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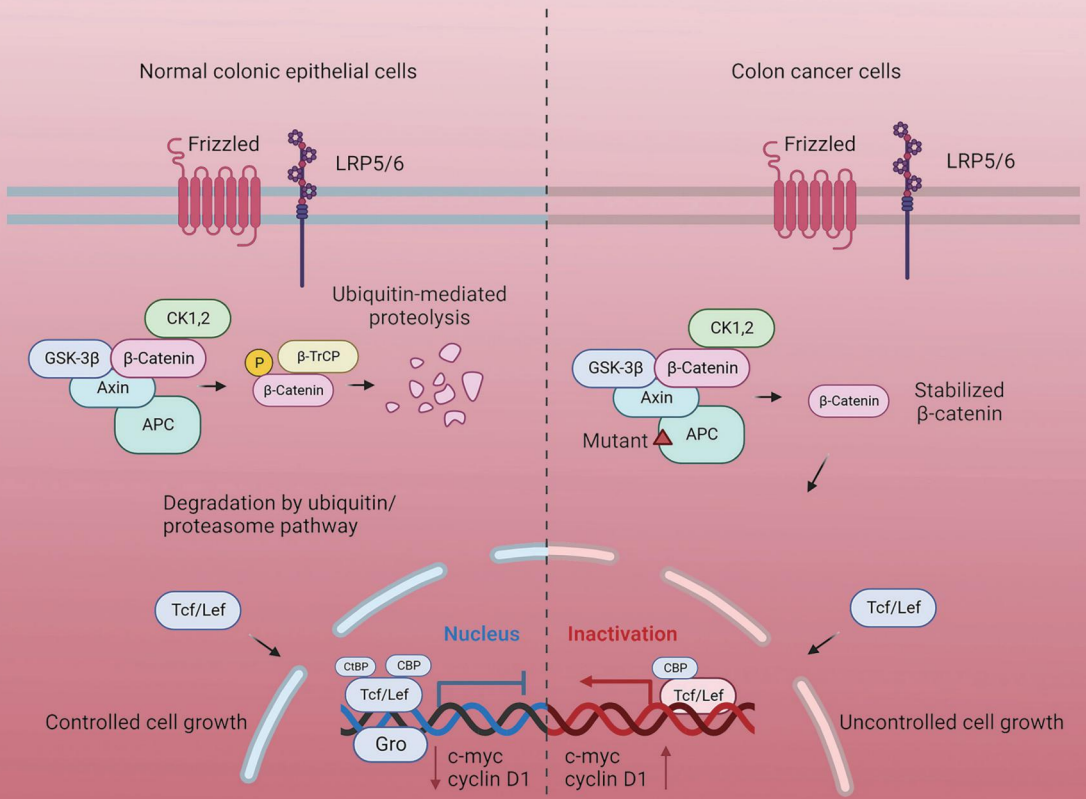

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Aims and Scope

Journal of Translational Gastroenterology (JTG) dedicates to improving clinical diagnosis and treatment, advancing understanding of the molecular mechanisms, and promoting translation from bench to bedside of gastrointestinal, hepatobiliary, and pancreatic diseases. The aim of JTG is to provide a forum for the exchange of ideas and concepts on basic, translational, and clinical aspects of gastroenterology, and promote cross-disciplinary research and collaboration.

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CONTENTS

2024 2(1):1–59

Original Articles

Review Articles

Mini Review

Journal of Translational Gastroenterology **2024** vol. 2(1) | 1–8
DOI: 10.14218/JTG.2023.00047

Original Article

The Effect of Measurement Depth and Technical Considerations in Performing Liver Attenuation Imaging

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Abstract

Background and objectives: Clinical unmet need in managing nonalcoholic fatty liver disease (NAFLD), a common liver disorder affecting 25–30% of American adults is to develop noninvasive and robust biomarkers.

Methods: We re-measured liver AC by placing a region of interest (ROI, 3 cm tall and 3 cm wide) at 4.5 cm, 6 cm, and 7.5 cm from the skin and a large ROI (6.0 cm tall and 7.3 cm wide) on pre-recorded ATI images from 117 participants screened for NAFLD. The difference in AC value at variable ROI depths was tested using one-way ANOVA (analysis of variance). Diagnostic performances of AC at variable depths in determining hepatic steatosis were examined by area under receiver operating characteristic curve (AUC) using MRI-proton density fat fraction (MRI-PDFF) as reference and were compared using paired-sample Z-test.

Results: Based on MRI-PDFF, 117 livers were divided to 27 normal livers (MRI-PDFF < 5%) or 90 steatotic livers (MRI-PDFF ≥ 5%). Differences in AUC and AC value at variable depths and size were statistically significant ($p < 0.01$). The best performance for determining hepatic steatosis was the AC measured at 6 cm from the skin (AUC = 0.92). Sources of errors in performing ATI included reverberation, blank color region, and acoustic shadowing within the measurement ROI.

Conclusions: ROI depth significantly influences liver AC estimation. The best ROI depth to measure liver AC in patients with BMI ≥ 30 may be at a depth of 6 cm from the skin. Technical considerations should be taken in performing liver ATI.

Introduction

The prevalence of nonalcoholic fatty liver disease (NAFLD) has seen a significant increase worldwide, with a 10% increase in a recent 5-year period.[1] NAFLD is now estimated to affect 25% of the general population, making it the most common chronic liver disorder in the world.[2] Moreover, there have been strong correlations between NAFLD and other metabolic syndromes such as diabetes mellitus and obesity, cardiovascular disease, and chronic kidney disease reported.[3,4] Therefore, NAFLD is an ever-increasing healthcare concern in which early detection can result in better clinical outcomes.

Hepatic steatosis, defined as an accumulation of lipids within the liver parenchyma (>5%), can cause liver tissue injury. This damage begins with inflammation that results in liver scarring, which ultimately develops fibrosis in the liver. If left untreated, progression of fibrosis can lead to cirrhosis, which significantly increases the risk for developing liver failure and hepatocellular carcinoma.[5] Early stages of NAFLD are reversible and can be managed with lifestyle changes and medications, however, once progression is made to later stages, there are no approved treatments other than liver transplantation.[6] The current gold standard in the diagnosis of NAFLD is liver biopsy, which is highly efficacious for diagnosis throughout all stages of NAFLD, specifically in determining nonalcoholic steatohepatitis (NASH).[7] The liver biopsy, as with any invasive procedure, has the associated risks of pain, infection, bleeding, and unintended comorbidities that are significant; in addition to variation in tissue sampling and interpretation.[8]

Alternatively, there are non-invasive imaging modalities available for assessing NAFLD including computed tomography (CT), magnetic resonance imaging (MRI), serologic testing, and ultrasound. CT has shown to be an effective measure in assessing more advanced liv-

Keywords: Attenuation coefficient; Liver; Magnetic resonance imaging-proton density fat fraction; Nonalcoholic fatty liver disease; Ultrasound.

Abbreviations: AC, attenuation coefficient; ANOVA, one-way analysis of variance; ATI, ultrasound attenuation imaging; AUC, area under receiver operating characteristic curve; CI, Confidence Interval; MRI, magnetic resonance imaging; MRI-PDFF, magnetic resonance imaging-based proton density fat fraction; NAFLD, nonalcohlic fatty liver disease; NASH, nonalcoholic steatohepatitis; ROC, receiver operating characteristic; ROI, region of interest.

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er disease but is insufficient in detecting earlier stages of steatosis and fibrosis. There is also the additional concern of radiation exposure to the patient.[9] Serological markers are available to assess inflammation and fibrosis developed in NAFLD without radiation exposure. However, these markers are not sensitive to stage hepatic steatosis.[10]

The current preferred imaging modality in the diagnosis of NAFLD is magnetic resonance imaging-based proton density fat fraction (MRI-PDFF). This technique is done by utilizing the multi-echo Dixon method, which discriminates between water and fat proton using the chemical inclusion and exclusion method.[11] Furthermore, MRI-PDFF has been proven to be more sensitive than histology-determined steatosis grading in quantifying fat content in the liver.[12] As such, MRI-PDFF has become a leading non-invasive imaging technique in managing NAFLD.[13] However, the limitations of MRI include high cost, contraindications (claustrophobia), and limited test access in rural areas.

Ultrasonography remains the most commonly used imaging modality to assess hepatic steatosis. This can be attributed to its high diagnostic utility, low cost, ability to be perform at bedside, wide availability, and overall patient tolerability.[14] However, underestimation of hepatic steatosis in individuals with <20% liver adiposity using conventional B-mode ultrasound criteria was reported.[15]

More recently, innovations in quantitative ultrasound biomarkers including two-dimensional attenuation imaging (ATI) have been made that allow for assessing hepatic steatosis with a widely available, cost-efficient, radiation free, and robust technique. ATI assesses the degree of ultrasound energy loss in a localized region of interest (ROI) on B-mode imaging. As reported, ultrasound attenuation coefficients (AC, dB/cm/MHz) assessed by ATI was closely correlated to MRI-PDFF in quantifying hepatic steatosis and intra- and inter-operator reliability in performing ATI was good.[16,17] Yet, the diagnostic scanning protocol of ATI in screening for NAFLD has not been standardized, and technical considerations in performing ATI need to be addressed.

We aimed to assess the variation in the value and diagnostic performance of AC measured at different depths using MRI-PDFF as the reference standard and elaborate on sources of errors in performing liver ATI to screen for NAFLD.

Materials and methods

The study was conducted through remeasuring AC values on pre-recorded ATI images in 117 adult participants who met inclusion criteria for screening for suspected NAFLD (age >18years old; suspicious or known NAFLD; alcohol intake <20g/day; no history of autoimmune, viral, drug, radiation, or metastasis related liver diseases, tolerant ultrasound and MRI scans) and underwent the ultrasound and MRI scans within 30 days each other in a previous pilot study. The initial study received ethical approval from the Institutional Review Board of Rocky Vista University (IRB#2019-0009) and was performed in accordance with the Declaration of Helsinki (as revised in 2013). All participants provided written informed consent upon enrollment. Additionally, the manuscript was prepared in accordance with Standards for Reporting of Diagnostic Accuracy Studies (STARD) study reporting guidelines. Initially, five liver ATI images were acquired for each participant using a commercial ultrasound scanner equipped with a curvilinear transducer (PVI-475BX, 1.8–6.2 MHz, Aplio i800, Canon Medical Systems USA, Tustin, CA, USA) after fasting 6–8 hours. Liver ACs were measured approximately 2.0 cm below the liver capsule. All ATI images were stored on the hard drive of the scanner. A senior operator with more than 30 years of experience in abdominal ultrasound and 4 years of experience in ATI performed all initial scans using manufacturer recommended machine settings and scanning protocol.[17] The liver MRI-PDFF were initially performed using a multipoint Dixon technique (Iterative decomposition of water and fat with echo asymmetry and least squares estimation (IDEAL) Intelligent Quotient (IQ), General Electric Healthcare (GE) Healthcare). The methods of MRI-PDFF acquisition used in the initial study included: noncontrast; breath-hold sequence; 3D complex gradient echo; low flip angle; 6 echo-imaging for T2* decay correction. The average of 9 MRI-PDFF values of the liver was used for analysis.[18] Hepatic steatosis was graded S0 or ≥S1 based on MRI-PDFF value <5% or ≥5%.[13] All liver images were interpreted by three radiologists who had more than 8 years of experience of clinical abdominal/liver imaging in the initial study.

Ultrasound attenuation imaging

Re-measurements of the liver AC were performed by two junior operators (C.A. and J.D.) who had training in abdominal ultrasound (2 years) and received instruction on how to measure attenuation coefficient of the liver. These two junior operators were blinded to the initial study results of liver AC, MRI-PDFF, and clinical information of the participants. Using the image review function on the ultrasound scanner (Aplio i800, Canon Medical Systems USA), each of 5 ATI images recorded for each liver in the initial scans was selected and displayed on the screen (one on one). The initial AC value and measurement ROI were automatically deleted once the AC measurement function was activated. As a result, a new AC value can be measured by manually placing a region of interest (ROI) in color-coded ATI image. The site of ROI placement for measuring liver AC was confirmed by both operators. The protocol for re-measuring AC of the liver with variable size at different depths was standardized: using depth scales on the ultrasound image as a guidance, the operator manually placed a trapezoid ROI (3.0 cm tall by 3.0 cm wide) in the liver at the depth of 4.5 cm (the distance from the skin to the center of ROI, Fig. 1a), 6 cm (Fig. 1b), 7.5 cm (Fig. 1c), and a large ROI (6.5 cm tall, upper border wide 4 cm, and lower border wide 7.3 cm) that encompassed the entire color-coded region on the ATI image (Fig. 1d). Five ATI images per participant were reviewed. The average of 5 AC values at each depth in the liver were used for analysis. The quality of each AC measurement was evaluated by the R^2 (coefficient of determination) value showed on the screen (Fig. 1a). AC measurements with $R^2 < 0.90$ were categorized as measurement failure. All measurements were then logged in a Microsoft Excel spread sheet for analysis.

Statistical Analysis

The Shapiro–Wilk test was used to test the normal distribution of quantitative variables. When quantitative variables were normally distributed, all variables including the distance from the skin to the liver capsule, body mass index (BMI), age of the participants, AC value measured at different ROI depth and size were expressed as mean and standard deviation (SD). Differences in age, BMI, and the distance from the skin to the liver capsule were examined using two-tailed *t*-test. The difference in mean AC value measured at variable ROI depth and size was tested using one-way analysis of variance (ANOVA). The diagnostic performance of AC measured at the different depths were examined using receiver operating characteristic (ROC) curve and displayed with area under ROC (AUC). The area difference under the ROC curves was compared using two- tailed paired-sample Z-test. The measurement failure rate (%) = (number of measurements with $R^2 < 0.90$ / total number of measurements) at each ROI depth was also calculated. A p value less than 0.05 was

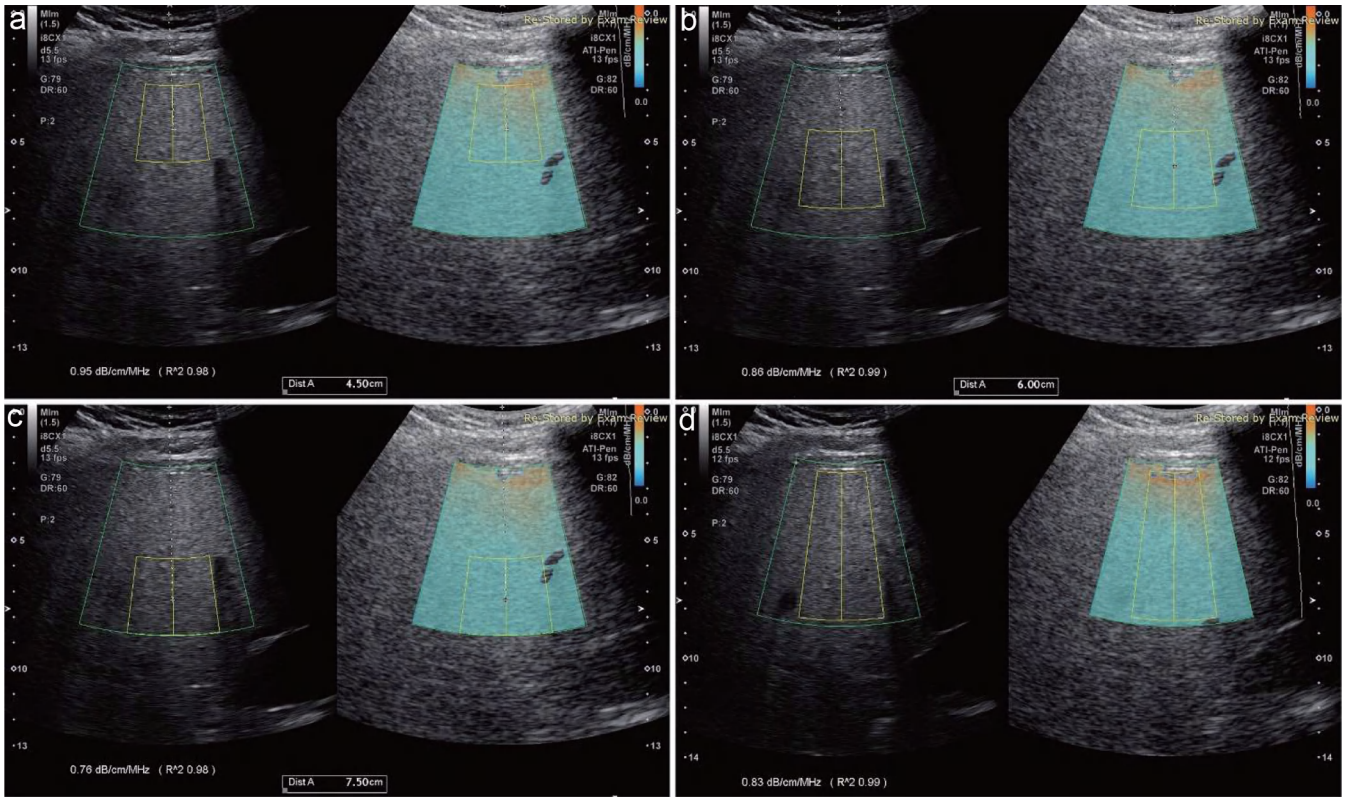

Fig. 1. Ultrasound attenuation coefficient (AC, dB/cm/MHz) is measured using two sizes of the region of interest (ROI). A ROI (3 cm tall × 3 cm wide) is placed at the depths of 4.5 cm (the distance from the skin to the center of ROI (a), 6 cm (b), and 7.5 cm (c) in the liver. A larger ROI (d), 6.5 cm tall, 4 cm top border, and 7.3 cm of bottom border) is also used to measure AC of the liver. The AC value is 1.06 dB/cm/MHz, 0.86 dB/cm/MHz, 0.66 dB/cm/MHz, and 0.85 dB/cm/MHz measured at the depths of 4.5 cm, 6 cm, 7.5 cm, and with a large ROI, respectively. AC, attenuation coefficient; ROI, region of interest.

considered statistically significant. Statistical analysis was conducted using the commercial software SPSS (Version 28.0, IBM).

Results

Total of 585 AC values (5 AC measurements for each liver) at each ROI depth were measured from 117 participants (49 men and 68 women, mean age 55 years, age range 20–81 years). Based on MRI-PDFF, 117 participants were divided to normal liver (MRI-PDFF< 5%, n = 27) group or steatotic liver (MRI-PDFF ≥ 5%, n = 90) group (Table 1) (Fig. 2). The difference in the age between the two groups was significant. Differences in BMI or the distance between the skin and the liver capsule between the two groups were not significant ($p > 0.05$, Table 1).

AC measured 0.88 ± 0.21 dB/cm/MHz, 0.73 ± 0.13 dB/cm/MHz, 0.57 ± 0.13 dB/cm/MHz, and 0.72 ± 0.13 dB/cm/MHz at

Table 1. Demographic information and AC values in 117 participants with and without NAFLD

Parameter	Normal liver	NAFLD	*P**
Participants (M/F)	27 (13/14)	90 (36/54)	
Age (Y)	60 ± 21	51 ± 13	0.04
Body mass index (kg/cm^2)	30.02 ± 7.51	32.34 ± 5.43	0.28
Distance from the skin to liver capsule (cm)	3.91 ± 0.55	4.08 ± 0.53	0.46
MRI-PDFF (%)	3.38 ± 0.96	14.55 ± 6.73	<0.001
AC measured with large ROI	0.66 ± 0.12	0.74 ± 0.22	<0.01
AC measured at 4.5 cm (dB/cm/MHz)	0.79 ± 0.24	0.92 ± 0.21	<0.01
AC measured at 6 cm (dB/cm/MHz)	0.63 ± 0.10	0.75 ± 0.12	<0.001
AC measured at 7.5 cm (dB/cm/MHz)	0.52 ± 0.13	0.57 ± 0.15	0.10

**P* is based on two-tailed *t*-test. AC, attenuation coefficient (dB/cm/MHz); MRI-PDFF, magnetic resonance imaging-based proton density fat fraction (%); NAFLD, nonalcoholic fatty liver disease based on MRI-PDFF ≥ 5%.

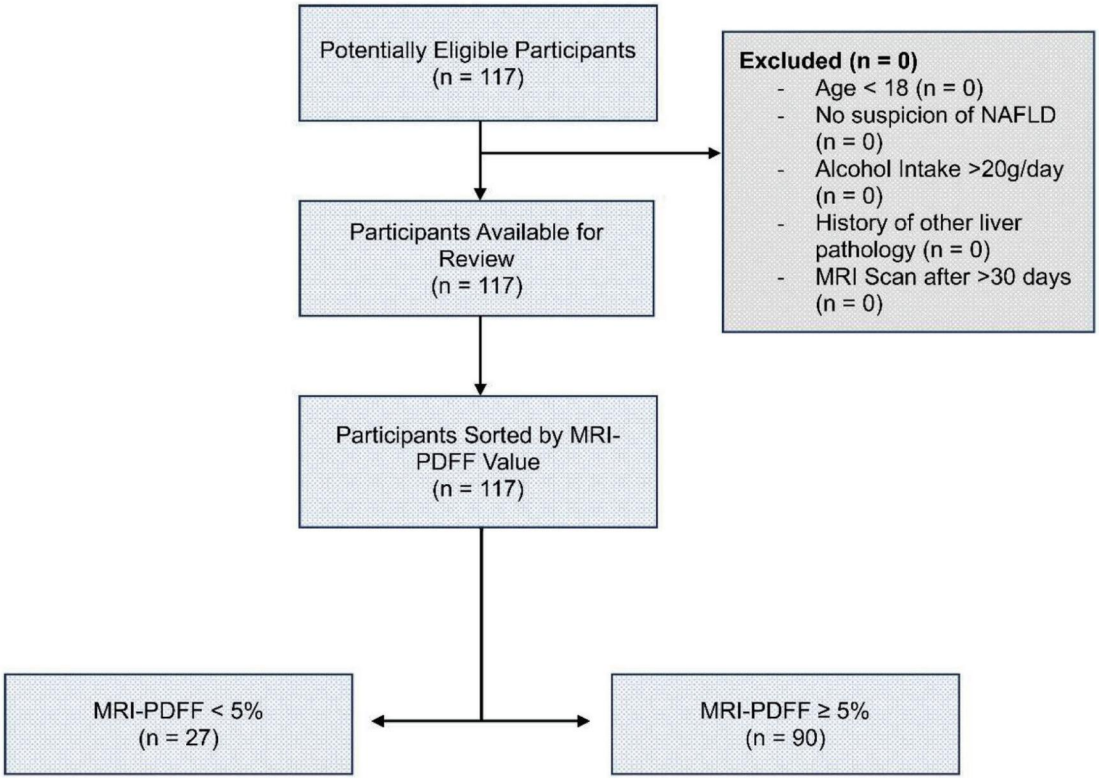

Fig. 2. Flow and organization of participants through our study. MRI-PDFF, magnetic resonance imaging-based proton density fat fraction; NAFLD, nonalcohlic fatty liver disease.

ROI depth of 4.5 cm, 6.0 cm, 7.5 cm from the skin and with the large ROI, respectively (Table 2). The difference in AC value measured at variable ROI depth and with different ROI size was significant ($p < 0.001$). The ATI quality represented by R^2 for AC estimation at different depths was listed in Table 2.

The diagnostic performance of AC measured at the different depths was listed in Table 2 and displayed in Figure 3. AC measured at 6 cm showed the highest AUC (AUC = 0.92). There is a significant difference in the area under ROC curves between AC value measured at 6 cm and those values measured at 4.5 cm, 7.5 cm, and large ROI ($p < 0.01$, Table 3). Common sources of pitfalls in performing ATI are discussed in Figure 4.

Discussion

We have observed significant differences in liver AC value, as well

Table 2. Analysis of AC measured at variable depth in screening for NAFLD

Parameter	ROI at 4.5 cm	ROI at 6 cm	ROI at 7.5 cm	Large ROI	ANOVA (*p*)
AC (dB/cm/MHz)	0.88 ± 0.21	0.73 ± 0.13	0.57 ± 0.13	0.72 ± 0.13	<0.001
ATI quality (R^2)	0.88 ± 0.09	0.95 ± 0.06	0.85 ± 0.11	0.91 ± 0.06	<0.001
Failure rate (%)	13/585* (2.2%)	3/585 (0.5%)	68/585 (12%)	7/585 (1.2%)	
ROC (S0 vs ≥ S1)					
Area under ROC	0.720	0.918	0.611	0.683	
(95% CI)	(0.593–0.847)	(0.854–0.982)	(0.501–0.721)	(0.563–0.803)	
Cutoff value	0.85	0.68	0.60	0.60	
Sensitivity	0.66	0.92	0.57	0.90	
Specificity	0.78	0.82	0.93	0.41	

AC, attenuation coefficient; ANOVA, one-way analysis of variance; ATI, attenuation imaging; CI, Confidence Interval; NAFLD, nonalcoholic fatty liver disease; ROI, region of interest; ROC, Receiver Operating Characteristic. failure rate (%) = (number of cases with $R^2 < 0.90$ / total number of measurement at each depth); Area under ROC (95% CI), area under the receiver operating characteristic curve (95% confidence interval); cutoff value is based on the maximum Kolmogorov-Smirnov (K-S) statistics and the largest one is reported; ROC (S0 vs ≥ S1), ROC of attenuation coefficient (AC) for determining ≥ mild hepatic steatosis; S0, MRI-PDFF < 5%; ≥S1, MRI-PDFF ≥ 5%; 585* values = 5 AC measurements/at each depth/per case × 117 cases.

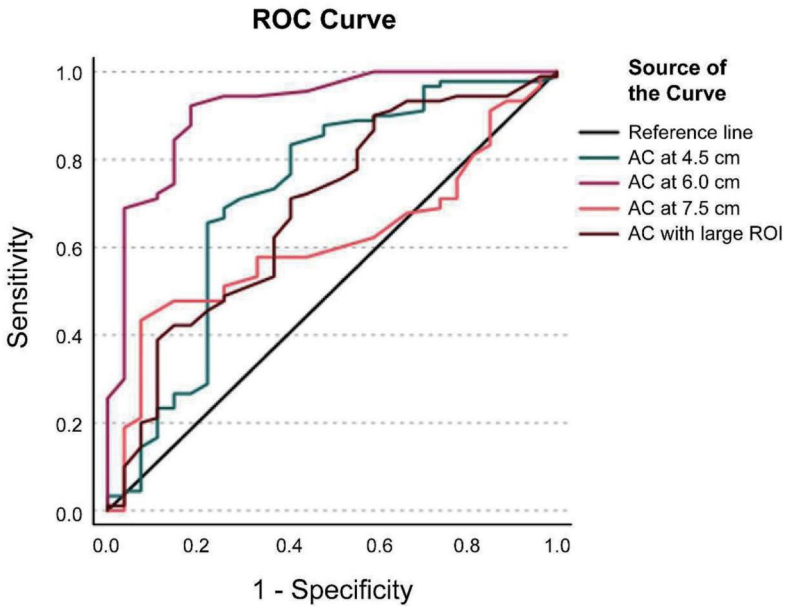

Fig. 3. The diagnostic performance of liver attenuation coefficient (AC, dB/cm/MHz) measured at different depths and sizes of the region of interest is analyzed using the area under receiver operating characteristic curve (AUC). AUC of AC measured at the depth of 4.5 cm (green curve), 6.0 cm (purple curve), 7.5 cm from the skin (orange curve), and with the large ROI (brown curve) in determining mild hepatic steatosis (≥S1, MRI-PDFF ≥ 5%) is 0.72, 0.92, 0.61, and 0.68, respectively. MRI-PDFF, magnetic resonance imaging-based proton density fat fraction; ROC, receiver operating characteristic; ROI, region of interest.

as in ATI quality, and diagnostic performance (AUC) for determining NAFLD among those measured at variable ROI depth and size. Importantly, re-measuring the AC value of the liver on the pre-recorded ATI images stored in the ultrasound scanner hard drive is an ideal method that allows radiologists to remeasure AC in different ROI location and correct technical errors in the AC measurement. As such, the accuracy of interpreting ATI images to assess hepatic steatosis can be improved without a requirement of re-scanning (callback) the patient.

In the study, the best ROI depth for measuring liver AC is at 6 cm from the skin (Fig. 1b) resulting in the highest diagnostic performance of AC to determine ≥ mild hepatic steatosis, ATI quality, and lowest failure rate compared with AC values measured at depths of 4.5 cm, 7.5 cm, and large ROI. The ROI depth at 4.5 cm seemed to be too close to the liver capsule to avoid the dark orange color area produced by high noise or reverberation artifact (Fig. 4a, b) in some patients. The ROI depth at 7.5 cm was often too deep from the skin to exclude the dark blue area (weak echo signal, Fig. 4c) due to less sound penetration,[19] which yielded the poor ATI quality, low diagnostic performance, and high failure rate. The utilization of a large ROI is able to assess tissue attenuation in relative larger region of liver parenchyma (6.5 cm × 7.3 cm vs. 3 cm × 3 cm). However, using a large ROI to measure liver AC magnifies technical challenges to place such a large ROI in a small liver (such as a cirrhotic liver) and avoid prominent hepatic vessels (e.g. dilatation of the hepatic veins in congestive heart failure or portal vein in significant portal hypertension). Further, AC measured at the depth of 7.5 failed to distinguish steatotic livers from normal livers as the difference in AC value between normal and steatotic livers was not significant ($p = 0.10$, Table 1).

Ultrasound attenuation-based fat quantification technique relies on the assessment of the energy loss of the acoustic signals while

Table 3. Comparison the AUC of AC in determining hepatic steatosis

Paired-sample area difference under the ROC curves						
Asymptotic			AUC difference	Std. error difference[b]	95% Confidence interval	
Test result pair(s)	z	Sig. (2-tail)[a]			Lower bound	Upper bound
4.5 cm: 6 cm	−3.622	0.000	−0.198	0.309	−0.305	−0.091
4.5 cm: 7.5 cm	1.715	0.086	0.109	0.343	−0.016	0.233
4.5 cm: large ROI	1.125	0.261	0.037	0.345	−0.027	0.101
6 cm: 7.5 cm	5.202	0.000	0.307	0.296	0.191	0.423
6 cm: large ROI	4.479	0.000	0.235	0.303	0.132	0.338
7.5 cm: large ROI	−1.586	0.113	−0.072	0.335	−0.161	0.017

AC, attenuation coefficient; AUC, area under register operating characteristic curve; ROI, region of interest. AUC comparison* is to test the area difference under the ROC curves using a two-tailed paired-sample Z-test; Sig, significance (*p* value); 4.5 cm, 6 cm, and 7.5 cm, the distance from the skin to the center of the region of interest for measuring liver attenuation coefficient (AC). Large ROI, the size of region of interest (6.5 cm × 7.3 cm) for measuring liver AC.

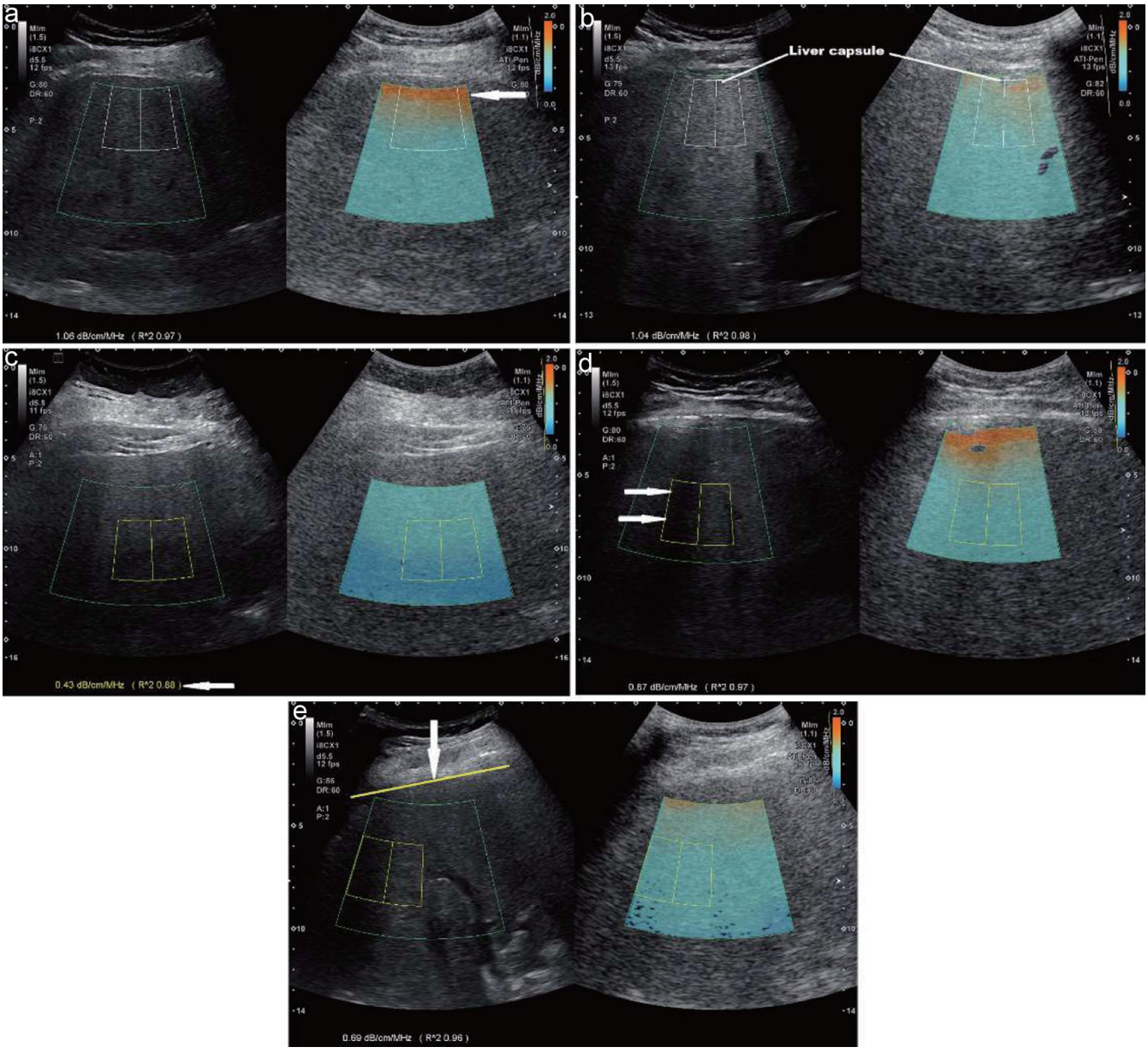

Fig. 4. Technical errors in measuring liver attenuation coefficient (AC). Common technical errors in performing liver ultrasound attenuation imaging (ATI) are dark orange area (white arrow, a), the liver capsule (b), the region with blank color at the depth of >10 cm (c, the white arrow points $R^2 < 0.90$), and acoustic shadowing (white arrows, d) included in the measurement ROI. In addition, placing measurement ROI out of the center of the ultrasound attenuation imaging (ATI) image and/or sound beam (white arrow) to liver capsule (yellow solid line) off 90 degrees (e) may also maximize scattering sound energy to various directions resulting in measurement errors.

travelling through the tissue.[20] The distance the sound beam travels, the scanning frequency, and the property of the tissue evaluated effect the ultrasound signal that returns to the transducer.[16,20] As reported, an AC value reflects the degree of acoustic attenuation produced by fat content in the liver and the liver AC estimation is depth dependent.[21] Therefore, it is important to place the ROI at a standardized depth to minimize intra- and inter-observer variation in performing ATI and technical errors among follow up scans for monitoring hepatic steatosis.

Best practices for ATI (Canon Medical Systems) measurement and reporting are still evolving. Besides manufacturer's recommendation, there is no standardized consensus available to guide performing ATI of the liver.[20] It is important to standardize prescan preparation (fasting 6–8 hours), machine settings (scanning frequency), scanning protocols (breath-holding maneuver, intercostal approach), and operator training for performing liver ATI. Further, some technical considerations should be taken when attempting to optimize the efficacy and utility of ATI in the diagnosis and monitoring of hepatic steatosis. There are sources of errors and pitfalls in performing ATI of the liver noted in the study.

1. The region below the liver capsule appearing dark orange color on ATI is produced by ultrasound reverberation artifact

(Fig. 4a). Therefore, dark-orange color below the liver capsule should be excluded from ROI for measuring liver AC.[22]
2. The liver capsule should be excluded from the measurement ROI (Fig. 4b).
3. The posterior region with dark blue (Fig. 4c) or blank color should be avoided from measurement ROI.[23]
4. Acoustic shadowing behind the ribs and/or lung (Fig. 4d) should be avoided from the measurement ROI.
5. The propagation direction of the ultrasound beam is not perpendicular to the liver capsule. Angling of the liver capsule (Fig. 4e) may cause stronger sound beam reflection and refraction once the angle between sound beam and the liver capsule is off 90 degrees,[16] which may affect AC estimation.

This study has several limitations. First, liver biopsy was not available as the reference to assess the accuracy of AC in quantifying hepatic steatosis. We employed MRI-PDFF as the reference standard, which has been used as an acceptable non-invasive alternative measure for quantifying fat content in the liver.[16,24] Second, only one senior operator (J.G.) performed all the ultrasound scans and interobserver variability was not tested in this study, however, good to excellent reproducibility was demonstrated in a training session prior to the study.[17] Third, our study included a large number of participants with obesity (54% participants with BMI > 30 kg/cm^2; 90% participants with BMI >25 kg/cm^2). Obesity can significantly alter the placement of ROI within the liver parenchyma due to varying amounts of subcutaneous adipose tissue. Therefore, the recommended placement of ROI at a depth of 6 cm from skin surface may be suitable for patients with BMI ≥ 30 based on our results. However, the ROI placement for estimating liver AC should be adjusted according to the level of comorbid obesity and the thickness of the subcutaneous adipose tissue. As such, measurement failure rate at the measurement depth of 7.5 cm was higher than at depths of 4.5 cm and 6.0 cm. Additionally, the placement of ROI for estimating liver AC should be adjusted according to varying levels of subcutaneous adipose tissue, especially in thin patients with NAFLD. Fourth, we did not analyze confounding factors, such as liver inflammation and fibrosis that may affect liver AC measurement because of the lack of biopsy pathology as areference. Fifth, we only measured liver AC at the depths of 4.5 cm, 6.0 cm, and 7.5 cm. However, AC measured at the other depths (such as 6.5 cm, 7.0 cm) may be more appropriate than the introduced protocol for individual participant based on his/her body habitus. Sixth, the sample size of the study was small and patient population utilized in this study demonstrated a significant difference in age of participants between the NAFLD and normal liver groups. A low inverse correlation between the age and liver MRI-PDFF was observed (Pearson correlation r = −0.18, p = 0.08), which is consistent with a previously reported inverse correlation between the age and patients with NAFLD in the general population.[25] Thus, an age matched study in populations with and without NAFLD is warranted. Lastly, the ultrasound scanner hardware and software used in the study were designed by a single ultrasound vendor. The variation in measuring liver attenuation coefficient by using ultrasound scanners and software designed by different vendors needs further investigation. Clinical and biomedical engineering researchers at the American Institute of Ultrasound in Medicine (AIUM)-RSNA Quantitative Imaging Biomarkers Alliance (QIBA) Pulse-Echo Quantitative Ultrasound (PEQUS) initiative for fat quantification are working on standardization of ultrasound attenuation coefficient technique for clinical application.[20] NAFLD is a common disorder affecting liver and cardiovascular systems. Following the validation of multiple quantitative imaging including ultrasound and MRI biomarkers to assess hepatic steatosis, the development and implementation of artificial intelligence and machine learning models in performing ultrasound attenuation imaging in NAFLD management is encouraged.

In conclusion, the ROI depth significantly influences the diagnostic performance and value of liver AC estimation. The best ROI location to measure liver AC in patients with BMI ≥ 30 may be at a depth of 6 cm from the skin. Technical considerations should be taken in performing ATI for assessing hepatic steatosis in patients with variable thickness of the subcutaneous tissue. Excluding reverberation, the region with blank color, and acoustic shadowing from measurement ROI, and AC value with R^2 <0.90 should be taken into consideration when scanning and interpreting ATI to screen for NAFLD. The study results provide the reference to develop a standardized protocol in performing ATI.

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Conflict of interest

All other authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (JG), acquisition of data (CA, JD, JG), analysis and interpretation of data (JG, ML), drafting of the manuscript (CA, JD), critical revision of the manuscript for important intellectual content (JG, JJ, LDH, ML), technical, or material support (LDH, JJ), and study supervision (JG). All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical statement

The initial study received ethical approval from the Institutional Review Board of Rocky Vista University (IRB#2019-0009) and was performed in accordance with the Declaration of Helsinki (as revised in 2013). All participants provided written informed consent upon enrollment.

Data sharing statement

Study data are available from the corresponding author upon reasonable requests.

