [38–41]. To the best of our knowledge, the relationship between CTSZ

and diverticulosis has not been extensively documented in the current literature, underscoring the novelty of our findings and the potential for these associations to provide new insights for clinical studies.

We also observed a positive correlation between CTSZ and CG, aligning with previous findings by Teller et al., which reported a significant upregulation of CTSZ in CG patients infected with Helicobacter pylori, a condition that may contribute to further disease progression, potentially culminating in gastric cancer [42]. It is hypothesized that CTSZ does not participate in the degradation of the extracellular matrix. Instead [43], it is involved in the immune response induced by Helicobacter pylori, as well as subsequent inflammatory responses. In the gastric mucosa during the late stage of Helicobacter pylori infection, ribosomal protein P0 (RPLP0) and CTSZ are strongly colocalized and highly expressed, with distinct distribution selectivity in epithelial and inflammatory cells. Their interaction weakens G1-phase arrest, disrupts the cell apoptosis pathway, and promotes cell proliferation [42]. Among the cells mentioned above, H. pylori upregulates CTSZ in macrophages using the extracellular signal-regulated kinase 1/2 signaling pathway and in N87 cells via the JUN N-terminal Kinase pathway pathway [15]. Therefore, in the pathological process of chronic gastritis, CTSZ is not only involved in the immuno-inflammatory mechanisms but also related to cell apoptosis dysregulation. Overall, CTSZ emerges as a key factor in the intricate mechanism of chronic gastritis development induced by H. pylori.

Furthermore, we found that CTSL2 levels promote NAFLD. We hypothesize that CTSL2 might exert effects similar to cathepsin L (CTSL) in the context of NAFLD, potentially owing to their genetic proximity and functional similarities. Specifically, a positive association was observed between CTSL2 expression and NAFLD. While CTSL2 is predominantly expressed in the cornea, thymus, heart, brain, and skin [44], its relationship with liver-related diseases in humans has been less explored than that of CTSL, which has been proven to be linked to NAFLD [45]. We hypothesized that the similar functions of CTSL2 and CTSL in liver disease may be related to their highly adjacent gene loci on chromosomes, as well as their remarkably high homology of up to 78% [46]. Furthermore, it is noteworthy that despite the close relationship between human CTSL2 and human CTSL, mouse CTSL is the functional equivalent of human CTSL2, instead of human CTSL [44]. Thus, Manchanda M's mouse experiment may corroborate our findings, as it observed specific upregulation of CTSL in an induced liver fibrosis model, with diffuse and strong cytoplasmic staining of CTSL in hepatocytes and fibroblasts [47]. At present, there are relatively few papers on the direct report of the relationship between CTSL2 and NAFLD in humans, and the mechanism is not clear, and our findings need to be further explored and verified.

Interestingly, our results suggest that CTSG may play a protective role against NAFLD. In contrast, Toonen et al. observed no CTSG overexpression in NAFLD-affected mouse livers and noted that NAFLD mice with neutrophil elastase/CTSG double knockout showed improved metabolic profiles [48]. These findings suggest that CTSG is a potential risk factor rather than a protective factor, which is inconsistent with our results. This situation may be due to the fact that mice cannot fully mimic humans and it is difficult to simulate the in vivo physiological changes of humans as a whole. Perhaps it is necessary to pay attention to relevant clinical cases and conduct research. Traditionally, CTSG has been associated with intestinal inflammatory

processes, such as Crohn's disease [21] and UC [22]. However, recent findings indicate that it is also biosynthesized in non-inflamed intestinal mucosa, particularly within specialized epithelial cells [49]. This might explain the lack of a causal association between CTSG and intestinal inflammatory diseases in our study.

The primary strength of our study lies in the use of genetic markers as instrumental variables, which reduces confounding and reverse causality [50] and overcomes some of the limitations of RCTs. Genetic variants, which are inherited and unaffected by environmental factors [51], offer a more reliable proxy for exposure than traditional observational data. Moreover, while previous MR analyses were mainly occupied with cancerous digestive diseases, understanding non-cancerous digestive diseases is of utmost importance for public health. Our study rises to this challenge. This MR study has expanded the disease research scope allowing us to explore the relationships between cathepsins and a wider range of non-cancerous digestive diseases. It provides a more thorough understanding of the complex associations in this field. Besides, we conducted a MVMR analysis, which helps control the interference from other exposure factors when multiple exposure factors are incorporated simultaneously and more accurately assess the causal relationships between a single cathepsin and different diseases. However, there were several limitations to our study. First, the analysis mainly included individuals with European heritage, which limited the generalizability of the findings. Additionally, although we selected relatively reasonable instrumental variables based on the selection norms in existing literature, this does not completely rule out the impact of selection bias. Genetic data complexity and resource constraints may have led to the exclusion of potentially effective instrumental variables. If these overlooked variables are related to exposure and outcome variables, it could bias the estimated causal link between cathepsins and non-cancerous digestive diseases [27,52].

