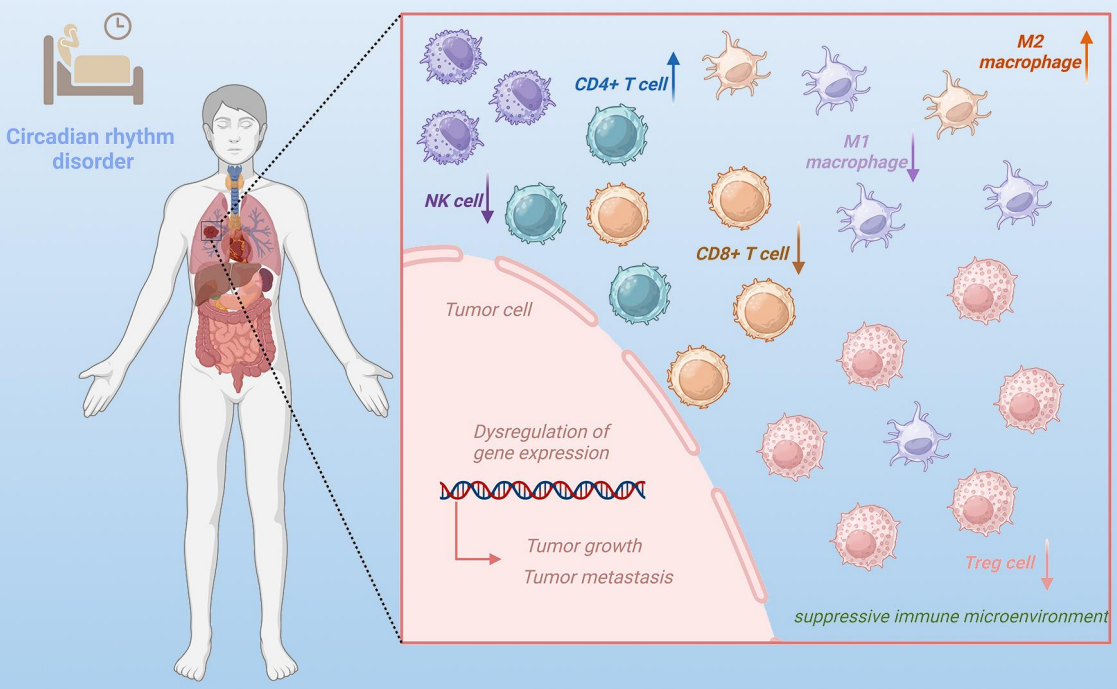
ISSN 2472-0712

Exploratory Research and Hypothesis in Medicine

2025 Volume 10 Issue 2
April



Circadian Rhythms in Tumor Regulation: Impacts on Tumor Progression and the Immune Microenvironment page 135

ERHM

PUBLISHED BY XIA & HE PUBLISHING INC.
eISSN: 2472-0712
Frequency: Quarterly
Launch date: March 6, 2016 (Volume 1, Issue 1)

Contact Information

Editorial Office

Executive Editor: Hua He
Managing Editor: Shi-Bing Zhang
Telephone: +1-409-420-2868
E-mail: erhm@xiahepublishing.com
Postal Address: 14090 Southwest Freeway, Suite 300, Sugar Land, Texas, 77478, USA

Publisher

Xia & He Publishing Inc.
Website: www.xiahepublishing.com
E-mail: service@xiahepublishing.com
Postal Address: 14090 Southwest Freeway, Suite 300, Sugar Land, Texas, 77478, USA

Current Issue: Volume 10, Issue 2

Publication date: April 30, 2025

Aims and Scope

Exploratory Research and Hypothesis in Medicine (ERHM) publishes original exploratory research articles and state-of-the-art reviews that focus on novel findings and the most recent scientific advances that support new hypotheses in medicine, with an emphasis on epidemiology, infectious diseases, cancers, cardiovascular diseases, artificial intelligence, and machine learning. The journal accepts a wide range of topics, including innovative diagnostic and therapeutic modalities as well as insightful theories related to the practice of medicine. ERHM welcomes and encourages article submission of original exploratory research as well as novel and timely state-of-the-art full reviews, mini-reviews, and opinion articles from leading scientists and scholars around the world. Topics of particular interest include but are not limited to: drug mechanism of action and functions; new methods and concepts in the diagnosis, treatment, or prevention of disease; novel discoveries in all diseases and disease mechanisms; associations among diseases; interesting findings in signal transduction; changes in clinical paradigms and treatment approaches; cutting edge technologies that can help improve scientific understanding; preliminary findings that may change current thinking in a particular field.