References

[1] Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J Gastroenterol 2017;23(47):8263–8276. doi:10.3748/wjg.v23.i47.8263, PMID:29307986.
[2] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, *et*

al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67(1):328–357. doi:10.1002/hep.29367, PMID:28714183.
[3] Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol 2015;62:S47–S64. doi:10.1016/j.jhep.2014.12.012, PMID:25920090.
[4] Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, *et al*. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50(8):1844–1850. doi:10.2337/diabetes.50.8.1844, PMID:11473047.
[5] Adams LA, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. CMAJ 2005;172(7):899–905. doi:10.1503/cmaj.045232, PMID:15795412.
[6] Rinella ME, Sanyal AJ. Management of NAFLD: a stage-based approach. Nat Rev Gastroenterol Hepatol 2016;13(4):196–205. doi:10.1038/nrgastro.2016.3, PMID:26907882.
[7] Li Q, Dhyani M, Grajo JR, Sirlin C, Samir AE. Current status of imaging in nonalcoholic fatty liver disease. World J Hepatol 2018;10(8):530–542. doi:10.4254/wjh.v10.i8.530, PMID:30190781.
[8] Salva-Pastor N, Chávez-Tapia NC, Uribe M, Nuño-Lámbarri N. The diagnostic and initial approach of the patient with non-alcoholic fatty liver disease: role of the primary care provider. Gastroenterol Hepatol Bed Bench 2019;12(4):267–277. PMID:31749914.
[9] Lee SS, Park SH, Kim HJ, Kim SY, Kim MY, Kim DY, *et al*. Non-invasive assessment of hepatic steatosis: prospective comparison of the accuracy of imaging examinations. J Hepatol 2010;52(4):579–585. doi:10.1016/j.jhep.2010.01.008, PMID:20185194.
[10] Dumitrascu DL, Neuman MG. Non-alcoholic fatty liver disease: an update on diagnosis. Clujul Med 2018;91(2):147–150. doi:10.15386/cjmed-993, PMID:29785151.
[11] Reeder SB, Cruite I, Hamilton G, Sirlin CB. Quantitative Assessment of Liver Fat with Magnetic Resonance Imaging and Spectroscopy. J Magn Reson Imaging 2011;34(4):729–749. doi:10.1002/jmri.22775, PMID:22025886.
[12] Noureddin M, Lam J, Peterson MR, Middleton M, Hamilton G, Le TA, *et al*. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. Hepatology 2013;58(6):1930–1940. doi:10.1002/hep.26455, PMID:23696515.
[13] Middleton MS, Heba ER, Hooker CA, Bashir MR, Fowler KJ, Sandrasegaran K, *et al*. Agreement Between Magnetic Resonance Imaging Proton Density Fat Fraction Measurements and Pathologist-Assigned Steatosis Grades of Liver Biopsies From Adults With Nonalcoholic Steatohepatitis. Gastroenterology 2017;153(3):753–761. doi:10.1053/j.gastro.2017.06.005, PMID:28624576.
[14] Mishra P, Younossi ZM. Abdominal ultrasound for diagnosis of nonalcoholic fatty liver disease (NAFLD). Am J Gastroenterol 2007;102(12):2716–2717. doi:10.1111/j.1572-0241.2007.01520.x, PMID:18042105.
[15] Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. J Hepatol 2009;51(6):1061–1067. doi:10.1016/j.jhep.2009.09.001, PMID:19846234.
[16] Jeon SK, Lee JM, Joo I, Yoon JH, Lee DH, Lee JY, *et al*. Prospective Evaluation of Hepatic Steatosis Using Ultrasound Attenuation Imaging in Patients with Chronic Liver Disease with Magnetic Resonance Imaging Proton Density Fat Fraction as the Reference Standard. Ultrasound Med Biol 2019;45(6):1407–1416. doi:10.1016/j.ultrasmedbio.2019.02.008, PMID:30975533.
[17] Gao J, Lee R, Trujillo M. Reliability of Performing Multiparametric Ultrasound in Adult Livers. J Ultrasound Med 2022;41(3):699–711. doi:10.1002/jum.15751, PMID:33982805.
[18] Gao J. Ultrasound attenuation coefficient of the liver and spleen in adults: A preliminary observation. Clin Imaging 2022;84:140–148. doi:10.1016/j.clinimag.2022.02.010, PMID:35217283.
[19] Ferraioli G, Berzigotti A, Barr RG, Choi BI, Cui XW, Dong Y, *et al*. Quantification of Liver Fat Content with Ultrasound: A WFUMB Position Paper. Ultrasound Med Biol 2021;47(10):2803–2820. doi:10.1016/j.ultrasmedbio.2021.06.002, PMID:34284932.
[20] Ferraioli G, Kumar V, Ozturk A, Nam K, de Korte CL, Barr RG. US Attenuation for Liver Fat Quantification: An AIUM-RSNA QIBA Pulse-Echo Quantitative Ultrasound Initiative. Radiology 2022;302(3):495–506. doi:10.1148/radiol.210736, PMID:35076304.
[21] Bae JS, Lee DH, Lee JY, Kim H, Yu SJ, Lee JH, *et al*. Assessment of hepatic steatosis by using attenuation imaging: a quantitative, easy-to-perform ultrasound technique. Eur Radiol 2019;29(12):6499–6507. doi:10.1007/s00330-019-06272-y, PMID:31175413.
[22] Ferraioli G, Maiocchi L, Raciti MV, Tinelli C, De Silvestri A, Nichetti M, *et al*. Detection of Liver Steatosis With a Novel Ultrasound-Based Technique: A Pilot Study Using MRI-Derived Proton Density Fat Fraction as the Gold Standard. Clin Transl Gastroenterol 2019;10(10):e00081. doi:10.14309/ctg.0000000000000081, PMID:31609745.
[23] Ferraioli G, Maiocchi L, Savietto G, Tinelli C, Nichetti M, Rondanelli M, *et al*. Performance of the Attenuation Imaging Technology in the Detection of Liver Steatosis. J Ultrasound Med 2021;40(7):1325–1332. doi:10.1002/jum.15512, PMID:32960457.
[24] Cassidy FH, Yokoo T, Aganovic L, Hanna RF, Bydder M, Middleton MS, *et al*. Fatty liver disease: MR imaging techniques for the detection and quantification of liver steatosis. Radiographics 2009;29(1):231–260. doi:10.1148/rg.291075123, PMID:19168847.
[25] Lin Y, Feng X, Cao X, Miao R, Sun Y, Li R, *et al*. Age patterns of non-alcoholic fatty liver disease incidence: heterogeneous associations with metabolic changes. Diabetol Metab Syndr 2022;14(1):181. doi:10.1186/s13098-022-00930-w, PMID:36443867.

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Original Article

A Systematic Exploration of Key Candidate Genes and Pathways in the Biogenesis of Human Gastric Cancer: A Comprehensive Bioinformatics Investigation

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Abstract

Background and objectives: Gastric cancer (GC) is a prevalent gastrointestinal malignancy, yet its early detection remains hindered due to the lack of available genetic markers. This study aimed to identify alternative genetic markers for the early prognosis and prevention of GC.

Methods: This objective was achieved through the analysis of differentially expressed genes (DEGs) from three datasets obtained from the Gene Expression Omnibus (GEO). By doing so, our goal was to identify hub genes associated with gastric adenocarcinoma that could serve as potential biomarkers for the early detection and management of GC. Three GEO datasets (GSE172032, GSE179581, and GSE181492), consisting of 10 normal and 10 GC samples were analyzed using the Galaxy web server. The visualizations of DEGs, including heatmaps, volcano plots, and MD plots, were generated via the same tool. ShinyGO performed Gene Ontology and KEGG enrichment analysis, while NetworkAnalyst identified a protein-protein interaction (PPI) network and screened 10 potential hub genes. Kaplan Meier plotter was used to analyze overall survival analysis for key hub genes, and NetworkAnalyst was used to assess protein-drug interactions for the top 10 hub genes.

Results: A total of 1,079 common DEGs emerged across datasets, concentrating on significant GC-related pathways. Ten hub genes (*H2BC21, H3C12, H2BC17, H3C2, H3C10, ERBB4, H2AC8, H3C8, H2BC14,* and *MAPT*) were found to be linked to GC via PPI analysis. Survival analysis revealed that higher expression levels of *ERBB4* and *MAPT* were associated with poor overall survival in GC patients. Furthermore, protein-drug interaction analysis revealed that the protein product of the *MAPT* gene exhibited a robust connection with drug compounds, specifically docetaxel and paclitaxel. These findings suggested that these drugs have the potential to inhibit the function of MAPT.

Conclusions: In summary, our findings provide putative candidate biomarkers, provide insights into GC treatment strategies, and highlight avenues for further research, contributing to a better understanding of the pathogenesis of GC.

Keywords: Gastric adenocarcinoma; Survival analysis; Differentially expressed gene; Biomarker.

Abbreviations: DEG, differentially expressed gene; GC, gastric cancer; GEO, gene expression omnibus: GO, gene ontology; logFC, log fold change; MSI, microsatellite instability; PPI, protein-protein interaction; TCGA, the cancer genome atlas.

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Introduction

Cancer initiation occurs when cells in the body undergo unregulated growth. Gastric cancer (GC), commonly termed stomach cancer, originates from the uncontrolled growth of cells within the stomach. Approximately 95% of cases involve the stomach lining and exhibit a gradual progression of cell mass. If left untreated, it can progress into a tumor, infiltrating deeper layers of the stomach wall. This tumor has the potential to metastasize to adjacent organs, including the liver and pancreas.[1,2] GC is a major contributor to global cancer-related fatalities. Functionally, the stomach aids digestion by secreting enzymes, gastric acid, and the intrinsic factor essential for vitamin B12 absorption. Its

lining comprises mucous membrane housing columnar epithelial cells and glands. Unfortunately, these cells are susceptible to inflammation, known as gastritis, which can progress to peptic ulcers and, ultimately, culminate in GC.[3] In recent years, stomach cancer has become a prevalent malignancy with significant morbidity and mortality rates making it a pressing concern in global medical research.[4]

GC is estimated to rank as the fifth most common cancer and the third leading cause of cancer-related deaths worldwide. Each year GC accounts for approximately 783,000 deaths, constituting about 8% of all cancer-related deaths.[3,5,6] The notable frequency of late-stage diagnosis, resistance to treatment, and the tendency to metastasize in GC significantly contribute to the low survival rate, with less than 20% achieving 5-year survival, and elevated recurrence rates in GC patients. Current treatment relies primarily on surgical interventions complemented by conventional chemotherapy, yet the outlook for GC patients remains discouraging.[7–9] Consequently, there is an urgent need to determine the molecular intricacies and potential biomarkers associated with GC. This approach is crucial not only for diagnosing GC but also for inhibiting metastasis and advancing effective treatment strategies, addressing a substantial and urgent demand in this field.[10]

Genetic factors, such as polymorphisms, can serve as promising biomarker candidates due to their potential contribution to GC risk. For instance, a study by Jing He *et al*. revealed that individuals with the rs873601A variant genotype in the nucleotide excision repair gene XPG are at an elevated risk of developing gastric adenocarcinoma.[11] Another study investigated the association of eight SNPs in the mammalian target of rapamycin complex 1 gene with GC in a cancer-control study and revealed that one of them (rs1883965A) had a significant correlation.[12] Similarly, a study in a Chinese population revealed an association between the rs2298881 CA variant in the nucleotide excision repair pathway gene ERCC1 and an elevated risk of GC.[13] However, it is important to note that these studies had limitations, such as a hospital-based case-control design and limited investigation of gene variants. Therefore, further studies are needed to confirm these findings and explore other genetic variants and risk factors. Additionally, the provided sources do not specifically mention the use of these genetic variations as candidate biomarkers.

In the modern landscape of biology, high-throughput data, including gene expression information obtained from RNA sequencing or microarrays, have gained broad utility in deciphering the underlying molecular dynamics driving tumor progression. Among these tools, mRNA expression microarray platforms stand out for their capacity to identify aberrant mRNA expression patterns and uncover differential expression genes (DEGs).[14] Recently, many researchers have utilized gene expression microarray platforms to explore the gene expression profiles characterizing various grades of GC tissues, aiming to identify genes intricately linked to the oncogenic processes underlying GC.[15] With these platforms, the Gene Expression Omnibus (GEO) database offers methods for the bioinformatics mining of gene expression profiles in a variety of tumors.[16] In this study, we identified DEGs between GC tissues and adjacent normal tissues by integrating three microarray datasets from the GEO database to find promising novel biomarkers. These biomarkers may provide new insights into the underlying molecular mechanisms and help understand the occurrence, progression, and pathogenesis of GC. The complete workflow followed to identify DEGs and perform *in silico* analysis is depicted in Figure 1.

Materials and methods

Retrieval of microarray data

RNA-Seq data from three datasets—GSE172032, GSE179581, and GSE181492—comprising human GC and corresponding adjacent normal tissue specimens, were included in our analysis. The datasets included 20 tissue samples, including 10 gastric carcinoma tissues and 10 adjacent non-tumorous tissues explored in our *in-silico* analysis. All gene expression profiles were pair-ended secondary data downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information.[17,18]

Expression analysis of DEGs

Galaxy (https://usegalaxy.org/) online analysis software was used to analyze the DEGs in the two concerned conditions: human GC and matched adjacent normal tissue specimens.[19] Three datasets were uploaded to the Galaxy web server to identify the DEGs. The count table generated in Galaxy after the limma command was subsequently converted into an Excel file and used to identify DEGs between tumor tissues and adjacent non-tumorous tissue samples. A p-value of 0.05 or lower was considered to indicate statistical significance. Genes with log fold change (log2FC) > 1 and log2FC < −1 and a p-value of 0.05 or lower were considered upregulated and downregulated, respectively.

Construction of heatmap, volcano plot, and MD plot of DEGs

Galaxy, a web-based platform, provides tools for researchers, even those lacking informatics expertise, to conduct computational analyses on extensive biomedical datasets.[20] In this study, the Galaxy web server's limma package was used for visualizing heatmaps, volcano plots, and MD plots.[21,22]

Gene ontology (GO) and KEGG enrichment analysis of DEGs

ShinyGO (http://bioinformatics.sdstate.edu/go/) served as a web-based tool for exploring GO term enrichment in genomic datasets. It enables the comparison of uploaded data to reference sets of gene or protein annotations. The tool visualizes the results of the enrichment analysis in an interactive and user-friendly way, making it easy for researchers to identify overrepresented functional categories in their data. ShinyGO is built on the R programming language and can be run locally or accessed through a web interface. ShinyGO online software was used for GO and KEGG enrichment analysis of DEGs.[23]

Protein-protein interaction (PPI) network construction and module analysis

NetworkAnalyst (https://www.networkanalyst.ca) is a user-friendly online tool that interprets gene expression data in the context of PPI networks. NetworkAnalyst 3.0 includes features for meta-analysis, allowing users to visually compare multiple gene lists through interactive heatmaps, enrichment networks, and Venn diagrams.[24,25] It is a powerful internet tool with a natural online interface that enables researchers to perform PPIs easily.[25,26] This online tool was used to construct the PPI network in our analysis.[24,26]

Prediction of the hub genes

PPIs play a crucial role in biological processes including gene expression, cell growth, proliferation, and apoptosis.[27,28] Understanding protein interactions provides an efficient approach for screening hub genes. Hub genes pinpointed through a PPI net-

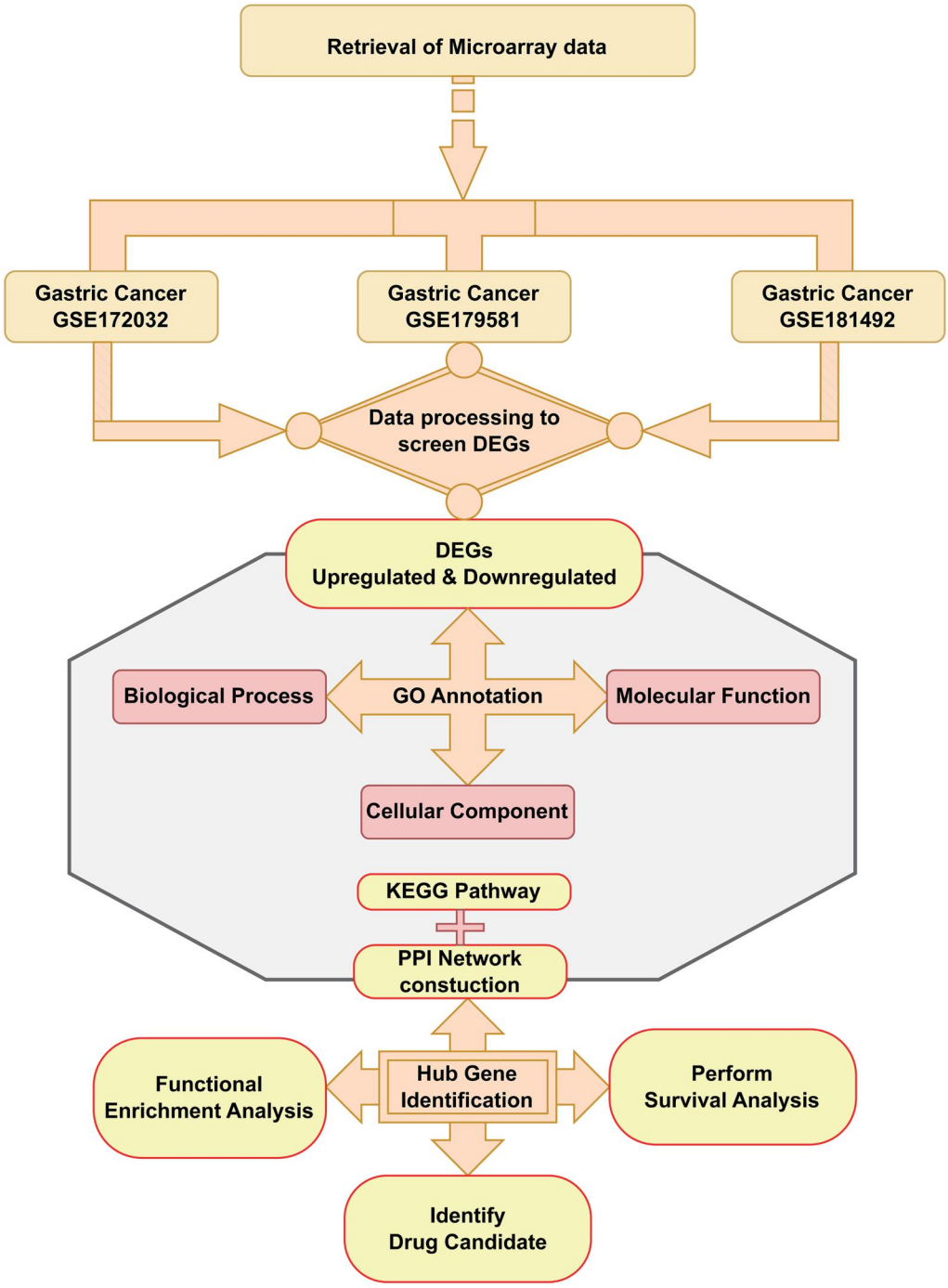

Fig. 1. The complete workflow followed to identify DEGs and to perform their *in-silico* analysis. DEG, differentially expressed gene; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction.

work-based approach have been documented in various cancers, including breast cancer[29] liver cancer[30] and GC.[31] Hub genes obtained from the PPI subnet were more meaningful than individual genes screened without network information.[32] Therefore, potential hub genes of GC were identified using PPI networks. According to the degree levels of PPIs, the top hub nodes were selected as hub genes.

Functional enrichment analysis of the hub genes

ExpressAnalyst is a web-based platform that focuses on gene expression profiling and meta-analysis. Functional enrichment analysis is a commonly used approach to identify the biological functions or pathways associated with a set of genes of interest. In this case, we were interested in performing functional enrichment analysis of the hub genes on https://www.expressanalyst.ca,

Table 1. Top 100 upregulated and top 100 downregulated gene identified in GC

DEGs	Genes
Upregulated Top 100 genes	*CXCL8, CXCL1, CCL20, ELF3, FCGR1A, LOC100128770, LGR5, SBSN, H2BC6, SLC26A3, GJB4, H2BC14, ZSCAN10, OVOL1, CFAP276, FUT3, SGK2, NECTIN4, TNFRSF9, TTC24, H2AC18, SLC7A4, QPCT, IL13, H3C2, OR2B6, CXCL2, LRRC25, SLC7A9, IL24, PI3, ALDOB, CILP2, CXCL3, LOC101928844, SOX30, DSG3, SP6, RAB33A, GPR25, GUCY1B2, H2AC13, H2BC7, SLC17A4, SLC43A2, VPREB3, ARMH1, ABCG8, XIRP1, SI, LAG3, PATL2, ADAMTS18, H2BU1, EREG, ZFP42, LINC00528, LUCAT1, HAPLN4, H2BC8, CYP27A1, GJB5, KRT4, TINAG, MAJIN, ASIC4, OR13H1, H2AC19, H2BC17, LINC00520, LHFPL3, H3C10, BCAR4, H3C8, MEFV, H2BC21, H2BC18, GPR84, C6orf52, FUT5, LOC105372412, PAGE2B, TULP2, H2AC17, PKP1, H2AC8, SLC3A1, LINC00628, TRIM54, BAAT, H1-6, ARL14, SLC5A2, PRKCG, H3C12, INHBA, CCL25, CST6, TNNC2, DNAJB5-DT*
Downregulated Top 100 genes	*KANK4, CHRNA4, ADCYAP1R1, LMO1, MRO, SYT10, CCKAR, GRIA2, DAND5, DPP10, DPP6, PRRT4, ASB11, SLITRK4, AQP4, RIMS1, ANKRD63, REEP1, CACNA2D3, CLCNKB, EPHA6, ACADL, PDILT, TAFA4, TUBB4A, CTB-178M22.2, OLFML1, RBPMS2, SLITRK3, FOXN4, PRIMA1, LRRTM1, LINC01018, DIRAS1, C2CD4C, OLFM3, CTNNA2, FAXC, LINC00908, LGI1, FUT1, MRGPRF, ERBB4, GABRA5, PTH1R, PTGER2, LGI3, SORCS3, GNAZ, SERTM1, FGFBP2, MGAT4C, SYT4, SLITRK5, MAPT, SMIM1, ENTPD8, EPHA5-AS1, LUZP2, LOC349160, TLR3, TMOD1, GABRG2, MTUS2, TSPAN18, ADCY8, NT5C1A, HMGCLL1, SACS-AS1, KCNIP3, HPN-AS1, HSPB7, HCN1, ONECUT1, LRP1B, PTENP1-AS, PKD1L2, PHLDB2, VLDLR, NPPC, AK4, RGMB-AS1, SEPTIN3, SNTB1, CPB1, PDGFD, LINC01625, NPAP1, WFDC1, NCAM1-AS1, NWD2, SLC16A7, SHISAL1, SLC38A3, LINC02060, WHAMMP2, MASP1, PITPNM3, FGF14-AS1, SPART*

DEG, differentially expressed gene; GC, gastric cancer.

an online tool for analyzing gene expression and gene network data. ExpressAnalyst visualizes enriched functional categories in a particular network.[33]

Overall survival (OS) analysis of key Hub genes

The Kaplan Meier Plotter serves as a robust tool for evaluating the association between gene expression (mRNA, miRNA, protein) and survival across a vast dataset encompassing over 30,000 samples derived from 21 distinct tumor types, such as breast, ovarian, lung, and GCs. The information is curated from diverse sources including GEO, the European Genome-phenome Archive, and The Cancer Genome Atlas (TCGA) databases. Its primary utility lies in conducting meta-analysis-driven identification and validation of survival-related biomarkers in cancer research. Utilizing this tool, we conducted an OS analysis of genes linked to these hub genes through the Kaplan–Meier Plotter online database.[34]

Identification of drug candidates based on hub genes

Understanding drug-protein binding is an essential step and is routinely investigated in the pre-clinical stages of drug discovery for determining the activity and consequences of the drug.[35] NetworkAnalysit, a powerful internet tool with a natural online interface, enables researchers to perform protein-drug interactions with ease.[25] This online tool was used to construct the protein-drug interactions in our analysis.[24]

Results

Exploring DEGs in GC: heatmap, volcano plot, and MD plot analysis

Galaxy web analysis identified a total of 1,079 DEGs, including 638 upregulated genes and 441 downregulated genes (Table 1). An expression heatmap, volcano plot, and MD plot (Fig. 2) were constructed to visualize the identified DEGs.

The heatmap, volcano plot, and MD plot show the expression profiles of the GSE172032, GSE179581, and GSE181492 datasets. A heatmap of DEGs is a useful visualization tool for analyzing gene expression data. The heatmap displays gene expression values as a color-coded matrix, with each row representing a gene and each column representing a sample or experimental condition. The color of each cell in the matrix corresponds to the expression level of a gene in a particular sample or condition, with higher expression levels represented by warmer colors (e.g., red) and lower expression levels by cooler colors (e.g., blue).[36] Figure 2a shows the heatmap of the top 10 DEGs in the three datasets. Gene expression levels are indicated by colors, as shown by the red arrow representing a high expression level and blue representing a low expression level. The top 10 DEGs based on log2FC and *p*-value obtained from the heatmap are presented in Table 2.

The ENSG00000077684 gene, also known as *JADE1*, was excluded from the table due to no statistical significance, as indicated by a log2FC of 0.862011258 and a *p*-value of 2.06E-05.

A volcano plot is a graphical representation commonly used to visualize the results of differential expression analysis. The x-axis of the volcano plot represents the log2FC in expression levels between two groups (such as treatment vs. control). The y-axis represents the negative logarithm of the *p*-value or the adjusted *p*-value, reflecting the statistical significance of the differential expression.

Figure 2b presents the volcano plot for the three aforementioned datasets. Each dot within the plot corresponds to a gene. Dots situated towards the positive end of the log2FC spectrum denote genes exhibiting elevated expression levels, while those positioned towards the negative end signify genes with reduced expression levels. Dots situated precisely at a log2FC score of zero indicate genes that, based on the criteria of a p-value < 0.05 and $|\log2\ FC| > 1$, show no significant differential expression.

Figure 2c shows the MD plot of DEGs in the three datasets. A red dot indicates genes with high levels of expression, a blue dot indicates genes with low levels of expression, and a black dot indicates genes with no differential expression based on the criteria of p-value < 0.05 and $|\log2\ FC| > 1$.

Functional enrichment analysis reveals diverse biological signatures of DEGs in GC

To identify the pathways that had the most significant involvement in the genes identified, the top 100 upregulated and top 100 downregulated DEGs were submitted to ShinyGO for GO and KEGG pathway analysis. GO analysis revealed that in biological process terms, the DEGs were mainly enriched in the interleukin-7-mediated signaling pathway, innate immune response in the mucosa, DNA replication-dependent nucleosome assembly, presynaptic

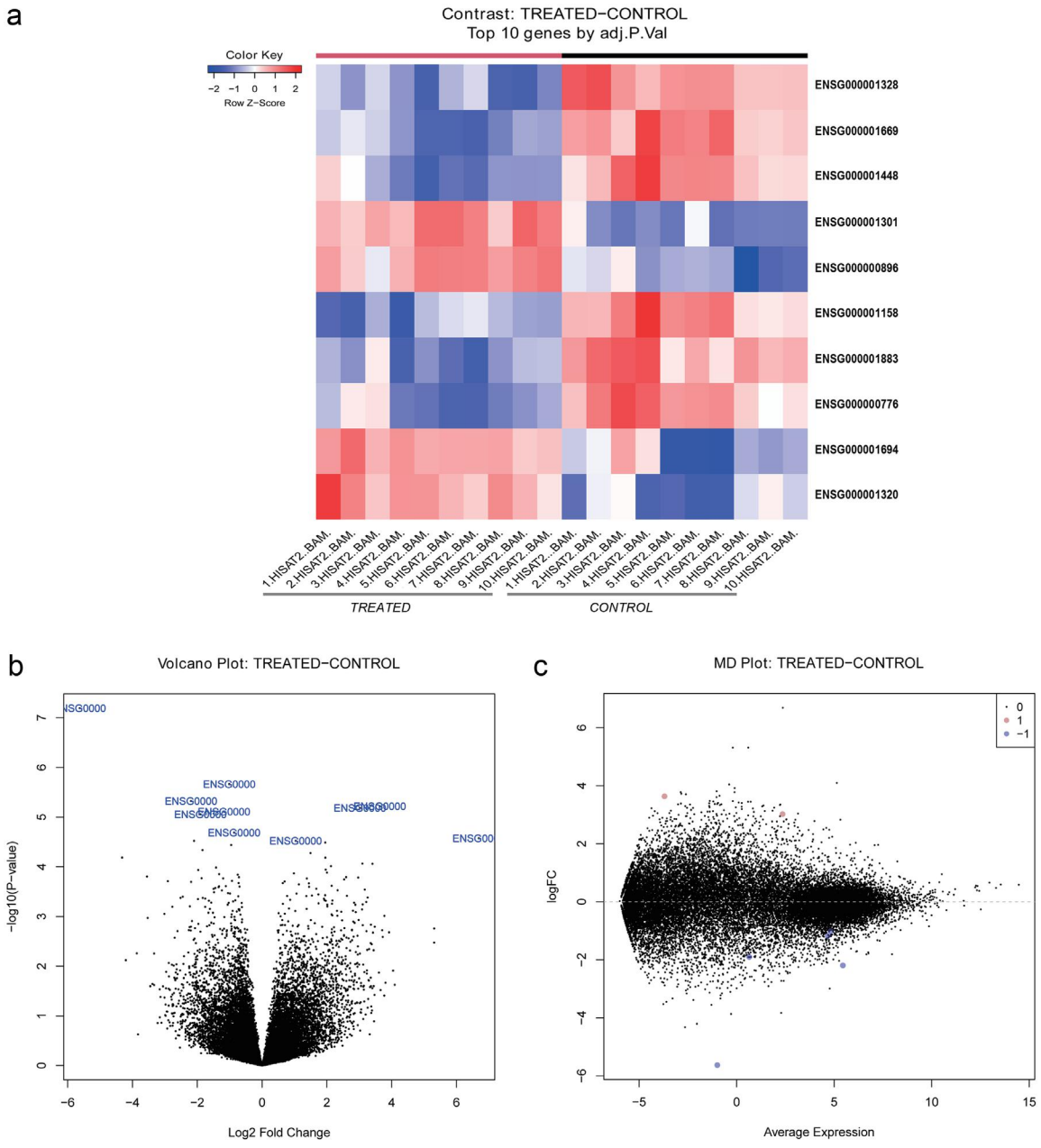

Fig. 2. Differential gene expression in GC. (a) Heatmap of the top 10 differentially expressed genes. (b) Volcano plot of Treated-Control. (c) MD plot of Treated-Control. MD, Mean-Difference; LogFC, Log Fold Change.

organization, antimicrobial humoral immune response mediated by an antimicrobial peptide, nucleosome assembly, chromatin assembly, nucleosome organization, chemokine-mediated signaling pathway, chromatin assembly or disassembly, antimicrobial humoral response, DNA packaging, negative regulation of inflammatory response to an antigenic stimulus, chromatin remodeling, protein–DNA complex assembly, DNA conformation change, and protein–DNA complex subunit organization (Fig. 3a).

The GO analysis further unveiled that, with regard to cellular components, the DEGs exhibited prominent enrichment in various categories. These included Nucleosome, DNA packaging complex, Protein-DNA complex, Cornified envelope, Brush border membrane, GABA-ergic synapse, Integral component of postsynaptic specialization membrane, Postsynaptic specialization membrane, Ion channel complex, Receptor complex, Transmembrane transporter complex, Synaptic membrane, Transporter complex, Integral component of the plasma membrane, Plasma membrane protein complex, Chromatin, Plasma membrane region, Synapse (Fig. 3b).

The molecular functions of DEGs included l-cystine transmembrane transporter activity, 4-galactosyl-N-acetylglucosaminide 3-alpha-L-fucosyltransferase activity, fucosyltransferase activity, CXCR chemokine receptor binding, basic amino acid transmembrane transporter activity, chemokine activity, peptide hormone

Table 2. The top 10 DEGs based on log2FC and *p*-value obtained from the heatmap

Gene ID	Gene Name	log2FC	*p*-value
ENSG00000132854	*KANK4*	−5.629	6.41E-08
ENSG00000166948	*TGM6*	1.209	0.012455544
ENSG00000144824	*PHLDB2*	−2.193	4.75E-06
ENSG00000130182	*ZSCAN10*	3.635	6.05E-06
ENSG00000089692	*LAG3*	3.021	6.55E-06
ENSG00000115850	*LCT*	1.572	0.034674889
ENSG00000188373	*C10orf99*	1.363	0.034141968
ENSG00000169429	*CXCL8*	6.689	2.66E-05
ENSG00000132000	*PODNL1*	1.828	0.027691668

DEG, differentially expressed gene.

binding, potassium channel regulator activity, chemokine receptor binding, protein heterodimerization activity, ligand-gated ion channel activity, cytokine activity, receptor ligand activity, signaling receptor activator activity, channel activity, passive transmembrane transporter activity, protein dimerization activity, transmembrane transporter activity, and transporter activity (Fig. 3c).

KEGG pathway analysis demonstrated that DEGs were significantly enriched in systemic lupus erythematosus, glycosphingolipid biosynthesis, neutrophil extracellular trap formation, alcoholism, nicotine addiction, viral protein interaction with cytokine and cytokine receptor, legionellosis, IL-17 signaling pathway, epithelial cell signaling in *Helicobacter pylori* infection, GABAergic synapse, rheumatoid arthritis, pancreatic secretion, amoebiasis, insulin secretion, retrograde endocannabinoid signaling, necroptosis, chemokine signaling pathway, viral carcinogenesis, cytokine–cytokine receptor interaction, transcriptional misregulation in cancer (Fig. 3d).

PPI network construction and module analysis unveil molecular insights into DEGs

By evaluating the relationships between various DEGs, a PPI network was constructed to assess the significance of these DEGs. This strategy enables researchers to concentrate on the most pertinent interactions and pinpoint crucial functional DEG modules, illuminating the molecular mechanisms underlying the studied illness or disease. Interactions between the identified DEGs revealed a total of 664 nodes and 1,892 edges in 29 subnetworks (Fig. 4).

Prediction of top hub genes through PPI network analysis

Hub gene prediction aimed to identify the hub genes based on the PPI network and uncover their clinical value. Hub genes were identified using PPI networks. According to the degree levels of PPIs, the top hub nodes were selected as hub genes. Our study identified a total of 30 hub nodes and among them, the top 10 hub nodes were predicted as hub genes for further analysis as shown in Table 3.

Functional enrichment analysis of predicted hub genes unveils insights into molecular mechanisms

Subsequent functional enrichment analysis, visualizing functional categories enriched in a network, revealed that the genes in this module were mainly enriched in systemic lupus erythematosus, alcoholism, viral carcinogenesis, necroptosis, transcriptional misregulation in cancer, gastric acid secretion, thyroid hormone synthesis, calcium signaling pathway, ERBB4 signaling pathway, insulin, and salivary secretion, etc. (Fig. 5)

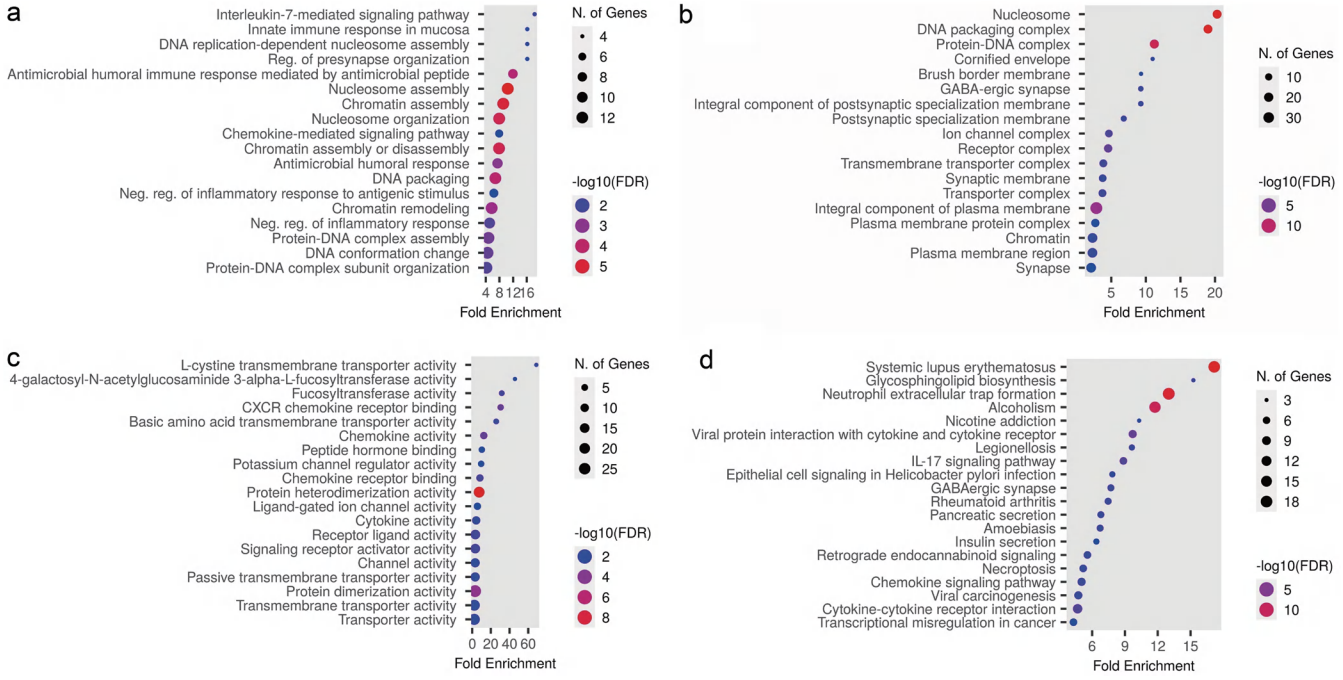

Fig. 3. Functional enrichment analysis of DEGs in GC. GO analysis revealed that DEGs were significantly enriched in (a) biological process terms (b) cellular component terms (c) molecular function terms (d) significantly enriched KEGG terms obtained from KEGG analysis. DEG, differentially expressed gene; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

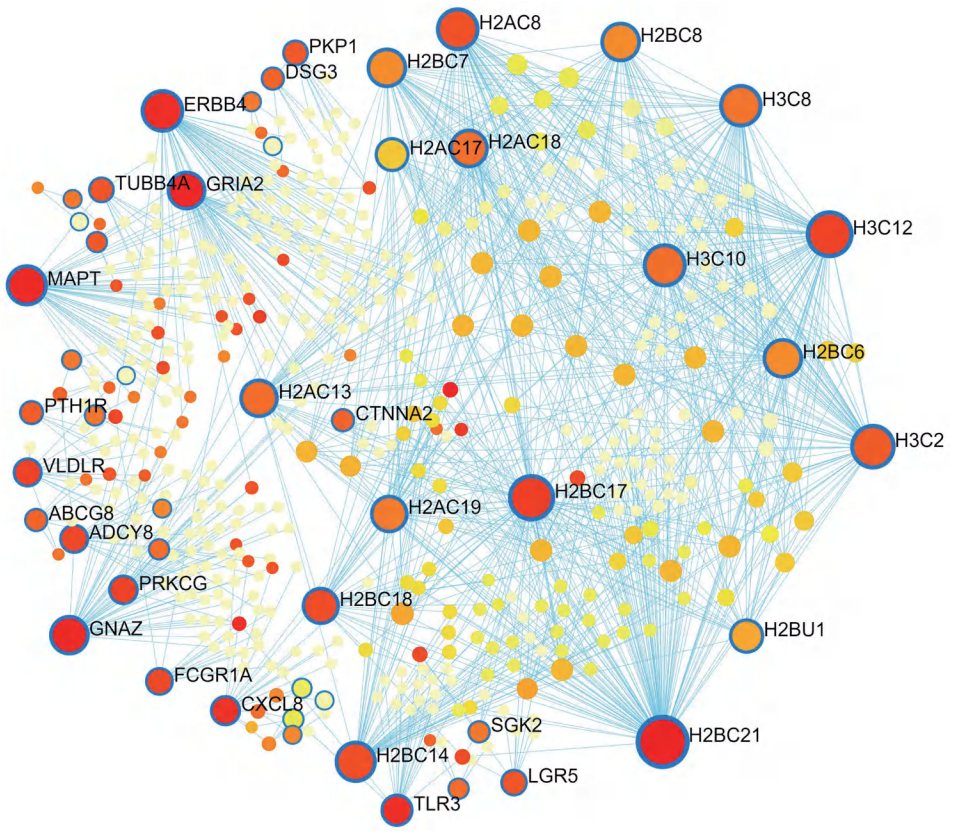

Fig. 4. PPI network of the top 100 upregulated and top 100 downregulated genes identified in GC. PPI, protein-protein interaction; GC, gastric cancer.

OS analysis reveals prognostic significance of hub genes in GC patients

The outcomes from Kaplan–Meier plotting underscored the impact of two central genes (*ERBB4* and *MAPT*) on GC prognosis. This analysis included 875 patients. Our findings indicate that *ERBB4* and *MAPT* exhibit favorable associations with the overall survival of GC patients. Conversely, the remaining hub genes (*H2BC21, H3C12, H2BC17, H3C2, H3C10, H2AC8, H3C8, H2BC14*) were not present in the Kaplan-Meier Plotter database (Fig. 6).