In addition, horizontal pleiotropy is another issue that cannot be ignored in MR studies. In this study, we adopted multiple methods to detect horizontal pleiotropy. When pleiotropy was detected, we re-ran MR analysis by excluding relevant SNPs. If pleiotropy persisted, we discarded the data, even if positive results were obtained. Although the methods we used attempted to correct the biases caused by pleiotropy to a certain extent, due to the complexity of the underlying mechanisms of horizontal pleiotropy, we were unable to completely eliminate its impact [29]. MR studies typically require larger sample sizes owing to their reduced statistical power [53]. Independent replication and experimental validation are essential for strengthening our findings.

5. Conclusion

In conclusion, our study sheds light on the potential roles of specific cathepsins in non-cancerous digestive diseases and identifies several novel associations that could inform future research:

Our research findings indicate that CTSB seemingly promotes the occurrence of both cholecystitis and cholelithiasis, which may be linked to the activation of the NLRP3 inflammasome. CTSZ has been observed to promote chronic gastritis and diverticulosis. The underlying mechanism is associated with immune-inflammatory processes. Diverticulosis is a novel discovery, as there has been no previous report on it. Higher levels of CTSL2 have been found to promote NAFLD and liver cirrhosis. Although we can assume that, in terms of function, CTSL in mice is equivalent to CTSL2 in humans, thus finding some support for our conclusion. However, considering the differences between mice and humans, further research is required to demonstrate the direct causal effect of CTSL2 on NAFLD in humans. In addition, we have discovered that CTSG reduces the risk of NAFLD, which contradicts the existing literature on mouse models. This discrepancy may also be attributed to the differences between mice and humans, suggesting that targeted research in this area is essential. Overall, these results highlight the intricate relationships between genes and digestive diseases, as well as the significance of further investigation, especially taking into account the limitations of animal models in mimicking human physiological conditions.

The current two-sample Mendelian randomization study has revealed some potential associations. However, to further confirm the causal relationships, future experimental validations are indispensable. In vitro experiments, like culturing relevant cell lines to manipulate gene expressions, can offer initial insights into the underlying mechanisms. Meanwhile, in vivo animal models, such as genetically modified mice, will help us assess these associations in a more physiological context. These additional experimental studies are expected to strengthen the reliability of our findings and fill the gap between genetic evidence and biological reality.

Supplementary materials: Supplementary **Table S1**: Information of selected SNPs of cathepsins. Supplementary **Table S2**: The results of forward MR analysis between various cathepsins and digestive disorders. Supplementary **Table S3**: The results of reverse MR analysis between various cathepsins and digestive disorders.

Author contributions: Conceptualization, RO; methodology, RO; software, RO; validation, RO, SH and YL; formal analysis, RO; investigation, RO; resources, RO and SH; data curation, RO; writing—original draft preparation, RO; writing—review and editing, RO, SH and YL; visualization, RO and SH; supervision, RO; project administration, RO and YL. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: We sincerely thank all participants for their valuable contributions to this study, which utilized publicly available data from prior research studies.

Data availability: The genetic summary statistics for the nine cathepsins analyzed in this study, derived from the INTERVAL study, are publicly available on the IEU OpenGWAS project website (https://www.ebi.ac.uk). Additionally, summary statistics for 23 non-cancerous digestive diseases were sourced from the FinnGen database, which can be accessed via https://www.finngen.fi/en. For data that required additional privacy protection, we adhered to all relevant data protection regulations and ensured participant anonymity. In compliance with these regulations, access to such data can be obtained by contacting project administrators.

Ethical approval: Not applicable.

Informed consent statement: Informed consent was obtained from all subjects involved in the study.

Conflict of interest: The authors declare no conflict of interest.