Indexing & Abstracting

ERHM is now indexed in Scopus, Google Scholar, Semantic Scholar, Scilit, and Dimensions.

Open Access

Disclaimer

Links

Journal Home: https://www.xiahepublishing.com/journal/erhm
Editorial Board: https://www.xiahepublishing.com/journal/erhm/editors
Archive: https://www.xiahepublishing.com/journal/erhm/archive
Instructions for Authors: https://www.xiahepublishing.com/journal/erhm/instruction
Online Submission System: https://mc03.manuscriptcentral.com/erhm

Editorial Board

ERHM

Associate Editors

Editorial Board Members

ERHM

EXPLORATORY RESEARCH AND HYPOTHESIS IN MEDICINE

CONTENTS . . . . . 2025 10(2):77–145

Exploratory Research and Hypothesis in Medicine **2025** vol. 10(2) | 77–86
DOI: 10.14218/ERHM.2025.00013

Original Article

Oral Microbiota Transmission Partially Mediates Depression and Anxiety in Newlywed Couples

Reza Rastmanesh[1*], Balachandar Vellingiri[2], Ciro Gargiulo Isacco[3], Abolfazl Sadeghinejad[4] and Neil Daghnall[5]

[1]Independent Researcher, # 6 Physicians Building, Sarshar Alley, Tajrish, Tehran, Iran; [2]Human Cytogenetics and Stem Cell Laboratory, Department of Zoology, Central University of Punjab, Bathinda, Punjab, India; [3]Department of Interdisciplinary Medicine, U.O.C. Microbiology and Virology, University-Hospital of Bari, Bari, Italy; [4]Otorhinolaryngology Department, Private Clinic, Tehran, Iran; [5]Deapartment of Psychology and Social Change, Manchester Metropolitan University, Manchester, UK

Received: March 07, 2025 | **Revised:** April 19, 2025 | **Accepted:** April 28, 2025 | **Published online:** April 30, 2025

Abstract

Background and objectives: Oral microbiota dysbiosis and altered salivary cortisol levels have been linked to depression and anxiety. Given that bacterial transmission can occur between spouses, this study aimed to investigate whether the transmission of oral microbiota between newlywed couples mediates symptoms of depression and anxiety.

Methods: Validated Persian versions of the Pittsburgh Sleep Quality Index, Beck Depression Inventory-II, and Beck Anxiety Inventory were administered to 1,740 couples who had been married for six months. The researchers compared 268 healthy control spouses with 268 affected cases in a cross-sectional study. Data were analyzed using appropriate statistical methods.

Results: After six months, healthy spouses married to an insomniac with the depression-anxiety (DA) phenotype scored significantly higher on the Pittsburgh Sleep Quality Index, Beck Depression Inventory-II, and Beck Anxiety Inventory compared to their baseline scores. This indicates that their sleep quality, depression, and anxiety scores became more similar to those of their affected spouses. Additionally, the composition of their oral microbiota changed significantly, becoming increasingly similar to that of their spouses. Specifically, in couples where one partner had the DA phenotype, the oral microbiota of the healthy spouse mirrored that of the affected partner ($p < 0.001$). These microbial changes correlated with alterations in salivary cortisol levels as well as depression and anxiety scores. Linear discriminant analysis revealed that the relative abundances of *Clostridia*, *Veillonella*, *Bacillus*, and *Lachnospiraceae* were significantly higher in insomniacs with the DA phenotype compared to healthy controls ($p < 0.001$).

Conclusions: Oral microbiota transmission between individuals in close contact partially mediates symptoms of depression and anxiety.

Introduction

Oral microbiota dysbiosis is significantly correlated with various neuropsychiatric disorders, including autism spectrum disorder, dementia, Parkinson's disease, schizophrenia, anxiety, epilepsy, and depression.[1] Wingfield *et al*.[2] demonstrated that the composition of the oral microbiota is significantly associated with depression in young adults who met the criteria for depression outlined in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (hereinafter referred to as DSM). They found that 21 bacterial taxa exhibited significantly different levels of abundance in depressed young adults. Specifically, there was an increase in Prevotella nigrescens and Neisseria spp., while 19 taxa showed a decrease in abundance. Oral microbiota exhibits variability influenced by individual factors, including oral hygiene practices, substance use, and tobacco consumption.[1]

Recently, Simpson *et al*.[3] demonstrated that the composition of the oral microbiota significantly correlates with symptoms of anxiety and depression in adolescents. Similar patterns have been observed in other populations, including pregnant women,[4] patients experiencing burning mouth syndrome with psychiatric

Keywords: Oral microbiome; Bacterial transmission; Depression; Anxiety; Salivary cortisol; Psychology; Bacterial establishment.