Prediction of drug candidates for the top 10 hub genes

The NetworkAnalyst tool (www.networkanalyst.ca/) was employed to scrutinize potential drug candidates for the top 10 hub genes through protein-drug interaction analysis. This analysis leveraged the DrugBank database (version 5.0), which is exclusively personalized for human data. (25). The analysis concluded that only two drugs interact with the protein product of the MAPT hub gene. In contrast, other hub genes did not show any interaction with the enlisted drugs in the database. Figure 7 shows the protein-drug interaction network between the hub proteins of MAPT, and the proposed drugs were obtained with the help of the NetworkAnalyst tool, where the degree of interaction is represented by the area of the nodes. The tool suggested that docetaxel and paclitaxel from the DrugBank database (version 5.0) play a role in the treatment of many cancers, including GC, and are associated with the regulation of *MAPT* expression. Docetaxel is a taxoid antineoplastic agent used to treat various cancers, such as locally advanced or metastatic breast cancer, metastatic prostate cancer, gastric adenocarcinoma, and head and neck cancer.[37,38] Similarly, paclitaxel is a taxoid chemotherapeutic agent used as a first-line and subsequent therapy for the treatment of advanced carcinoma of the ovary, and other various cancers, including breast and lung cancer.[39]

Discussion

The TCGA research network has devised a genetic classification system for GC, encompassing four distinct subtypes: Epstein Barr virus positive, microsatellite instability (MSI), genomically stable, and chromosomally unstable (CIN). This classification is rooted in the analysis of genetic alterations within GC samples, offering valuable insights into the molecular basis of the malignancy. The TCGA classification has been popularly utilized in both preclinical and clinical studies to settle on treatment approaches and patient prognosis. For example, it aids in identifying specific therapeutic targets for different GC subtypes. A case in point would opt for immune checkpoint inhibitors for MSI-high tumors. Furthermore, it has proven to be instrumental in creating prognostic models for patient survival and guiding personalized treatment methods.[40]

The PD1/PDL1 pathway plays a critical role in the immune checkpoint system in GC. The PD1 receptors on immune cells interact with PDL1 ligands, which are expressed in both tumor cells and immune cells. This interaction curbs immune activity causing subsequent immune suppression and evasion of tumor. High PDL1 expression is usually connected to poor prognosis in GC patients, indicating its potential as a prognostic factor. Moreover, the PD1/PDL1 pathway has already been a target for immunotherapy in

Table 3. The top 20 hub nodes according to degree levels

ENTREZ ID	ENSEMBL ID	GENE Symbol	Degree	Betweenness
8349	ENSG00000184678	*H2BC21*	116	46,206.28
8356	ENSG00000197153	*H3C12*	74	8,226.37
8348	ENSG00000274641	*H2BC17*	71	8,981.81
8358	ENSG00000286522	*H3C2*	62	2,602.36
8357	ENSG00000278828	*H3C10*	55	1,211.2
2066	ENSG00000178568	*ERBB4*	53	23,502.95
3012	ENSG00000277075	*H2AC8*	53	4,246.52
8355	ENSG00000273983	*H3C8*	52	1,163.28
4137	ENSG00000186868	*MAPT*	47	28,396.16
8342	ENSG00000273703	*H2BC14*	47	4,428.13
2781	ENSG00000128266	*GNAZ*	41	28,934.41
8343	ENSG00000277224	*H2BC7*	41	415.45
8344	ENSG00000274290	*H2BC6*	41	415.45
8339	ENSG00000273802	*H2BC8*	41	410.57
2891	ENSG00000120251	*GRIA2*	38	42,220.87
8337	ENSG00000203812	*H2AC18*	38	1,288.19
8329	ENSG00000196747	*H2AC13*	37	1,468.81
723790	ENSG00000272196	*H2AC19*	34	795.73
440689	ENSG00000203814	*H2BC18*	33	4,941.79
128312	ENSG00000196890	*H2BU1*	25	157.58

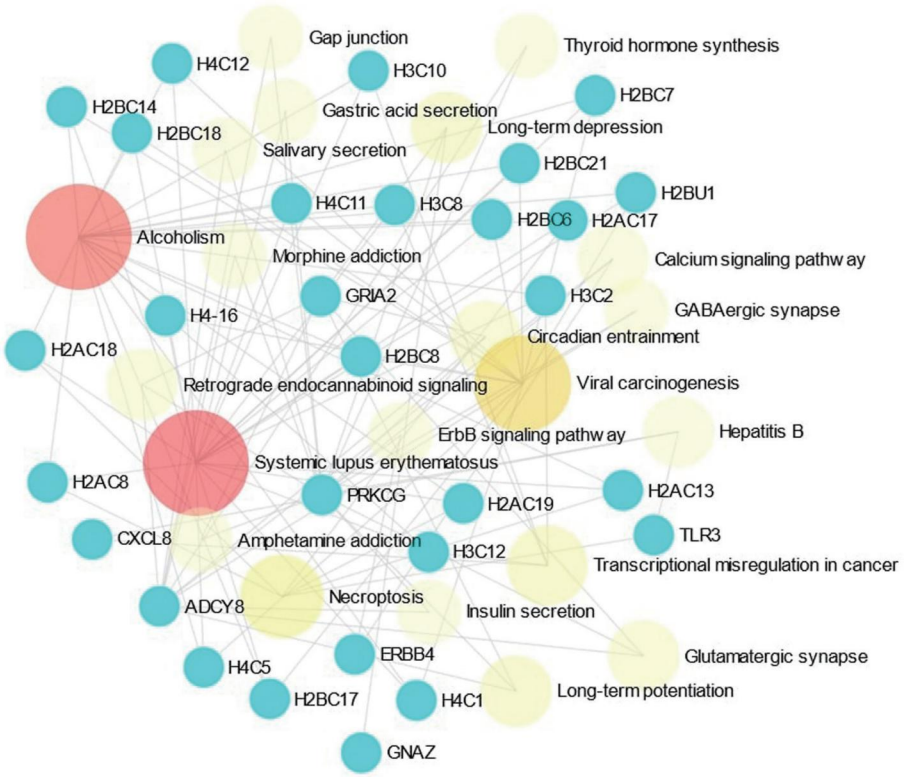

Fig. 5. Functional enrichment analysis of predicted hub genes.

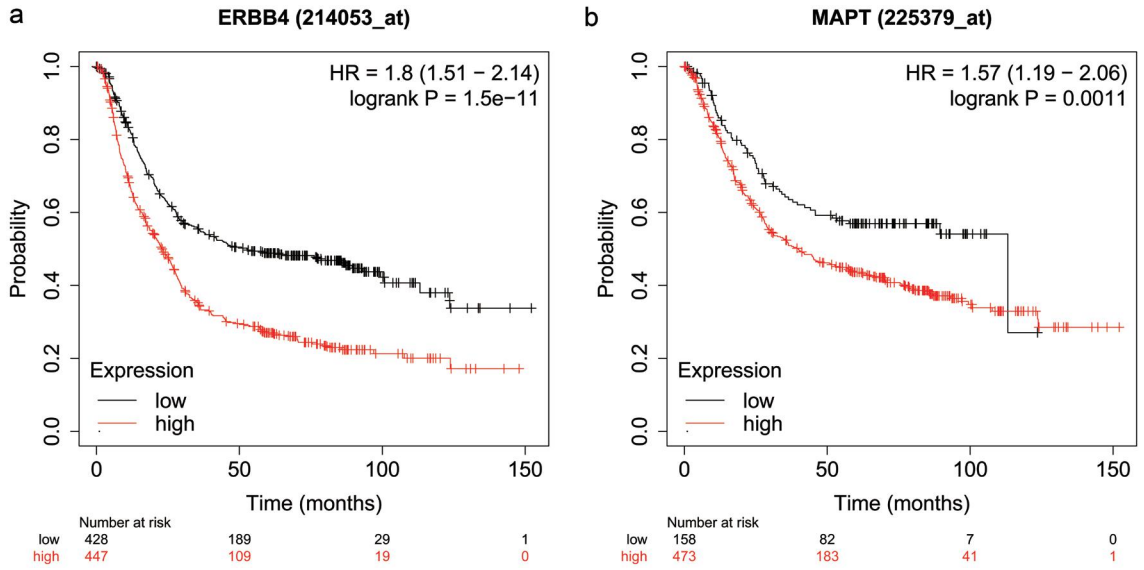

Fig. 6. Overall survival analysis of GC patients. Here, (a) *ERBB4* and (b) *MAPT* expression data-based (microarray) association study in the survival rate of patients with gastric cancer. A log-rank test was performed to evaluate the survival differences between the two curves. HR, Hazard Ratio; ERBB4, erythroblastic oncogene B; MAPT, microtubule-associated protein Tau.

GC, with promising results from clinical trials using the PD1/PDL1 inhibitors—pembrolizumab and nivolumab for advanced cancer patients. This pathway is important because it regulates the immune response and serves as a target for personalized treatment options. However, further research is required to identify additional predictive markers, as not all patients with increased PDL1 expression respond to its inhibitors.[40]

The present study employed a comprehensive bioinformatics approach to identify key candidate genes and pathways associated with human GC. Through the integration of gene expression profiling, PPI analysis, pathway enrichment, and functional annotation analysis, the study identified 10 hub genes that may serve as potential biomarkers for GC. The identified hub genes included *H2BC21, H3C12, H2BC17, H3C2, H3C10, ERBB4, H2AC8, H3C8, H2BC14*, and *MAPT*.

One of the important hub genes, *ERBB4* (also known as *HER4*) is a member of the epidermal growth factor receptor family of receptor tyrosine kinases (RTKs). This receptor has been implicated in the development and progression of various cancers, including GC.[41] Several studies have shown that *ERBB4* can promote the proliferation of GC cells through the PI3K/Akt signaling pathway.[42–44] This pathway is a key regulator of cell growth, survival, and metabolism, and is frequently dysregulated in cancer. Upon ligand binding, ERBB4 undergoes activation, subsequently recruiting and activating PI3K, which, in turn, triggers Akt activation. The activated Akt pathway fosters cell survival and growth by phosphorylating downstream targets involved in essential processes such as cell cycle regulation, protein synthesis, and metabolism. In GC cells, ERBB4 has been found to promote proliferation by activating the PI3K/Akt pathway. Inhibition of ERBB4 or its downstream effectors, such as PI3K or Akt, can significantly reduce cell proliferation and induce apoptosis in GC cells. Therefore, targeting the ERBB4/PI3K/Akt pathway may represent a promising strategy for the treatment of GC.[42–44]

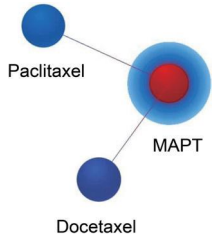

Fig. 7. Protein-drug Interactions analysis with the products of *MAPT* hub genes. MAPT, microtubule-associated protein Tau.

Another pivotal hub gene, known as the clustered histone gene group H3 (*H3C2, H3C8, H3C10, H3C12*), plays a crucial role in chromatin remodeling and is intricately associated with gastric adenocarcinoma.[45] Numerous investigations have indicated that modifications in the expression of H3 cluster histone genes could play a pivotal role in the initiation and advancement of GC. For instance, Mitani *et al.*[46] found that the tumor suppressor gene P21 WAP1/CIP1, which has a low level of H3 acetylation on promoter, resulted in its down-regulation in GC. Additionally, a study revealed a significant upregulation of the H3 cluster of histone genes in GC tissues.[47] Furthermore, alterations in the post-translational modifications of histone proteins have also been implicated in GC. As an illustration, the dysregulation of histone H3 acetylation on lysine residues has been demonstrated in GC. Elevated levels of histone H3 acetylation have been connected to tumor progression and an unfavorable prognosis.[46,48] In addition, alterations in the post-translational modifications of histone proteins have also been implicated in GC. For example, the acetylation of lysine residues on histone H3 has been shown to be dysregulated in GC, and increased levels of histone H3 acetylation are associated with tumor progression and poor prognosis.

Collectively, these studies suggest that alterations in the expression and modification of H3 cluster histone genes may play a role in the development and progression of GC. Further extensive investigations are needed to gain deeper insights into the intricate molecular mechanisms that underlie these findings and to pave the way for innovative therapeutic approaches aimed at both prevent-

ing and treating GC.

Another pivotal hub gene, *MAPT*, is closely linked to GC due to its expression pattern. Tau actively contributes to the stabilization and assembly of microtubules. Its primary expression is observed in neurons, where it crucially maintains axonal structure and function. However, recent studies have suggested that tau expression may also be involved in the development and progression of certain types of cancer, including GC.[41] In one study, it was reported that there was a notable upregulation of tau expression in GC tissues when compared to adjacent noncancerous tissues.[47] Furthermore, elevated tau expression was associated with advanced tumor stage, lymph node metastasis, and an unfavorable patient prognosis.[49] The precise mechanisms that underlie the link between tau expression and GC remain partly elusive. However, it is plausible that these mechanisms encompass interactions with other proteins or modulation of signaling pathways that oversee critical cellular processes such as proliferation, survival, and migration. Overall, these studies suggest that the expression of *MAPT* may be associated with GC. However, further research is needed to better understand the role of tau in GC pathogenesis and to develop novel therapeutic strategies targeting tau for the prevention and treatment of this disease.

The hub mentioned above genes have previously been reported to be involved in various cellular processes, including nucleosome and chromatin assembly, ligand-gated ion channel activity, CXCR signaling receptor activity, systemic lupus erythematous, glycosphingolipid biosynthesis, IL-17 signaling pathway, pancreatic secretion, and viral carcinogenesis, which are recognized to be crucial in the emergence and progression of stomach cancer.[50–52] The investigation additionally identified several novel genes, including *H2BC21, H2BC17, H3BC14, and H2AC8* which have not previously been implicated in GC.

Through pathway enrichment analysis, a cluster of pivotal pathways correlated with GC emerged. These include gastric acid secretion, alcoholism, salivary secretion, ErbB4 signaling pathway, viral carcinogenesis, and retrograde endocannabinoid signaling pathways. These pathways, which are dysregulated across diverse cancers, including GC, play a significant role in crucial processes, such as cell proliferation and survival.

The findings of this study provide valuable insights into the molecular mechanisms underlying GC development and progression. The identified hub genes and pathways may serve as potential therapeutic targets for the development of novel therapies for GC treatment. Furthermore, the identified hub genes may serve as potential biomarkers for the early detection of GC. This study has several limitations. Limitations and potential directions for future research are that the stomach region from which the tumor samples were taken was not specified before collecting the pair-ended microarray datasets used in this analysis, and samples taken from the same disease stage are preferable for a better study of each form of cancer. However, the source of the microarray data was not mentioned, and all the linear correlations between gene expression levels that were known to exist were used in this investigation. Future research that incorporates nonlinear relationships more thoroughly may produce more accurate information about the interactions between proteins and possibly recommend new medicines. To quantify gene expression, RNA-Seq technology may provide more accurate data. However, paired RNA-Seq data were not available for this study, paired microarray data were used instead, which matched better and might yield more reliable results. In addition, the study did not investigate the regulatory mechanisms of the identified hub genes in GC, which warrants further investigation.

Conclusions

This study identified 1079 DEGs, with 638 upregulated and 441 downregulated, between human GC tissues and matched adjacent normal tissue specimens based on the GSE172032, GSE179581, and GSE181492 datasets. Further analysis of DEGs suggested that three types of hub genes namely, H3 Clustered Histone genes (*H3C2, H3C8, H3C10, H3C12*), *HER4*, and *MAPT*, could play critical roles in the progression of GC. The strong association of these predicted hub genes with the progression of GC has been identified in many studies by researchers. In summary, the present study provides a comprehensive analysis of key candidate genes and pathways in human GC using a bioinformatics approach. The identified hub genes and pathways provide valuable insights into the molecular mechanisms underlying GC development and progression and may serve as potential therapeutic targets and biomarkers for the early detection of GC.

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Conflict of interest

The authors declare that they have no conflict of interests related to this publication.

Author contributions

Conceptualization: SDG, RKD; data curation: SH & SDG; formal analysis: SH; methodology: SH; writing – original draft: SH, IH; review and editing: SDG, RKD.

Data sharing statement

No additional data are available.

References

[1] Zali H, Rezaei-Tavirani M, Azodi M. Gastric cancer: prevention, risk factors and treatment. Gastroenterol Hepatol Bed Bench 2011;4(4):175–185. doi:10.22037/ghfbb.v4i4.193, PMID:24834180.

[2] Nagini S. Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention. World J Gastrointest Oncol 2012;4(7):156–169. doi:10.4251/wjgo.v4.i7.156, PMID:22844547.

[3] Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. Prz Gastroenterol 2019;14(1):26–38. doi:10.5114/pg.2018.80001, PMID:30944675.

[4] Peleteiro B, Severo M, La Vecchia C, Lunet N. Model-based patterns in stomach cancer mortality worldwide. Eur J Cancer Prev 2014;23(6):524–531.doi:10.1097/CEJ.0b013e328364f2b6,PMID:25259885.

[5] Hou R, Mu Z, Kang W, Liu Z, Na B, Niu W. Cancer mortality in 2020 and its trend analysis in Inner Mongolia during four time periods from 1973 to 2020. Front Oncol 2023;13:1096968. doi:10.3389/

fonc.2023.1096968, PMID:36798823.
[6] Garattini SK, Basile D, Cattaneo M, Fanotto V, Ongaro E, Bonotto M, *et al*. Molecular classifications of gastric cancers: Novel insights and possible future applications. World J Gastrointest Oncol 2017;9(5):194–208. doi:10.4251/wjgo.v9.i5.194, PMID:28567184.
[7] Wang FH, Shen L, Li J, Zhou ZW, Liang H, Zhang XT, *et al*. The Chinese Society of Clinical Oncology (CSCO): clinical guidelines for the diagnosis and treatment of gastric cancer. Cancer Commun (Lond) 2019;39(1):10. doi:10.1186/s40880-019-0349-9, PMID:30885279.
[8] Wang F, Xue Q, Xu D, Jiang Y, Tang C, Liu X. Identifying the hub gene in gastric cancer by bioinformatics analysis and in vitro experiments. Cell Cycle 2020;19(11):1326–1337. doi:10.1080/15384101.2020.1749789, PMID:32293980.
[9] Lee YC, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, *et al*. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. Gastroenterology 2016;150(5):1113–1124.e5. doi:10.1053/j.gastro.2016.01.028, PMID:26836587.
[10] Lei ZN, Teng QX, Tian Q, Chen W, Xie Y, Wu K, *et al*. Signaling pathways and therapeutic interventions in gastric cancer. Signal Transduct Target Ther 2022;7(1):358. doi:10.1038/s41392-022-01190-w, PMID:36209270.
[11] He J, Qiu LX, Wang MY, Hua RX, Zhang RX, Yu HP, *et al*. Polymorphisms in the XPG gene and risk of gastric cancer in Chinese populations. Hum Genet 2012;131(7):1235–1244. doi:10.1007/s00439-012-1152-8, PMID:22371296.
[12] He J, Wang MY, Qiu LX, Zhu ML, Shi TY, Zhou XY, *et al*. Genetic variations of mTORC1 genes and risk of gastric cancer in an Eastern Chinese population. Mol Carcinog 2013;52(Suppl 1):E70–E79. doi:10.1002/mc.22013, PMID:23423739.
[13] He J, Zhuo ZJ, Zhang A, Zhu J, Hua RX, Xue WQ, *et al*. Genetic variants in the nucleotide excision repair pathway genes and gastric cancer susceptibility in a southern Chinese population. Cancer Manag Res 2018;10:765–774. doi:10.2147/CMAR.S160080, PMID:29695933.
[14] Li T, Gao X, Han L, Yu J, Li H. Identification of hub genes with prognostic values in gastric cancer by bioinformatics analysis. World J Surg Oncol 2018;16(1):114. doi:10.1186/s12957-018-1409-3, PMID:29921304.
[15] Wang K, Yuen ST, Xu J, Lee SP, Yan HH, Shi ST, *et al*. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. Nat Genet 2014;46(6):573–582. doi:10.1038/ng.2983, PMID:24816253.
[16] Jiang P, Liu XS. Big data mining yields novel insights on cancer. Nat Genet 2015;47(2):103–104. doi:10.1038/ng.3205, PMID:25627899.
[17] Barrett T, Suzek TO, Troup DB, Wilhite SE, Ngau WC, Ledoux P, *et al*. NCBI GEO: mining millions of expression profiles—database and tools. Nucleic Acids Res 2005;33(Database issue):D562–D566. doi:10.1093/nar/gki022, PMID:15608262.
[18] Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002;30(1):207–210. doi:10.1093/nar/30.1.207, PMID:11752295.
[19] Batut B, van den Beek M, Doyle MA, Soranzo N. RNA-Seq Data Analysis in Galaxy. RNA Bioinformatics 2021;2284:367–392. doi:10.1007/978-1-0716-1307-8_20, PMID:33835453.
[20] Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, *et al*. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 2018;46(W1):W537–W544. doi:10.1093/nar/gky379, PMID:29790989.
[21] Vandel J, Gheeraert C, Staels B, Eeckhoute J, Lefebvre P, Dubois-Chevalier J. GIANT: galaxy-based tool for interactive analysis of transcriptomic data. Sci Rep 2020;10(1):19835. doi:10.1038/s41598-020-76769-w, PMID:33199699.
[22] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, *et al*. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43(7):e47. doi:10.1093/nar/gkv007, PMID:25605792.
[23] Ge SX, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics 2020;36(8):2628–2629. doi:10.1093/bioinformatics/btz931, PMID:31882993.
[24] Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Res 2019;47(W1):W234–W241. doi:10.1093/nar/gkz240, PMID:30931480.
[25] Xia J, Gill EE, Hancock RE. NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. Nat Protoc 2015;10(6):823–844. doi:10.1038/nprot.2015.052, PMID:25950236.
[26] Xia J, Benner MJ, Hancock RE. NetworkAnalyst—integrative approaches for protein-protein interaction network analysis and visual exploration. Nucleic Acids Res 2014;42(Web Server issue):W167–W174. doi:10.1093/nar/gku443, PMID:24861621.
[27] Xu J, Li Y. Discovering disease-genes by topological features in human protein-protein interaction network. Bioinformatics 2006;22(22):2800–2805. doi:10.1093/bioinformatics/btl467, PMID:16954137.
[28] Hu Y, Zhang Y, Ren J, Wang Y, Wang Z, Zhang J. Statistical Approaches for the Construction and Interpretation of Human Protein-Protein Interaction Network. Biomed Res Int 2016;2016:5313050. doi:10.1155/2016/5313050, PMID:27648447.
[29] Zhuang DY, Jiang L, He QQ, Zhou P, Yue T. Identification of hub subnetwork based on topological features of genes in breast cancer. Int J Mol Med 2015;35(3):664–674. doi:10.3892/ijmm.2014.2057, PMID:25573623.
[30] Jin B, Wang W, Du G, Huang GZ, Han LT, Tang ZY, *et al*. Identifying hub genes and dysregulated pathways in hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2015;19(4):592–601. PMID:25753876.
[31] Chang W, Ma L, Lin L, Gu L, Liu X, Cai H, *et al*. Identification of novel hub genes associated with liver metastasis of gastric cancer. Int J Cancer 2009;125(12):2844–2853. doi:10.1002/ijc.24699, PMID:19569046.
[32] Langfelder P, Mischel PS, Horvath S. When is hub gene selection better than standard meta-analysis? PLoS One 2013;8(4):e61505. doi:10.1371/journal.pone.0061505, PMID:23613865.
[33] Liu P, Ewald J, Pang Z, Legrand E, Jeon YS, Sangiovanni J, *et al*. ExpressAnalyst: A unified platform for RNA-sequencing analysis in non-model species. Nat Commun 2023;14(1):2995. doi:10.1038/s41467-023-38785-y, PMID:37225696.
[34] Lánczky A, Győrffy B. Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. J Med Internet Res 2021;23(7):e27633. doi:10.2196/27633, PMID:34309564.
[35] Sharma H, Navalkar A, Maji SK, Agrawal A. Analysis of drug–protein interaction in bio-inspired microwells. SN Applied Sciences 2019;1(8):819. doi:10.1007/s42452-019-0778-8.
[36] Grant GR, Manduchi E, Stoeckert CJ Jr. Analysis and management of microarray gene expression data. Curr Protoc Mol Biol 2007;77:19.6.1–19.6.30. doi:10.1002/0471142727.mb1906s77, PMID:18265395.
[37] Imran M, Saleem S, Chaudhuri A, Ali J, Baboota S. Docetaxel: An update on its molecular mechanisms, therapeutic trajectory and nanotechnology in the treatment of breast, lung and prostate cancer. J Drug Deliv Sci Tec 2020;60:101959. doi:10.1016/j.jddst.2020.101959.
[38] Ilson DH. Advances in the treatment of gastric cancer: 2019. Curr Opin Gastroenterol 2019;35(6):551–554. doi:10.1097/MOG.0000000000000577, PMID:31436556.
[39] Sharifi-Rad J, Quispe C, Patra JK, Singh YD, Panda MK, Das G, *et al*. Paclitaxel: Application in Modern Oncology and Nanomedicine-Based Cancer Therapy. Oxid Med Cell Longev 2021;2021:3687700. doi:10.1155/2021/3687700, PMID:34707776.
[40] Zhang Y, Yang Y, Chen Y, Lin W, Chen X, Liu J, *et al*. PD-L1: Biological mechanism, function, and immunotherapy in gastric cancer. Front Immunol 2022;13:1060497. doi:10.3389/fimmu.2022.1060497, PMID:36505487.
[41] Wu H, Huang M, Lu M, Zhu W, Shu Y, Cao P, *et al*. Regulation of microtubule-associated protein tau (MAPT) by miR-34c-5p determines the chemosensitivity of gastric cancer to paclitaxel. Cancer Chemother Pharmacol 2013;71(5):1159–1171. doi:10.1007/s00280-013-2108-y, PMID:23423488.
[42] Xu J, Gong L, Qian Z, Song G, Liu J. ERBB4 promotes the proliferation of gastric cancer cells via the PI3K/Akt signaling pathway. Oncol Rep 2018;39(6):2892–2898. doi:10.3892/or.2018.6343, PMID:29620274.
[43] Song G, Zhang H, Chen C, Gong L, Chen B, Zhao S, *et al*. miR-551b regulates epithelial-mesenchymal transition and metastasis of gastric cancer by inhibiting ERBB4 expression. Oncotarget 2017;8(28):45725–45735. doi:10.18632/oncotarget.17392, PMID:28501849.
[44] El-Gamal MI, Mewafi NH, Abdelmotteleb NE, Emara MA, Tarazi H,

Sbenati RM, *et al*. A Review of HER4 (ErbB4) Kinase, Its Impact on Cancer, and Its Inhibitors. Molecules 2021;26(23):7376. doi:10.3390/molecules26237376, PMID:34885957.
[45] Bilgiç F, Gerçeker E, Boyacıoğlu SÖ, Kasap E, Demirci U, Yıldırım H, *et al*. Potential role of chromatin remodeling factor genes in atrophic gastritis/gastric cancer risk. Turk J Gastroenterol 2018;29(4):427–435. doi:10.5152/tjg.2018.17350, PMID:30249557.
[46] Mitani Y, Oue N, Hamai Y, Aung PP, Matsumura S, Nakayama H, *et al*. Histone H3 acetylation is associated with reduced p21(WAF1/CIP1) expression by gastric carcinoma. J Pathol 2005;205(1):65–73. doi:10.1002/path.1684, PMID:15586362.
[47] Rashid M, Shah SG, Verma T, Chaudhary N, Rauniyar S, Patel VB, *et al*. Tumor-specific overexpression of histone gene, H3C14 in gastric cancer is mediated through EGFR-FOXC1 axis. Biochim Biophys Acta Gene Regul Mech 2021;1864(4-5):194703. doi:10.1016/j.bbagrm.2021.194703, PMID:33727172.
[48] Wang GG, Allis CD, Chi P. Chromatin remodeling and cancer, Part I: Covalent histone modifications. Trends Mol Med 2007;13(9):363–372. doi:10.1016/j.molmed.2007.07.003, PMID:17822958.
[49] Callari M, Sola M, Magrin C, Rinaldi A, Bolis M, Paganetti P, *et al*. Cancer-specific association between Tau (MAPT) and cellular pathways, clinical outcome, and drug response. Sci Data 2023;10(1):637. doi:10.1038/s41597-023-02543-y.
[50] Yu C, Chen J, Ma J, Zang L, Dong F, Sun J, *et al*. Identification of Key Genes and Signaling Pathways Associated with the Progression of Gastric Cancer. Pathol Oncol Res 2020;26(3):1903–1919. doi:10.1007/s12253-019-00781-3, PMID:31848941.
[51] Dey L, Mukhopadhyay A. A systems biology approach for identifying key genes and pathways of gastric cancer using microarray data. Gene Reports 2021;22:101011. doi:10.1016/j.genrep.2020.101011.
[52] Li Z, Zhou Y, Tian G, Song M. Identification of Core Genes and Key Pathways in Gastric Cancer using Bioinformatics Analysis. Russian Journal of Genetics 2021;57(8):963–971. doi:10.1134/S1022795421080081.

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Review Article

Liver Fibrosis as an Independent Cardiovascular Risk Factor in Non-alcoholic Fatty Liver Disease

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Abstract

This review summarizes the current investigations that confirm the significance of liver fibrosis (LF) as an independent cardiovascular risk factor in non-alcoholic fatty liver disease (NAFLD). PubMed, Google Scholar, Web of Science platform, Reference Citation Analysis, and Cochrane Systematic Reviews were searched for articles published between 2008 and 2023. Relevant articles were identified using the following keywords: "cardiovascular diseases", "cardiovascular risk factors", "non-alcoholic fatty liver disease", "nonalcoholic steatohepatitis", and "liver fibrosis". The reference lists of the identified articles were also searched for other relevant publications. The investigations that described LF as a cardiovascular risk factor in NAFLD met the inclusion criteria. NAFLD occupies a leading position among liver diseases worldwide. Cardiovascular disorders are the most significant cause of unfavorable outcomes in NAFLD patients. Currently, the relationship between them is well established. The pathophysiological mechanisms predisposing to the development of cardiovascular disorders in NAFLD include atherogenic dyslipidemia, impaired glucose metabolism and liver insulin resistance, low-grade systemic inflammation, endothelial dysfunction, cardiovascular remodeling, as well as gut dysbiosis, which are influenced by numerous genetic and epigenetic factors. Identification of cardiovascular risk factors in NAFLD is an important public health issue. At present, there is evidence that the presence of advanced LF may be a strong independent predictor and risk factor for cardiovascular disorders in NAFLD. It is obvious that early diagnosis of LF will allow to stratify NAFLD patients by cardiovascular risk groups and thereby determine the most optimal therapeutic interventions.

Introduction

Non-alcoholic fatty liver disease (NAFLD) occupies a leading position among liver diseases worldwide. The ubiquitous NAFLD incidence increased from 25.26% in 1990–2006 to 38.00% in 2016–2019.[1] NAFLD is described as a condition in which ≥5% of hepatocytes accumulate fat in patients who do not abuse alcohol. There are two main manifestations: simple steatosis without liver fibrosis (LF) (nonalcoholic fatty liver) and nonalcoholic steatohepatitis (NASH). NASH, in addition to steatosis, is characterized by lobular inflammation, hepatocyte ballooning, and various LF stages.[2]

The correlation between NAFLD and cardiovascular diseases (CVDs) is established by numerous clinical studies.[3] NAFLD and NASH are accompanied by an increase in the frequency of cardiovascular events, particularly coronary artery disease, hypertension, atherosclerosis,[4] myocardial infarction, ischemic stroke, atrial fibrillation, and heart failure.[5] The risk of these events escalates with the progression of NAFLD, especially in advanced LF.[6,7] As a result, CVDs are currently the predominant cause of death in NAFLD patients.[8] This problem is compounded by an increase in the number of NAFLD patients with CVDs, who may have cardiovascular risk factors.[9] Therefore, the most commonly used assessment systems, such as the Framingham risk score for hard coronary heart disease, may underestimate the cardiovascular risk associated with NAFLD.[10] Nevertheless, serious cardiovascular disorders can occur in all clinical forms of NAFLD regardless of established cardiovascular risk factors.[11] For example, the relationships between NAFLD, insulin resistance, metabolic syndrome, and CVDs have been well established.[12] It is known that the metabolic syndrome is characterized by a combination of signs such as abdominal obesity, dyslipidemia,

Keywords: Cardiovascular diseases; Cardiovascular risk factors; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis; Liver fibrosis.

Abbreviations: ALT, alanine aminotransferase; APRI, AST to Platelet Ratio; AST, aspartate aminotransferase; CRN, Clinical Research Network; CVDs, cardiovascular diseases; FIB-4, fibrosis-4; GLS, global longitudinal strain; LF, liver fibrosis; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NASH, nonalcoholic steatohepatitis; NFS, NAFLD fibrosis score; RV, right ventricular; T2DM, type 2 diabetes mellitus.

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glucose metabolism disorders and hypertension.[13] However, even normoponderal NAFLD patients have an increased risk of CVDs.[14] This is likely due to the presence of other independent cardiovascular risk factors in NAFLD patients. At present, there is evidence that the presence of advanced LF may be a strong independent predictor and risk factor for cardiovascular disorders in NAFLD.[15,16]

This review summarizes the current investigations that confirm the significance of LF as an independent cardiovascular risk factor in NAFLD.

Literature search

PubMed, Google Scholar, Web of Science platform, Reference Citation Analysis, and Cochrane Systematic Reviews were searched for articles published between 2008 and 2023. Relevant articles were identified using the following keywords: "cardiovascular diseases", "cardiovascular risk factors", "non-alcoholic fatty liver disease", "nonalcoholic steatohepatitis", and "liver fibrosis". The reference lists of the identified articles were also searched for other relevant publications. The investigations that described LF as a cardiovascular risk factor in NAFLD met the inclusion criteria.

Pathophysiological mechanisms of cardiovascular disorders in NAFLD

The pathophysiological mechanisms predisposing to the development of cardiovascular disorders in NAFLD are complex and multifactorial.[17] These mechanisms include atherogenic dyslipidemia, impaired glucose metabolism, liver insulin resistance, low-grade systemic inflammation, endothelial dysfunction, as well as gut dysbiosis, all of which are influenced by numerous genetic and epigenetic factors.[18] In addition, advanced LF/cirrhosis in NASH may contribute to cardiovascular disorders as a result of cardiovascular remodeling in response to the hyperdynamic circulatory state associated with portal hypertension. The term "remodeling" began to be used in cardiology in the 1980s, and in strict interpretation, means the process of reorganization of the existing structure, during which new material is attached to it, or it is completely changed (Fig. 1).[19] In particular, left ventricular concentric remodeling, which was an unfavorable prognostic sign, was revealed in NASH patients.[20] LF in NASH may also be associated with CVDs by a more expressed profile of systemic inflammation affecting various organs and systems and the interactions between them, leading to further inflammation and immune response activation.[21]

Noninvasive tests of liver fibrosis to assess cardiovascular risk in NAFLD

Given the known limitations of performing a liver biopsy, noninvasive tests of LF have been used in most investigations to assess cardiovascular risk in NAFLD (Table 1).[22,23,24–44] The Fibrosis-4 (FIB-4) score is an index based on aspartate aminotransferase (AST) level, alanine aminotransferase (ALT) level, platelet count, and age to evaluate LF. When evaluating LF in NAFLD patients, a FIB-4 score <1.3 is categorized as low risk, while a FIB-4 score ≥2.67 is categorized as high risk of LF.[45] The NAFLD fibrosis score (NFS) is a combined assessment of age, hyperglycemia, body mass index, platelet count, albumin, and the AST/ALT ratio to evaluate LF. The following NFS thresholds for evaluating LF are proposed: <−1.455 - predictor of absence of significant LF (F0-F2); ≤−1.455 to ≤0.675 - indeterminate score; >0.675 - predictor of presence of significant LF (F3-F4).[46] The BARD score includes three variables: AST/ALT ratio ≥0.8–2 points; a body mass index ≥28—1 point; and the presence of type 2 diabetes mellitus (T2DM)—1 point. The possible score ranges from 0 to 4 points. A total score of ≥2 is associated with advanced LF.[47] The APRI index is calculated by using the formula AST/upper limit of normal × 100/platelet count. APRI index values of ≤0.3 and ≤0.5 rule out significant LF and cirrhosis, respectively, and a value of ≥ 1.5 rules out significant LF.[48] The Forns index, calculated based on the following four parameters: patient age, total cholesterol, gamma-glutamyl transferase, and platelet count, has the cut-off points for the LF assessment <4.2 and >6.9.[49] Transient elastography is the most commonly used imaging-based LF assessment method. To exclude advanced LF in NAFLD patients, the recommended values of liver stiffness measured by transient elastography are <8 kPa. The general limitations of noninvasive tests include insufficient verification accuracy for mild and moderate LF and inadequate differences in adjacent LF stages; in addition, there are not enough noninvasive tests to diagnose subclinical hepatic inflammation and ballooning, as well as to accurately determine the severity of portal hypertension in compensated advanced chronic liver disease. There are also specific advantages and limitations of individual noninvasive tests. Finally, the test-retest reliability of noninvasive tests has not been fully studied, warranting future research. Nevertheless, the use of noninvasive tests in scientific research for evaluating liver disease severity and prognosis is supported by the current guidelines.[50]

Impact of liver fibrosis on cardiovascular risk in NAFLD

It has been shown that patients with NASH or advanced LF are at a higher risk of atherosclerotic CVDs compared to non-LF NAFLD patients, independent of established cardiovascular metabolic risk factors.[22] In a study by Labenz *et al.*,[51] the overall 10-year CVDs risk, according to the Framingham risk scale, was high among patients with histologically confirmed NAFLD, with the highest risk observed in those with advanced LF. Noninvasive LF markers in NAFLD patients may be predictors of an increased risk of cardiovascular events, regardless of metabolic syndrome.[23] For example, a FIB-4 score ≥2.67 was found to be a strong independent prognostic criterion for major adverse cardiovascular events in NAFLD and was invariably associated with unstable angina, myocardial infarction, heart failure, percutaneous coronary intervention, and coronary artery bypass grafting in addition to known cardiovascular risk factors.[24] In a study by Hanson *et al.*,[52] the NFS in NAFLD patients with advanced LF without prior CVDs was found to be an independent predictor of cardiovascular events, even after adjusting for the relevant covariates, which included cardiovascular risk indicators such as the Framingham risk score and atherosclerotic CVDs indicators. In the Alimentazione, Benessere Cardiovascolare e Diabete study, the LF severity assessed by transient elastography was an independent factor for a higher atherosclerotic CVDs risk in addition to steatosis after adjusting for obesity.[25] Multivariate adjusted logistic regression models that were used in 3,276 adult participants of the Framingham Heart Study showed a significant association between advanced LF assessed by transient elastography and obesity-related signs, namely, hypertension, low high-density lipoprotein cholesterol and most notably, T2DM. This association persisted with a 2.5-fold increase even after accounting for controlled attenuation parameters. This suggests a link between LF and cardiometabolic

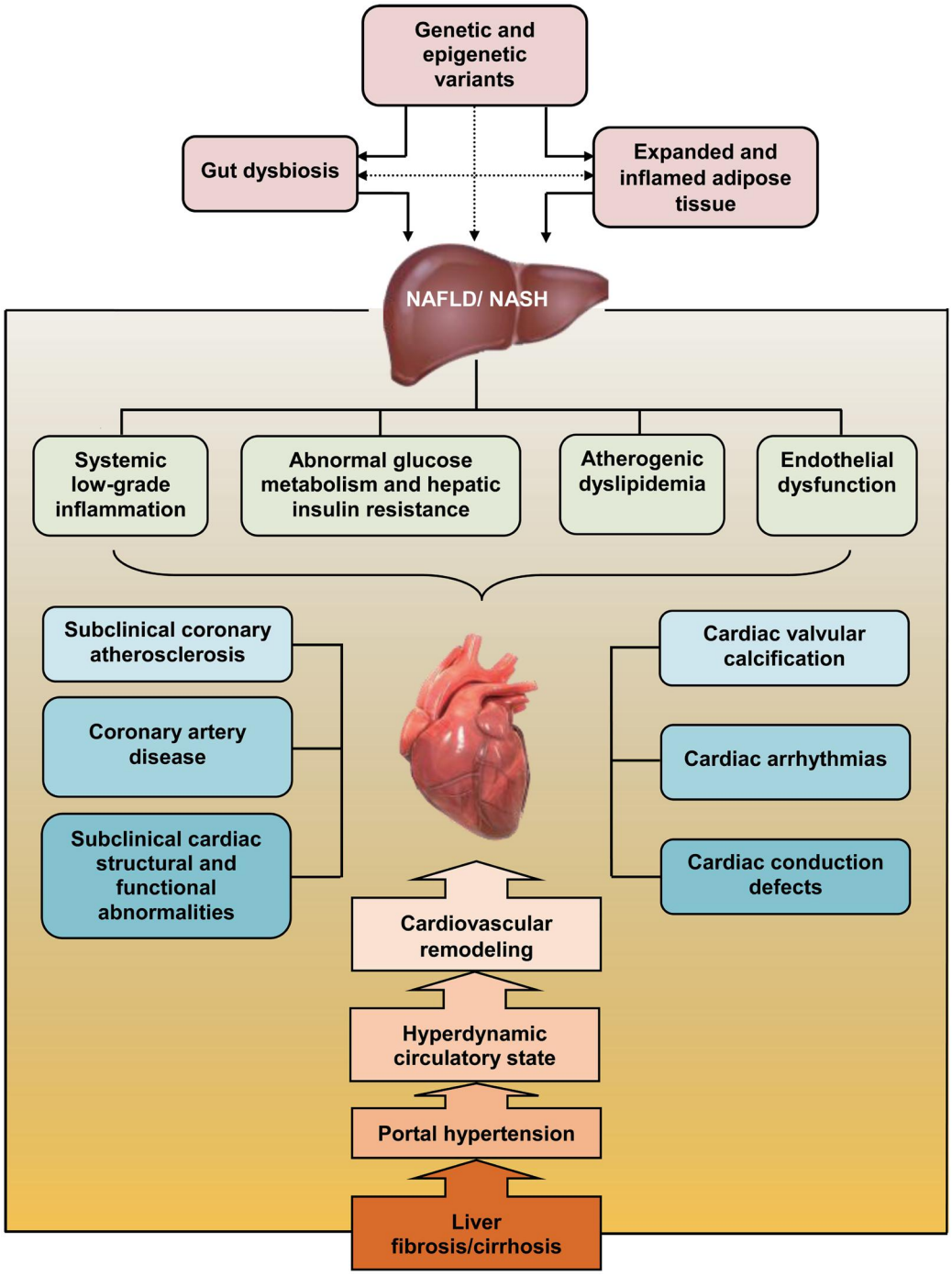

Fig. 1. Potential pathophysiological mechanisms of cardiovascular disorders in non-alcoholic fatty liver disease. NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

diseases in addition to an association with liver steatosis.[26]

Impact of liver fibrosis on the cardiovascular outcome in NAFLD

Although liver-related complications are a significant cause of mortality in NAFLD, CVDs accounts for at least 40% of the total number of deaths in NAFLD, making it the predominant cause of mortality.[53] According to a meta-analysis by Younossi *et al.*,[1] the pooled CVDs-related mortality rate in NAFLD patients was 4.2 per 1,000 person-years. The NAFLD severity is the main factor determining the increased risk of CVDs. Therefore, patients with NASH and progressive LF can be classified as a special risk group.[54] In a large study involving 11,154 patients, 34% of whom

Table 1. Main characteristics of investigations on the significance of liver fibrosis as an independent cardiovascular risk factor in non-alcoholic fatty liver disease

First author, year, ref.	Design	Liver fibrosis assessment	Main findings*
Impact of liver fibrosis on the cardiovascular risk in NAFLD			
Park, 2021[22]	A prospective cross-sectional study	Liver biopsy	NASH or advanced LF was independently associated with a higher risk of atherosclerotic CVDs.
Baratta, 2020[23]	A prospective cohort study	FIB-4 score, NFS	FIB-4 score >2.67, and NFS >0.676 in NAFLD patients were independently associated with risk of incident cardiovascular events.
Vieira Barbosa, 2022[24]	A prospective cohort study	FIB-4 score	FIB-4 score ≥2.67 in NAFLD patients was the strongest predictor of major adverse cardiovascular events overall and was consistently associated with myocardial infarction, hospitalization for unstable angina, hospitalization for heart failure, coronary artery bypass graft, and percutaneous coronary intervention.
Pennisi, 2021[25]	A prospective cohort study	Transient elastography	LF severity in NAFLD patients were independent factors for a higher atherosclerotic cardiovascular risk after adjusting for obesity.
Long, 2021[26]	A prospective cohort study	Transient elastography	LF in NAFLD patients was associated with multiple cardiovascular risk factors, including increased odds of obesity, metabolic syndrome, T2DM, hypertension, and low high-density lipoprotein cholesterol.
Impact of liver fibrosis on the cardiovascular outcome in NAFLD			
Kim, 2013[27]	A retrospective cohort study	NFS, APRI index, FIB-4 score	Compared to NAFLD patients without LF, those with a high probability of advanced LF had a 69% increase in mortality after adjustment for other known predictors of mortality. These increases in mortality were almost entirely from cardiovascular causes.
Park, 2021[28]	A prospective cohort study	BARD score	NAFLD patients with advanced LF demonstrated a significantly higher incidence of heart failure, hospitalized heart failure, all–cause mortality and cardiovascular mortality compared to NAFLD patients without advanced LF.
Impact of liver fibrosis on the cardiovascular comorbidities in NAFLD Subclinical coronary atherosclerosis			
Jamalinia, 2023[29]	Systematic review and meta-analysis	No data available	A significant association of subclinical atherosclerosis with LF in NAFLD patients was revealed, as well as its correlation with LF stages.
Song, 2019[30]	A retrospective, cross-sectional study	NFS, FIB-4 score, Forns index, APRI index	An association of coronary atherosclerosis with LF in NAFLD patients was revealed.
Tsai, 2022[31]	A retrospective cohort study	NFS, FIB-4 score, Forns index, APRI index	The male gender, diastolic blood pressure, and NFS in NAFLD patients were independently associated with coronary segment stenosis score progression.
Chen, 2015[32]	A prospective cross-sectional study	NFS	Compared to NAFLD patients without advanced LF, presence of advanced LF associated with a 303% increased risk for elevated carotid intima-media thickness, a 398% increased risk of prevalence of carotid plaque, and a 456% increased risk for prevalence of arterial stiffness.
Kim, 2022[33]	A retrospective, cross-sectional study	NFS, BARD score	Patients with lean NAFLD and advanced LF had a significantly higher risk for atherosclerotic CVDs than those with obese NAFLD with or without advanced LF.
Coronary artery disease			
Sinn, 2020[34]	A retrospective cohort study	NFS, FIB-4 score	NAFLD patients with advanced LF may have an increased risk of myocardial infarction.