Abbreviations

AG	Acute gastritis	IVs	Instrumental variables
BE	Barrett's esophagus	IVW	Inverse variance weighting
CD	Celiac disease	MR	Mendelian randomization
CG	Chronic gastritis	NAFLD	Non-alcoholic fatty liver disease
CIs	Confidence intervals	NASH	Non-alcoholic steatohepatitis
СР	Chronic pancreatitis	OR	Odds ratios
CTS	Cathepsin	RCTs	Randomized controlled trials
DU	Duodenal ulcer	SNPs	Single-nucleotide polymorphisms
GERD	Gastroesophageal reflux	UC	Ulcerative colitis
GWAS	Genome-wide associated studies	WM	Weighted median

References

- 1. Ricciardiello L. Digestive diseases: Big burden, low funding? Results of the new United European Gastroenterology White Book on digestive diseases. United European Gastroenterology Journal. 2022; 10(7): 627-628. doi: 10.1002/ueg2.12297
- Rose TC, Pennington A, Kypridemos C, et al. Analysis of the burden and economic impact of digestive diseases and investigation of research gaps and priorities in the field of digestive health in the European Region—White Book 2: Executive summary. United European Gastroenterology Journal. 2022; 10(7): 657-662. doi: 10.1002/ueg2.12298
- 3. Farthing M, Roberts SE, Samuel DG, et al. Survey of digestive health across Europe: Final report. Part 1: The burden of gastrointestinal diseases and the organisation and delivery of gastroenterology services across Europe. United European Gastroenterology Journal. 2014; 2(6): 539-543. doi: 10.1177/2050640614554154
- 4. Wang Y, Huang Y, Chase RC, et al. Global Burden of Digestive Diseases: A Systematic Analysis of the Global Burden of Diseases Study, 1990 to 2019. Gastroenterology. 2023; 165(3): 773-783.e15. doi: 10.1053/j.gastro.2023.05.050
- 5. Hanauer SB. The burdens of digestive diseases. Nature Reviews Gastroenterology & Hepatology. 2009; 6(7): 377-377. doi: 10.1038/nrgastro.2009.104
- 6. Yadati T, Houben T, Bitorina A, et al. The Ins and Outs of Cathepsins: Physiological Function and Role in Disease Management. Cells. 2020; 9(7): 1679. doi: 10.3390/cells9071679
- da Costa Fernandes C, Rodríguez VMO, Soares-Costa A, et al. Cystatin-like protein of sweet orange (CsinCPI-2) modulates pre-osteoblast differentiation via β-Catenin involvement. Journal of Materials Science: Materials in Medicine. 2021; 32(4). doi: 10.1007/s10856-021-06504-y
- Fonović M, Turk B. Cysteine cathepsins and extracellular matrix degradation. Biochimica et Biophysica Acta (BBA) -General Subjects. 2014; 1840(8): 2560-2570. doi: 10.1016/j.bbagen.2014.03.017
- 9. Vasiljeva O, Reinheckel T, Peters C, et al. Emerging Roles of Cysteine Cathepsins in Disease and their Potential as Drug Targets. Current Pharmaceutical Design. 2007; 13(4): 387-403. doi: 10.2174/138161207780162962
- de Almeida Chuffa LG, Freire PP, dos Santos Souza J, et al. Aging whole blood transcriptome reveals candidate genes for SARS-CoV-2-related vascular and immune alterations. Journal of Molecular Medicine. 2021; 100(2): 285-301. doi: 10.1007/s00109-021-02161-4
- 11. Turk B, Turk D, Turk V. Protease signalling: the cutting edge. The EMBO Journal. 2012; 31(7): 1630-1643. doi: 10.1038/emboj.2012.42
- 12. Patel S, Homaei A, El-Seedi HR, et al. Cathepsins: Proteases that are vital for survival but can also be fatal. Biomedicine & Pharmacotherapy. 2018; 105: 526-532. doi: 10.1016/j.biopha.2018.05.148
- 13. Barrera C, Ye G, Espejo R, et al. Expression of cathepsins B, L, S, and D by gastric epithelial cells implicates them as antigen presenting cells in local immune responses. Hum Immunol. 2001; 62(10): 1081-1091.