(continued)

Table 1. - *(continued)*

First author, year, ref.	Design	Liver fibrosis assessment	Main findings*
Higashiura, 2022[35]	A prospective cohort study	FIB-4 score	LF stage in NAFLD patients correlated with new onset of ischemic heart disease in the group with "fatty liver", but not in the group without "fatty liver".
Subclinical cardiac structural and functional abnormalities			
Lee, 2018[36]	A prospective cohort study	Transient elastography, NFS	Compared to those without NAFLD, NAFLD patients had alterations in cardiac remodeling, manifested by increased left ventricular mass index, left ventricular end-diastolic diameter, and left atrial volume index. NAFLD patients with advanced LF demonstrated higher values of left ventricular filling pressure and tended to increase it.
Chung, 2018[37]	A prospective cross-sectional study	NFS	The risk of diastolic dysfunction in NAFLD patients gradually increases according to the LF severity.
Canada, 2019[38]	A prospective cross-sectional study	Liver biopsy	On stress echocardiography in NAFLD patients a significant stepwise increase in stress left ventricular filling pressure with increasing LF stage was noted. A trend between impaired left ventricular relaxation with exercise and increasing LF stages was also noted.
Lee, 2020[39]	A retrospective cohort study	NFS	LF in NAFLD patients was independently associated with diastolic dysfunction after adjusting for insulin resistance and cardiometabolic risk factors.
Sunbul, 2015[40]	A prospective cohort study	Liver biopsy	NAFLD patients with LF had significantly lower RV function assessed by GLS compared to patients without LF. NASH CRN score ≥5 was associated with lower RV-GLS. NASH CRN score inversely correlated with RV-GLS such as patients with impaired RV-GLS (<19%) showed significantly higher NASH CRN score compared to normal RV-GLS group.
Cardiac arrhythmias			
Käräjämäki, 2017[41]	A prospective cross-sectional study	Transient elastography, NFS	LF severity was highest in NAFLD patients with atrial fibrillation.
Park, 2020[42]	A retrospective cohort study	FIB-4 score	LF severity in NAFLD patients had a significant correlation with atrial fibrillation.
Kang, 2020[43]	A retrospective, cross-sectional study	NFS, FIB-4 score	LF severity in NAFLD patients was associated with atrial fibrillation.
Cardiac conduction defects			
Mantovani, 2017[44]	A retrospective cross-sectional study	FIB-4 score, APRI index	NAFLD patients with advanced LF had a substantially greater prevalence of heart block as compared to NAFLD patients with mild and moderate LF or persons without NAFLD.

*The research results are statistically significant. APRI, AST to Platelet Ratio; CRN, Clinical Research Network; CVDs, cardiovascular diseases; FIB-4, fibrosis-4; GLS, global longitudinal strain; LF, liver fibrosis; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NFS, NAFLD fibrosis score; RV, right ventricular; T2DM, Type 2 Diabetes Mellitus.

were diagnosed with NAFLD, higher values of noninvasive LF tests, such as the APRI index, FIB-4 score, and NFS, were associated with a progressive increase in CVDs mortality after correction for other predictors of death.[27] In a study by Mann *et al.*,[55] NAFLD patients with liver cirrhosis had higher mortality regardless of known cardiovascular risk factors. Additionally, liver steatosis and/or advanced LF in NAFLD patients assessed by the fatty liver index as well as the BARD score and NFS significantly correlated with the risk of heart failure and mortality.[28,56]

Impact of liver fibrosis on cardiovascular comorbidities in NAFLD

NAFLD can negatively affect both the coronary arteries and other heart anatomical structures, contributing to an increase in morbidity and mortality from CVDs among NAFLD patients.[57] In particular, there is strong evidence linking NAFLD with the risk of developing coronary atherosclerosis and coronary artery disease, cardiac structural and functional abnormalities, cardiac valvular calcification, cardiac arrhythmias, and conduction defects.[58]

Subclinical coronary atherosclerosis

For a long time, NAFLD was not considered a probable cause of atherosclerosis but was recognized as a valuable indicator of the early stages of its development.[59] Moreover, well-planned and controlled studies conducted in recent years have provided very valuable information that allows one to take a fresh look at the relationships among these pathological conditions.[60] In particular, the association of LF in NAFLD with subclinical atherosclerosis was shown, and LF severity aggravated this relationship.[29]

Coronary artery calcium scoring via computerized tomography is usually used to determine the degree of coronary atherosclerosis. In a study involving 665 NAFLD patients, noninvasive LF markers, such as APRI index, NFS, and FIB-4 score, made it possible to reliably predict the values of the coronary calcium index >100 via computerized tomography.[30] In a study by Tsai *et al.*,[31] NAFLD patients with basal coronary plaques had higher NFSs, FIB-4 scores and Forns index, suggesting the possibility of their use for early identification of coronary plaques and prediction of the risk of adverse cardiovascular events. According to a study by Chen *et al.*,[32] NAFLD patients with advanced LF assessed by the NFS had a higher probability of carotid artery intima-media thickening, the presence of carotid plaque and arterial stiffness, regardless of known metabolic factors, prior cardiovascular events, or insulin resistance. It was found that NASH patients have higher carotid artery intima-media thickness than nonalcoholic fatty liver patients. In addition, NASH patients had high levels of high-sensitivity C-reactive protein, and the levels of high-sensitivity C-reactive protein were significantly correlated with LF. It is known that high levels of highly sensitive C-reactive protein are associated with an increased risk of heart attack.[61] Interestingly, lean NAFLD patients with advanced LF are more likely to have atherosclerotic CVDs than obese subjects.[33]

Coronary artery disease

The presence, severity and prevalence of coronary artery disease may be associated with NAFLD, regardless of well-known risk factors. In addition, the relationship between coronary artery disease and NAFLD may be attributed to the formation of atherosclerotic coronary plaques characteristic of both diseases. Their calcium content according to computerized tomography data is a clinically significant sign of subclinical coronary artery disease.[62] In a study by Wong *et al.*,[63] NAFLD patients prevailed among those with significant coronary artery stenosis. An association between NAFLD and an increased risk of acute myocardial infarction has also been shown, regardless of known risk factors.[34] An independent correlation was shown between the FIB-4 score in NAFLD patients and the risk of coronary artery disease.[35] In a study by Ghoneim *et al.*,[64] it was found that NASH is associated with acute myocardial infarction regardless of the established risk factors. The probability of acute myocardial infarction in young NASH patients was higher than that in older subjects. Acute myocardial infarction is a frequent outcome in NASH patients.

Subclinical cardiac structural and functional abnormalities

Recent studies have identified NAFLD as a risk factor not only for premature coronary artery disease and cardiovascular events but also for early cardiac structural and functional abnormalities. For example, in a study by Lee *et al.*,[36] it was demonstrated that advanced LF in NAFLD patients without a history of CVDs correlates with an increase in left ventricular filling pressure, which is associated with diastolic dysfunction associated with impaired myocardial glucose uptake. It was noted that left ventricular diastolic dysfunction in advanced LF was significant only in NAFLD patients without obesity.[37] Alterations in myocardial structure and in the load dependence of left ventricular diastolic function parameters were also observed in NASH patients without a history of CVDs.[65] Another study revealed that NASH patients with liver cirrhosis had an increased prevalence of diastolic dysfunction compared with patients with other causes of liver cirrhosis.[66] Diastolic dysfunction in NASH patients leads to a decrease in physical performance. The severity of these disorders correlates with the LF stage.[38] In a study by Lee *et al.*[39] including T2DM patients aged ≥50 years, participants with NAFLD had changes in left ventricular structure and diastolic dysfunction compared to non-NAFLD patients. Advanced LF significantly correlated with left ventricular diastolic dysfunction after correction for cardiovascular risk factors, especially in patients without insulin resistance. Although NASH is accompanied by a higher frequency of left ventricular diastolic dysfunction, this does not affect the immediate post-transplant outcome or 30-day mortality from all causes.[67] Sunbul *et al.*[40] have shown that NAFLD patients with LF have significantly lower right ventricular function compared to patients without LF. They used the NASH CRN histological scoring system as an independent predictor. It turned out that the NASH CRN score ≥5 was associated with lower right ventricular global longitudinal strain. The NASH CRN score inversely correlated with right ventricular global longitudinal strain. Patients with impaired right ventricular global longitudinal strain had a higher NASH CRN score than did those with normal right ventricular global longitudinal strain. Cardiac structural and functional abnormalities contribute to the development of heart failure, which, in NAFLD, occurs with a preserved ejection fraction. The relationship between more advanced heart failure and LF stage was evident in NAFLD patients. Left atrial dilatation and more pronounced diastolic dysfunction were observed in NAFLD patients with advanced LF.[68]

Cardiac arrhythmias

Atrial fibrillation is an extremely important social problem due to its large prevalence and high morbidity and mortality rates.[69] Atrial fibrillation often occurs in NAFLD patients, in whom it usually has a permanent (chronic) form.[70] In a study by Whitsett *et al.*,[71] atrial fibrillation was found to be twice as common in NASH patients than in the general population. An Oulu Project Elucidating

the Risk of Atherosclerosis study revealed a link between atrial fibrillation and liver stiffness measured by transient elastography in elderly NAFLD patients.[41] A number of studies have shown an independent association between atrial fibrillation and advanced LF assessed by NFS and FIB-4 score in NAFLD patients.[42,43]

Cardiac conduction defects

Cardiac conduction defects are a well-established risk factor for general and cardiac mortality in NAFLD patients.[72] In a study by Mantovani *et al.*,[43] persistent heart block was found to be most common in NAFLD patients with T2DM in the presence of advanced LF, assessed by the FIB-4 score.

Conclusions

NAFLD occupies a leading position among liver diseases worldwide. Given that cardiovascular disorders are the most significant cause of unfavorable outcomes in NAFLD patients, identifying cardiovascular risk factors is an important public health issue. There is much evidence that LF can considerably increase morbidity and mortality from CVDs in NAFLD patients. Early diagnosis of LF will allow to stratify NAFLD patients by cardiovascular risk groups and thereby determine the most optimal therapeutic interventions.

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Conflict of interest

The author has no conflict of interests related to this publication.

References

[1] Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. Hepatology 2023;77(4):1335–1347. doi:10.1097/HEP.0000000000000004, PMID:36626630.

[2] Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, *et al*. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology 2023;77(5):1797–1835. doi:10.1097/HEP.0000000000000323, PMID:36727674.

[3] Bisaccia G, Ricci F, Khanji MY, Sorella A, Melchiorre E, Iannetti G, *et al*. Cardiovascular Morbidity and Mortality Related to Non-alcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis. Curr Probl Cardiol 2023;48(6):101643. doi:10.1016/j.cpcardiol.2023.101643, PMID:36773944.

[4] Wu S, Wu F, Ding Y, Hou J, Bi J, Zhang Z. Association of non-alcoholic fatty liver disease with major adverse cardiovascular events: A systematic review and meta-analysis. Sci Rep 2016;6:33386. doi:10.1038/srep33386, PMID:27633274.

[5] Alon L, Corica B, Raparelli V, Cangemi R, Basili S, Proietti M, *et al*. Risk of cardiovascular events in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis. Eur J Prev Cardiol 2022;29(6):938–946. doi:10.1093/eurjpc/zwab212, PMID:34939092.

[6] Mantovani A, Csermely A, Petracca G, Beatrice G, Corey KE, Simon TG, *et al*. Non-alcoholic fatty liver disease and risk of fatal and non-fatal cardiovascular events: an updated systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2021;6(11):903–913. doi:10.1016/S2468-1253(21)00308-3, PMID:34555346.

[7] Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. J Hepatol 2016;65(3):589–600. doi:10.1016/j.jhep.2016.05.013, PMID:27212244.

[8] European Association for the Study of the Liver (EASL)., European Association for the Study of Diabetes (EASD)., European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64(6):1388–1402. doi:10.1016/j.jhep.2015.11.004, PMID:27062661.

[9] Polyzos SA, Kechagias S, Tsochatzis EA. Review article: non-alcoholic fatty liver disease and cardiovascular diseases: associations and treatment considerations. Aliment Pharmacol Ther 2021;54(8):1013–1025. doi:10.1111/apt.16575, PMID:34416040.

[10] Chiriac S, Stanciu C, Girleanu I, Cojocariu C, Sfarti C, Singeap AM, *et al*. Nonalcoholic Fatty Liver Disease and Cardiovascular Diseases: The Heart of the Matter. Can J Gastroenterol Hepatol 2021;2021:6696857. doi:10.1155/2021/6696857, PMID:33505944.

[11] Byrne CD, Targher G. Non-alcoholic fatty liver disease-related risk of cardiovascular disease and other cardiac complications. Diabetes Obes Metab 2022;24(Suppl 2):28–43. doi:10.1111/dom.14484, PMID:34324263.

[12] Muzurović E, Mikhailidis DP, Mantzoros C. Non-alcoholic fatty liver disease, insulin resistance, metabolic syndrome and their association with vascular risk. Metabolism 2021;119:154770. doi:10.1016/j.metabol.2021.154770, PMID:33864798.

[13] Godoy-Matos AF, Silva Júnior WS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. Diabetol Metab Syndr 2020;12:60. doi:10.1186/s13098-020-00570-y, PMID:32684985.

[14] Yoshitaka H, Hamaguchi M, Kojima T, Fukuda T, Ohbora A, Fukui M. Nonoverweight nonalcoholic fatty liver disease and incident cardiovascular disease: A post hoc analysis of a cohort study. Medicine (Baltimore) 2017;96(18):e6712. doi:10.1097/md.0000000000006712, PMID:28471965.

[15] Mullish BH, Forlano R, Manousou P, Mikhailidis DP. Non-alcoholic fatty liver disease and cardiovascular risk: an update. Expert Rev Gastroenterol Hepatol 2018;12(12):1175–1177. doi:10.1080/17474124.2018.1533117, PMID:30791787.

[16] Tamaki N, Kurosaki M, Takahashi Y, Itakura Y, Inada K, Kirino S, *et al*. Liver fibrosis and fatty liver as independent risk factors for cardiovascular disease. J Gastroenterol Hepatol 2021;36(10):2960–2966. doi:10.1111/jgh.15589, PMID:34154037.

[17] Garbuzenko DV, Belov DV. Non-alcoholic fatty liver disease as an independent factor of cardiometabolic risk of cardiovascular diseases. Exp Clin Gastroenterol 2021;194:22–34, (in Russian)doi:10.31146/1682-8658-ecg-194-10-22-34.

[18] Garbuzenko DV. Pathophysiological mechanisms of cardiovascular disorders in non-alcoholic fatty liver disease. Gastroenterol Hepatol Bed Bench 2022;15(3):194–203. doi:10.22037/ghfbb.v15i3.2549, PMID:36311966.

[19] Garbuzenko DV, Arefyev NO, Belov DV. Restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension. World J Hepatol 2016;8(36):1602–1609. doi:10.4254/wjh.v8.i36.1602, PMID:28083082.

[20] Styczynski G, Kalinowski P, Michałowski Ł, Paluszkiewicz R, Ziarkiewicz-Wróblewska B, Zieniewicz K, *et al*. Cardiac Morphology, Function, and Hemodynamics in Patients With Morbid Obesity and Nonalcoholic Steatohepatitis. J Am Heart Assoc 2021;10(8):e017371. doi:10.1161/JAHA.120.017371, PMID:33847141.

[21] Campos-Murguía A, Ruiz-Margáin A, González-Regueiro JA, Macías-Rodríguez RU. Clinical assessment and management of liver fibrosis in non-alcoholic fatty liver disease. World J Gastroenterol 2020;26(39):5919–5943. doi:10.3748/wjg.v26.i39.5919, PMID:33132645.

[22] Park JH, Koo BK, Kim W, Kim WH, Innovative Target Exploration of NAFLD (ITEN) Consortium. Histological severity of nonalcoholic fatty liver disease is associated with 10-year risk for atherosclerotic cardi-

ovascular disease. Hepatol Int 2021;15(5):1148–1159. doi:10.1007/s12072-021-10209-3, PMID:34081289.
[23] Baratta F, Pastori D, Angelico F, Balla A, Paganini AM, Cocomello N, *et al*. Nonalcoholic Fatty Liver Disease and Fibrosis Associated With Increased Risk of Cardiovascular Events in a Prospective Study. Clin Gastroenterol Hepatol 2020;18(10):2324–2331.e4. doi:10.1016/j.cgh.2019.12.026, PMID:31887443.
[24] Vieira Barbosa J, Milligan S, Frick A, Broestl J, Younossi Z, Afdhal N, *et al*. Fibrosis-4 Index Can Independently Predict Major Adverse Cardiovascular Events in Nonalcoholic Fatty Liver Disease. Am J Gastroenterol 2022;117(3):453–461. doi:10.14309/ajg.0000000000001606, PMID:35041626.
[25] Pennisi G, Di Marco V, Buscemi C, Mazzola G, Randazzo C, Spatola F, *et al*. Interplay between non-alcoholic fatty liver disease and cardiovascular risk in an asymptomatic general population. J Gastroenterol Hepatol 2021;36(9):2389–2396. doi:10.1111/jgh.15523, PMID:33871081.
[26] Long MT, Zhang X, Xu H, Liu CT, Corey KE, Chung RT, *et al*. Hepatic Fibrosis Associates With Multiple Cardiometabolic Disease Risk Factors: The Framingham Heart Study. Hepatology 2021;73(2):548–559. doi:10.1002/hep.31608, PMID:33125745.
[27] Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. Hepatology 2013;57(4):1357–1365. doi:10.1002/hep.26156, PMID:23175136.
[28] Park J, Kim G, Kim H, Lee J, Lee YB, Jin SM, *et al*. The association of hepatic steatosis and fibrosis with heart failure and mortality. Cardiovasc Diabetol 2021;20(1):197. doi:10.1186/s12933-021-01374-8, PMID:34583706.
[29] Jamalinia M, Zare F, Lankarani KB. Systematic review and meta-analysis: Association between liver fibrosis and subclinical atherosclerosis in nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2023;58(4):384–394. doi:10.1111/apt.17617, PMID:37345533.
[30] Song DS, Chang UI, Kang SG, Song SW, Yang JM. Noninvasive Serum Fibrosis Markers are Associated with Coronary Artery Calcification in Patients with Nonalcoholic Fatty Liver Disease. Gut Liver 2019;13(6):658–668. doi:10.5009/gnl18439, PMID:30970434.
[31] Tsai TY, Hsu PF, Wu CH, Huang SS, Chan WL, Lin SJ, *et al*. Association between Coronary Artery Plaque Progression and Liver Fibrosis Biomarkers in Population with Low Calcium Scores. Nutrients 2022;14(15):3163. doi:10.3390/nu14153163, PMID:35956339.
[32] Chen Y, Xu M, Wang T, Sun J, Sun W, Xu B, *et al*. Advanced fibrosis associates with atherosclerosis in subjects with nonalcoholic fatty liver disease. Atherosclerosis 2015;241(1):145–150. doi:10.1016/j.atherosclerosis.2015.05.002, PMID:25988358.
[33] Kim Y, Han E, Lee JS, Lee HW, Kim BK, Kim MK, *et al*. Cardiovascular Risk Is Elevated in Lean Subjects with Nonalcoholic Fatty Liver Disease. Gut Liver 2022;16(2):290–299. doi:10.5009/gnl210084, PMID:34238770.
[34] Sinn DH, Kang D, Chang Y, Ryu S, Cho SJ, Paik SW, *et al*. Non-alcoholic fatty liver disease and the incidence of myocardial infarction: A cohort study. J Gastroenterol Hepatol 2020;35(5):833–839. doi:10.1111/jgh.14856, PMID:31512278.
[35] Higashiura Y, Tanaka M, Mori K, Mikami T, Hosaka I, Ohnishi H, *et al*. High fibrosis-4 index predicts the new onset of ischaemic heart disease during a 10-year period in a general population. Eur Heart J Open 2022;2(3):oeac030. doi:10.1093/ehjopen/oeac030, PMID:35919342.
[36] Lee YH, Kim KJ, Yoo ME, Kim G, Yoon HJ, Jo K, *et al*. Association of non-alcoholic steatohepatitis with subclinical myocardial dysfunction in non-cirrhotic patients. J Hepatol 2018;68(4):764–772. doi:10.1016/j.jhep.2017.11.023, PMID:29175242.
[37] Chung GE, Lee JH, Lee H, Kim MK, Yim JY, Choi SY, *et al*. Nonalcoholic fatty liver disease and advanced fibrosis are associated with left ventricular diastolic dysfunction. Atherosclerosis 2018;272:137–144. doi:10.1016/j.atherosclerosis.2018.03.027, PMID:29604480.
[38] Canada JM, Abbate A, Collen R, Billingsley H, Buckley LF, Carbone S, *et al*. Relation of Hepatic Fibrosis in Nonalcoholic Fatty Liver Disease to Left Ventricular Diastolic Function and Exercise Tolerance. Am J Cardiol 2019;123(3):466–473. doi:10.1016/j.amjcard.2018.10.027, PMID:30502049.
[39] Lee H, Kim G, Choi YJ, Huh BW, Lee BW, Kang ES, *et al*. Association between Non-Alcoholic Steatohepatitis and Left Ventricular Diastolic Dysfunction in Type 2 Diabetes Mellitus. Diabetes Metab J 2020;44(2):267–276. doi:10.4093/dmj.2019.0001, PMID:30877708.
[40] Sunbul M, Kivrak T, Durmus E, Akin H, Aydin Y, Ergelen R, *et al*. Non-alcoholic Steatohepatitis Score is an Independent Predictor of Right Ventricular Dysfunction in Patients with Nonalcoholic Fatty Liver Disease. Cardiovasc Ther 2015;33(5):294–299. doi:10.1111/1755-5922.12145, PMID:26202098.
[41] Käräjämäki AJ, Kettunen O, Lepojärvi S, Koivurova OP, Kesäniemi YA, Huikuri H, *et al*. Presence of atrial fibrillation is associated with liver stiffness in an elderly Finnish population. PLoS One 2017;12(3):e0173855. doi:10.1371/journal.pone.0173855, PMID:28288202.
[42] Park HE, Lee H, Choi SY, Kim HS, Chung GE. The risk of atrial fibrillation in patients with non-alcoholic fatty liver disease and a high hepatic fibrosis index. Sci Rep 2020;10(1):5023. doi:10.1038/s41598-020-61750-4, PMID:32193478.
[43] Kang MK, Park JG, Kim MC. Association between Atrial Fibrillation and Advanced Liver Fibrosis in Patients with Non-Alcoholic Fatty Liver Disease. Yonsei Med J 2020;61(10):860–867. doi:10.3349/ymj.2020.61.10.860, PMID:32975060.
[44] Mantovani A, Rigolon R, Pichiri I, Bonapace S, Morani G, Zoppini G, *et al*. Nonalcoholic fatty liver disease is associated with an increased risk of heart block in hospitalized patients with type 2 diabetes mellitus. PLoS One 2017;12(10):e0185459. doi:10.1371/journal.pone.0185459, PMID:28981521.
[45] Tokushige K, Ikejima K, Ono M, Eguchi Y, Kamada Y, Itoh Y, *et al*. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. J Gastroenterol 2021;56(11):951–963. doi:10.1007/s00535-021-01796-x, PMID:34533632.
[46] Blond E, Disse E, Cuerq C, Drai J, Valette PJ, Laville M, *et al*. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease in severely obese people: do they lead to over-referral? Diabetologia 2017;60(7):1218–1222. doi:10.1007/s00125-017-4264-9, PMID:28352941.
[47] Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008;57(10):1441–1447. doi:10.1136/gut.2007.146019, PMID:18390575.
[48] Loaeza-del-Castillo A, Paz-Pineda F, Oviedo-Cárdenas E, Sánchez-Avila F, Vargas-Voráckova F. AST to platelet ratio index (APRI) for the noninvasive evaluation of liver fibrosis. Ann Hepatol 2008;7(4):350–357. PMID:19034235.
[49] European Association for Study of Liver., Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol 2015;63(1):237–264. doi:10.1016/j.jhep.2015.04.006, PMID:25911335.
[50] European Association for the Study of the Liver, Clinical Practice Guideline Panel; Chair:; EASL Governing Board representative:; Panel members. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis—2021 update. J Hepatol 2021;75(3):659–689. doi:10.1016/j.jhep.2021.05.025, PMID:34166721.
[51] Labenz C, Prochaska JH, Huber Y, Nagel M, Straub BK, Wild P, *et al*. Cardiovascular Risk Categories in Patients With Nonalcoholic Fatty Liver Disease and the Role of Low-Density Lipoprotein Cholesterol. Hepatol Commun 2019;3(11):1472–1481. doi:10.1002/hep4.1428, PMID:31701071.
[52] Henson JB, Simon TG, Kaplan A, Osganian S, Masia R, Corey KE. Advanced fibrosis is associated with incident cardiovascular disease in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2020;51(7):728–736. doi:10.1111/apt.15660, PMID:32043602.
[53] Przybyszewski EM, Targher G, Roden M, Corey KE. Nonalcoholic Fatty Liver Disease and Cardiovascular Disease. Clin Liver Dis (Hoboken) 2021;17(1):19–22. doi:10.1002/cld.1017, PMID:33552481.
[54] Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, Castellanos M, Aller-de la Fuente R, Metwally M, *et al*. Fibrosis Severity as a Determinant of Cause-Specific Mortality in Patients With Advanced Nonalcoholic Fatty Liver Disease: A Multi-National Cohort Study. Gastroenterology 2018;155(2):443–457.e17. doi:10.1053/j.gastro.2018.04.034,

PMID:29733831.
[55] Mann JP, Carter P, Armstrong MJ, Abdelaziz HK, Uppal H, Patel B, *et al*. Hospital admission with non-alcoholic fatty liver disease is associated with increased all-cause mortality independent of cardiovascular risk factors. PLoS One 2020;15(10):e0241357. doi:10.1371/journal.pone.0241357, PMID:33108366.
[56] Yoshihisa A, Sato Y, Yokokawa T, Sato T, Suzuki S, Oikawa M, *et al*. Liver fibrosis score predicts mortality in heart failure patients with preserved ejection fraction. ESC Heart Fail 2018;5(2):262–270. doi:10.1002/ehf2.12222, PMID:28967709.
[57] Stahl EP, Dhindsa DS, Lee SK, Sandesara PB, Chalasani NP, Sperling LS. Nonalcoholic Fatty Liver Disease and the Heart: JACC State-of-the-Art Review. J Am Coll Cardiol 2019;73(8):948–963. doi:10.1016/j.jacc.2018.11.050, PMID:30819364.
[58] Targher G, Byrne CD, Tilg H. NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. Gut 2020;69(9):1691–1705. doi:10.1136/gutjnl-2020-320622, PMID:32321858.
[59] Trovato GM. Non-alcoholic fatty liver disease and Atherosclerosis at a crossroad: The overlap of a theory of change and bioinformatics. World J Gastrointest Pathophysiol 2020;11(3):57–63. doi:10.4291/wjgp.v11.i3.57, PMID:32435522.
[60] VanWagner LB. New insights into NAFLD and subclinical coronary atherosclerosis. J Hepatol 2018;68(5):890–892. doi:10.1016/j.jhep.2018.01.023, PMID:29410378.
[61] Ozturk K, Uygun A, Guler AK, Demirci H, Ozdemir C, Cakir M, *et al*. Nonalcoholic fatty liver disease is an independent risk factor for atherosclerosis in young adult men. Atherosclerosis 2015;240(2):380–386. doi:10.1016/j.atherosclerosis.2015.04.009, PMID:25875390.
[62] Patil R, Sood GK. Non-alcoholic fatty liver disease and cardiovascular risk. World J Gastrointest Pathophysiol 2017;8(2):51–58. doi:10.4291/wjgp.v8.i2.51, PMID:28573067.
[63] Wong VW, Wong GL, Yip GW, Lo AO, Limquiaco J, Chu WC, *et al*. Coronary artery disease and cardiovascular outcomes in patients with non-alcoholic fatty liver disease. Gut 2011;60(12):1721–1727. doi:10.1136/gut.2011.242016, PMID:21602530.
[64] Ghoneim S, Dhorepatil A, Shah AR, Ram G, Ahmad S, Kim C, *et al*. Non-alcoholic steatohepatitis and the risk of myocardial infarction: A population-based national study. World J Hepatol 2020;12(7):378–388. doi:10.4254/wjh.v12.i7.378, PMID:32821336.
[65] Simon TG, Bamira DG, Chung RT, Weiner RB, Corey KE. Nonalcoholic Steatohepatitis is Associated with Cardiac Remodeling and Dysfunction. Obesity (Silver Spring) 2017;25(8):1313–1316. doi:10.1002/oby.21879, PMID:28745025.
[66] Izzy M, Soldatova A, Sun X, Angirekula M, Mara K, Lin G, *et al*. Cirrhotic Cardiomyopathy Predicts Posttransplant Cardiovascular Disease: Revelations of the New Diagnostic Criteria. Liver Transpl 2021;27(6):876–886. doi:10.1002/lt.26000, PMID:33533556.
[67] Marella HK, Kamal F, Peravali R, Jacob J, Nair SP. Left ventricular diastolic dysfunction in liver transplantation: a stronger association with non-alcoholic steatohepatitis. Clin Exp Hepatol 2020;6(2):158–162. doi:10.5114/ceh.2020.95893, PMID:32728634.
[68] Miller A, McNamara J, Hummel SL, Konerman MC, Tincopa MA. Prevalence and staging of non-alcoholic fatty liver disease among patients with heart failure with preserved ejection fraction. Sci Rep 2020;10(1):12440. doi:10.1038/s41598-020-69013-y, PMID:32709942.
[69] Essien UR, Kornej J, Johnson AE, Schulson LB, Benjamin EJ, Magnani JW. Social determinants of atrial fibrillation. Nat Rev Cardiol 2021;18(11):763–773. doi:10.1038/s41569-021-00561-0, PMID:34079095.
[70] Anstee QM, Mantovani A, Tilg H, Targher G. Risk of cardiomyopathy and cardiac arrhythmias in patients with nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2018;15(7):425–439. doi:10.1038/s41575-018-0010-0, PMID:29713021.
[71] Whitsett M, Wilcox J, Yang A, Zhao L, Rinella M, VanWagner LB. Atrial fibrillation is highly prevalent yet undertreated in patients with biopsy-proven nonalcoholic steatohepatitis. Liver Int 2019;39(5):933–940. doi:10.1111/liv.14018, PMID:30536602.
[72] Wijarnpreecha K, Panjawatanan P, Kroner PT, Cheungpasitporn W, Ungprasert P. Association between cardiac conduction defect and nonalcoholic fatty liver disease: a systematic review and meta-analysis. Ann Gastroenterol 2020;33(6):661–666. doi:10.20524/aog.2020.0535, PMID:33162743.

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Review Article

Obesity and Current Treatment Approaches: A Comprehensive Review

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Abstract

Obesity is a global health burden and is closely associated with severe chronic co-morbidities, which remain the leading causes of death. Significant progress has been made in the treatment of hypertension, diabetes, and hyperlipidemia over the last half-century. However, advancements in the management of obesity have been slow, with some medications exhibiting inadequate efficacy and dangerous side effects. Improved understanding of the gut-brain axis has inspired the pursuit of novel medications aiming to provide sustainable and safe weight loss. Current evidence-based practices for obesity management involve multi-modal approaches, including lifestyle modification, mechanical gastric restriction, modulation in the secretion of multiple gut hormones, alteration in the composition and secretion of bile acids, and alterations of the gut microbiome. Each physician is responsible for recognizing obesity as a disease and assisting patients in appropriate management based on strong evidence and a good safety profile, aligned with the patient's goals. Through this review, we aim to inform the readers of recent approaches for managing obesity and comparing their beneficial effects and efficacy on obesity and its long-term co-morbidities.

Introduction

The relationship between diet and chronic diseases such as hypertension, diabetes, colon cancer, and obesity has undergone extensive investigation, supported by a large number of data, indicating a causal relationship between them. Globally, mortality has shown strong associations with diets low in whole grains, high in sodium, and low in fruits.[1] Recent increases in obesity rates have been attributed to unhealthy eating habits and food choices leading to excessive energy intake.[2] Many studies have recognized the positive correlations between energy density, weight, and other markers of metabolic syndrome.[3] The problem of obesity or overweight accounts for two-thirds of the U.S. population. Obesity, a global health burden, is associated with comorbidities, such as diabetes mellitus, coronary artery disease, hypertension, and other systemic health issues, which are the leading causes of death.[4] In the modern era, obesity is typically defined as a body mass index (BMI) ≥30 kg/m^2, while a BMI value of 25–29.9 kg/m^2 is classified as overweight. Dietary factors, lifestyle, genetics, and environmental factors significantly contribute to obesity. A recent analysis revealed a near doubling of worldwide obesity prevalence since 1985, affecting half a billion people worldwide, and accounting for 4 million deaths annually worldwide.[5] However, the awareness of available therapeutic options remains low, prompting us to provide insights into these options through this article.

Obesity has significant effects on the gastrointestinal system. It contributes to esophageal diseases through both mechanical and humoral factors, with proinflammatory cytokines playing a crucial role in other digestive diseases.[6] Munch *et al*. demonstrated in an experiment on L2-IL1B mice (a transgenic mouse model of Barrett's esophagus) that a high-fat diet accelerated esophageal dysplasia by enhancing local pro-inflammatory immune responses and altering intestinal microbiota, irrespective of body weight.[7] Lower esophageal sphincter abnormalities, increased risk of hiatal hernia, and increased intragastric pressure are other mechanical causes of obesity directly influencing Barrett's esophagus and adenocarcinoma.[6] Obesity is also an important risk factor for colorectal adenoma and cancer. Several factors contribute to the increased risk of colon cancer in individuals with obesity, including alterations in systemic growth factors, visceral adipose tissue, the microbiome, bile acids, inflammation, and a diet rich in fat, sugar,

Keywords: Obesity; Weight loss; Bariatric surgery; Glucagon like peptide 1; Orlistat; Probiotics; Intragastric balloon; Endoscopy.

Abbreviations: BMI, body mass index; ESG, endoscopic sleeve gastroplasty; EWL, excess weight loss; FDA, food and drug administration; GLP-1, glucagon like peptide 1; IGB, intragastric balloon; LRYGB, laparoscopic roux-en-y gastric bypass; LSG, laparoscopic sleeve gastrectomy; POSE, primary obesity surgery endoluminal; TBE, transcatheter bariatric embolization; TRL, triglyceride rich lipoproteins.

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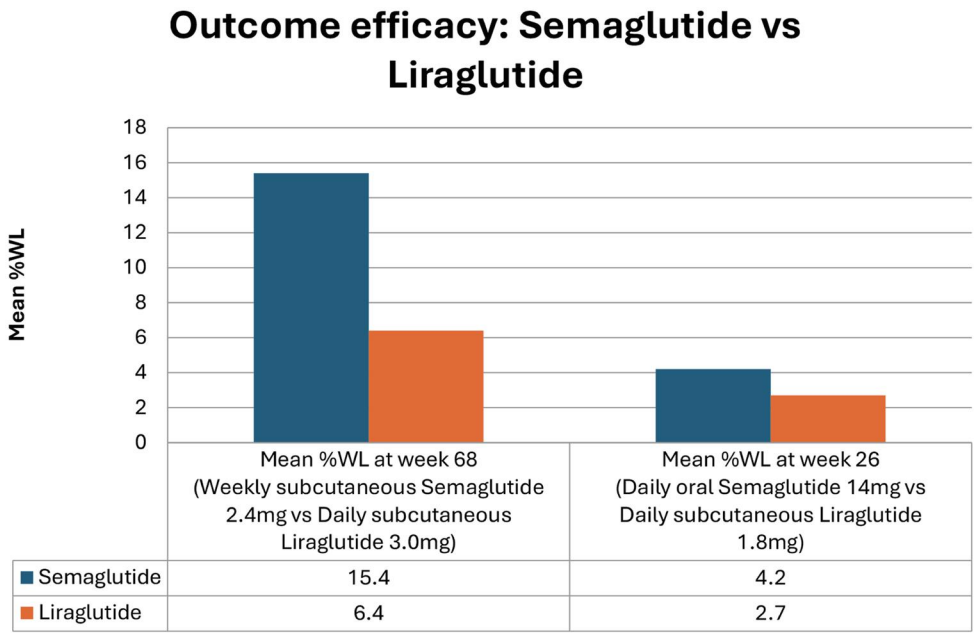

Fig. 1. Outcome efficacy of semaglutide and liraglutide. The left graph shows the mean percent weight loss (%WL) at week 68 by comparing weekly subcutaneous semaglutide to daily subcutaneous liraglutide. The right graph shows the mean percent weight loss (%WL) at week 26 by comparing daily oral semaglutide to daily subcutaneous liraglutide. %WL, percent weight loss.

high fructose corn syrup, or low vitamin D.[8] Studies have indicated that visceral adipose tissue may lead to higher circulating levels of insulin growth factor through worsening insulin resistance, thereby increasing the risk of carcinogenesis.[6] Furthermore, a high-fat diet induces colon and intestinal tumorigenesis by promoting the proliferation of intestinal stem cells.[9]

Multiple modalities, including lifestyle modification, mechanical gastric restriction, modulation in the secretion of multiple gut hormones, alteration in the composition and secretion of bile acids, and alterations of the gut microbiome, have been explored in obesity management.[10] Previous studies have primarily focused on pharmaceutical therapies, including combination therapies using different medical or interventional therapies with multiple targets for treating obesity.[11] Recently, bariatric surgical procedures have been extensively adopted and demonstrated efficacy in treating obesity.[12] As the prevalence of obesity increases, novel therapeutic approaches such as probiotics,[13,14] laparoscopic surgery,[15] topical lotions and subcutaneous medication,[16,17] transcatheter bariatric embolization,[18] low insulin method,[19] or gene therapy[20] have gained attention.

This comprehensive review aims to consolidate the recently applied medical, endoscopic, and surgical approaches for managing obesity and compare their beneficial effects and efficacy on obesity and its long-term comorbidities. We particularly aim to highlight newer experimental techniques for the management of obesity, including transcatheter bariatric embolization, intragastric balloon therapies, primary obesity surgery endoluminal procedures, and the Endobarrier procedure, which have shown promise in recent studies.

Medical management

Glucagon-like peptide 1 agonist

Long-acting glucagon-like peptide 1 (GLP-1) agonists such as semaglutide, liraglutide, and tirzepatide are currently available in the U.S. for the management of obesity, especially in patients with impaired glucose tolerance.[21,22] The primary outcome of a recent study indicated that the mean weight loss with weekly subcutaneous injections of semaglutide 2.4 mg was 15.4% at week 68, compared to a mean weight loss of 6.4% in those receiving daily subcutaneous liraglutide 3.0 mg.[23] Another analysis compared daily oral semaglutide 14 mg with daily subcutaneous liraglutide 1.8 mg for obesity management in diabetic patients whose glycemic indicators were stable on metformin. The outcomes indicated a placebo-subtracted average weight loss of 4.2% with oral semaglutide compared to a placebo-subtracted mean weight loss of 2.7% with subcutaneous liraglutide at the end of the 26th week.[24] Thus, whether administered orally or subcutaneously, semaglutide appears to be superior to subcutaneous liraglutide for the management of obesity. Figure 1 shows a comparison of the results from these two studies. Tirzepatide is a newer dual glucose-dependent insulinotropic polypeptide and GLP-1 receptor agonist.[25] Although trials comparing the efficacy of tirzepatide and other GLP-1 are still underway, recent studies have demonstrated encouraging outcomes. An open-label, 40-week, phase III randomized trial comparing weekly tirzepatide and semaglutide in type 2 diabetes mellitus patients indicated that reductions in body weight were greater and statistically significant with tirzepatide than with semaglutide in the secondary endpoints.[25] A more recent phase III placebo-controlled, double-blind, randomized trial comparing percentage weight loss for three different doses of weekly tirzepatide showed a significant and sustained reduction in weight, with a higher percentage of weight loss observed with higher doses.[26]

Orlistat

Orlistat is a reversible inhibitor of gastrointestinal lipases, traditionally employed for obesity management.[21,22] Orlistat, combined with lifestyle changes, contributed to a reduction in weight by 5.8

kg compared to 3.3 kg with placebo over 4 years.[27] A 37.3% reduction in the risk of diabetes mellitus was observed in patients treated with orlistat vs. placebo. Orlistat has an excellent long-term safety profile, and serious adverse events are rare.[28] Despite this, a high rate of gastrointestinal side effects such as oily stools, diarrhea, abdominal pain, and fecal spotting, as well as interactions with several drugs affecting their bioavailability and effectiveness, limits its adherence and makes it a less popular option.[29]

Lorcaserin

Lorcaserin is a serotonin 2C receptor agonist. Research indicates that it contributes to a reduction in body weight of 5.8 kg in 47.5% of the subjects over a year, compared to a weight reduction of 2.2 kg in 20.3% of the subjects in the placebo group. Weight loss was sustained in a significantly greater number of patients in the Lorcaserin group during the second year.[30] The CAMELLIA–TIMI 61 trial (Cardiovascular and Metabolic Effects of Lorcaserin in Overweight and Obese Patients–Thrombolysis in Myocardial Infarction 61) investigated the long-term cardiovascular safety and efficacy of lorcaserin in obese or overweight patients with cardiovascular disease or risk factors. The rates of several cardiovascular and metabolic risk factors, such as blood pressure, heart rate, low density lipoprotein, and triglycerides were slightly lower in the intervention group than in the placebo group. At one year, the rate of cardiovascular events was similar in both groups.[31] A safety review of this study also identified a potential signal for increased cancer incidence, however, the study was not powered for cancer end-points.[32] A review conducted by the Food and Drug Administration (FDA) in 2020, based on a large post-marketing clinical trial revealed a higher frequency of cancer diagnosis for 13 types of cancer, including colorectal cancer, pancreatic cancer, and lung cancer, in the lorcaserin group compared to the placebo group.[32] Consequently, the FDA requested manufacturers to voluntarily withdraw their products from the market due to these safety concerns.

Combination therapies

Combination pharmacotherapy is increasingly being adopted worldwide for obesity treatment due to its heightened efficacy and beneficial outcomes.[21] The combined implication of pramlintide and phentermine was found to be eight times more efficacious than pramlintide monotherapy in reducing human weight. This combined pharmacotherapy resulted in a weight reduction of approximately 10.5%, compared to 2.5% for pramlintide alone after 24 weeks.[11] Exenatide once weekly, combined with daily dapagliflozin, induced greater weight reduction than either of the individual therapies, with results sustained over a year, suggesting long-term sustainable benefits in weight reduction.[11] The combination of phentermine and topiramate resulted in an overall placebo-subtracted weight loss of 3.5% at low doses and 9.3% at higher doses. Major studies leading to the approval of naltrexone/bupropion reported an average placebo-subtracted weight loss of 3.7% at a dose of 16/360 mg, and 4.8% at a dose of 32/360 mg.[33] Similarly, co-infusion of sub-anorectic doses of GLP-1 and glucagon demonstrated a 13% reduction in food intake[34] while simultaneously increasing energy expenditure, thus improving obesity and glycemia.[35] Therefore, combination therapies are not only more efficacious in treating obesity but also have more long-lasting effects than monotherapies. Some of the commonly prescribed medications for the management of obesity are summarized in Table 1.

Probiotics

Probiotics can modify gut microbiota and have been shown to contribute to body weight reduction in experimental animal studies. In an 8-week-old Apoe knock-out mouse model, the group of mice receiving *Lactobacillus reuteri* strain ATCC PTA 4659 indicated a significant reduction in body weight, adipose, and liver weight, and decreased serum insulin levels, attributing to increased β-oxidation.[13] Another study demonstrated that the oral administration of *Saccharomyces boulardii* over 4 weeks resulted in a 15% reduction in body weight gain, accompanied by a significant decrease in whole-body fat mass, without altering food intake in a mouse model.[14] Additionally, supplementation with S. boulardii and superoxide dismutase for 60 days in obese population led to significant weight loss and fat loss, while preserving fat-free mass in a randomized clinical trial (RCT).[36]

Herbal supplements

The use of herbal weight loss supplements has recently attracted increased amounts of attention due to the increasing prevalence of obesity. Garcinia cambogia supplements containing hydroxycitric acid are marketed for weight loss;[37] however, the FDA has recently issued a warning following post-marketing surveillance indicating an increased risk of hepatotoxicity associated with garcinia cambogia. Conjugated linoleic acid supplementation has shown limited evidence for weight loss, but studies have demonstrated an increase in oxidative stress and insulin resistance with regular consumption of conjugated linoleic acid, which limits its utilization.[38] L-carnitine, an amino acid naturally produced in the liver and kidneys, is thought to aid in managing obesity through its effects on glycemic control and lipid-lowering activities. However, analyses have shown that it produces only a moderate effect on weight loss.[39]

Other novel medical approaches

There are several other promising medical approaches for the management of obesity. The administration of transforming growth factor beta superfamily ligands, including GDF15 and MIC-1, has been shown to reduce body weight and food intake in mouse and human models, respectively, making them advantageous in the treatment of obesity.[40,41] Similarly, twice-daily topical application of a lotion containing aminophylline, caffeine, yohimbe, L-carnitine, and gotu kola, combined with exercise and restricted calorie intake for 28 days effectively reduced body mass, fat mass, and circumference in the treated area.[16]

Surgical management

The surgical approach for managing obesity has long been used to achieve sustainable results, especially in obese patients resistant to pharmacotherapy. Bariatric procedures are widely employed surgical interventions for treating obesity and its associated morbidities, consistently yielding desirable outcomes. Bariatric surgeries are considered the treatment of choice for patients with a BMI >40 kg/m2 or BMI >35 kg/m2 with severe associated comorbidities.[10]

Two major surgical approaches are laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass (LRYGB). Figure 2 illustrates a compilation of studies comparing the post-surgical benefits and metabolic effects of LSG and LRYGB. Peterli *et al*. compared the post-surgical effects of LSG and LRYGB over 3 years in an RCT.[15] The study concluded that both LSG and LRYGB groups demonstrated statistically equal efficacy in reducing excessive body mass index and improving quality of life up to 3 years after surgery. After 3 years, the improvement in comorbidities was similar for both groups, except for dyslipidemia

Table 1. Commonly prescribed medications for obesity management

Drug Class	Generic Names	Doses	Comments
Glucagon-like Peptide 1 agonist	Semaglutide	Start with 0.25 mg subcutaneous (SC) once a week. Increase the dose every 4 weeks by 0.25 mg till a maximum of 2.4 mg is reached.	Monitor for eye complications in patients with Diabetic retinopathy.
	Liraglutide	Start with 0.6 mg SC daily and increase at weekly intervals by 0.6 until maximum 3 mg.	
	Tirzepatide	Start with 2.5 mg weekly and increase by 2.5 every 4 weeks to maximum 15 mg.	Currently approved for type 2 diabetes and obesity management.
			All: Hypoglycemia if co-administered with other diabetes medications. Rarely reported: pancreatitis. Contraindicated in pregnancy and patients with a family history of medullary thyroid cancer (based on murine models) or multiple endocrine neoplasia.
Gastric/pancreatic lipase inhibitors	Orlistat	120 mg TID with fat containing meals (60 mg TID for those who cannot tolerate 120 mg).	Good safety profile for long-term use. GI side effects could be the limiting factor.
Combination Therapies	Phentermine and Topiramate	Start with 3.75 phentermine and 23 mg topiramate daily for 14 days, increase by 3.75/23 for 12 weeks. Then increase based on response to a maximum of 15/92.	Phentermine has abuse potential. Side effects include dry mouth, paresthesia, cognitive deficits, anxiety, insomnia, etc. Contraindicated in pregnancy (note topiramate is teratogenic), hyperthyroidism, glaucoma, and co-administration with MAO inhibitors.
	Naltrexone and bupropion	Start with 8 mg naltrexone and 90 mg bupropion daily (1 combination pill). Increase by 1 pill every week to a maximum of 4 tablets daily.	Nausea, vomiting, insomnia, dry mouth, increase in blood pressure. Contraindicated in poorly controlled hypertension, seizure disorder, opioid use disorder, opioid agonist therapy, pregnancy, and breastfeeding.
Noradrenergic sympathomimetics	phentermine	15 mg to 37.5 mg daily.	Several side effects and usually avoided unless it is short term only (<12 weeks).

MAO, monoamine oxidase; SC, subcutaneous; TID, ter in die.

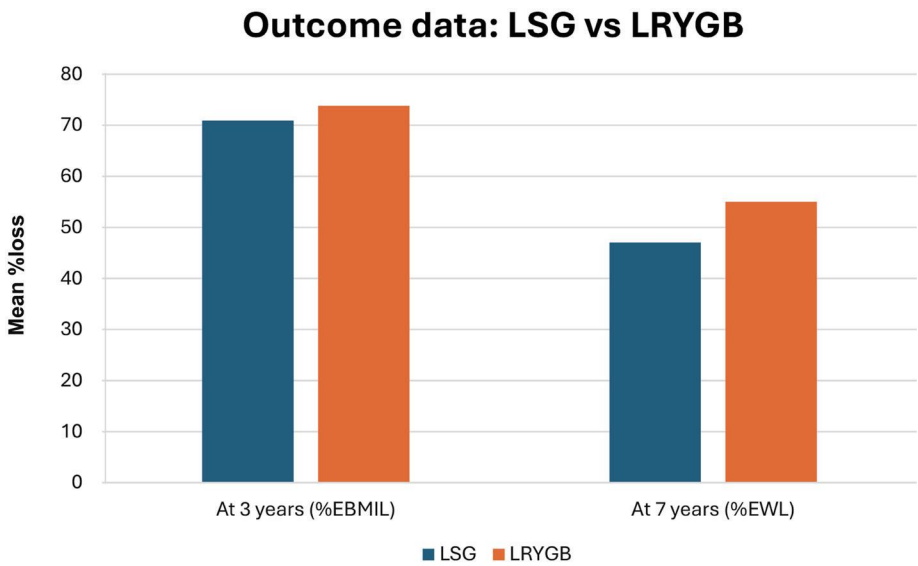

Fig. 2. Outcomes of laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass (LRYGB). The left graph shows the mean percent excess BMI loss (%EBMIL) between LSG and LRYGB at 3 years. The right graph displays the mean percent excess weight loss (%EWL) between LSG and LRYGB at 7 years. %EBMIL, percent excess body mass index loss; %EWL, excess weight loss; LRYGB, laparoscopic Roux-en-Y gastric bypass; LSG, laparoscopic sleeve gastrectomy.

and gastroesophageal reflux disease, which responded more effectively to LRYGB treatment. Gronroos *et al*. performed another RCT comparing the post-surgical effects of LSG and LRYGB over a 7-year period.[42] The results indicated that in a follow-up after 7 years, the mean percentage of excess weight loss was higher after LRYGB (55%) than after LSG (47%). Although LRYGB resulted in greater weight loss, it was associated with a 4.6% higher total morbidity rate. The long-term quality of life was similar after both procedures.

In a study comparing the metabolic effects of LSG and LRYGB, the number of significantly altered lipid metabolites was higher following LSG than LRYGB, mainly due to anatomical differences between the two surgeries and factors related to gut microbiota.[43] LSG was associated with alterations in amino acid metabolism, while LRYGB was associated with changes in bile acids. Studies conducted on triglyceride-rich lipoproteins (TRL) 6 months after surgery revealed that both TRL-apoB-100 and TRL-apoB-48 declined after LSG due to decreased production rates of both lipoproteins and an increased fractional catabolic rate of TRL-apoB-100 only. In contrast, the TRL-apoB-48 level did not significantly decrease after LRYGB.[44]

Laparoscopic vertical banded gastroplasty is another bariatric procedure effective in reducing body fat; however, it is less efficacious than LRYGB.[45]

Endoscopic management

As minimally invasive surgery is favored by patients, there has been significant development in endoscopic weight reduction procedures and devices. The major endoscopic procedures currently available are listed as follows:

Transcatheter bariatric embolization

Transcatheter bariatric embolization (TBE) uses a balloon microcatheter to occlude the left gastric artery, thereby promoting weight loss. The LOSEIT study (The Lowering Weight in Severe Obesity by Embolization of the Gastric Artery Trial) was a randomized pilot study that established the proof-of-principle demonstrating that TBE is well-tolerated and effective in weight reduction.[18] In the intention-to-treat population, total body weight loss was 7.4 kg with TBE (6.4% reduction) compared to 3.0 kg with sham (2.8% reduction) at 6 months after the procedure. Subjects treated with TBE had significant improvements in physical function, self-esteem, and overall quality of life at 6 and 12 months.

Endoscopic sleeve gastroplasty

Endoscopic sleeve gastroplasty (ESG) is a minimally invasive procedure that effectively induces a reduction in body weight by decreasing the size of the gastric reservoir. Subjects who underwent ESG experienced a significant reduction in excess body weight of 53% at 6 months.[46] In a physiological analysis, there was a 59% decrease in caloric intake to reach gastric fullness, along with decreased gastric emptying time for solids and increased insulin sensitivity.

Percutaneous gastrostomy devices

In a recent RCT by Thompson *et al*., an endoscopic device comprising an endoscopically placed percutaneous gastrostomy tube and an external device to facilitate drainage was utilized. The study demonstrated that 58.6% of participants in the intervention group lost 25% of their excess body weight, compared to 15.3% of participants in the control group. Notably, only 3.6% of the intervention group participants developed serious postoperative adverse effects.[47]

Primary obesity surgery endoluminal procedure

The primary obesity surgery endoluminal (POSE) procedure is an endoscopic incision procedure aimed to reduce the size of the stomach and decrease hunger cravings. A recent study reported that 79% of patients who underwent POSE procedures had a mean percent excess weight loss of approximately 50% after 1 year, with no development of any serious side effects.[48]

Endoluminal endoscopic gastric jejunal bypass sleeve

Gastro-duodeno-jejunal bypass sleeve is a novel technique that serves as an alternative to bariatric surgery in patients with morbid obesity. It consists of a 120 cm long sleeve device, placed endoscopically to create an endoluminal bypass tract from the lower gastroesophageal junction to the jejunum. A prospective trial designed to study the effectiveness of endoluminal, endoscopic gastric bypass sleeve implants in morbidly obese individuals concluded that almost half of the participants experienced a mean percentage excess weight loss (EWL) of 54% after 12 months and sustained a mean %EWL of 30% at the 14-month post-explant follow-up, while the remaining required explantation or experienced partial cuff detachment before completing 1 year.[49] This trial demonstrated that the gastro-duodenal-jejunal bypass sleeve could be an effective treatment option for the long-term management of morbid obesity.

Intragastric balloon therapy

Intragastric balloon (IGB) therapy has become an attractive tool for weight loss, owing to its sustained efficacy, low complication rate, and broad application, extending to class I and II obesity. This therapy involves a space-occupying device that alters gastric emptying and gastrointestinal neurohumoral pathways, leading to early satiety.[50] Several different types of IGBs are commercially available in the U.S. Among patients with a BMI range of 30–40 kg/m^2, IGB has shown superior outcomes in terms of weight loss compared to lifestyle modification alone. IGBs lead to greater weight loss at 6, 9, and 12 months after initial balloon placement; however, the amount of weight loss decreases during each successive time-period.[51] A pooled analysis of 7 RCTs revealed that the percent total body weight loss (%TBWL) at the end of 6–8 months was 7.4–14.9% for patients with IGB compared to 2.4–5.4% for those receiving standard care.[50]

IGB use is associated with the improvement in various metabolic parameters and medical conditions compared with noninvasive measures for weight loss.[51] IGB decreased the incidence of metabolic syndrome from 34.8% (pre-IGB) to 11.6% at 12 months post-IGB removal. The incidences of type 2 diabetes mellitus, hypertriglyceridemia, hypercholesterolemia, and hypertension decreased from 32.6%, 37.7%, 33.4%, and 44.9% (pre-IGB) to 21.3%, 17.4%, 18.9%, and 34.8% respectively at 12 months post IGB removal.[52] Among patients undergoing bio-enteric IGB placement, the prevalence of hypertension, diabetes, hypercholesterolemia, and osteoarthropathy decreased from 29%, 15%, 32%, and 25% (pre-IGB), respectively, to 16%, 10%, 21%, and 13% at 3 years post-IGB removal.[53] Device intolerance (sense of fullness) and symptomatic intolerance (including epigastric pain, reflux, nausea, or emesis) remain the primary reasons for early IGB removal, occurring in approximately 9.4% of patients. More serious adverse events, such as gastrointestinal perforation (0.3%), esophageal mucosal injury (0.8%), gastric ulcer/bleeding (0.76%),

and gastric outlet/bowel obstruction (0.12%), are relatively rare. No mortality was reported during the 6–8 month period following balloon placement.[51]

Endoluminal duodenal-jejunal bypass liner (endobarrier)

Procedure

The application of endoluminal duodenal-jejunal bypass liner (DJBL), commonly referred to as endobarrier, has demonstrated effectiveness in managing chronic morbid obesity.[54] In patients with class I obesity and long-term type 2 diabetes mellitus, the DJBL procedure resulted in a 15% reduction in total body weight and a 0.6% reduction in Hb1Ac at 12 months. Only 9.5% of the patients with the DJBL procedure experienced major side effects, including severe abdominal pain in one patient and acute cholecystitis with duodenal fistula due to bulbar transmural penetration and gall bladder impaction by one of the anchors.[54] In an RCT for DJBL in patients with type 2 diabetes mellitus and obesity, 24% of the patients in the DJBL group achieved a ≥15% reduction in body weight compared to 4% in the control group at 12 months. DJBL demonstrated superior reductions in serum cholesterol, systolic blood pressure, and alanine transaminase levels at 12 months, while there was no significant difference in glycemic control.[55]

Duodenal mucosal resurfacing

Duodenal mucosal resurfacing (DMR) is a minimally invasive endoscopic procedure for circumferential hydrothermal ablation. DMR, particularly when combined with hypocaloric intake, has long-lasting efficacy in controlling diabetes and reducing both intramyocellular and intrahepatocellular lipids, while favoring the mobilization of abdominal fat and improving glycemia.[56]

Conclusions

Obesity has been a primary target for medical and surgical therapy. Various monotherapy options, such as GLP-1 agonists, have shown success in reducing weight. The combination pharmacotherapies have demonstrated significantly greater efficacy in weight loss compared to the individual drugs. Bariatric surgical methods provide more effective and long-lasting outcomes and carry a relatively higher risk of complications, which limits their widespread adoption. Several novel endoscopic devices and procedures are promising due to their satisfactory results, relatively lower cost, and lower risk. Further studies assessing the safety, effectiveness, and sustainability of these novel endoscopic techniques are warranted.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (KMa, MR and SJ), drafting of manuscript (KMa, MR), proofreading (SJ and KMu), critical revision of the manuscript (KMu).

References

[1] GBD 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2019;393(10184):1958–1972. doi:10.1016/S0140-6736(19)30041-8, PMID:30954305.
[2] Smethers AD, Rolls BJ. Dietary Management of Obesity: Cornerstones of Healthy Eating Patterns. Med Clin North Am 2018;102(1):107–124. doi:10.1016/j.mcna.2017.08.009, PMID:29156179.
[3] Vernarelli JA, Mitchell DC, Rolls BJ, Hartman TJ. Dietary energy density is associated with obesity and other biomarkers of chronic disease in US adults. Eur J Nutr 2015;54(1):59–65. doi:10.1007/s00394-014-0685-0, PMID:24664188.
[4] Heymsfield SB, Wadden TA. Mechanisms, Pathophysiology, and Management of Obesity. N Engl J Med 2017;376(3):254–266. doi:10.1056/NEJMra1514009, PMID:28099824.
[5] Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, *et al*. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med 2017;377(1):13–27. doi:10.1056/NEJMoa1614362, PMID:28604169.
[6] Nam SY. Obesity-Related Digestive Diseases and Their Pathophysiology. Gut Liver 2017;11(3):323–334. doi:10.5009/gnl15557, PMID:27890867.
[7] Münch NS, Fang HY, Ingermann J, Maurer HC, Anand A, Kellner V, *et al*. High-Fat Diet Accelerates Carcinogenesis in a Mouse Model of Barrett's Esophagus via Interleukin 8 and Alterations to the Gut Microbiome. Gastroenterology 2019;157(2):492–506.e2. doi:10.1053/j.gastro.2019.04.013, PMID:30998992.
[8] Mana MD, Hussey AM, Tzouanas CN, Imada S, Barrera Millan Y, Bahceci D, *et al*. High-fat diet-activated fatty acid oxidation mediates intestinal stemness and tumorigenicity. Cell Rep 2021;35(10):109212. doi:10.1016/j.celrep.2021.109212, PMID:34107251.
[9] van Driel MS, van Neerven SM, Vermeulen L. High-Fat Diet Impacts on Tumor Development in the Gut. Trends Cancer 2021;7(8):664–665. doi:10.1016/j.trecan.2021.06.005, PMID:34219052.
[10] Gadde KM, Martin CK, Berthoud HR, Heymsfield SB. Obesity: Pathophysiology and Management. J Am Coll Cardiol 2018;71(1):69–84. doi:10.1016/j.jacc.2017.11.011, PMID:29301630.
[11] Camilleri M, Acosta A. Combination Therapies for Obesity. Metab Syndr Relat Disord 2018;16(8):390–394. doi:10.1089/met.2018.0075, PMID:29993319.
[12] Meneses E, Zagales I, Fanfan D, Zagales R, McKenney M, Elkbuli A. Surgical, metabolic, and prognostic outcomes for Roux-en-Y gastric bypass versus sleeve gastrectomy: a systematic review. Surg Obes Relat Dis 2021;17(12):2097–2106. doi:10.1016/j.soard.2021.06.020, PMID:34642101.
[13] Fåk F, Bäckhed F. Lactobacillus reuteri prevents diet-induced obesity, but not atherosclerosis, in a strain dependent fashion in Apoe-/- mice. PLoS One 2012;7(10):e46837. doi:10.1371/journal.pone.0046837, PMID:23056479.
[14] Everard A, Matamoros S, Geurts L, Delzenne NM, Cani PD. Saccharomyces boulardii administration changes gut microbiota and reduces hepatic steatosis, low-grade inflammation, and fat mass in obese and type 2 diabetic db/db mice. mBio 2014;5(3):e01011–e01014. doi:10.1128/mBio.01011-14, PMID:24917595.
[15] Peterli R, Wölnerhanssen BK, Vetter D, Nett P, Gass M, Borbély Y, *et al*. Laparoscopic Sleeve Gastrectomy Versus Roux-Y-Gastric Bypass for Morbid Obesity-3-Year Outcomes of the Prospective Randomized Swiss Multicenter Bypass Or Sleeve Study (SM-BOSS). Ann Surg 2017;265(3):466–473. doi:10.1097/SLA.0000000000001929, PMID:28170356.
[16] Escalante G, Bryan P, Rodriguez J. Effects of a topical lotion containing aminophylline, caffeine, yohimbe, l-carnitine, and gotu kola on thigh circumference, skinfold thickness, and fat mass in sedentary females. J Cosmet Dermatol 2019;18(4):1037–1043. doi:10.1111/jocd.12801, PMID:30456780.
[17] Wadden TA, Bailey TS, Billings LK, Davies M, Frias JP, Koroleva A, *et al*. Ef-

fect of Subcutaneous Semaglutide vs Placebo as an Adjunct to Intensive Behavioral Therapy on Body Weight in Adults With Overweight or Obesity: The STEP 3 Randomized Clinical Trial. JAMA 2021;325(14):1403–1413. doi:10.1001/jama.2021.1831, PMID:33625476.

[18] Reddy VY, Neužil P, Musikantow D, Sramkova P, Rosen R, Kipshidze N, *et al*. Transcatheter Bariatric Embolotherapy for Weight Reduction in Obesity. J Am Coll Cardiol 2020;76(20):2305–2317. doi:10.1016/j.jacc.2020.09.550, PMID:33183504.

[19] Röhling M, Martin K, Ellinger S, Schreiber M, Martin S, Kempf K. Weight Reduction by the Low-Insulin-Method-A Randomized Controlled Trial. Nutrients 2020;12(10):3004. doi:10.3390/nu12103004, PMID:33007918.

[20] Angelidi AM, Belanger MJ, Kokkinos A, Koliaki CC, Mantzoros CS. Novel Noninvasive Approaches to the Treatment of Obesity: From Pharmacotherapy to Gene Therapy. Endocr Rev 2022;43(3):507–557. doi:10.1210/endrev/bnab034, PMID:35552683.

[21] Garvey WT, Mechanick JI, Brett EM, Garber AJ, Hurley DL, Jastreboff AM, *et al*. American Association of Clinical Endocrinologists and American College of Endocrinology Comprehensive Clinical Practice Guidelines For Medical Care of Patients with Obesity. Endocr Pract 2016;22(Suppl 3):1–203. doi:10.4158/EP161365.GL, PMID:27219496.

[22] Son JW, Kim S. Comprehensive Review of Current and Upcoming Anti-Obesity Drugs. Diabetes Metab J 2020;44(6):802–818. doi:10.4093/dmj.2020.0258, PMID:33389955.

[23] Rubino DM, Greenway FL, Khalid U, O'Neil PM, Rosenstock J, Sørrig R, *et al*. Effect of Weekly Subcutaneous Semaglutide vs Daily Liraglutide on Body Weight in Adults With Overweight or Obesity Without Diabetes: The STEP 8 Randomized Clinical Trial. JAMA 2022;327(2):138–150. doi:10.1001/jama.2021.23619, PMID:35015037.

[24] Pratley R, Amod A, Hoff ST, Kadowaki T, Lingvay I, Nauck M, *et al*. Oral semaglutide versus subcutaneous liraglutide and placebo in type 2 diabetes (PIONEER 4): a randomised, double-blind, phase 3a trial. Lancet 2019;394(10192):39–50. doi:10.1016/S0140-6736(19)31271-1, PMID:31186120.

[25] Frías JP, Davies MJ, Rosenstock J, Pérez Manghi FC, Fernández Landó L, Bergman BK, *et al*. Tirzepatide versus Semaglutide Once Weekly in Patients with Type 2 Diabetes. N Engl J Med 2021;385(6):503–515. doi:10.1056/NEJMoa2107519, PMID:34170647.

[26] Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, *et al*. Tirzepatide Once Weekly for the Treatment of Obesity. N Engl J Med 2022;387(3):205–216. doi:10.1056/NEJMoa2206038, PMID:35658024.

[27] Torgerson JS, Hauptman J, Boldrin MN, Sjöström L. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care 2004;27(1):155–161. doi:10.2337/diacare.27.1.155, PMID:14693982.

[28] Sahebkar A, Simental-Mendía LE, Reiner Ž, Kovanen PT, Simental-Mendía M, Bianconi V, *et al*. Effect of orlistat on plasma lipids and body weight: A systematic review and meta-analysis of 33 randomized controlled trials. Pharmacol Res 2017;122:53–65. doi:10.1016/j.phrs.2017.05.022, PMID:28559211.

[29] Filippatos TD, Derdemezis CS, Gazi IF, Nakou ES, Mikhailidis DP, Elisaf MS. Orlistat-associated adverse effects and drug interactions: a critical review. Drug Saf 2008;31(1):53–65. doi:10.2165/00002018-200831010-00005, PMID:18095746.

[30] Smith SR, Weissman NJ, Anderson CM, Sanchez M, Chuang E, Stubbe S, *et al*. Multicenter, placebo-controlled trial of lorcaserin for weight management. N Engl J Med 2010;363(3):245–256. doi:10.1056/NEJMoa0909809, PMID:20647200.

[31] Bohula EA, Wiviott SD, McGuire DK, Inzucchi SE, Kuder J, Im K, *et al*. Cardiovascular Safety of Lorcaserin in Overweight or Obese Patients. N Engl J Med 2018;379(12):1107–1117. doi:10.1056/NEJMoa1808721, PMID:30145941.

[32] Sharretts J, Galescu O, Gomatam S, Andraca-Carrera E, Hampp C, Yanoff L. Cancer Risk Associated with Lorcaserin - The FDA's Review of the CAMELLIA-TIMI 61 Trial. N Engl J Med 2020;383(11):1000–1002. doi:10.1056/NEJMp2003873, PMID:32905671.

[33] Pilitsi E, Farr OM, Polyzos SA, Perakakis N, Nolen-Doerr E, Papathanasiou AE, *et al*. Pharmacotherapy of obesity: Available medications and drugs under investigation. Metabolism 2019;92:170–192. doi:10.1016/j.metabol.2018.10.010, PMID:30391259.

[34] Cegla J, Troke RC, Jones B, Tharakan G, Kenkre J, McCullough KA, *et al*. Coinfusion of low-dose GLP-1 and glucagon in man results in a reduction in food intake. Diabetes 2014;63(11):3711–3720. doi:10.2337/db14-0242, PMID:24939425.

[35] Tan TM, Field BC, McCullough KA, Troke RC, Chambers ES, Salem V, *et al*. Coadministration of glucagon-like peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia. Diabetes 2013;62(4):1131–1138. doi:10.2337/db12-0797, PMID:23248172.

[36] Rondanelli M, Miraglia N, Putignano P, Castagliuolo I, Brun P, Dall'Acqua S, *et al*. Effects of 60-Day Saccharomyces boulardii and Superoxide Dismutase Supplementation on Body Composition, Hunger Sensation, Pro/Antioxidant Ratio, Inflammation and Hormonal Lipo-Metabolic Biomarkers in Obese Adults: A Double-Blind, Placebo-Controlled Trial. Nutrients 2021;13(8):2512. doi:10.3390/nu13082512, PMID:34444671.

[37] Golzarand M, Omidian M, Toolabi K. Effect of Garcinia cambogia supplement on obesity indices: A systematic review and dose-response meta-analysis. Complement Ther Med 2020;52:102451. doi:10.1016/j.ctim.2020.102451, PMID:32951714.

[38] Benjamin S, Prakasan P, Sreedharan S, Wright AD, Spener F. Pros and cons of CLA consumption: an insight from clinical evidences. Nutr Metab (Lond) 2015;12:4. doi:10.1186/1743-7075-12-4, PMID:25972911.

[39] Talenezhad N, Mohammadi M, Ramezani-Jolfaie N, Mozaffari-Khosravi H, Salehi-Abargouei A. Effects of l-carnitine supplementation on weight loss and body composition: A systematic review and meta-analysis of 37 randomized controlled clinical trials with dose-response analysis. Clin Nutr ESPEN 2020;37:9–23. doi:10.1016/j.clnesp.2020.03.008, PMID:32359762.

[40] Johnen H, Lin S, Kuffner T, Brown DA, Tsai VW, Bauskin AR, *et al*. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. Nat Med 2007;13(11):1333–1340. doi:10.1038/nm1677, PMID:17982462.

[41] Mullican SE, Lin-Schmidt X, Chin CN, Chavez JA, Furman JL, Armstrong AA, *et al*. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. Nat Med 2017;23(10):1150–1157. doi:10.1038/nm.4392, PMID:28846097.

[42] Grönroos S, Helmiö M, Juuti A, Tiusanen R, Hurme S, Löyttyniemi E, *et al*. Effect of Laparoscopic Sleeve Gastrectomy vs Roux-en-Y Gastric Bypass on Weight Loss and Quality of Life at 7 Years in Patients With Morbid Obesity: The SLEEVEPASS Randomized Clinical Trial. JAMA Surg 2021;156(2):137–146. doi:10.1001/jamasurg.2020.5666, PMID:33295955.

[43] Lee G, Park YS, Cho C, Lee H, Park J, Park DJ, *et al*. Short-term changes in the serum metabolome after laparoscopic sleeve gastrectomy and Roux-en-Y gastric bypass. Metabolomics 2021;17(8):71. doi:10.1007/s11306-021-01826-y, PMID:34355282.

[44] Padilla N, Maraninchi M, Béliard S, Berthet B, Nogueira JP, Wolff E, *et al*. Effects of bariatric surgery on hepatic and intestinal lipoprotein particle metabolism in obese, nondiabetic humans. Arterioscler Thromb Vasc Biol 2014;34(10):2330–2337. doi:10.1161/ATVBAHA.114.303849, PMID:25104797.

[45] Olbers T, Björkman S, Lindroos A, Maleckas A, Lönn L, Sjöström L, *et al*. Body composition, dietary intake, and energy expenditure after laparoscopic Roux-en-Y gastric bypass and laparoscopic vertical banded gastroplasty: a randomized clinical trial. Ann Surg 2006;244(5):715–722. doi:10.1097/01.sla.0000218085.25902.f8, PMID:17060764.

[46] Abu Dayyeh BK, Acosta A, Camilleri M, Mundi MS, Rajan E, Topazian MD, *et al*. Endoscopic Sleeve Gastroplasty Alters Gastric Physiology and Induces Loss of Body Weight in Obese Individuals. Clin Gastroenterol Hepatol 2017;15(1):37–43.e1. doi:10.1016/j.cgh.2015.12.030, PMID:26748219.

[47] Thompson CC, Abu Dayyeh BK, Kushner R, Sullivan S, Schorr AB, Amaro A, *et al*. Percutaneous Gastrostomy Device for the Treatment of Class II and Class III Obesity: Results of a Randomized Controlled Trial. Am J Gastroenterol 2017;112(3):447–457. doi:10.1038/ajg.2016.500, PMID:27922026.

[48] López-Nava G, Bautista-Castaño I, Jimenez A, de Grado T, Fernandez-Corbelle JP. The Primary Obesity Surgery Endolumenal (POSE) proce-

dure: one-year patient weight loss and safety outcomes. Surg Obes Relat Dis 2015;11(4):861–865. doi:10.1016/j.soard.2014.09.026, PMID:25701201.

[49] Sandler BJ, Rumbaut R, Swain CP, Torres G, Morales L, Gonzales L, *et al*. One-year human experience with a novel endoluminal, endoscopic gastric bypass sleeve for morbid obesity. Surg Endosc 2015;29(11):3298–3303. doi:10.1007/s00464-015-4081-5, PMID:25631114.

[50] Shah R, Davitkov P, Abu Dayyeh BK, Saumoy M, Murad MH. AGA Technical Review on Intragastric Balloons in the Management of Obesity. Gastroenterology 2021;160(5):1811–1830. doi:10.1053/j.gastro.2021.02.043, PMID:33832658.

[51] Muniraj T, Day LW, Teigen LM, Ho EY, Sultan S, Davitkov P, *et al*. AGA Clinical Practice Guidelines on Intragastric Balloons in the Management of Obesity. Gastroenterology 2021;160(5):1799–1808. doi:10.1053/j.gastro.2021.03.003, PMID:33832655.

[52] Crea N, Pata G, Della Casa D, Minelli L, Maifredi G, Di Betta E, *et al*. Improvement of metabolic syndrome following intragastric balloon: 1 year follow-up analysis. Obes Surg 2009;19(8):1084–1088. doi:10.1007/s11695-009-9879-6, PMID:19506981.

[53] Genco A, López-Nava G, Wahlen C, Maselli R, Cipriano M, Sanchez MM, *et al*. Multi-centre European experience with intragastric balloon in overweight populations: 13 years of experience. Obes Surg 2013;23(4):515–521. doi:10.1007/s11695-012-0829-3, PMID:23224509.

[54] Vilarrasa N, de Gordejuela AG, Casajoana A, Duran X, Toro S, Espinet E, *et al*. Endobarrier® in Grade I Obese Patients with Long-Standing Type 2 Diabetes: Role of Gastrointestinal Hormones in Glucose Metabolism. Obes Surg 2017;27(3):569–577. doi:10.1007/s11695-016-2311-0, PMID:27468906.

[55] Ruban A, Miras AD, Glaysher MA, Goldstone AP, Prechtl CG, Johnson N, *et al*. Duodenal-Jejunal Bypass Liner for the management of Type 2 Diabetes Mellitus and Obesity: A Multicenter Randomized Controlled Trial. Ann Surg 2022;275(3):440–447. doi:10.1097/SLA.0000000000004980, PMID:34647708.

[56] Garvey WT. Ablation of the Duodenal Mucosa as a Strategy for Glycemic Control in Type 2 Diabetes: Role of Nutrient Signaling or Simple Weight Loss. Diabetes Care 2016;39(12):2108–2110. doi:10.2337/dc16-1611, PMID:27879354.

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Review Article

Nonalcoholic Fatty Liver Disease and Gut-liver Axis: Role of Intestinal Microbiota and Therapeutic Mechanisms

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Abstract

The correlation between gut, secreted metabolites, and hepatic diseases has strengthened over the last decade. Interactions of intestinal permeability, gut microbes, and derived metabolites influence the development and progression of nonalcoholic fatty liver disease (NAFLD), a prevalent disease that affects more than 30% of the global population. NAFLD is now called metabolic dysfunction-associated steatotic liver disease (MASLD) to better reflect the disease process. Here, we describe mechanisms of NAFLD development, the role of gut bacteria, gut metabolites, interventions for diagnosis, and the prognosis of NAFLD. We discuss new paradigms that challenge the conventional, addressing disease etiology and translational approaches in a new dimension. Previous studies shed light on intricate relationships of the gut microbiome with the liver, or the gut-liver axis. Bidirectional communication between the gut and the liver involves exchange of metabolites, immune signaling, and inflammatory responses that has potential for novel NAFLD/nonalcoholic steatohepatitis (NASH) treatments. In this review, we propose exploring functional metagenomics to develop NAFLD diagnostic methods and risk assessment. The prospects of genetic engineering, fecal transplants, and specialized diet as targets of novel therapeutic regimes to combat NAFLD/NASH are discussed. Changes in lifestyle and diet in the population, combined with genetic predisposition, have led to an increasing number of cases of NAFLD. The microbiome responds to diet, exercise, and the environment, and can modulate NAFLD in cases with surgical impediments. It is thus vital to explore its emerging roles in human healthcare and not only liver disease.

Introduction

In 1980, Dr. Jürgen Ludwig was the first to describe nonalcoholic fatty liver disease (NAFLD).[1] As a result of severe changes in our lifestyles, NAFLD has become the most common liver condition in China and other parts of the world, with no established therapeutic interventions but only prevention in the form of lifestyle and nutrition adjustments.[2,3] Clinical symptoms of NAFLD are expected to impact around 25% of the population worldwide, making it a worldwide burden.[4,5] The disease encompasses a wide range of liver conditions, such as simple steatosis that progresses to nonalcoholic steatohepatitis (NASH), severe liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC).[6] Western and Eastern nations are predicted to have a two- to three-fold increase in the burden of end-stage liver disease by 2030.[5,6] Recently, using a two-stage Delphi consensus, NAFLD has been renamed metabolic dysfunction-associated steatotic liver disease (MASLD), which refers to a chronic and progressive condition that affects 30–40% of the global population and is strongly associated with features of metabolic syndrome, including obesity and type 2 diabetes mellitus.[7] MASLD is caused by accumulation of fat in the liver and includes a range of disease states, from isolated lipid accumulation or steatosis (i.e. MASL), and its active inflammatory form, metabolic dysfunction-associated steatohepatitis.[8] MASLD includes patients with hepatic steatosis along with cardiometabolic risk.