- 14. Bühling F, Peitz U, Krüger S, et al. Cathepsins K, L, B, X and W are differentially expressed in normal and chronically inflamed gastric mucosa. Biological Chemistry. 2004; 385(5). doi: 10.1515/bc.2004.051
- 15. Krueger S, Kuester D, Bernhardt A, et al. Regulation of cathepsin X overexpression in H. pylori-infected gastric epithelial cells and macrophages. The Journal of Pathology. 2008; 217(4): 581-588. doi: 10.1002/path.2485
- 16. Zdravkova K, Mijanovic O, Brankovic A, et al. Unveiling the Roles of Cysteine Proteinases F and W: From Structure to Pathological Implications and Therapeutic Targets. Cells. 2024; 13(11): 917. doi: 10.3390/cells13110917
- 17. Iwama H, Mehanna S, Imasaka M, et al. Cathepsin B and D deficiency in the mouse pancreas induces impaired autophagy and chronic pancreatitis. Scientific Reports. 2021; 11(1). doi: 10.1038/s41598-021-85898-9
- Aghdassi AA, John DS, Sendler M, et al. Cathepsin D regulates cathepsin B activation and disease severity predominantly in inflammatory cells during experimental pancreatitis. Journal of Biological Chemistry. 2018; 293(3): 1018-1029. doi: 10.1074/jbc.m117.814772
- 19. Fusco R, Cordaro M, Siracusa R, et al. Biochemical Evaluation of the Antioxidant Effects of Hydroxytyrosol on Pancreatitis-Associated Gut Injury. Antioxidants. 2020; 9(9): 781. doi: 10.3390/antiox9090781
- 20. Baghy K, Ladányi A, Reszegi A, et al. Insights into the Tumor Microenvironment—Components, Functions and Therapeutics. International Journal of Molecular Sciences. 2023; 24(24): 17536. doi: 10.3390/ijms242417536
- 21. Mayet WJ, Hermann E, Finsterwalder J, et al. Antibodies to Cathepsin G in Crohn's disease. European Journal of Clinical Investigation. 1992; 22(6): 427-433. doi: 10.1111/j.1365-2362.1992.tb01485.x
- 22. Kuwana T, Sato Y, Saka M, et al. Anti-cathepsin G antibodies in the sera of patients with ulcerative colitis. Journal of Gastroenterology. 2000; 35(9): 682-689. doi: 10.1007/s005350070047
- 23. Yang Z, Liu Y, Qin L, et al. Cathepsin H–Mediated Degradation of HDAC4 for Matrix Metalloproteinase Expression in Hepatic Stellate Cells. The American Journal of Pathology. 2017; 187(4): 781-797. doi: 10.1016/j.ajpath.2016.12.001
- 24. Fløyel T, Brorsson C, Nielsen LB, et al. CTSH regulates β-cell function and disease progression in newly diagnosed type 1 diabetes patients. Proceedings of the National Academy of Sciences. 2014; 111(28): 10305-10310. doi: 10.1073/pnas.1402571111
- 25. Steimle A, Gronbach K, Beifuss B, et al. Symbiotic gut commensal bacteria act as host cathepsin S activity regulators. Journal of Autoimmunity. 2016; 75: 82-95. doi: 10.1016/j.jaut.2016.07.009
- Nørgaard M, Ehrenstein V, Vandenbroucke JP. Confounding in observational studies based on large health care databases: problems and potential solutions – a primer for the clinician. Clinical Epidemiology. 2017; 9: 185-193. doi: 10.2147/clep.s129879
- 27. Walker V, Sanderson E, Levin MG, et al. Reading and conducting instrumental variable studies: guide, glossary, and checklist. BMJ. 2024; e078093. doi: 10.1136/bmj-2023-078093
- 28. Burgess S, Davey Smith G, Davies NM, et al. Guidelines for performing Mendelian randomization investigations. Wellcome Open Research. 2019; 4: 186. doi: 10.12688/wellcomeopenres.15555.1
- 29. Sanderson E, Glymour MM, Holmes MV, et al. Mendelian randomization. Nature Reviews Methods Primers. 2022; 2(1). doi: 10.1038/s43586-021-00092-5
- Hartwig FP, Davies NM, Hemani G, et al. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. International Journal of Epidemiology. 2016; 45(6): 1717-1726. doi: 10.1093/ije/dyx028
- Sun BB, Maranville JC, Peters JE, et al. Genomic atlas of the human plasma proteome. Nature. 2018; 558(7708): 73-79. doi: 10.1038/s41586-018-0175-2
- 32. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. International Journal of Epidemiology. 2017; 46(6): 1734-1739. doi: 10.1093/ije/dyx034
- 33. Verbanck M, Chen CY, Neale B, et al. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nature Genetics. 2018; 50(5): 693-698. doi: 10.1038/s41588-018-0099-7
- 34. Zhu R, Zhang N, Zhu H, et al. Major depressive disorder and the risk of irritable bowel syndrome: A Mendelian randomization study. Molecular Genetics & Genomic Medicine. 2024; 12(3). doi: 10.1002/mgg3.2413
- 35. Tong T, Zhu C, Farrell JJ, et al. Blood-derived mitochondrial DNA copy number is associated with Alzheimer disease, Alzheimer-related biomarkers and serum metabolites. Alzheimer's Research & Therapy. 2024; 16(1). doi: 10.1186/s13195-024-01601-w