As mentioned above, many NAFLD patients have metabolic issues that further increase their risk of cardiovascular disease, diabetes, chronic renal disease, and cancer, which severely degrade health.[9] The mechanisms underlying the progression of MASLD

Keywords: Gut microbiota; NAFLD; MASLD; Metabolites; Dysbiosis; FMT.
Abbreviations: BA, bile acid; FA, fatty acid; FFA, free fatty acid; FMT, fecal microbiota transplantation; HCC, hepatocellular carcinoma; IL, interleukin; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SCFA, short-chain fatty acid.
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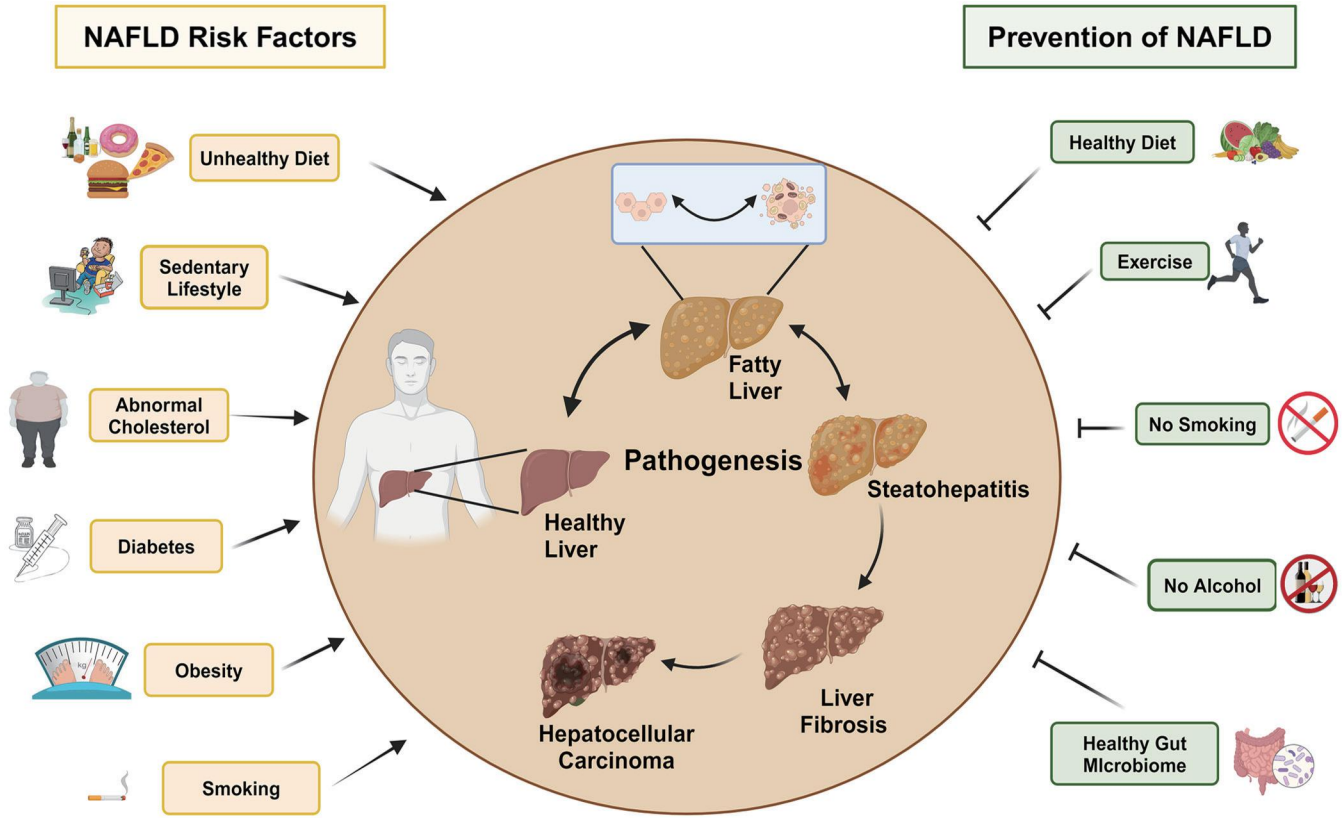

Fig. 1. Illustration of common risks and the prevention of NAFLD. NAFLD, nonalcoholic fatty liver disease.

to NASH and other severe liver disorders are largely unknown. This review explores various avenues to understand the complex interplay between intestinal microbiota and NAFLD progression.

The presence of the liver in the foregut in early development demonstrates that the gut and the liver are connected fundamentally by development stages.[7,10] Patients with NAFLD have higher levels of intestinal permeability, and it is linked with an increase in bacterial population inside the intestines.[11,12] Considering the high prevalence and morbidity of NAFLD, a better understanding of the underlying pathogenic mechanisms is essential for disease management.[13,14] This review aims to summarize significant findings on the association of the intestinal microbiota, gut-liver axis, crosstalk, and balance within the gut microbiota that in turn maintains intestinal permeability and tissue homeostasis. The goal is to present an overview depicting the impact of the intestinal microbiota on NAFLD development. The review describes recent advances in precision medicine offered by creative and emerging ideas from fecal microbiota transplantation (FMT), prebiotics, synbiotics, and probiotics. This review focuses on information that can help answer questions of the effects of alterations in microbiota composition and microbial function in NAFLD, molecular mechanisms underlying disease pathogenesis, comparative assessment of widely used diagnostic biochemical and biophysical methods, the causal relationship of gut microenvironment and progression of NAFLD, and laying the foundation for gut microbiota-targeted therapeutic regimes in NAFLD/NASH treatment. Previous reviews have discussed the role of the gut-brain axis in the onset of NAFLD, our review is focused more on the molecular mechanism of this association and investigating the key mediators of the process.

NAFLD

NAFLD is affiliated with a wide variety of liver disorders caused by lipid deposits in the hepatocytes with no causal connection to alcoholic drinks and/or drug consumption, as well as acquired or hereditary metabolic abnormalities that increase the risk of cirrhosis and HCC.[15,16] NAFLD is defined clinico-pathologically as the deposition of lipids in > 5% of hepatocytes and the exclusion of other sources of fat accumulation (Fig. 1).[17] This illness is linked to diabetes, cardiovascular disease, stroke, and liver damage. It is an implication of the hepatic metabolic syndrome that is supported by a two-hit approach in pathogenesis, as suggested and evidenced by the role of lipid peroxidation. The first hit is directed at the progression of hepatic steatosis by causing accumulation of triglycerides in hepatocytes and facilitates a second hit directed at minor and major inflammation, fibrosis, and lipoapoptosis.[18,19] Although the intrahepatic etiology is still under investigation and the interactions of immune responses are not clear, many potential pathophysiological mechanisms are proposed. It is well-established that an inflammatory cascade is activated by hepatocytic injury caused by oxidative stress and mitochondrial dysfunction. It further activates hepatic stellate cells, and infiltration of immune cells occurs as a downstream consequence that results in NASH.[20] Its prevalence is linked to obesity, insulin resistance, hypertension, hyperglycemia, and hyperlipidemia.[21] Insulin resistance and obesity contribute to chronic inflammation, NASH, and altered lipid metabolism, all of which contribute to procarcinogenic circumstances that promote HCC formation, the fifth most frequent cancer and the leading cause of death globally.[22] Type 2 diabetes occurrence signifies faster progression of NAFLD to NASH, advanced fibrosis, or cirrhosis, explaining

Table 1. Available diagnostic tools for detecting NAFLD

S. no.	Detection method	Advantage	Disadvantage	Reference
1.	Metagenomics and metabolomics	Stool specimens, easy collection, noninvasive tool in the differential diagnosis	Unsatisfactory results from long-term analysis	26
2.	Biopsy/ histopathology	Histological spectrum differentiating steatosis and fibrosis	Invasive, potentially harmful, sampling error, expensive, extreme cases lead to morbidity and mortality	27–29
3.	Liver enzymes and related scoring systems. FIB-4 index, NFS(NAFLD fibrosis score), NASH test, Fibro test, Steato test	Early detection of NAFLD, ability to grade the diseases into stages, better pathogenesis	Not sensitive for NAFLD diagnosis, validation required	30,31
4.	Liver ultrasound or ultrasonography	Noninvasive, time-saving, well tolerated	Insensitive, operator dependent, reliably diagnose NAFLD only if steatosis is >33%, less accuracy in patients of obesity and coexistent renal disease	11
5.	Magnetic resonance imaging, elastography, and magnetic resonance spectroscopy	Sufficient sensitivity, specifies the stages of the disease	Limited availability, needs expertise prescription, difficult data collection, requires spectral analysis	25,32
6.	Magnetic resonance imaging proton-density fat traction	More sensitive than liver histology, early detection	Unable to assess liver inflammation, ballooning, or the resolution of NASH	33,34
7.	Computed tomography	Sensitive techniques, easier quantification of steatosis	Radiation exposure, high cost, limited accuracy	35

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

why its treatment might prove beneficial for lowering the risks of NAFLD/NASH.[23] It is further reported that extra-hepatic cancers such as lung, breast, gynecological, or urinary system cancer are linked with NAFLD prevalence in large cohorts. Yet, the mechanism is not yet deciphered.[24] That may be because obesity and diabetes are synergistic with fatty liver pathogenesis in harming the immune system and in hindering cell signaling and affecting apoptosis, the cell cycle, and proliferation.

NAFLD nomenclature is now updated and associated to link to a state of generalized metabolic disarrangement and is therefore renamed to MASLD as a more appropriate term according to its multisystem and multifactorial characteristics, based on proven data from *in vitro* and *in vivo* research that relate NAFLD to metabolic dysfunction.[25] This undefined set of adverse conditions is characterized by hepatocellular ballooning, an increase in Mallory–Denk bodies and inflammation, glycogenated nuclei, lipogranulomas, and acidophil bodies, as indicated in Takahashi's histological research.[26] Clinical manifestations include high serum triglyceride, low serum high-density lipoprotein, and high aminotransferase, gamma-glutamyl transferase,[27] and total bile acid (BA) levels.[28] However, the enzyme activities may provide a false indication for clinical conduct; thus, liver biopsy has been deemed a reliable yet invasive approach for diagnosing the stages of steatosis and fibrosis. Ultrasound can be used as a standardized method for observing the development of simple steatosis to NASH but cannot be used to investigate occurrence.[15] Noninvasive tests for fibrosis, steatosis, and steatohepatitis, such as the Fibro-Test, Steato-Test, Nash-Test, and Acti-Test, are also in extensive use.[27] However, these tests are neither sophisticated nor completely reliable. Among studies of total antioxidant capacity, products of oxidative damage including total oxidant status and malondialdehyde, and DNA/RNA oxidative damage in human serum samples, researchers reported that advanced glycation end products were a potential noninvasive biomarker of NAFLD.[29] Magnetic resonance imaging and magnetic resonance elastography have been used for noninvasive quantitative assessment of hepatic steatosis and fibrosis in NAFLD,[30,31] but more advancement in these imaging modalities is needed for future prospects. As a result, noninvasive approaches for early identification and treatment of progressive fibrosis are required. Table 1 depicts the various diagnostic tools available for detecting liver disease.[11,25–35]

Various mechanisms underlying development of NAFLD

The cellular and immunological mechanisms underlying the development of NAFLD toward NASH might include endoplasmic reticulum stress,[32] mitochondrial dysfunction,[33] lipotoxicity, and the release of pro-inflammatory cytokines responsible for liver inflammation, such as TNF-α, interleukin (IL)-6, leptin, and resistin in enhanced amounts and decreased secretion of adiponectin.[34,35] The molecular insights primarily suggest that the root causes are increase in fat supply or excessive adipose lipolysis as well as a reduction in fat export such as very low-density lipoprotein, a decrease in free fatty beta-oxidation and elevation in *de novo* lipogenesis, which leads to decreased insulin sensitivity, the most common manifestation of NAFLD.[36]

Effects of fatty acids (FAs)

The majority of fats are stored in hepatocytes as triglycerides, while the remaining fats are stored as a combination of free fatty acids (FFAs), triglycerides, diacylglycerol, cholesterol esters, free cholesterol, and phospholipids.[37] Insulin acts as an antagonist for lipolysis by inhibiting hormone-sensitive lipase, which controls the release of FFAs from adipose tissue, resulting in the accumulation of triglycerides.[38–40] Saturated FAs induce hepatocyte apoptosis by mediating activation of the JNK pathway.[41] TNF-α was pro-

posed to play an important role in insulin resistance[42] and was also the first pro-inflammatory cytokine discovered in adipose tissue. The sterol response element binding protein gene, which regulates lipogenesis, is upregulated when dietary fat, particularly saturated fat, is consumed.[43] When the amount of calories in our diet exceeds our liver's ability to export triglycerides, lipid droplets form in parenchymal hepatocytes, signaling the start of NAFLD.[44]

Role of insulin

The progression of NAFLD to NASH involves insulin resistance caused by aberrant insulin post-receptor signaling, which leads to dysregulated lipolysis and excessive FA delivery to the liver. FFA is a key player in NAFLD development via its role in inducement of TNF expression mediated by an activation of nuclear factor-kappa B.[45] The carbohydrate response element binding protein is activated by fructose, independent of insulin, and promotes hepatic steatosis. There is a more significant release of blood glucose by the liver as a result of increased carbohydrate consumption and decreased glucose uptake by insulin-resistant muscle and adipose tissue because a high-carbohydrate diet activates several lipogenic enzymes like acetyl CoA carboxylase and FA synthase, resulting in hyperglycemia and other health-threatening symptoms.[40]

Association between mitochondrial dysfunction and NAFLD

Mitochondrial dysfunction is a central abnormality underlying the progression from simple steatosis to steatohepatitis in NAFLD.[35] NAFLD is characterized by a metabolic infestation that often includes large, swollen, multilamellar mitochondria, often without cristae, and paracrystalline inclusion bodies.[36,46] FAs are β-oxidized in mitochondria or esterified to be excreted as very low-density lipoprotein or stored as lipid droplets.[47] When mitochondrial activity is disrupted, ATP concentrations are reduced, which causes FA metabolism to be downregulated, causing NAFLD patients to progress from steatosis to steatohepatitis.[33,48] Cell proliferation induced in NAFLD and NASH in obesity-associated HCC is promoted by elevated IL6 and TNF-β.[32] Along with hepatic stellate cells, also known as multifunctional cells of the liver, which are most closely related to immune cells, hepatic cells also play a significant role in the production of fibrogenic stimuli and reactive oxygen species,[49] which might signify the induction of mitochondria-mediated apoptosis.[50] By creating myofibroblast-like cells in the liver, reactive oxygen species' damage of the liver gradually leads to liver fibrosis. Adipokines and myokines regulate the activation and fibrosis of hepatic stellate cells. Iron accumulation catalyzes oxidative stress, which leads to fibrosis and eventually NASH, in a process known as haemochromatosis.[51] Along with anatomical changes in the liver, NAFLD patients show narrowed tight junctions and irregularly arranged microvilli, which depicts a change in the alignment of intact tight junctions and extensive microvilli in their duodenum. The structural backbone of the small intestine, occludin proteins are present in far larger quantities in healthy intestines than in NAFLD-affected counterparts.[52]

Link between BAs and NAFLD

BAs have an essential role in cholesterol homeostasis, lipid metabolism, and absorption of fat and fat-soluble vitamins. BA homeostasis disruption is another important prognostic factor of NAFLD.[53] The progression of NAFLD to HCC can be accelerated by intestinal BA deconjugation and hepatocyte exposure to more toxic BAs. In studies, increased secondary BAs, taurine, and glycine-conjugated BAs have been linked to steatohepatitis.[54] Changes in the pathway associated with the farnesoid X receptor, which plays a role in many important systems responsible for BA regulation, glucose regulation, and lipid regulation can lead to imbalances in energy balance, exacerbating inflammation and fibrosis. Cholic acid, a secondary BA, has been shown in studies to protect mice from hepatic lipogenesis by inhibiting sterol regulatory element-binding protein 1 and its target genes.[55] In human gallstone patients, chenodeoxycholic acid administration lowers the production of elevated hepatic very low-density lipoprotein and plasma triglyceride levels. Obeticholic acid (6α-ethyl-chenodeoxycholic acid), a semisynthetic form of chenodeoxycholic acid, has been shown to be very protective in obese rats in Phase-2a and Phase-2b trials. It helps reduce the risk of liver steatosis as well as fibrosis.[56,57] Intrahepatic accumulation of tauro-beta-muricholic acid, a farnesoid X receptor nuclear receptor antagonist which is involved in the regulation of BA, lipid, and glucose metabolism, showed contribution in decreasing risk to NAFLD in antibiotic and temporal treated mice by inhibiting farnesoid X receptor signaling in the intestine.[58,59] Significant decreases in serum palmitoyl-, stearoyl-, and oleoyl-lysophosphatidylcholine were detected in mice with NASH.[60]

Gut-liver axis

The gut-liver axis is the bidirectional link between the gut, its bacteria, and the liver. The gut barrier is an integral secure system with an army of tight junctional complexes. These goblet cells form the mucus layer, Paneth cells that regulate antimicrobial defense, and a network of innate and adaptive immune cells.[61] It maintains homeostasis by interacting with nuclear receptors to control metabolic activities and forming a feedback loop for BAs and antibodies via the portal circulation between the liver and the gut.[62] The gut mucosal barrier comprising intestinal epithelial cells segregating gut microbiota and host immune cells maintains gut homeostasis. The balance and smooth maintenance are due to the integrated action of the protective layer of defensins on the intraluminal surface, tight junction proteins, and gut immune cells. If the mucosal membrane is disrupted, the resulting altered intestinal permeability induces local inflammation. Bacterial products, if translocated to various cell types such as Kupfer cells, will initiate a fibrotic response resulting in harmful effects in hepatocytes and to host immunity. It also facilitates pathogen-associated molecular patterns, lipopolysaccharides, and microbiome-derived metabolites to enter the liver through the portal circulation, triggering a pro-inflammatory cascade that exacerbates hepatic inflammation.[63] IL22 is reported to regulate gut epithelial cells and, thereby, related immune functions.[64] As a result, lipopolysaccharide reduction and tight junction restoration may be effective as a treatment for reducing NAFLD and its development.[65] To gain insight into explaining the progression of NAFLD, alterations of gut bacteria abundance that are involved in NAFLD pathogenesis.

Gut microbiota

The human gut microbiome contains 10–100 trillion microorganisms, mostly bacteria, which outweigh our human cells by a factor of 10.[66] Alpha-diversity (among samples) and beta-diversity (between samples) are two types of microbiome diversity (comparison of samples from a given population).[67] The microbiome's bacterial component has received the most attention so far. *Bacteroidetes* and *Firmicutes* are the two most prevalent bacterial groups, and *Euryarchaeota* is the most common of the *Archaea*.[68] Nonbacterial species, such as resident archaeal, fungal, and viral populations,

are predicted to have roles in the microbiome, especially in their interactions with other microbiome populations. Gut colonization begins at birth, and a complex combination of dietary habits, ethnicity, and genetic variables influences microbiota composition. In humans, the gut microbiota can define the host condition, whether it is in homeostasis or illness. The gut microbiota interacts with the immune system and actively absorbs food substances into the portal and systemic circulation. Gut microbiota may affect NAFLD by improving energy production, maintaining gut permeability, regulating inflammation, modifying choline and BA metabolism, and enhancing endogenous ethanol synthesis. As a result, it may influence the host, even if it is not present, by modulating immune cells and the production of metabolites.[69] Many studies have evaluated various samples, such as fecal matter and animal tissues, to explore the roles of different bacteria in the progression of NAFLD/NASH.

The clear relationship between microorganisms and the human host makes the human a superorganism.[70] This diversity that establishes a life-long, bidirectional, symbiotic association between the gut and microorganisms is called the intestinal microbiota and is favored by the food that passes through the tract, affecting the integrity of the digestive tract and other linked systems.[71] These commensal bacteria help the host metabolize the dietary fibers that cannot be processed due to a lack of enzymes.[72] *Veillonellaceae* and *Rhinococcacea* were selected as the most representative and significant fibrosis-related bacterial taxa as shown in Table 2.[9,73-91]

Gut metabolites: keystone component

Fermentation of dietary fiber and choline yields metabolites such as short-chain fatty acids (SCFAs), including acetic acid, propionate, butyrate, and succinate, hydrogen sulfide, and other proteolytic metabolites. SCFAs mediate the regulatory effect on the gut microbiota and host inflammatory responses, such as modulating adiponectin and resistin transcriptional expression by modifying DNA methylation in obese mice.[92] Butyrate, the most potent anti-inflammatory mediator, has been shown to be effective in reducing local inflammation in the intestine and preventing the progression of inflammatory responses to the systemic circulation.[93] SCFAs enter the liver directly through the portal vein, where they help to reduce inflammation and steatosis. Though SCFAs regulate the health of visceral adipose tissue and FA, lipid, and glucose metabolism, combining their advantages while preserving intestinal homeostasis is complex, and the overall effect of SCFAs on NAFLD etiology is yet unknown.[92]

Colonic bacteria also ferment nondigestible carbohydrates to SCFAs. SCFAs have been proposed to contribute to obesity and liver steatosis as they provide approximately 10% of the daily caloric consumption and may enhance nutrient absorption by promoting the expression of glucagon-like peptides.[94] However, trimethylamine-N-oxide is only derived from gut microbial metabolism.[73] Trimethylamine-N-oxide, a gut microbe-generated metabolite produced by the flavin monooxygenase 3 produced in the liver, is detrimental to liver health. Cystathionine β-synthase/cystathionine γ-lyase regulates trans-sulphuration and desulfuration reactions in the liver, kidney, small intestine, pancreas, and brain.[74] The trans-sulphuration pathway is linked to the methionine cycle through homocysteine, a nonprotein sulfur-containing amino acid. Homocysteine is irreversibly metabolized via the trans-sulphuration pathway to support endogenous cysteine synthesis. Cystathionine β-synthase and cystathionine γ-lyase catalyze alternative desulphuration reactions in addition to the trans-sulphuration pathway.[75] H_2S is synthesized endogenously by these alternative reactions. Homocysteine and cysteine may catalyze these alternative reactions.[76,77] It has been shown that cystathionine β-synthase and cystathionine γ-lyase are highly expressed in hepatocytes, leading to their high expression in the parenchyma tissue.[78] In patients with NAFLD and its associated comorbidities, there are changes in circulating homocysteine and hydrogen sulfide levels. Homocysteine has been proposed as a risk marker for NAFLD.[79]

Gut microbiota dysbiosis

In dysbiosis, the normal flora in the gut microbiome is disturbed, resulting in increased microbial translocation and the development of alcoholic liver disease. This affects the abundance of species such as *Streptococcus*, *Shuttleworthia*, and *Rothia*.[80] Small metabolites are produced by healthy gut microbiota, including SCFAs, which provide energy to colonic epithelia. When the microbiota starts to produce toxic metabolites that interfere with the gut-liver axis and cause metabolic dysfunction, dysbiosis is confirmed, and eventually, chronic disease development occurs. In patients with NAFLD, decreased abundance of *Faecalibacterium prausnitzii* and increased abundance of *Proteobacteria*, *Escherichia coli,* and *Enterobacteriaceae* have been reported.[81] NASH patients had decreased fecal *Bacteroidetes* and increased *Clostridium coccoides*.[82] At the same time, chronic alcohol consumption can cause leaky gut and reduced gut bacterial diversity, which might be the leading cause of alcoholic liver disease.[83]

NAFLD patients had fewer *Bacteroidetes*, *Ruminococcaceae*, *Faecalibacterium prausnitzii*, and more *Prevotella*, *Porphyromas*, *Lactobacillus*, *Escherichia*, and *Streptococcus* bacteria than healthy subjects.[53,84] However, increased levels of *Veillonella*, *Megasphaera*, *Dialister*, *Atopobium*, and *Prevotella* have been observed in cirrhotic patients. Several mechanisms may contribute to NAFLD pathogenesis as a result of the influence of the gut microbiota influence, including (1) increased production and absorption of gut SCFAs, (2) altered dietary choline metabolism by the microbiota, (3) altered BA pools by the microbiota, (4) increased delivery of microbiota-derived ethanol to the liver, (5) gut permeability alterations and endotoxin release, and (6) interaction between specific diet and microbiota.[47] Chronic kidney disease may aggravate NAFLD and associated metabolic disturbances through multiple mechanisms, including altered intestinal barrier function and microbiome composition.[85] 3-phenylpropionate, a metabolite generated by anaerobic bacteria, plays a crucial part in the process.[86,87] NASH development is linked to gut microbiome-derived products of branched-chain and aromatic amino acid metabolism, such as phenylacetic acid and 3-(4-hydroxyphenyl) lactate, which are linked to insulin resistance.

Pathogen-associated molecular patterns develop when the gut microbiota is out of equilibrium (dysbiosis). Dysbiosis is also linked to increased exposure to bacterial compounds found in the intestine, such as lipopolysaccharides. Hepatic cells have a variety of cellular receptors that react to molecular pattern molecules (e.g., damage-associated molecular patterns and pathogen-associated molecular patterns), which attract neutrophils, macrophages, and other innate immune system components. Pathogen-associated molecular patterns, elevated lipopolysaccharide levels, and damage-associated molecular patterns activate Kupfer cells, which detect liver tissue injury. When Kupfer cells are activated, they release pro-inflammatory cytokines and chemotactic factors, such as the chemokine C-C motif ligand. Consequently, hepatic stellate cells are activated, which leads to the modulation of key extracellular matrix components and functional interactions with a

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Table 2. Alterations of gut bacteria abundance involved in NAFLD pathogenesis

S. no.	Genus	Phylum	Role in the progression of NAFLD/NASH	Type of sample/study	Altered abundance in NAFLD population compared with control	Reference
1.	*Blautia*	Firmicutes	Dysregulation of mucosal immunity, promotes lymphocyte activation, increases intestinal permeability	Fecal	Increase	73–75
2.	*Roseburia*	Firmicutes	Positively associated with tauro ursodeoxycholic acid and tauro chenodeoxycholic acid, reduces gut inflammation, improves intestinal barrier function, decreases intestinal fat transport	Fecal	Increase	76,77
3.	*Lactobacillus*	Firmicutes	Reduces IL17 and other angiogenesis factors, decreases pro-inflammatory (chemokine C-C motif ligand 2, CCR2, TNF) and lipopolysaccharide, increases intestinal barrier function permeability	Fecal	Increase	9,78,79
4.	*Clostridium*	Firmicutes	Modifies BAs from primary to secondary BAs	Animal models	Increase	80
5.	*Ruminococcus*	Firmicutes	Increases fibrosis	Fecal, biopsy	Increase	73
6.	*Flavonifractor*	Firmicutes	Attenuates the increase in TNF-α transcription	Animal model	Decrease	81
7.	*Coprococcus*	Firmicutes	Butyrate-producing bacteria	Fecal	Decrease	9
8.	*Prevotella*	Bacteroidetes	Reduces the Th17 polarization, and promotes differentiation of anti-inflammatory Treg/Tr1 cells in the gut	Fecal	Decrease	82
9.	*Escherichia*	Proteobacteria	Increases gut permeability	Stool study, animal model, biopsy	Decrease	83
10.	*Bifidobacterium*	Actinobacteria	Reduces lipopolysaccharide/TLR-4 axis, affects humoral and cellular inflammatory markers	Fecal	Decrease	9,84
11.	*Oscillospira*	Firmicutes	Decrease is coupled to 2-butanone upregulation	Fecal	Decrease	85
12.	*Akkermansia*		Promotes the growth of other bacteria with anti-inflammatory properties, reverses fat gain, disruptions in serum lipopolysaccharide levels and gut barrier function, and insulin resistance, increases endocannabinoids and gut peptides	Biopsy mucus layer	Decrease	86,87
13.	*Helicobacter*	Proteobacteria	Associated with tauro ursodeoxycholic acid, glycocholic acid, and tauro chenodeoxycholic acid	Ultrasonography	Increase	88
14.	*Oscillibacter*	Firmicutes	Reduces Th17 polarization, and promotes the differentiation of anti-inflammatory Treg/Tr1 cells in the gut	Fecal	Decrease	82
15.	*Erysipelotrich*	Firmicutes	Bacterial predictor of susceptibility to choline deficiency-induced fatty liver disease	Fecal, animal study	Increase	89
16.	*Klebsiella*	Proteobacteria	Predictor of susceptibility to choline deficiency-induced fatty liver disease	Fecal	Increase	90
17.	*Desulfovibrio*	Proteobacteria	Produces both H_2S and acetic acid, modulates the hepatic gene expression pattern of lipids metabolism, suppresses hepatic fatty acid synthase and CD36 protein expression	Fecal	Increase	91
18.	*Mucispirillum*	Deferribacteres	Predicts susceptibility to choline deficiency-induced fatty liver disease	Fecal	Increase	88

BA, bile acid; IL, interleukin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TLR, Toll-like receptor.

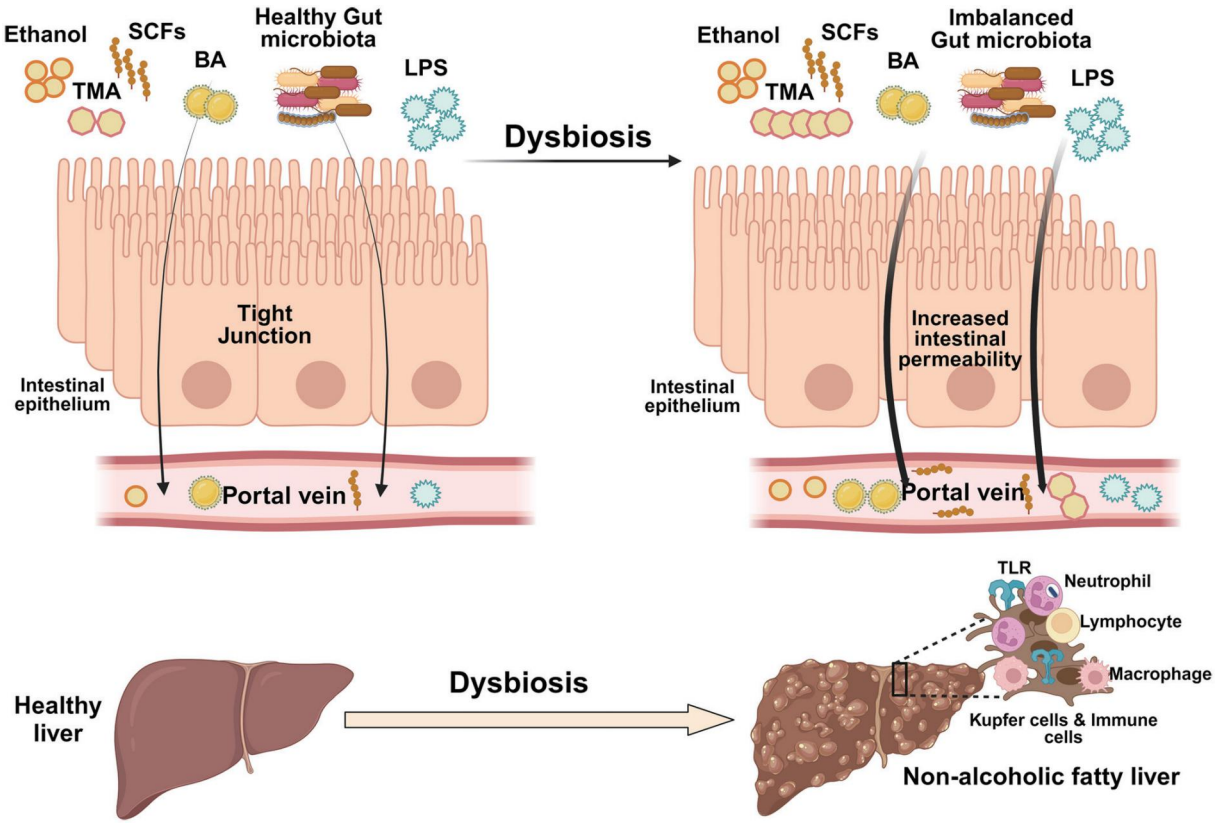

Fig. 2. Schematic representation of how the gut microbiota contributes to the development of NAFLD. In the left panel, the gut-liver axis components are functioning normally. NAFLD is depicted in the right panel. The dysbiotic microbiome, together with the changed intestinal barrier due to the malfunction of the tight junctions, facilitates the translocation of some bacterial products into the portal vein. These bacterial products interact with TLRs on the surface of the hepatic cells, which leads to inflammation and NAFLD development. NAFLD, nonalcoholic fatty liver disease; TLR, Toll-like receptor.

microRNA implicated in NAFLD fibrosis as shown in Figure 2.[88] We have highlighted various metabolites of the gut microbiota and their roles in NAFLD progression in Table 3.[88–91,95-114]

Therapeutic interventions

Gaining insights into the role of gut microbiota, microbe-associated molecular patterns, and metabolites produced by microbiota in the development of NAFLD may pave the way for innovative diagnostic and therapeutic strategies. NAFLD encompasses a diverse range of disorders, each with distinct subtypes resulting from different combinations of the aforementioned factors. Thus, it is crucial to incorporate this knowledge into both the diagnosis and treatment of NAFLD.

Currently, the diagnosis and monitoring of liver disease require a liver biopsy. Therefore, it is crucial to find reliable noninvasive methods to assess NAFLD. Recent research on gut microbiota has found that certain bacterial species and metabolites were useful as diagnostic and prognostic indicators. Loomba *et al*. have identified a panel of 37 bacterial strains from the gut microbiota that accurately diagnose advanced fibrosis in NAFLD patients. Additionally, several metabolites derived from the microbiota show promise as indicators of NAFLD. Phenylacetic acid, succinate, and 3-(4-hydroxyphenyl) lactate are among the most promising. NAFLD patients often have a decreased microbial gene richness, which affects the metabolism of aromatic and branched-chain amino acids. For example, 3-(4-hydroxyphenyl) lactate, which is associated with liver fibrosis, is a byproduct of aromatic amino acid metabolism. The level of phenylacetic acid in the blood is correlated with the severity of liver steatosis. Succinate, produced by bacteria associated with NAFLD like *Bacteroidaceae* and *Prevotella*, is elevated in feces, serum, and liver samples of NAFLD patients.[31]

On numerous levels, a comprehensive understanding of gut microbiota might be employed for therapeutic purposes, as illustrated in Figure 3. The utility of precision medicine encompassing tailored probiotics, prebiotics, synbiotics, and FMT to target dysbiosis of the gut microbiota in individual patients provides a new avenue for microbial-derived therapeutics. Another exciting prospect is the modulation of the production of beneficial metabolites and blocking the synthesis of harmful ones. FMT is emerging as a potential treatment for various gastrointestinal disorders and offers a way to restore a healthy gut microbiota composition and function in patients. FMT is a medical procedure where fecal matter from a healthy donor is transplanted into a recipient's gut to restore a healthy gut microbiome. It can help restore a balanced and diverse gut microbiota in NAFLD patients, potentially mitigating dysbiosis by the introduction of *Lactobacillus*, *Bifidobacterium*, and *Pediococcus* species.[115] FMT has been shown to enhance gut barrier function, reducing the translocation of harmful bacterial products like lipopolysaccharides into the liver and reducing inflammation.[116] FMT may influence BA composition and metabolism in the gut, which can impact liver health, inflammation, and

Table 3. Role of various metabolites in NAFLD progression

Metabolites		Role	References
Short-chain fatty acids			
1.	Propionate	Activates AMP-activated protein kinase, increases expression of the fatty acid oxidation gene, suppresses macrophage pro-inflammatory activation, inhibits isoproterenol and adenosine deaminase-stimulated lipolysis	89,90
2.	Butyrate	Activates AMPK activation, increases expression of the fatty acid oxidation gene, suppresses macrophage pro-inflammatory activation, upregulates glucagon-like peptide-1 receptor expression to improve NAFLD	94,95
3.	Acetate	Regulates hepatic lipid metabolism and insulin sensitivity via FFA receptor 2 in hepatocytes	96
Indole derivatives			
4.	Indole-3-acetic acid (IAA)	Improves lipid metabolism, insulin resistance, and inflammatory and oxidative stress	97
5.	Indole	Reduces the lipopolysaccharide-induced upregulation of -pro-inflammatory mediators	98
6.	Indican: indoxyl-3- sulfate	Reduces gut permeability in high fat diet-fed mice	99
7.	Indigo	Development of obesity, white adipose tissue, inflammation, and insulin resistance	100
8.	IPA: indole-3-propionate	Increases expression of the intestinal mucosa and tight junction proteins	101,102
9.	Ethanol	Oxidative stress and inflammation, increases gut permeability and levels of lipopolysaccharide, decreases the gut barrier	103
10.	2-butanone	Regulates insulin sensitivity	85
11.	Ceramides	Induces sterol regulatory element-binding protein regulator, increases TAG (Triacyl glycerol) synthesis and lipid droplet storage	104
Bile acids			
12.	Primary bile acids chenodeoxycholic acid, cholic acid, deoxycholic and lithocholic acid	Increases insulin sensitivity, inhibits gluconeogenesis and lipogenesis, anti-inflammatory and antifibrotic properties, regulates the gut microbiota, enhances fatty acid translocation and uptake, promotes CD36 translocation to the plasma membrane	105,106
13.	Choline	Regulates mitochondrial bioenergetics and fatty acid beta-oxidation, phosphorylcholine synthesis, loss of apoptotic mechanisms, reactive oxygen species generation, endoplasmic reticulum stress	107–109
14.	Trimethylamine N-oxide	Suppresses the BA-mediated hepatic farnesoid C receptor signaling, increases inflammatory cytokine C-C motif chemokine ligand 2 and insulin resistance	110
15.	Homocysteine	Increases hepatic oxidative stress, induces expression of inflammatory cytokines and profibrogenic factors, activates the aryl hydrocarbon receptor/CD36 pathway	111–113
16.	Serotonin	Inhibits energy expenditure of brown adipose tissue, blocks mitochondrial uncoupling protein	114

FFA, free fatty acid; NAFLD, nonalcoholic fatty liver disease.

fat accumulation in hepatocytes.[117] FMT from a healthy donor may increase the production of beneficial SCFAs in the recipient's gut. SCFAs have anti-inflammatory properties and can improve insulin sensitivity. FMT can facilitate communication between the host and the gut microbiota, leading to positive changes in metabolic pathways. Clinical trials exploring the efficacy of FMT in NAFLD patients are needed to validate its potential therapeutic role.[115] The identification of specific gut microbial markers associated with NAFLD progression could lead to the development of noninvasive diagnostic tools. These tools may rely on fecal-, blood-, or breath-based biomarkers that enable early detection and monitoring of NAFLD without the need of invasive liver biopsies. Further research on the interaction between gut microbiota and metabolites could shed light on the underlying mechanisms that drive NAFLD progression. Moreover, single beneficial strains or groups of beneficial strains (probiotics) can be introduced into the gut microbiota to restore lost functionality, while harmful or undesirable strains can be removed with antimycotics, antibiotics, or bacteriophages. Finally, microbial pathways might be targeted to minimize or prevent the formation of harmful metabolites while

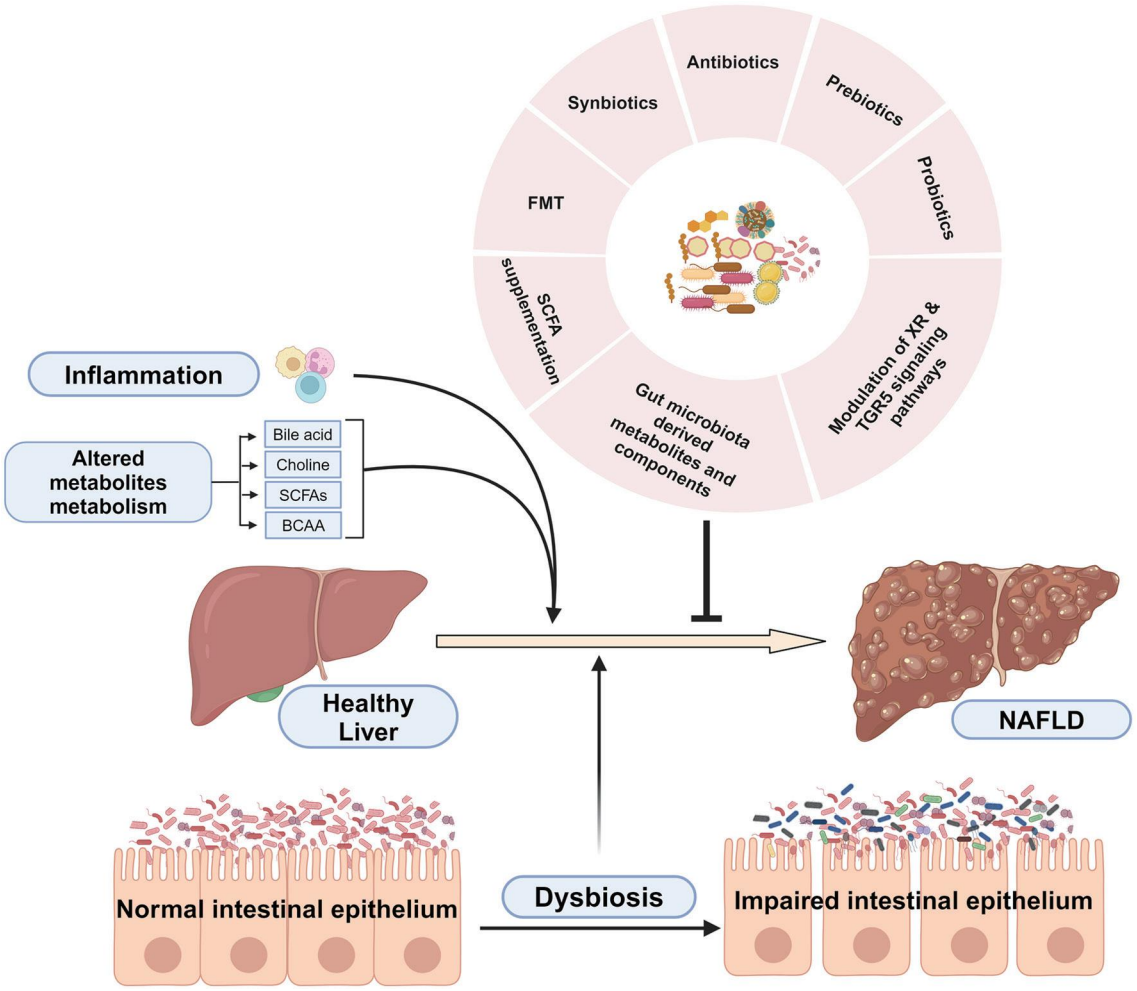

Fig. 3. Gut microbiome-centered therapeutic strategies against NAFLD. Dysbiosis promotes the process of NAFLD via multiple pathways. Gut microbiome-targeted therapeutic strategies include probiotic, prebiotic, synbiotic, and FMT that can reverse dysbiosis and mitigate the process of NAFLD. BCAA, branched-chain amino acid; FMT, fecal microbiota transplantation; NAFLD, nonalcoholic fatty liver disease; SCFA, short-chain fatty acid.

enhancing the production of beneficial ones.