- 36. Ma H, Song D, Zhang H, et al. Phenotypic insights into genetic risk factors for immune-related adverse events in cancer immunotherapy. Cancer Immunology, Immunotherapy. 2024; 74(1). doi: 10.1007/s00262-024-03854-8
- Au Yeung SL, Gill D. Standardizing the reporting of Mendelian randomization studies. BMC Medicine. 2023; 21(1). doi: 10.1186/s12916-023-02894-8
- Rajamäki K, Lappalainen J, Öörni K, et al. Cholesterol Crystals Activate the NLRP3 Inflammasome in Human Macrophages: A Novel Link between Cholesterol Metabolism and Inflammation. PLoS ONE. 2010; 5(7): e11765. doi: 10.1371/journal.pone.0011765
- 39. Hornung V, Bauernfeind F, Halle A, et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nature Immunology. 2008; 9(8): 847-856. doi: 10.1038/ni.1631
- 40. Haasken S, Sutterwala FS. Damage control: Management of cellular stress by the NLRP3 inflammasome. European Journal of Immunology. 2013; 43(8): 2003-2005. doi: 10.1002/eji.201343848
- Zheng Z, Xiong H, Zhao Z, et al. Tibetan medicine Si-Wei-Qiang-Wei Powder ameliorates cholecystitis via inhibiting the production of pro-inflammatory cytokines and regulating the MAPK signaling pathway. Journal of Ethnopharmacology. 2023; 303: 116026. doi: 10.1016/j.jep.2022.116026
- 42. Teller A, Jechorek D, Hartig R, et al. Dysregulation of apoptotic signaling pathways by interaction of RPLP0 and cathepsin X/Z in gastric cancer. Pathology Research and Practice. 2015; 211(1): 62-70. doi: 10.1016/j.prp.2014.09.005
- 43. Kos J, Sekirnik A, Premzl A, et al. Carboxypeptidases cathepsins X and B display distinct protein profile in human cells and tissues. Experimental Cell Research. 2005; 306(1): 103-113. doi: 10.1016/j.yexcr.2004.12.006
- 44. Lecaille F, Chazeirat T, Saidi A, et al. Cathepsin V: Molecular characteristics and significance in health and disease. Molecular Aspects of Medicine. 2022; 88: 101086. doi: 10.1016/j.mam.2022.101086
- 45. Fukuo Y, Yamashina S, Sonoue H, et al. Abnormality of autophagic function and cathepsin expression in the liver from patients with non-alcoholic fatty liver disease. Hepatology Research. 2014; 44(9): 1026-1036. doi: 10.1111/hepr.12282
- 46. He Y, Xu M, Zhou C, et al. The Prognostic Significance of CTSV Expression in Patients with Hepatocellular Carcinoma. International Journal of General Medicine. 2024; 17: 4867-4881. doi: 10.2147/ijgm.s467179
- 47. Manchanda M, Das P, Gahlot GPS, et al. Cathepsin L and B as Potential Markers for Liver Fibrosis: Insights From Patients and Experimental Models. Clinical and Translational Gastroenterology. 2017; 8(6): e99. doi: 10.1038/ctg.2017.25
- Toonen EJM, Mirea AM, Tack CJ, et al. Activation of Proteinase 3 Contributes to Nonalcoholic Fatty Liver Disease and Insulin Resistance. Molecular Medicine. 2016; 22(1): 202-214. doi: 10.2119/molmed.2016.00033
- 49. Zamolodchikova TS, Tolpygo SM, Svirshchevskaya EV. Cathepsin G—Not Only Inflammation: The Immune Protease Can Regulate Normal Physiological Processes. Frontiers in Immunology. 2020; 11. doi: 10.3389/fimmu.2020.00411
- 50. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018; k601. doi: 10.1136/bmj.k601
- Ai S, Zhang J, Zhao G, et al. Causal associations of short and long sleep durations with 12 cardiovascular diseases: linear and nonlinear Mendelian randomization analyses in UK Biobank. European Heart Journal. 2021; 42(34): 3349-3357. doi: 10.1093/eurheartj/ehab170
- Yang Q, Sanderson E, Tilling K, et al. Exploring and mitigating potential bias when genetic instrumental variables are associated with multiple non-exposure traits in Mendelian randomization. European Journal of Epidemiology. 2022; 37(7): 683-700. doi: 10.1007/s10654-022-00874-5
- 53. Li J, Tang M, Gao X, et al. Mendelian randomization analyses explore the relationship between cathepsins and lung cancer. Communications Biology. 2023; 6(1). doi: 10.1038/s42003-023-05408-7