FMT can reconstruct whole microbial ecosystems. Moreover, single beneficial strains or groups of beneficial strains (probiotics) can be introduced into the gut microbiota to restore lost functionality, while harmful or undesirable strains can be removed with antimycotics, antibiotics, or bacteriophages. Finally, microbial metabolic pathways might be targeted to minimize or prevent the formation of harmful metabolites while enhancing the production of beneficial ones.

Data on the efficacy of FMT in the treatment of NAFLD are scarce. FMT has been shown to be effective in treating cirrhotic individuals with hepatic encephalopathy.[118] and alcoholic hepatitis.[119] NAFLD has also been treated using prebiotics, probiotics, and synbiotics. Prebiotics are indigestible food components such as that selectively increase the development and activity of helpful gut bacteria.[120] This concept was eventually broadened to encompass fiber-based probiotics and other substrates that the host bacteria use selectively and provide health advantages. Not only indigestible carbohydrates like galacto-oligosaccharides, fructo-oligosaccharides, and trans-galacto-oligosaccharides but also other substances like polyphenols and polyunsaturated FAs that can modulate the gut microbiota are included in the new definition.[121] Probiotics are living, nonpathogenic bacteria that, when ingested, can improve the host's health. *Lactobacilli, Streptococci,* and *Bifidobacteria are* the most widely used probiotics in clinical studies.[122]

Synbiotics are a combination of probiotics and prebiotics that positively impact the host. According to animal and human trials data, synbiotics may help alleviate NAFLD-related dysbiosis and liver disease. In NAFLD patients, for example, a recent meta-analysis discovered that taking synbiotics/probiotics was linked to improvement of liver-specific indicators of hepatic stiffness, inflammation, and steatosis.[123] The therapeutic strategy of using a bacteriophage to target a specific strain, especially cytolytic *E. faecalis*, was efficacious in treating ethanol-induced liver injury in humanized mice.

Emerging therapeutic methods can change gut microbiota composition to promote the synthesis of beneficial metabolites and decrease the production of toxic metabolites. For example, 3, 3-dimethyl-1-butanol can prevent microbial trimethylamine lyases from converting dietary choline to trimethylamine. Trimethylamine is a well-known toxic metabolite that can induce inflammation in

gut, and prolonged inflammation can induce IBD and colorectal cancer.[124] Other studies have determined that increased levels of beneficial metabolites such as SCFA can improve liver steatosis. Another drug, tributyrin, which is a butyrate prodrug, is reported to protect mice from insulin resistance, obesity, and hepatic steatosis, whereas acetate and propionate supplementation prevented diet-induced weight gain, insulin resistance, and hepatic steatosis.[125] XR and TGR5 signaling pathways that modulate BA metabolism are also interesting therapeutic targets, such as obeticholic acid is shown to improve fibrosis, portal hypertension, and hepatic steatosis in animal models and improved histological features in NASH patients. In addition, fibroblast growth factor has been established as a therapeutic agent for metabolic diseases because of its role in lipid and carbohydrate metabolism. Clinical trials of fibroblast growth factor-based therapies have shown its efficacy in patients with NAFLD. These treatments contain fibroblast growth factor analogues that can reduce liver inflammation and fibrosis.[126] NGM282, counterpart of fibroblast growth factor 19 that modulates BA synthesis and glucose balance, has been identified as having the potential to reduce hepatic steatosis in NASH patients.[127] Farnesoid X receptor agonist, obeticholic acid, is a first-in-class approved agonist for noncirrhotic primary biliary cholangitis treatment; however, second-generation farnesoid X receptor agonists are in development to overcome the side effects of the first-in-class drug. For example, MET409 is a second-generation farnesoid X receptor agonist which has shown better efficacy and less side effects such as pruritus and increase in low-density lipoprotein than obeticholic acid.[128] Tropifexor and cilofexor are farnesoid X receptor agonists possessing distinct structures from obeticholic acid and MET409. A study reported that administration of 30 mg cilofexor for 12 weeks in NASH and fibrosis patients decreased liver stiffness and hepatic fat and improved liver biochemistry.[129] Additionally, under development for NAFLD treatment are specific agonists for the thyroid hormone receptor-beta, namely resmetirom and VK2809. Resmetirom is the pioneer oral, liver-targeted thyroid hormone receptor-beta 1-selective agonist. In a 36-week phase II randomized clinical study, resmetirom achieved NASH resolution in a subgroup of patients with control biopsies. Simultaneously, improvements were recorded in liver steatosis, liver stiffness, lipid serum profile, and fibrosis biomarkers like Pro-C3 and hepatic enzymes. This was coupled with a marked reduction in NAFLD activity.[130] VK2809, an alternative thyroid hormone receptor-beta agonist, undergoes hepatic metabolism through the action of CYP450 enzymes. It had a highly favorable tolerability profile, and a substantial decrease in hepatic fat was detected by magnetic resonance imaging following a 12 weeks of treatment.[131]

Conclusions

A growing body of evidence indicates that the microbiome unifies and explains the divergent findings in liver disease-related investigations. The broad interplay between the gut microbiota via specialized chemicals such as trimethylamine, acetaldehyde, and lipopolysaccharide, and the host immune system via Kupffer-cell-mediated liver inflammation is now widely accepted as playing a role in liver damage. However, we still do not completely understand the interactions between the microbiota and the liver. Many critical molecular processes in the etiology of liver disease have been elucidated primarily in animal models, notably rodents. Including the microbiome in these models will give researchers a more comprehensive picture of the liver ecosystem. Because technical heterogeneity can hide underlying biological signals in microbiome research, there is a need for uniformity in technological platforms and standardized methods so that results from diverse laboratories and model species can be replicated and confirmed. It is also crucial to find an animal model that closely resembles human illness in all physiological and metabolic aspects because studies have constantly been finding evidence of an association between NAFLD risk and extra-hepatic cancer development in both sexes. Furthermore, this review highlights the importance of placing more attention on developing biomarkers based on microbiome and metabolic profile that can diagnose the stage of NAFLD, assess the risk, and help in the selection of a specific treatment approach.

We are gradually moving away from observational studies in people as research lays the groundwork for microbiome-based treatment modalities like FMT and probiotic therapies. However, effectively translating and applying results from animal models to humans demands well-designed, large-scale clinical studies encompassing a wide range of illness etiologies and health status. We underline the necessity of concentrating on microbiome-aware initiatives to efficiently confront the socio-economic burden of this range of liver disorders as the microbiota functions in hepatic disease development, prognosis, and therapy become better understood.

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Conflict of interest

The authors declare that there are no competing interests.

Author contributions

Drafted the manuscript (AJ, AS), contributed to critical discussions (RJ, NB), and conceptualized the study and managed the manuscript process (RD, SK).

References

[1] Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55(7):434–438. PMID:7382552.
[2] Lu R, Liu Y, Hong T. Epidemiological characteristics and management of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis in China: A narrative review. Diabetes Obes Metab 2023;25(Suppl 1):13–26. doi:10.1111/dom.15014, PMID:36775938.
[3] Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, *et al*. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr Disord 2022;22(1):63. doi:10.1186/s12902-022-00980-1, PMID:35287643.
[4] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64(1):73–84. doi:10.1002/hep.28431, PMID:26707365.
[5] Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugi-

anesi E, *et al*. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Hepatology 2019;69(6):2672–2682. doi:10.1002/hep.30251, PMID:30179269.
[6] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, *et al*. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41(6):1313–1321. doi:10.1002/hep.20701, PMID:15915461.
[7] Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, *et al*. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. J Hepatol 2018;69(4):896–904. doi:10.1016/j.jhep.2018.05.036, PMID:29886156.
[8] Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 2018;67(1):123–133. doi:10.1002/hep.29466, PMID:28802062.
[9] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, *et al*. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol 2020;73(1):202–209. doi:10.1016/j.jhep.2020.03.039, PMID:32278004.
[10] Gofton C, Upendran Y, Zheng MH, George J. MAFLD: How is it different from NAFLD? Clin Mol Hepatol 2023;29(Suppl):S17–S31. doi:10.3350/cmh.2022.0367, PMID:36443926.
[11] Chacko KR, Reinus J. Extrahepatic Complications of Nonalcoholic Fatty Liver Disease. Clin Liver Dis 2016;20(2):387–401. doi:10.1016/j.cld.2015.10.004, PMID:27063276.
[12] Yu Y, Cai J, She Z, Li H. Insights into the Epidemiology, Pathogenesis, and Therapeutics of Nonalcoholic Fatty Liver Diseases. Adv Sci (Weinh) 2019;6(4):1801585. doi:10.1002/advs.201801585, PMID:30828530.
[13] Ponziani FR, Bhoori S, Castelli C, Putignani L, Rivoltini L, Del Chierico F, *et al*. Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease. Hepatology 2019;69(1):107–120. doi:10.1002/hep.30036, PMID:29665135.
[14] Arab JP, Karpen SJ, Dawson PA, Arrese M, Trauner M. Bile acids and nonalcoholic fatty liver disease: Molecular insights and therapeutic perspectives. Hepatology 2017;65(1):350–362. doi:10.1002/hep.28709, PMID:27358174.
[15] Ballestri S, Romagnoli D, Nascimbeni F, Francica G, Lonardo A. Role of ultrasound in the diagnosis and treatment of nonalcoholic fatty liver disease and its complications. Expert Rev Gastroenterol Hepatol 2015;9(5):603–627.doi:10.1586/17474124.2015.1007955,PMID:25694178.
[16] Ge X, Zheng L, Wang M, Du Y, Jiang J. Prevalence trends in non-alcoholic fatty liver disease at the global, regional and national levels, 1990-2017: a population-based observational study. BMJ Open 2020;10(8):e036663. doi:10.1136/bmjopen-2019-036663, PMID:32747349.
[17] Mazzolini G, Sowa JP, Atorrasagasti C, Kücükoglu Ö, Syn WK, Canbay A. Significance of Simple Steatosis: An Update on the Clinical and Molecular Evidence. Cells 2020;9(11):2458. doi:10.3390/cells9112458, PMID:33187255.
[18] Berson A, De Beco V, Lettéron P, Robin MA, Moreau C, El Kahwaji J, *et al*. Steatohepatitis-inducing drugs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes. Gastroenterology 1998;114(4):764–774. doi:10.1016/s0016-5085(98)70590-6, PMID:9516397.
[19] Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114(4):842–845. doi:10.1016/s0016-5085(98)70599-2, PMID:9547102.
[20] Manne V, Handa P, Kowdley KV. Pathophysiology of Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis. Clin Liver Dis 2018;22(1):23–37. doi:10.1016/j.cld.2017.08.007, PMID:29128059.
[21] Dumas ME, Kinross J, Nicholson JK. Metabolic phenotyping and systems biology approaches to understanding metabolic syndrome and fatty liver disease. Gastroenterology 2014;146(1):46–62. doi:10.1053/j.gastro.2013.11.001, PMID:24211299.
[22] Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J Gastroenterol 2017;23(47):8263–8276. doi:10.3748/wjg.v23.i47.8263, PMID:29307986.
[23] Targher G, Corey KE, Byrne CD, Roden M. The complex link between NAFLD and type 2 diabetes mellitus - mechanisms and treatments. Nat Rev Gastroenterol Hepatol 2021;18(9):599–612. doi:10.1038/s41575-021-00448-y, PMID:33972770.
[24] Mantovani A, Petracca G, Beatrice G, Csermely A, Tilg H, Byrne CD, *et al*. Non-alcoholic fatty liver disease and increased risk of incident extrahepatic cancers: a meta-analysis of observational cohort studies. Gut 2022;71(4):778–788. doi:10.1136/gutjnl-2021-324191, PMID:33685968.
[25] Xian YX, Weng JP, Xu F. MAFLD vs. NAFLD: shared features and potential changes in epidemiology, pathophysiology, diagnosis, and pharmacotherapy. Chin Med J (Engl) 2020;134(1):8–19. doi:10.1097/CM9.0000000000001263, PMID:33323806.
[26] Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol 2014;20(42):15539–15548. doi:10.3748/wjg.v20.i42.15539, PMID:25400438.
[27] Lassailly G, Caiazzo R, Hollebecque A, Buob D, Leteurtre E, Arnalsteen L, *et al*. Validation of noninvasive biomarkers (FibroTest, SteatoTest, and NashTest) for prediction of liver injury in patients with morbid obesity. Eur J Gastroenterol Hepatol 2011;23(6):499–506. doi:10.1097/MEG.0b013e3283464111, PMID:21499110.
[28] Chen J, Deng W, Wang J, Shao Y, Ou M, Ding M. Primary bile acids as potential biomarkers for the clinical grading of intrahepatic cholestasis of pregnancy. Int J Gynaecol Obstet 2013;122(1):5–8. doi:10.1016/j.ijgo.2013.02.015, PMID:23562588.
[29] Świderska M, Maciejczyk M, Zalewska A, Pogorzelska J, Flisiak R, Chabowski A. Oxidative stress biomarkers in the serum and plasma of patients with non-alcoholic fatty liver disease (NAFLD). Can plasma AGE be a marker of NAFLD? Oxidative stress biomarkers in NAFLD patients. Free Radic Res 2019;53(8):841–850. doi:10.1080/10715762.2019.1635691, PMID:31234658.
[30] Dulai PS, Sirlin CB, Loomba R. MRI and MRE for non-invasive quantitative assessment of hepatic steatosis and fibrosis in NAFLD and NASH: Clinical trials to clinical practice. J Hepatol 2016;65(5):1006–1016. doi:10.1016/j.jhep.2016.06.005, PMID:27312947.
[31] Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, *et al*. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. Cell Metab 2017;25(5):1054–1062.e5. doi:10.1016/j.cmet.2017.04.001, PMID:28467925.
[32] Nakagawa H, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, Ogata H, *et al*. ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. Cancer Cell 2014;26(3):331–343. doi:10.1016/j.ccr.2014.07.001, PMID:25132496.
[33] Caldwell SH, Chang CY, Nakamoto RK, Krugner-Higby L. Mitochondria in nonalcoholic fatty liver disease. Clin Liver Dis 2004;8(3):595–617. doi:10.1016/j.cld.2004.04.009, PMID:15331066.
[34] Bugianesi E, Vanni E, Marchesini G. NASH and the risk of cirrhosis and hepatocellular carcinoma in type 2 diabetes. Curr Diab Rep 2007;7(3):175–180. doi:10.1007/s11892-007-0029-z, PMID:17547834.
[35] Harrison SA. Liver disease in patients with diabetes mellitus. J Clin Gastroenterol 2006;40(1):68–76. doi:10.1097/01.mcg.0000190774.91875.d2, PMID:16340637.
[36] Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, *et al*. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120(5):1183–1192. doi:10.1053/gast.2001.23256, PMID:11266382.
[37] Cheung O, Sanyal AJ. Abnormalities of lipid metabolism in nonalcoholic fatty liver disease. Semin Liver Dis 2008;28(4):351–359. doi:10.1055/s-0028-1091979, PMID:18956291.
[38] Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. J Clin Invest 2004;114(2):147–152. doi:10.1172/JCI22422, PMID:15254578.
[39] Carmen GY, Víctor SM. Signalling mechanisms regulating lipolysis. Cell Signal 2006;18(4):401–408. doi:10.1016/j.cellsig.2005.08.009, PMID:16182514.
[40] Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest 2008;118(3):829–838. doi:10.1172/

JCI34275, PMID:18317565.
[41] Malhi H, Bronk SF, Werneburg NW, Gores GJ. Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. J Biol Chem 2006; 281(17):12093–12101. doi:10.1074/jbc.M510660200, PMID:16505490.
[42] Alzamil H. Elevated Serum TNF-α Is Related to Obesity in Type 2 Diabetes Mellitus and Is Associated with Glycemic Control and Insulin Resistance. J Obes 2020;2020:5076858. doi:10.1155/2020/5076858, PMID:32089876.
[43] Chen CH, Jiang T, Yang JH, Jiang W, Lu J, Marathe GK, *et al*. Low-density lipoprotein in hypercholesterolemic human plasma induces vascular endothelial cell apoptosis by inhibiting fibroblast growth factor 2 transcription. Circulation 2003;107(16):2102–2108. doi:10.1161/01.CIR.0000065220.70220.F7, PMID:12695302.
[44] Kolb R, Sutterwala FS, Zhang W. Obesity and cancer: inflammation bridges the two. Curr Opin Pharmacol 2016;29:77–89. doi:10.1016/j.coph.2016.07.005, PMID:27429211.
[45] Cordeiro A, Costa R, Andrade N, Silva C, Canabrava N, Pena MJ, *et al*. Does adipose tissue inflammation drive the development of non-alcoholic fatty liver disease in obesity? Clin Res Hepatol Gastroenterol 2020;44(4):394–402. doi:10.1016/j.clinre.2019.10.001, PMID:32044284.
[46] Wei Y, Rector RS, Thyfault JP, Ibdah JA. Nonalcoholic fatty liver disease and mitochondrial dysfunction. World J Gastroenterol 2008;14(2):193–199. doi:10.3748/wjg.14.193, PMID:18186554.
[47] Yu J, Marsh S, Hu J, Feng W, Wu C. The Pathogenesis of Nonalcoholic Fatty Liver Disease: Interplay between Diet, Gut Microbiota, and Genetic Background. Gastroenterol Res Pract 2016;2016:2862173. doi:10.1155/2016/2862173, PMID:27247565.
[48] Fromenty B, Robin MA, Igoudjil A, Mansouri A, Pessayre D. The ins and outs of mitochondrial dysfunction in NASH. Diabetes Metab 2004;30(2):121–138. doi:10.1016/s1262-3636(07)70098-8, PMID:15223984.
[49] Nieto N, Friedman SL, Cederbaum AI. Cytochrome P450 2E1-derived reactive oxygen species mediate paracrine stimulation of collagen I protein synthesis by hepatic stellate cells. J Biol Chem 2002;277(12):9853–9864. doi:10.1074/jbc.M110506200, PMID:11782477.
[50] Chavez-Tapia NC, Rosso N, Tiribelli C. Effect of intracellular lipid accumulation in a new model of non-alcoholic fatty liver disease. BMC Gastroenterol 2012;12:20. doi:10.1186/1471-230X-12-20, PMID:22380754.
[51] Philippe MA, Ruddell RG, Ramm GA. Role of iron in hepatic fibrosis: one piece in the puzzle. World J Gastroenterol 2007;13(35):4746–4754. doi:10.3748/wjg.v13.i35.4746, PMID:17729396.
[52] Jiang W, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, *et al*. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci Rep 2015;5:8096. doi:10.1038/srep08096, PMID:25644696.
[53] Wang C, Zhu C, Shao L, Ye J, Shen Y, Ren Y. Role of Bile Acids in Dysbiosis and Treatment of Nonalcoholic Fatty Liver Disease. Mediators Inflamm 2019;2019:7659509. doi:10.1155/2019/7659509, PMID:31341422.
[54] Aranha MM, Cortez-Pinto H, Costa A, da Silva IB, Camilo ME, de Moura MC, *et al*. Bile acid levels are increased in the liver of patients with steatohepatitis. Eur J Gastroenterol Hepatol 2008;20(6):519–525. doi:10.1097/MEG.0b013e3282f4710a, PMID:18467911.
[55] Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, *et al*. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J Clin Invest 2004;113(10):1408–1418. doi:10.1172/JCI21025, PMID:15146238.
[56] Leiss O, von Bergmann K. Different effects of chenodeoxycholic acid and ursodeoxycholic acid on serum lipoprotein concentrations in patients with radiolucent gallstones. Scand J Gastroenterol 1982;17(5):587–592. doi:10.3109/00365528209181063, PMID:7178821.
[57] Mudaliar S, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, *et al*. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. Gastroenterology 2013;145(3):574–82.e1. doi:10.1053/j.gastro.2013.05.042, PMID:23727264.
[58] Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, *et al*. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab 2013;17(2):225–235. doi:10.1016/j.cmet.2013.01.003, PMID:23395169.
[59] Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, *et al*. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. J Clin Invest 2015;125(1):386–402. doi:10.1172/jci76738, PMID:25500885.
[60] Tanaka N, Matsubara T, Krausz KW, Patterson AD, Gonzalez FJ. Disruption of phospholipid and bile acid homeostasis in mice with nonalcoholic steatohepatitis. Hepatology 2012;56(1):118–129. doi:10.1002/hep.25630, PMID:22290395.
[61] Kolodziejczyk AA, Zheng D, Shibolet O, Elinav E. The role of the microbiome in NAFLD and NASH. EMBO Mol Med 2019;11(2):e9302. doi:10.15252/emmm.201809302, PMID:30591521.
[62] Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. J Hepatol 2020;72(3):558–577. doi:10.1016/J.JHEP.2019.10.003, PMID:31622696.
[63] Tilg H, Moschen AR, Szabo G. Interleukin-1 and inflammasomes in alcoholic liver disease/acute alcoholic hepatitis and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Hepatology 2016;64(3):955–965. doi:10.1002/hep.28456, PMID:26773297.
[64] Rendon JL, Li X, Akhtar S, Choudhry MA. Interleukin-22 modulates gut epithelial and immune barrier functions following acute alcohol exposure and burn injury. Shock 2013;39(1):11–18. doi:10.1097/SHK.0b013e3182749f96, PMID:23143063.
[65] Douhara A, Moriya K, Yoshiji H, Noguchi R, Namisaki T, Kitade M, *et al*. Reduction of endotoxin attenuates liver fibrosis through suppression of hepatic stellate cell activation and remission of intestinal permeability in a rat non-alcoholic steatohepatitis model. Mol Med Rep 2015;11(3):1693–1700. doi:10.3892/mmr.2014.2995, PMID:25421042.
[66] Ursell LK, Clemente JC, Rideout JR, Gevers D, Caporaso JG, Knight R. The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites. J Allergy Clin Immunol 2012;129(5):1204–1208. doi:10.1016/j.jaci.2012.03.010, PMID:22541361.
[67] Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486(7402):207–214. doi:10.1038/nature11234, PMID:22699609.
[68] Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature 2012;489(7415):242–249. doi:10.1038/nature11552, PMID:22972297.
[69] Aron-Wisnewsky J, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, *et al*. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. Nat Rev Gastroenterol Hepatol 2020;17(5):279–297. doi:10.1038/s41575-020-0269-9, PMID:32152478.
[70] Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. Worlds within worlds: evolution of the vertebrate gut microbiota. Nat Rev Microbiol 2008;6(10):776–788. doi:10.1038/nrmicro1978, PMID:18794915.
[71] Tancrède C. Role of human microflora in health and disease. Eur J Clin Microbiol Infect Dis 1992;11(11):1012–1015. doi:10.1007/BF01967791, PMID:1295753.
[72] Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, *et al*. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr 2011;94(1):58–65. doi:10.3945/ajcn.110.010132, PMID:21543530.
[73] Zhu Q, Jin Z, Wu W, Gao R, Guo B, Gao Z, *et al*. Analysis of the intestinal lumen microbiota in an animal model of colorectal cancer. PLoS One 2014;9(6):e90849. doi:10.1371/journal.pone.0090849, PMID:24603888.
[74] Finkelstein JD. Metabolic regulatory properties of S-adenosylmethionine and S-adenosylhomocysteine. Clin Chem Lab Med 2007;45(12):1694–1699. doi:10.1515/CCLM.2007.341, PMID:17963455.
[75] Ratnam S, Maclean KN, Jacobs RL, Brosnan ME, Kraus JP, Brosnan JT. Hormonal regulation of cystathionine beta-synthase expression in liver. J Biol Chem 2002;277(45):42912–42918. doi:10.1074/jbc.M206588200, PMID:12198128.
[76] Singh S, Banerjee R. PLP-dependent H(2)S biogenesis. Biochim Bio-

phys Acta 2011;1814(11):1518–1527. doi:10.1016/j.bbapap.2011.02.004, PMID:21315854.
[77] Zhang L, Yang G, Untereiner A, Ju Y, Wu L, Wang R. Hydrogen sulfide impairs glucose utilization and increases gluconeogenesis in hepatocytes. Endocrinology 2013;154(1):114–126. doi:10.1210/en.2012-1658, PMID:23183179.
[78] Siebert N, Cantré D, Eipel C, Vollmar B. H2S contributes to the hepatic arterial buffer response and mediates vasorelaxation of the hepatic artery via activation of K(ATP) channels. Am J Physiol Gastrointest Liver Physiol 2008;295(6):G1266–G1273. doi:10.1152/ajpgi.90484.2008, PMID:18974309.
[79] Polyzos SA, Kountouras J, Patsiaoura K, Katsiki E, Zafeiriadou E, Deretzi G, *et al*. Serum homocysteine levels in patients with nonalcoholic fatty liver disease. Ann Hepatol 2012;11:68–76. doi:10.1016/S1665-2681(19)31488-7, PMID:22166563.
[80] Maccioni L, Gao B, Leclercq S, Pirlot B, Horsmans Y, De Timary P, *et al*. Intestinal permeability, microbial translocation, changes in duodenal and fecal microbiota, and their associations with alcoholic liver disease progression in humans. Gut Microbes 2020;12(1):1782157. doi:10.1080/19490976.2020.1782157, PMID:32588725.
[81] Fukui H. Role of Gut Dysbiosis in Liver Diseases: What Have We Learned So Far? Diseases 2019;7(4):58. doi:10.3390/diseases7040058, PMID:31726747.
[82] Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, *et al*. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology 2013;58(1):120–127. doi:10.1002/hep.26319, PMID:23401313.
[83] Li F, McClain CJ, Feng W. Microbiome dysbiosis and alcoholic liver disease(). Liver Res 2019;3(3-4):218–226. doi:10.1016/j.livres.2019.09.001, PMID:33868760.
[84] Adolph TE, Grander C, Moschen AR, Tilg H. Liver-Microbiome Axis in Health and Disease. Trends Immunol 2018;39(9):712–723. doi:10.1016/j.it.2018.05.002, PMID:29843959.
[85] Benten D, Wiest R. Gut microbiome and intestinal barrier failure—the "Achilles heel" in hepatology? J Hepatol 2012;56(6):1221–1223. doi:10.1016/j.jhep.2012.03.003, PMID:22406521.
[86] Musso G, Cassader M, Cohney S, Pinach S, Saba F, Gambino R. Emerging Liver-Kidney Interactions in Nonalcoholic Fatty Liver Disease. Trends Mol Med 2015;21(10):645–662. doi:10.1016/j.molmed.2015.08.005, PMID:26432021.
[87] Moss CW, Lambert MA, Goldsmith DJ. Production of hydrocinnamic acid by clostridia. Appl Microbiol 1970;19(2):375–378. doi:10.1128/am.19.2.375-378.1970, PMID:5437307.
[88] Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, *et al*. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A 2009;106(10):3698–3703. doi:10.1073/pnas.0812874106, PMID:19234110.
[89] Dai X, Hou H, Zhang W, Liu T, Li Y, Wang S, *et al*. Microbial Metabolites: Critical Regulators in NAFLD. Front Microbiol 2020;11:567654. doi:10.3389/fmicb.2020.567654, PMID:33117316.
[90] Heimann E, Nyman M, Degerman E. Propionic acid and butyric acid inhibit lipolysis and de novo lipogenesis and increase insulin-stimulated glucose uptake in primary rat adipocytes. Adipocyte 2015;4(2):81–88. doi:10.4161/21623945.2014.960694, PMID:26167409.
[91] Endo H, Niioka M, Kobayashi N, Tanaka M, Watanabe T. Butyrate-producing probiotics reduce nonalcoholic fatty liver disease progression in rats: new insight into the probiotics for the gut-liver axis. PLoS One 2013;8(5):e63388. doi:10.1371/journal.pone.0063388, PMID:23696823.
[92] Yao H, Fan C, Lu Y, Fan X, Xia L, Li P, *et al*. Alteration of gut microbiota affects expression of adiponectin and resistin through modifying DNA methylation in high-fat diet-induced obese mice. Genes Nutr 2020;15(1):12. doi:10.1186/s12263-020-00671-3, PMID:32586265.
[93] Chen J, Vitetta L. The Role of Butyrate in Attenuating Pathobiont-Induced Hyperinflammation. Immune Netw 2020;20(2):e15. doi:10.4110/in.2020.20.e15, PMID:32395367.
[94] den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 2013;54(9):2325–2340. doi:10.1194/jlr.R036012, PMID:23821742.
[95] Kaji I, Karaki S, Kuwahara A. Short-chain fatty acid receptor and its contribution to glucagon-like peptide-1 release. Digestion 2014;89(1):31–36. doi:10.1159/000356211, PMID:24458110.
[96] Aoki R, Onuki M, Hattori K, Ito M, Yamada T, Kamikado K, *et al*. Commensal microbe-derived acetate suppresses NAFLD/NASH development via hepatic FFAR2 signalling in mice. Microbiome 2021;9(1):188. doi:10.1186/s40168-021-01125-7, PMID:34530928.
[97] Ji Y, Gao Y, Chen H, Yin Y, Zhang W. Indole-3-Acetic Acid Alleviates Nonalcoholic Fatty Liver Disease in Mice via Attenuation of Hepatic Lipogenesis, and Oxidative and Inflammatory Stress. Nutrients 2019;11(9):2062. doi:10.3390/nu11092062, PMID:31484323.
[98] Beaumont M, Neyrinck AM, Olivares M, Rodriguez J, de Rocca Serra A, Roumain M, *et al*. The gut microbiota metabolite indole alleviates liver inflammation in mice. FASEB J 2018;32(12):fj201800544. doi:10.1096/fj.201800544, PMID:29906245.
[99] Leong SC, Sirich TL. Indoxyl Sulfate-Review of Toxicity and Therapeutic Strategies. Toxins (Basel) 2016;8(12):358. doi:10.3390/toxins8120358, PMID:27916890.
[100] Lin YH, Luck H, Khan S, Schneeberger PHH, Tsai S, Clemente-Casares X, *et al*. Aryl hydrocarbon receptor agonist indigo protects against obesity-related insulin resistance through modulation of intestinal and metabolic tissue immunity. Int J Obes (Lond) 2019;43(12):2407–2421. doi:10.1038/s41366-019-0340-1, PMID:30944419.
[101] Li X, Zhang B, Hu Y, Zhao Y. New Insights Into Gut-Bacteria-Derived Indole and Its Derivatives in Intestinal and Liver Diseases. Front Pharmacol 2021;12:769501. doi:10.3389/fphar.2021.769501, PMID:34966278.
[102] Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, *et al*. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity 2014;41(2):296–310. doi:10.1016/j.immuni.2014.06.014, PMID:25065623.
[103] Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, *et al*. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57(2):601–609. doi:10.1002/hep.26093, PMID:23055155.
[104] Chaurasia B, Tippetts TS, Mayoral Monibas R, Liu J, Li Y, Wang L, *et al*. Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. Science 2019;365(6451):386–392. doi:10.1126/science.aav3722, PMID:31273070.
[105] Pellicciari R, Costantino G, Camaioni E, Sadeghpour BM, Entrena A, Willson TM, *et al*. Bile acid derivatives as ligands of the farnesoid X receptor. Synthesis, evaluation, and structure-activity relationship of a series of body and side chain modified analogues of chenodeoxycholic acid. J Med Chem 2004;47(18):4559–4569. doi:10.1021/jm049904b, PMID:15317466.
[106] Jiang X, Zheng J, Zhang S, Wang B, Wu C, Guo X. Advances in the Involvement of Gut Microbiota in Pathophysiology of NAFLD. Front Med (Lausanne) 2020;7:361. doi:10.3389/fmed.2020.00361, PMID:32850884.
[107] Soon RK Jr, Yan JS, Grenert JP, Maher JJ. Stress signaling in the methionine-choline-deficient model of murine fatty liver disease. Gastroenterology 2010;139(5):1730–1739.e1. doi:10.1053/j.gastro.2010.07.046, PMID:20682321.
[108] Corbin KD, Zeisel SH. Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. Curr Opin Gastroenterol 2012;28(2):159–165. doi:10.1097/MOG.0b013e32834e7b4b, PMID:22134222.
[109] Serviddio G, Giudetti AM, Bellanti F, Priore P, Rollo T, Tamborra R, *et al*. Oxidation of hepatic carnitine palmitoyl transferase-I (CPT-I) impairs fatty acid beta-oxidation in rats fed a methionine-choline deficient diet. PLoS One 2011;6(9):e24084. doi:10.1371/journal.pone.0024084, PMID:21909411.
[110] Gao X, Liu X, Xu J, Xue C, Xue Y, Wang Y. Dietary trimethylamine N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. J Biosci Bioeng 2014;118(4):476–481. doi:10.1016/j.jbiosc.2014.03.001, PMID:24721123.
[111] Robert K, Nehmé J, Bourdon E, Pivert G, Friguet B, Delcayre C, *et al*. Cystathionine beta synthase deficiency promotes oxidative stress, fibrosis, and steatosis in mice liver. Gastroenterology 2005;128(5):1405–1415. doi:10.1053/j.gastro.2005.02.034, PMID:

15887121.
[112] Hwang SY, Sarna LK, Siow YL, O K. High-fat diet stimulates hepatic cystathionine β-synthase and cystathionine γ-lyase expression. Can J Physiol Pharmacol 2013;91(11):913–919. doi:10.1139/cjpp-2013-0106, PMID:24117258.
[113] Dai H, Wang W, Tang X, Chen R, Chen Z, Lu Y, *et al*. Association between homocysteine and non-alcoholic fatty liver disease in Chinese adults: a cross-sectional study. Nutr J 2016;15(1):102. doi:10.1186/s12937-016-0221-6, PMID:27955646.
[114] Crane JD, Palanivel R, Mottillo EP, Bujak AL, Wang H, Ford RJ, *et al*. Inhibiting peripheral serotonin synthesis reduces obesity and metabolic dysfunction by promoting brown adipose tissue thermogenesis. Nat Med 2015;21(2):166–172. doi:10.1038/nm.3766, PMID:25485911.
[115] Gupta H, Min BH, Ganesan R, Gebru YA, Sharma SP, Park E, *et al*. Gut Microbiome in Non-Alcoholic Fatty Liver Disease: From Mechanisms to Therapeutic Role. Biomedicines 2022;10(3):550. doi:10.3390/biomedicines10030550, PMID:35327352.
[116] Witjes JJ, Smits LP, Pekmez CT, Prodan A, Meijnikman AS, Troelstra MA, *et al*. Donor Fecal Microbiota Transplantation Alters Gut Microbiota and Metabolites in Obese Individuals With Steatohepatitis. Hepatol Commun 2020;4(11):1578–1590. doi:10.1002/hep4.1601, PMID:33163830.
[117] Zheng L, Ji YY, Wen XL, Duan SL. Fecal microbiota transplantation in the metabolic diseases: Current status and perspectives. World J Gastroenterol 2022;28(23):2546–2560. doi:10.3748/wjg.v28.i23.2546, PMID:35949351.
[118] Hatton GB, Ran S, Tranah TH, Shawcross DL. Lessons Learned from Faecal Microbiota Transplantation in Cirrhosis. Curr Hepatology Rep 2020;19:159–167. doi:10.1007/s11901-020-00520-2.
[119] Qi X, Yang M, Stenberg J, Dey R, Fogwe L, Alam MS, *et al*. Gut microbiota mediated molecular events and therapy in liver diseases. World J Gastroenterol 2020;26(48):7603–7618. doi:10.3748/wjg.v26.i48.7603, PMID:33505139.
[120] Ziemer CJ, Gibson GR. An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. Int Dairy J 1998;8:473–479. doi:10.1016/s0958-6946(98)00071-5.
[121] Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, *et al*. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. Foods 2019;8(3):92. doi:10.3390/foods8030092, PMID:30857316.
[122] Williams NT. Probiotics. Am J Health Syst Pharm 2010;67(6):449–458. doi:10.2146/ajhp090168, PMID:20208051.
[123] Vitetta L, Henson JD. Probiotics and synbiotics targeting the intestinal microbiome attenuate non-alcoholic fatty liver disease. Hepatobiliary Surg Nutr 2020;9(4):526–529. doi:10.21037/hbsn.2019.11.24, PMID:32832510.
[124] Jalandra R, Makharia GK, Sharma M, Kumar A. Inflammatory and deleterious role of gut microbiota-derived trimethylamine on colon cells. Front Immunol 2022;13:1101429. doi:10.3389/fimmu.2022.1101429, PMID:36726978.
[125] Sato FT, Yap YA, Crisma AR, Portovedo M, Murata GM, Hirabara SM, *et al*. Tributyrin Attenuates Metabolic and Inflammatory Changes Associated with Obesity through a GPR109A-Dependent Mechanism. Cells 2020;9(9):2007. doi:10.3390/cells9092007, PMID:32882837.
[126] Tillman EJ, Rolph T. FGF21: An Emerging Therapeutic Target for Non-Alcoholic Steatohepatitis and Related Metabolic Diseases. Front Endocrinol (Lausanne) 2020;11:601290. doi:10.3389/fendo.2020.601290, PMID:33381084.
[127] Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, *et al*. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391(10126):1174–1185. doi:10.1016/S0140-6736(18)30474-4, PMID:29519502.
[128] Harrison SA, Bashir MR, Lee KJ, Shim-Lopez J, Lee J, Wagner B, *et al*. A structurally optimized FXR agonist, MET409, reduced liver fat content over 12 weeks in patients with non-alcoholic steatohepatitis. J Hepatol 2021;75(1):25–33. doi:10.1016/j.jhep.2021.01.047, PMID:33581174.
[129] Lawitz E, Herring R, Younes ZH, Gane E, Ruane P, Schall RA, *et al*. PS-105 - Proof of concept study of an apoptosis-signal regulating kinase (ASK1) inhibitor (selonsertib) in combination with an acetyl-CoA carboxylase inhibitor (GS-0976) or a farnesoid X receptor agonist (GS-9674) in NASH. J Hepatol 2018;68:S57. doi:10.1016/S0168-8278(18)30335-0.
[130] Harrison SA, Bashir M, Moussa SE, McCarty K, Pablo Frias J, Taub R, *et al*. Effects of Resmetirom on Noninvasive Endpoints in a 36-Week Phase 2 Active Treatment Extension Study in Patients With NASH. Hepatol Commun 2021;5(4):573–588. doi:10.1002/hep4.1657, PMID:33860116.
[131] Loomba R, Neutel J, Mohseni R, Bernard D, Severance R, Dao M, *et al*. LBP-20-VK2809, a Novel Liver-Directed Thyroid Receptor Beta Agonist, Significantly Reduces Liver Fat with Both Low and High Doses in Patients with Non-Alcoholic Fatty Liver Disease: A Phase 2 Randomized, Placebo-Controlled Trial. J Hepatol 2019;70:e150–151. doi:10.1016/S0618-8278(19)30266-X.

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Mini Review

Advances and Challenges in Targeted Therapy for Colorectal Cancer: A Focus on Adenomatous Polyposis Coli and Kirsten Rat Sarcoma Virus Mutations

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Abstract

The global burden of colorectal cancer (CRC) is a pressing concern, with a substantial impact on public health. Despite advancements in understanding the molecular mechanisms of CRC development, challenges remain in translating this knowledge into effective clinical interventions. Key genetic mutations, notably in the adenomatous polyposis coli (APC) and Kirsten rat sarcoma virus (KRAS) genes, are central to CRC initiation and progression. Current CRC treatments include surgery and chemotherapy, often combined with targeted agents. However, resistance and heterogeneity within CRC patients limit the effectiveness of these therapies. Promisingly, research has focused on targeting APC and KRAS mutations for therapy. Small molecules inhibiting the Wnt pathway and antibodies targeting specific components are under investigation. Targeting KRAS itself is challenging due to its conserved structure, but disrupting its membrane interactions and subcellular localization are potential therapeutic strategies. To address the limitations of single-drug therapy, combination approaches are gaining traction. Combination therapy not only minimizes off-target effects but also tackles drug resistance and diverse genetic alterations within tumors. The intricate interplay of mutations and pathways in CRC necessitates multifaceted therapeutic strategies. Although progress has been made in understanding CRC genetics and developing targeted therapies, there is still work to be done to translate these insights into effective clinical treatments for CRC patients. This review provides crucial information for novel combination treatments for CRC.

Keywords: Colorectal cancer; Adenomatous polyposis coli; Kirsten rat sarcoma virus; Combination therapy; CRC treatment; Drug resistant.

Abbreviations: 5-FU, 5-fluorouracil; APC, adenomatous polyposis coli; CEB, CCAAT enhancer binding protein; CRC, colorectal cancer; CtBP, C-terminal binding protein; EGFR, growth factor receptor; FZD, frizzled receptors; GDP, guanosine diphosphate; GTP, guanosine triphosphate; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PI3K, phosphatidylinositol 3-kinase; PORCN, porcupine O-Acyltransferase; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; TCF, T-cell factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WNT, wingless-related integration site.

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Introduction

Colorectal cancer (CRC) is a malignant tumor that originates in the colon or rectum. CRC is a significant global health concern, as demonstrated by statistics from 2020, where approximately 150,000 individuals worldwide received a CRC diagnosis, resulting in 53,200 fatalities.[1] Among these patients, 17,930 individuals under the age of 50 were diagnosed with CRC, leading to 3,640 deaths in this age group.[1] Gender differences are apparent, with CRC being more prevalent in males than in females, as evidenced by data from the World Health Organization database. Furthermore, variations in CRC incidence rates are evident globally. Countries such as Australia, New Zealand, Europe, and North America experience higher rates of the disease, while Africa and South-Central Asia exhibit lower rates (Global Burden of Disease Cancer Collaboration). These disparities may stem from factors such as dietary habits, environmental influences, and genetic variations.[2] The rising trend of CRC incidence is particularly evident in China, where the burden on the healthcare system has been steadily increasing, especially in developed regions. A similar

scenario has been observed in Hong Kong, where CRC remains a common form of cancer, as highlighted by 5,634 new cases reported in 2018. Furthermore, the mortality rate for males was 37 per 100,000, while for females, it was 22.2 per 100,000 (Centre for Health Protection 2020).

CRC is not solely attributed to a single genetic mutation; instead, it emerges from intricate molecular signaling pathways characterized by a complex interplay of mutations and disruptions. This process involves a gradual transition from adenoma to carcinoma and eventually to metastatic disease—a multistep journey driven by gene mutations and irregular pathways.[3] Recent advances in genome-wide sequencing have unveiled a comprehensive array of nearly 80 mutated genes implicated in CRC. Notably, among these are adenomatous polyposis coli (APC), Kirsten rat sarcoma 2 viral oncogene homolog (KRAS), and p53.[4] The APC gene mutation, occurring in 70–80% of CRC cases, plays a pivotal role within the Wnt/beta-catenin signaling pathway is significant.[5] In addition to APC, another recurrently observed mutation involves the RAS gene family, especially KRAS, a commonly altered oncogene affecting 30–50% of CRC patients.[6] The p53 gene, functioning as a tumor suppressor, influences the cell cycle, apoptosis, genetic stability, and angiogenesis control.[7] While specific mutations initiate tumorigenesis, it is important to recognize that the progression and development of tumors involve the intricate interplay of multiple genes.[8] Additionally, epigenetic factors such as DNA methylation, histone modifications, chromatin remodelers, and noncoding RNAs have emerged as significant contributors to the advancement and growth of CRC.[9]

This review explores APC and KRAS mutations in colorectal cancer, discusses prevailing treatment challenges, and outlines emerging combination therapies. We aim for this review to enhance comprehension of colorectal cancer's mutational landscape and therapeutic strategies, thereby fostering research and implementation of innovative combination therapies.

APC mutations in CRC

The APC gene holds substantial importance as a frequently mutated tumor suppressor gene within CRC.[10] Situated on chromosome 5q21-q22, this gene spans 8535 nucleotides and comprises 21 exons encoding a 310 kDa protein containing 2843 amino acids. A pivotal site for both germline and somatic mutations of APC lies within exon 15, encompassing 75% of the gene's coding sequence.[11] This finding is consistent with the central role of APC in governing the influence of the Wnt pathway on the proliferation and differentiation of gastrointestinal tract cells.[12] Mechanistically, APC plays a pivotal role in inhibiting β-catenin/T-cell factor (TCF)-dependent transcription through complex breakdown. This process involves stimulating the phosphorylation and subsequent ubiquitin-dependent degradation of β-catenin.[13] APC bolsters this degradation mechanism by promoting Axin multimerization and stabilizing the Axin complex.[14] Additional regulatory mechanisms include reducing nuclear β-catenin levels through the promotion of β-catenin export, direct binding to β-catenin to impede TCF interactions,[15] and facilitating C-terminal binding protein (CtBP)-mediated repression of Wnt-target genes through direct interaction with a repression complex.[16,17] Alterations in APC result in the activation of β-catenin/TCF transcriptional activity due to β-catenin accumulation. This attenuation of CtBP-mediated inhibition within the repression complex leads to elevated levels of downstream targets, including cyclin D1 and c-myc. These factors significantly influence tumor cell proliferation, apoptosis, and cell cycle regulation (Fig. 1).[18,19] Evidently, APC intricately interacts with critical signaling pathways and biological processes implicated in CRC development.[10] Recent investigations have shown that restoring APC functionality can promote tumor regression and restore crypt homeostasis in CRC, suggesting that the Wnt pathway is a promising therapeutic target for CRC treatment.[20]

KRAS mutation in CRC

KRAS is one of the most commonly mutated genes in human cancer and has significant implications for CRC treatment. Within this context, various forms of KRAS mutations have been categorized into three main groups based on the altered codon: G12 (mutations at codon 12), G13 (mutations at codon 13), and Q61 (mutations at codon 61).[21] Notably, KRAS mutations are prevalent in approximately 30–50% of CRC cases.[6] Among these mutations, 15 distinct point mutations are found to be particularly significant: G12A, G12D, G12F, G12K, G12N, G12S, G12V, G12Y, G12C, G12E, G12I, G12L, G12R, G12T, and G12W. Of these, G12D and G12V are the predominant subtypes, accounting for approximately 41% and 28%, respectively, of all G12 mutations.[22] Clinical investigations consistently indicate that CRC patients carrying KRAS mutations tend to experience reduced survival rates compared to those without such mutations.[23] Moreover, within the realm of KRAS mutations, G12D and G12V mutations have been associated with the poorest prognoses among CRC patients.[24] Additionally, research findings demonstrate that individuals with G13 mutations in CRC patients experience significantly lower survival rates when diagnosed at stage I or II than when diagnosed with wild-type KRAS.[6,25] Furthermore, for CRC patients harboring mutations at codon 12, the 5-year overall survival rate is notably lower than that for those carrying codon 13 mutations or wild-type KRAS.[26]

KRAS functions as a pivotal sensor that initiates a cascade of signaling molecules, facilitating the transmission of signals from the cell surface to the nucleus. This activation process significantly influences essential cellular functions, including cell differentiation, growth, chemotaxis, and apoptosis. Notably, KRAS plays a critical role in regulating key signaling pathways such as the PI3K-Akt and RAS-RAF-MAPK pathways, which play pivotal roles in cell proliferation.[27–29] KRAS functions as a downstream component of the epidermal growth factor receptor (EGFR) pathway. Upon EGFR activation, the intracellular tyrosine kinase phosphorylates and activates KRAS, subsequently triggering the RAS-RAF-MAPK pathway. After activation, KRAS transitions to its activated state, KRAS-GTP, which is later hydrolyzed by GTPase, returning to the inactive KRAS-GDP state. This dynamic equilibrium involves alternating between its active (KRAS-GTP) and inactive (KRAS-GDP) forms. However, mutations within KRAS lead to the abnormal activation of downstream pathways, such as RAS-RAF-MAPK or phosphoinositide 3-kinase (PI3K), regardless of EGFR activation status (Fig. 2).[30,31] Persistently active KRAS results in irregular and uncontrolled cell growth, cellular transformation, heightened cancer metastasis, and increased resistance to chemotherapy and EGFR-targeted therapies across various cancer types, including CRC.[32,33]

Clinical challenges

Surgery stands as the primary curative approach for patients with nonmetastatic CRC, while chemotherapy offers an alternative therapeutic avenue. Notable drugs utilized for CRC treatment include

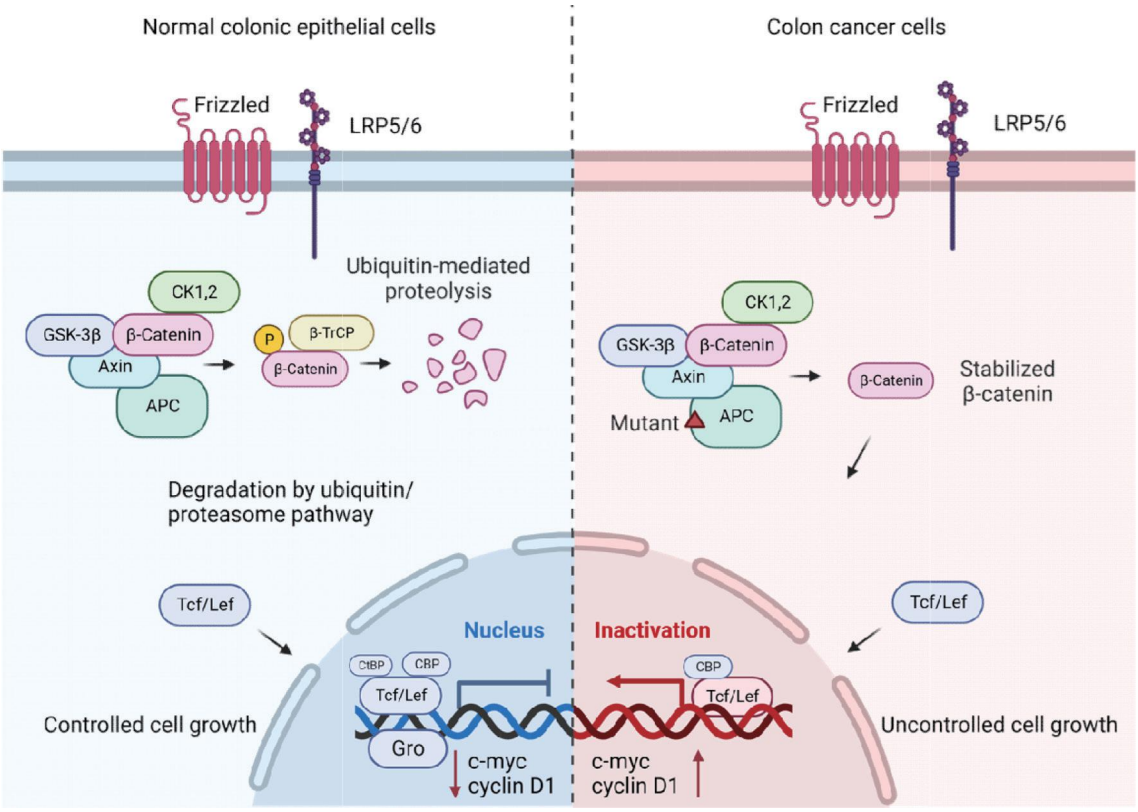

Fig. 1. A schematic diagram showing the Wnt signaling pathway in normal colonic epithelial cells and colon cancer cells. APC, adenomatous polyposis coli; CBP, cAMP-response element binding protein; CIBP, calcium- and integrin-binding protein; CK, creatine kinase. CtBP, C-terminal binding protein; GRO, growth-regulated oncogene alpha; GSK, glycogen synthase kinase LRP5/6, low-density lipoprotein receptor-related protein (LRP)5 and 6; Tcf/Lef, T-cell factor/lymphoid enhancer factor.

5-fluorouracil (5-FU), capecitabine, irinotecan, oxaliplatin, cetuximab, and panitumumab.[34] In addition to conventional chemotherapy, targeted agents play a role in treating metastatic CRC. For example, cetuximab, the first FDA-approved targeted drug for CRC, targets EGFR. Additionally, bevacizumab, focusing on VEGF, has gained approval. Other drugs like panitumumab, regorafenib, and ramucirumab, all targeting VEGF/VEGFR, have also been approved for CRC treatment. Notably, recent years have seen the approval of immune checkpoint inhibitors such as pembrolizumab, nivolumab, and ipilimumab.[35] However, the landscape of CRC is complex and characterized by multifaceted processes marked by a sequence of genetic alterations.[36] Notably, the pronounced occurrence of tumor heterogeneity in CRC, stemming from chromosomal instability or microsatellite instability,[37] collectively influences the efficacy of targeted therapies.

Despite these promising avenues, drugs specifically targeting APC and/or KRAS mutations have yet to receive FDA approval. CRC frequently involves APC and KRAS mutations, rendering them attractive therapeutic targets. However, it is important to note that medications aimed at targeting the APC/WNT/beta-catenin signaling pathways are currently in the preclinical development phase (Table 1).[38,39–46]

Over the past decade, a dedicated pursuit has aimed to advance therapeutic strategies against the APC/WNT/beta-catenin signaling pathway in CRC patients. This endeavor has led to the discovery of a range of small molecules that effectively inhibit this pathway by targeting various signaling molecules.[38,47,48] Notably, phase 1 and 2 clinical trials have been conducted for these inhibitors, including WNT974, ETC-1922159, RXC004, and CGX1321, which are PORCN inhibitors; OTSA101-DTPA-90Y, which functions as an FZD10 antagonist; OMP-18R5, a monoclonal antibody targeting FZD receptors; and PRI-724, a CEB/beta-catenin antagonist.[49] Despite these promising efforts, none have yet secured FDA approval for CRC treatment. The exceptional complexity of the APC/WNT/beta-catenin pathway plays a significant role in this process. Beyond APC mutations, beta-catenin can be further activated through alternate signaling pathways.[50–53] Numerous studies suggest that these supplementary regulatory processes contribute to the observed limitations in achieving satisfactory clinical outcomes with these inhibitors and antibodies. Moreover, the potential toxicity of these inhibitors on the intestinal epithelium, coupled with the risk of off-target effects, might have hindered their progress in clinical applications (Table 2).[54]

Presently, there is a lack of approved drugs specifically targeting KRAS for CRC treatment. Instead, approvals have been directed toward inhibitors of downstream signaling cascades, such as the RAF and MEK pathways (Table 1).[55] For example, selumetinib (AZD6244), functioning as a MEK 1/2 inhibitor, is designed to hinder the MEK enzyme within the RAS/MAPK pathway. Additionally, trametinib, a potent and selective ATP-independent inhibitor of MEK1/2 kinases, falls within this category.[56] Another example is GDC-0623, a MEK inhibitor that enhances BIM expression, which is currently under investigation in a phase I clinical trial.[57] However, concentrating solely on downstream cascades unrelated

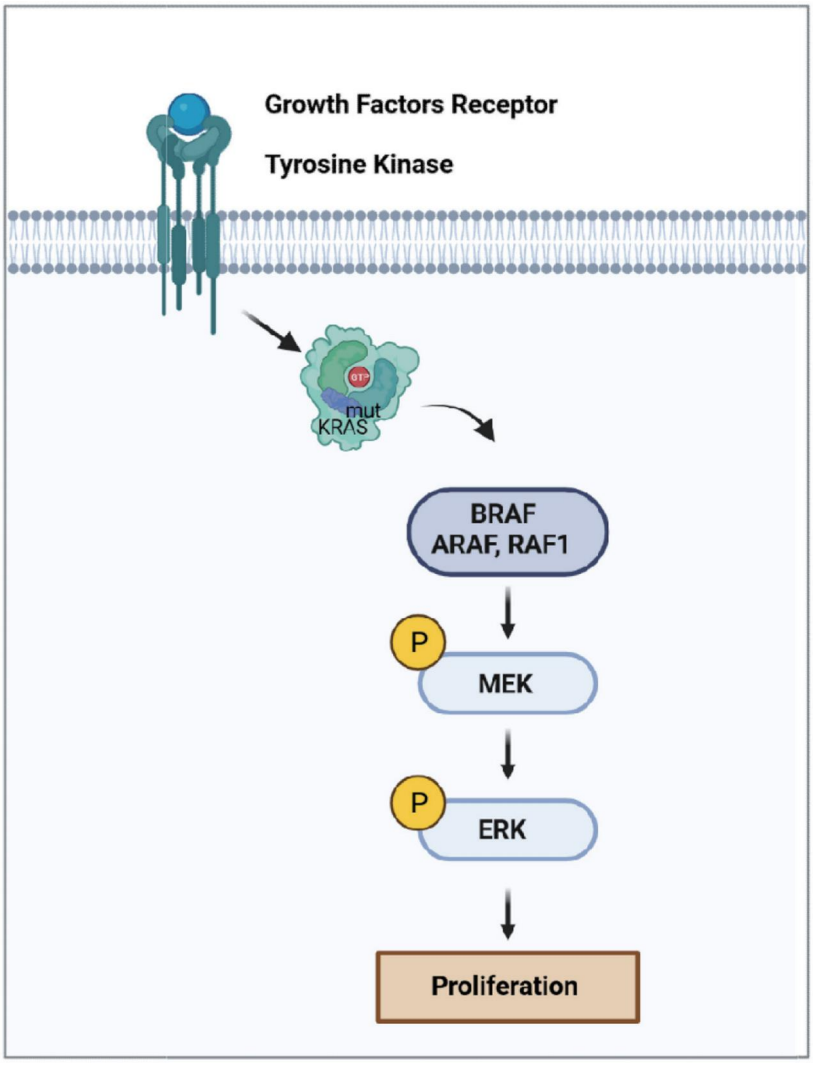

Fig. 2. A schematic diagram showing oncogenic signaling pathways associated with mutated KRAS. ARAF, serine/threonine-protein kinase A-Raf; BRAF, B-Raf proto-oncogene, serine/threonine kinase; ERK, extracellular signal-regulated kinase; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog; MEK, mitogen-activated protein kinase kinase; RAF1, rapidly accelerated fibrosarcoma 1.

to KRAS may not yield the desired effectiveness in cancer treatment. This challenge might arise from the inherent difficulty in pharmaceutically targeting KRAS.[58] Research has highlighted several obstacles in the quest for KRAS-targeted treatments (Table 2). These include the highly conserved nature of the GTPase catalytic domain on KRAS proteins, the competitive binding issues faced by small molecule drugs with substrates, and the limited number of binding sites on the KRAS protein surface for small molecule inhibitors.[59–64] Nevertheless, strategies aimed at disrupting KRAS-membrane interactions and altering KRAS subcellular localization continue to hold promise. Recent insights into functionally significant posttranslational modifications of the KRAS protein, including phosphorylation and ubiquitylation, introduce novel prospects for inhibiting KRAS activity.

Development of novel drug combinations for CRC treatment

The inception of combination therapy dates back to 1965 when Emil Frei and colleagues pioneered the inaugural utilization of combination chemotherapy in pediatric patients afflicted with acute leukemia.[65] The resounding success of this innovative therapeutic paradigm ushered in a transformative era within clinical oncology.[66] Subsequently, cancer research has increasingly focused on the exploration of combination therapies designed to concurrently target disparate molecular pathways, resulting in favorable anticancer outcomes. Concurrently, progress in cancer cell genomics, epigenomics, transcriptomics, and proteomics has facilitated the identification of novel molecular targets, underpinning the development of highly selective targeted anticancer interventions.[67] These targeted therapies have substantially diversified the arsenal of combinational anticancer modalities, capable of integration with other targeted therapies or conventional chemotherapeutic agents.[68]

The efficacy of single-drug therapy often encounters limitations, leading to the emergence of drug resistance.[69] In fact, resistance to 5-FU treatment occurs in approximately half of all CRC patients.[70] Recently, there has been a growing focus on combining

Table 1. Selected targeted therapy trials for colorectal cancer

Treatment	Trail	Sample size	Study groups	Response rate	Side effects	Reference
WNT974	Phase 1	94	BRAF-mutant CRC, BRAF-mutant CRC with RNF43 mutation and/or RSPO fusion	N.A	Dysgeusia, Decreased appetite, and Nausea	39
ETC-1922159	Phase 1	20	Metastatic solid tumors	N.A	Dysgeusia, β-CTX increase, Fatigue, Constipation, and Nausea	40
RXC004	Phase 2	20	RNF43 or RSPO aberrated, metastatic, microsatellite stable colorectal cancer	Ongoing	Ongoing	41
CGX1321	Phase 1	77	colorectal cancer or small bowel cancer carrying RSPO or RNF43 alterations	N.A	Dysgeusia, Bone resorption	42
OTSA101-DTPA-90Y	Phase 1	20	Progressive advanced Synovial Sarcomas	N.A	Reversible hematological disorders	43
OMP-18R5	Phase 1	18	Advanced solid tumors	N.A	Fatigue, Vomiting, Abdominal pain, Constipation, Diarrhea and Causea	44
PRI-724	Phase 1	18	Advanced solid tumors	N.A	Hyperbilirubinemia, Diarrhea, Bilirubin elevation, Hypophosphatemia, Nausea, Fatigue, Anorexia, Thrombocytopenia and Alkaline phosphatase elevation.	45
GDC-0623	phase 1	45	Advanced solid tumors	N.A	Rash, Gastrointestinal symptoms and Visual disturbance	46

BRAF, B-Raf proto-oncogene, serine/threonine kinase; CRC, colorectal cancer; RNF, ring finger protein; RSPO, R-spondin; β-CTX, serum C-terminal telopeptide of type I collagen.

drugs to leverage synergistic interactions. Combination therapy offers notable advantages. First, it allows for reduced drug dosages, thereby decreasing the risk of off-target side effects.[71] Second, this approach targets multiple facets, effectively curbing the development of drug resistance.[72] These attributes hold particular importance when addressing heterogeneous cancers such as CRC.[73] The intrinsic heterogeneity of CRC is well documented. In some cases, patients with the same tumor may display distinct genetic alterations, and even cells within a tumor might carry varying genetic mutations. Resistance to a single chemotherapeutic agent, whether innate or acquired, can stem from factors such as suppressed apoptosis or enhanced DNA repair, leading to cancer relapse or treatment resistance. Therefore, combination therapy is especially advantageous because diverse drugs can target different pathways or genes. This approach substantially reduces the number of cancer cells that can withstand treatment, effectively delaying cancer recurrence and, optimally, achieving complete eradication.

The utilization of combination chemotherapy has evolved into the prevailing standard of care within the field of medical oncology. Considering the profusion of available chemotherapeutic and targeted anticancer agents, forecasting and developing innovative drug combinations presents a formidable challenge. Thus, it is imperative to explore the requisite methodologies for prognosticating combinations that exhibit synergistic anticancer efficacy.

Conclusion

CRC represents a significant global health challenge, with considerable variations in incidence rates across regions and gender differences. Among numerous genes that contribute to CRC development, APC and KRAS mutations are pivotal factors driving tumorigenesis. Current research efforts are focused on inhibiting the APC/Wnt/beta-catenin and KRAS pathways. While progress has been made in the field of small molecules and inhibitors, their clinical application has encountered hurdles due to the complexity of these pathways and the emergence of alternative signaling mechanisms. Combination therapy has emerged as a promising approach to address the complexity and heterogeneity of CRC. By targeting multiple facets and pathways simultaneously, combination therapies can potentially enhance treatment efficacy, mitigate drug resistance, and ultimately improve patient outcomes.

Acknowledgments

None.

Table 2. Hurdles of development of targeted therapies

Target	Obstacles
APC	Potential toxicity; Off-target effects.
KRAS	Highly conserved nature of the GTPase; Catalytic domain on KRAS proteins; Competitive binding issues; Limited binding sites.

APC, adenomatous polyposis coli; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog; WNT, wingless-related integration site.

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Conflict of interest

All authors declared that there are no conflict of interests.

Author contributions

Conceptualization (GQC, SC, RHG), Data curation and original draft preparation (RHG, GQC, SC), Figures and Tables (RHG, GQC, SC), Review and editing (GQC, SC, RHG). All authors contributed to the article and approved the final manuscript.

References

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin 2021;71(1):7–33. doi:10.3322/caac.21654, PMID:33433946.
[2] Song M, Chan AT, Sun J. Influence of the Gut Microbiome, Diet, and Environment on Risk of Colorectal Cancer. Gastroenterology 2020;158(2):322–340. doi:10.1053/j.gastro.2019.06.048, PMID:31586566.
[3] Wu Z, Li Y, Zhang Y, Hu H, Wu T, Liu S, *et al*. Colorectal Cancer Screening Methods and Molecular Markers for Early Detection. Technol Cancer Res Treat 2020;19:1533033820980426. doi:10.1177/1533033820980426, PMID:33353503.
[4] Dunne PD, Arends MJ. Molecular pathological classification of colorectal cancer-an update. Virchows Arch 2024;484(2):273–285. doi:10.1007/s00428-024-03746-3, PMID:38319359.
[5] Hankey W, Frankel WL, Groden J. Functions of the APC tumor suppressor protein dependent and independent of canonical WNT signaling: implications for therapeutic targeting. Cancer Metastasis Rev 2018;37(1):159–172. doi:10.1007/s10555-017-9725-6, PMID:29318445.
[6] Zhu G, Pei L, Xia H, Tang Q, Bi F. Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer. Mol Cancer 2021;20(1):143. doi:10.1186/s12943-021-01441-4, PMID:34742312.
[7] Liebl MC, Hofmann TG. The Role of p53 Signaling in Colorectal Cancer. Cancers (Basel) 2021;13(9):2125. doi:10.3390/cancers13092125, PMID:33924934.
[8] Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013;339(6127):1546–1558. doi:10.1126/science.1235122, PMID:23539594.
[9] Jung G, Hernández-Illán E, Moreira L, Balaguer F, Goel A. Epigenetics of colorectal cancer: biomarker and therapeutic potential. Nat Rev Gastroenterol Hepatol 2020;17(2):111–130. doi:10.1038/s41575-019-0230-y, PMID:31900466.
[10] Kok SY, Nakayama M, Morita A, Oshima H, Oshima M. Genetic and nongenetic mechanisms for colorectal cancer evolution. Cancer Sci 2023;114(9):3478–3486. doi:10.1111/cas.15891, PMID:37357016.
[11] Béroud C, Soussi T. APC gene: database of germline and somatic mutations in human tumors and cell lines. Nucleic Acids Res 1996;24(1):121–4. doi:10.1093/nar/24.1.121, PMID:8594558.
[12] Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, *et al*. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther 2022;7(1):3. doi:10.1038/s41392-021-00762-6, PMID:34980884.
[13] Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P, *et al*. Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. Science 1996;272(5264):1023–1026. doi:10.1126/science.272.5264.1023, PMID:8638126.
[14] Pronobis MI, Rusan NM, Peifer M. A novel GSK3-regulated APC:Axin interaction regulates Wnt signaling by driving a catalytic cycle of efficient βcatenin destruction. Elife 2015;4:e08022. doi:10.7554/eLife.08022, PMID:26393419.
[15] Neufeld KL, Zhang F, Cullen BR, White RL. APC-mediated downregulation of beta-catenin activity involves nuclear sequestration and nuclear export. EMBO Rep 2000;1(6):519–23. doi:10.1093/embo-reports/kvd117, PMID:11263497.
[16] Hamada F, Bienz M. The APC tumor suppressor binds to C-terminal binding protein to divert nuclear beta-catenin from TCF. Dev Cell 2004;7(5):677–85. doi:10.1016/j.devcel.2004.08.022, PMID:15525529.
[17] Sierra J, Yoshida T, Joazeiro CA, Jones KA. The APC tumor suppressor counteracts beta-catenin activation and H3K4 methylation at Wnt target genes. Genes Dev 2006;20(5):586–600. doi:10.1101/gad.1385806, PMID:16510874.
[18] He TC, Sparks AB, Rago C, Hermeking H, Zawel L. Identification of c-MYC as a target of the APC pathway. Science 1998;281(5382):1509–1512. doi:10.1126/science.281.5382.1509, PMID:9727977.
[19] Yan H, Jiang F, Yang J. Association of β-Catenin, APC, SMAD3/4, Tp53, and Cyclin D1 Genes in Colorectal Cancer: A Systematic Review and Meta-Analysis. Genet Res (Camb) 2022;2022:5338956. doi:10.1155/2022/5338956, PMID:36072013.
[20] Dow LE, O'Rourke KP, Simon J, Tschaharganeh DF, van Es JH, Clevers H, *et al*. Apc Restoration Promotes Cellular Differentiation and Reestablishes Crypt Homeostasis in Colorectal Cancer. Cell 2015;161(7):1539–1552. doi:10.1016/j.cell.2015.05.033, PMID:26091037.
[21] Uprety D, Adjei AA. KRAS: From undruggable to a druggable Cancer Target. Cancer Treat Rev 2020;89:102070. doi:10.1016/j.ctrv.2020.102070, PMID:32711246.
[22] Christensen JG, Olson P, Briere T, Wiel C, Bergo MO. Targeting Krasg12c -mutant cancer with a mutation-specific inhibitor. J Intern Med 2020;288(2):183–191. doi:10.1111/joim.13057, PMID:32176377.
[23] Pang XL, Li QX, Ma ZP, Shi Y, Ma YQ, Li XX, *et al*. Association between clinicopathological features and survival in patients with primary and paired metastatic colorectal cancer and KRAS mutation. Onco Targets Ther 2017;10:2645–2654. doi:10.2147/OTT.S133203, PMID:28579802.
[24] Li W, Liu Y, Cai S, Yang C, Lin Z, Zhou L, *et al*. Not all mutations of KRAS predict poor prognosis in patients with colorectal cancer. Int J Clin Exp Pathol 2019;12(3):957–967. PMID:31933906.
[25] Chen J, Guo F, Shi X, Zhang L, Zhang A, Jin H, *et al*. BRAF V600E mutation and KRAS codon 13 mutations predict poor survival in Chinese colorectal cancer patients. BMC Cancer 2014;14:802. doi:10.1186/1471-2407-14-802, PMID:25367198.
[26] Abubaker J, Bavi P, Al-Haqawi W, Sultana M, Al-Harbi S, *et al*. Prognostic significance of alterations in KRAS isoforms KRAS-4A/4B and KRAS mutations in colorectal carcinoma. J Pathol 2009;219(4):435–445. doi:10.1002/path.2625, PMID:19824059.
[27] Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Fujimoto M, Kawakami F, *et al*. Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. J Thorac Oncol 2013;8(1):52–61. doi:10.1097/JTO.0b013e3182769aa8, PMID:23242438.
[28] Degirmenci U, Wang M, Hu J. Targeting Aberrant RAS/RAF/MEK/ERK Signaling for Cancer Therapy. Cells 2020;9(1):198. doi:10.3390/cells9010198, PMID:31941155.
[29] Castellano E, Sheridan C, Thin MZ, Nye E, Spencer-Dene B. Requirement for interaction of PI3-kinase p110α with RAS in lung tumor maintenance. Cancer Cell 2013;24(5):617–630. doi:10.1016/j.ccr.2013.09.012, PMID:24229709.
[30] Martelli V, Pastorino A, Sobrero AF. Prognostic and predictive molecular biomarkers in advanced colorectal cancer. Pharmacol Ther 2022;236:108239. doi:10.1016/j.pharmthera.2022.108239, PMID:35780916.
[31] Domagała P, Hybiak J, Sulżyc-Bielicka V, Cybulski C, Ryś J, Domagała W, *et al*. KRAS mutation testing in colorectal cancer as an example of the pathologist's role in personalized targeted therapy: a practical approach. Pol J Pathol 2012;63(3):145–64. doi:10.5114/pjp.2012.31499, PMID:23161231.
[32] Shingu T, Holmes L, Henry V, Wang Q, Latha K, Gururaj AE, *et al*. Suppression of RAF/MEK or PI3K synergizes cytotoxicity of receptor tyrosine kinase inhibitors in glioma tumor-initiating cells. J Transl Med 2016;14:46. doi:10.1186/s12967-016-0803-2, PMID:26861698.
[33] Van Schaeybroeck S, Kalimutho M, Dunne PD, Carson R, Allen W, Jithesh PV, *et al*. ADAM17-dependent c-MET-STAT3 signaling mediates resistance to MEK inhibitors in KRAS mutant colorectal cancer.

Cell Rep 2014;7(6):1940–1955. doi:10.1016/j.celrep.2014.05.032, PMID:24931611.
[34] Nussinov R, Tsai CJ, Jang H. Anticancer drug resistance: An update and perspective. Drug Resist Updat 2021;59:100796. doi:10.1016/j.drup.2021.100796, PMID:34953682.
[35] Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther 2020;5(1):22. doi:10.1038/s41392-020-0116-z, PMID:32296018.
[36] Lahouel K, Younes L, Danilova L, Giardiello FM, Hruban RH, Groopman J, *et al*. Revisiting the tumorigenesis timeline with a data-driven generative model. Proc Natl Acad Sci USA 2020;117(2):857–864. doi:10.1073/pnas.1914589117, PMID:31882448.
[37] Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. Gastroenterology 2010;138(6):2059–72. doi:10.1053/j.gastro.2009.12.065, PMID:20420946.
[38] Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, *et al*. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. Mol Cancer 2022;21(1):144. doi:10.1186/s12943-022-01616-7, PMID:35836256.
[39] Rodon J, Argilés G, Connolly RM, Vaishampayan U, de Jonge M, Garralda E, *et al*. Phase 1 study of single-agent WNT974, a first-in-class Porcupine inhibitor, in patients with advanced solid tumours. Br J Cancer 2021;125(1):28–37. doi:10.1038/s41416-021-01389-8, PMID:33941878.
[40] Tan SP, Matthew CH, Ng VS, Wells AM, John HS, Veronica D, *et al*. A phase 1B dose escalation study of ETC-159 in combination with pembrolizumab in advanced or metastatic solid tumours. J Clin Oncol 2023;41(suppl):2601–2601. doi:10.1200/JCO.2023.41.16_suppl.2601.
[41] Kopetz S, Morris VK, O'Neil B, Bridgewater JA, Graham J, Parkes EE, *et al*. A multi-arm, phase 2, open-label study to assess the efficacy of RXC004 as monotherapy and in combination with nivolumab in patients with ring finger protein 43 (RNF43) or R-spondin (RSPO) aberrated, metastatic, microsatellite stable colorectal cancer following standard treatments. J Clin Oncol 2022;40(suppl):TPS3637–TPS3637. doi:10.1200/JCO.2022.40.16_suppl.TPS3637.
[42] Giannakis M, Le DT, Pishvaian MJ, Weinberg BA, Papadopoulos KP, Shen L, *et al*. Phase 1 study of WNT pathway Porcupine inhibitor CGX1321 and phase 1b study of CGX1321 + pembrolizumab (pembro) in patients (pts) with advanced gastrointestinal (GI) tumors. J Clin Oncol 2023;41(suppl):3514. doi:10.1200/JCO.2023.41.16_suppl.3514.
[43] Giraudet AL, Cassier PA, Iwao-Fukukawa C, Garin G, Badel JN, Kryza D, *et al*. A first-in-human study investigating biodistribution, safety and recommended dose of a new radiolabeled MAb targeting FZD10 in metastatic synovial sarcoma patients. BMC Cancer 2018;18(1):646. doi:10.1186/s12885-018-4544-x, PMID:29884132.
[44] Smith DC, Rosen LS, Chugh R, Goldman JW, Xu L, Kapoun A, *et al*. First-in-human evaluation of the human monoclonal antibody vantictumab (OMP-18R5; anti-Frizzled) targeting the WNT pathway in a phase I study for patients with advanced solid tumors. J Clin Oncol 2013;31(suppl):2540. doi:10.1200/jco.2013.31.15_suppl.2540.
[45] El-Khoueiry AB, Ning Y, Yang D, Cole S, Kahn M, Zoghbi M, *et al*. A phase I first-in-human study of PRI-724 in patients (pts) with advanced solid tumors. J Clin Oncol 2013;31(suppl):2501. doi:10.1200/jco.2013.31.15_suppl.2501.
[46] El-Khoueiry A, Kurkjian C, Semrad T, Musib L, Gates M, Eppler S, *et al*. A first in-human phase I study to evaluate the MEK1/2 inhibitor GDC-0623 in patients with advanced solid tumors. Mol Cancer Ther 2013;12(Suppl.11):B75. doi:10.1007/s10637-016-0374-3.
[47] Huang SM, Temple R, Throckmorton DC, Lesko LJ. Drug interaction studies: study design, data analysis, and implications for dosing and labeling. Clin Pharmacol Ther 2007;81(2):298–304. doi:10.1038/sj.clpt.6100054, PMID:17259955.
[48] Thorne CA, Hanson AJ, Schneider J, Tahinci E, Orton D, Cselenyi CS, *et al*. Small-molecule inhibition of Wnt signaling through activation of casein kinase 1α. Nat Chem Biol 2010;6(11):829–836. doi:10.1038/nchembio.453, PMID:20890287.
[49] Jung YS, Park JI. Wnt signaling in cancer: therapeutic targeting of Wnt signaling beyond β-catenin and the destruction complex. Exp Mol Med 2020;52(2):183–191. doi:10.1038/s12276-020-0380-6, PMID:32037398.
[50] Jung YS, Wang W, Jun S, Zhang J, Srivastava M, Kim MJ, *et al*. Deregulation of CRAD-controlled cytoskeleton initiates mucinous colorectal cancer via β-catenin. Nat Cell Biol 2018;20(11):1303–1314. doi:10.1038/s41556-018-0215-z, PMID:30361697.
[51] Voloshanenko O, Erdmann G, Dubash TD, Augustin I, Metzig M, Moffa G, *et al*. Wnt secretion is required to maintain high levels of Wnt activity in colon cancer cells. Nat Commun 2013;4:2610. doi:10.1038/ncomms3610, PMID:24162018.
[52] Hao HX, Xie Y, Zhang Y, Charlat O, Oster E, Avello M, *et al*. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. Nature 2012;485(7397):195–200. doi:10.1038/nature11019, PMID:22575959.
[53] Horst D, Chen J, Morikawa T, Ogino S, Kirchner T, Shivdasani RA, *et al*. Differential WNT activity in colorectal cancer confers limited tumorigenic potential and is regulated by MAPK signaling. Cancer Res 2012;72(6):1547–56. doi:10.1158/0008-5472.CAN-11-3222, PMID:22318865.
[54] Kahn M. Can we safely target the WNT pathway? Nat Rev Drug Discov 2014;13(7):513–32. doi:10.1038/nrd4233, PMID:24981364.
[55] Moore AR, Rosenberg SC, McCormick F, Malek S. RAS-targeted therapies: is the undruggable drugged? Nat Rev Drug Discov 2020;19(8):533–552. doi:10.1038/s41573-020-0068-6, PMID:32528145.
[56] Cheng Y, Tian H. Current Development Status of MEK Inhibitors. Molecules 2017;22(10):1551. doi:10.3390/molecules22101551, PMID:28954413.
[57] Zaanan A, Okamoto K, Kawakami H, Khazaie K, Huang S, Sinicrope FA. The Mutant KRAS Gene Up-regulates BCL-XL Protein via STAT3 to Confer Apoptosis Resistance That Is Reversed by BIM Protein Induction and BCL-XL Antagonism. J Biol Chem 2015;290(39):23838–23849. doi:10.1074/jbc.M115.657833, PMID:26245900.
[58] Khan I, Rhett JM, O'Bryan JP. Therapeutic targeting of RAS: New hope for drugging the "undruggable". Biochim Biophys Acta Mol Cell Res 2020;1867(2):118570. doi:10.1016/j.bbamcr.2019.118570, PMID:31678118.
[59] Zimmerli D, Cecconi V, Valenta T, Hausmann G, Cantù C, Restivo G, *et al*. WNT ligands control initiation and progression of human papillomavirus-driven squamous cell carcinoma. Oncogene 2018;37(27):3753–3762. doi:10.1038/s41388-018-0244-x, PMID:29662191.
[60] Li H, Jiao S, Li X, Banu H, Hamal S, Wang X. Therapeutic effects of antibiotic drug tigecycline against cervical squamous cell carcinoma by inhibiting Wnt/β-catenin signaling. Biochem Biophys Res Commun 2015;467(1):14–20. doi:10.1016/j.bbrc.2015.09.140, PMID:26427870.
[61] Kahlert UD, Suwala AK, Koch K, Natsumeda M, Orr BA, Hayashi M, *et al*. Pharmacologic Wnt Inhibition Reduces Proliferation, Survival, and Clonogenicity of Glioblastoma Cells. J Neuropathol Exp Neurol 2015;74(9):889–900. doi:10.1097/NEN.0000000000000227, PMID:26222502.
[62] Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: Mission possible? Nat Rev Drug Discov 2014;13(11):828–851. doi:10.1038/nrd4389, PMID:25323927.
[63] Ledford H. Cancer studies clash over mechanisms of malignancy. Nature 2015;528(7582):317. doi:10.1038/528317a, PMID:26672533.
[64] Lu SSM, Mohammed Z, Häggström C, Myte R, Lindquist E, Gylfe Å, *et al*. Antibiotics Use and Subsequent Risk of Colorectal Cancer: A Swedish Nationwide Population-Based Study. J Natl Cancer Inst 2022;114(1):38–46. doi:10.1093/jnci/djab125, PMID:34467395.
[65] Frei E 3rd, Karon M, Levin RH, Freireich EJ, Taylor RJ, Hananian J, *et al*. The effectiveness of combinations of antileukemic agents in inducing and maintaining remission in children with acute leukemia. Blood 1965;26(5):642–656. PMID:5321112.
[66] Ismail M, Khan S, Khan F, Noor S, Sajid H, Yar S, *et al*. Prevalence and significance of potential drug-drug interactions among cancer patients receiving chemotherapy. BMC Cancer 2020;20(1):335. doi:10.1186/s12885-020-06855-9, PMID:32307008.
[67] Falzone L, Salomone S, Libra M. Evolution of Cancer Pharmacological Treatments at the Turn of the Third Millennium. Front Pharmacol 2018;9:1300. doi:10.3389/fphar.2018.01300, PMID:30483135.
[68] Gilad Y, Gellerman G, Lonard DM, O'Malley BW. Drug Combination in Cancer Treatment-From Cocktails to Conjugated Combinations. Cancers (Basel) 2021;13(4):669. doi:10.3390/cancers13040669, PMID:33562300.

[69] Huang L, Hu C, Di Benedetto M, Varin R, Liu J, Wang L, *et al*. Induction of multiple drug resistance in HMEC-1 endothelial cells after long-term exposure to sunitinib. Onco Targets Ther 2014;7:2249–2255. doi:10.2147/OTT.S67251, PMID:25587220.

[70] Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, *et al*. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000;355(9209):1041–1047. doi:10.1016/s0140-6736(00)02034-1, PMID:10744089.

[71] Mokhtari RB, Qorri B, Baluch N, Sparaneo A, Fabrizio FP, Muscarella LA, *et al*. Next-generation multimodality of nutrigenomic cancer therapy: sulforaphane in combination with acetazolamide actively target bronchial carcinoid cancer in disabling the PI3K/Akt/mTOR survival pathway and inducing apoptosis. Oncotarget 2021;12(15):1470–1489. doi:10.18632/oncotarget.28011, PMID:34316328.

[72] Saputra EC, Huang L, Chen Y, Tucker-Kellogg L. Combination Therapy and the Evolution of Resistance: The Theoretical Merits of Synergism and Antagonism in Cancer. Cancer Res 2018;78(9):2419–2431. doi:10.1158/0008-5472.CAN-17-1201, PMID:29686021.

[73] Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature 2013;501(7467):328–337. doi:10.1038/nature12624, PMID:24048065.

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