[*]**Correspondence to:** Reza Rastmanesh, Independent Researcher, # 6 Physicians Building, Sarshar Alley, Tajrish, Tehran 1961835555, Iran. ORCID: https://orcid.org/0000-0002-6221-9062. Tel: +98-22750414, E-mail: r.rastmanesh@gmail.com

How to cite this article: Rastmanesh R, Vellingiri B, Isacco CG, Sadeghinejad A, Daghnall N. Oral Microbiota Transmission Partially Mediates Depression and Anxiety in Newlywed Couples. *Explor Res Hypothesis Med* 2025;10(2):77–86. doi: 10.14218/ERHM.2025.00013.

symptoms,[5] individuals with anxiety, mood disorders, trauma- and stress-related disorders,[6] and those with irritable bowel syndrome,[7] among others.

Salivary cortisol does not directly cause depression or anxiety but serves as an indicator of these disorders. The suggested mechanism of action is that the oral microbiota may directly compromise the blood-brain barrier or exert indirect effects through the oral microbiota-brain axis.[1]

On the other hand, a diminished cortisol awakening response is linked to an increased likelihood of experiencing a negative and persistent progression of depression and/or anxiety disorders.[8,9]

The first author has previously discussed how closeness may facilitate bacterial transmission and, consequently, impact microbiota-related research.[10] In various statistical simulations, I demonstrated significant differences in the occurrence of microbiota phenotypes between two groups of otherwise healthy spouses of individuals with a specific disease condition. These differences can largely be attributed to varying levels of social closeness. It is important to note that social closeness refers to one's sense of belonging and physical and psychological bonds in personal relationships. More specifically, it is defined as the average distance from a given node (a small group of individuals) to all other nodes (a higher number of small groups or nodes). A network consists of a collection of nodes (representing variables or the same small group of individuals) and edges (representing connections between or among these small groups) that link the nodes. The width of an edge indicates the strength of the relationship between the connected nodes, with wider edges signifying stronger associations. To assess the significance of each node within the network, three centrality measures—strength, betweenness, and closeness—are utilized.[11]

Prior research has identified various forms of physiological synchrony between couples, including synchrony in diurnal cortisol patterns,[12] cardiac synchrony,[13] and sleep concordance.[14] The bidirectional associations between sleep disturbances and ocular surface parameters,[15,16] in conjunction with other physiological synchronies observed in couples,[12–14] lead the authors to hypothesize that the transmission of oral microbiota partially mediates depression and anxiety. Furthermore, cortisol is recognized as a biomarker for anxiety and depressive states in couples and partners.[8,9]

Based on these interconnected premises, we hypothesized that oral microbiota partially mediates psychometric parameters in newlywed couples through person-to-person contact. To assess this hypothesis, the researchers enrolled couples in which one spouse simultaneously experienced depression and insomnia (referred to as the depression-anxiety (DA) phenotype,[17] see *Psychometric assessments* under Materials and methods). To facilitate contact, the spouses lived in the same household. We aimed to investigate whether oral bacterial transmission among newlywed couples partially mediates depression and anxiety.

Materials and methods

Participants

This is a cross-sectional longitudinal study. Data were collected prospectively from two private sleep clinics in Tehran, Iran, between February and October 2024. Our primary outcome measures included the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Pittsburgh Sleep Quality Index (PSQI), serum cortisol levels, and the composition of oral microbiota.

The sample size required for subgroup comparisons was determined by Placzek *et al*.[18] Accordingly, 422 samples (211 in the case group and 211 in the control group) were deemed sufficient. However, we enrolled 268 participants as cases and 268 as controls, as we had sufficient budget and resources. This ensured even greater statistical power than initially calculated.

We sent invitations to all 1,740 couples at the two private clinics to attend briefing sessions about the study. Data were collected using paper-based self-administered questionnaires, which were distributed by a healthcare assistant. Both otherwise healthy controls and individuals with insomnia and/or hypersomnia were screened based on their scores on the BAI, BDI, and PSQI at baseline and at the six-month follow-up.

Screening for insomnia, depression, and anxiety

For the case group, inclusion criteria consisted of having insomnia and/or hypersomnia, as determined by the self-administered PSQI,[19] and experiencing depressive or anxiety states based on the validated Persian versions of the BDI-II and the BAI.[20,21] Exclusion criteria included the use of medications known to affect gut, oral, or ocular microbiota composition, pregnancy, divorce during the study, antibiotic use in the past month, ongoing active ocular infections (including conjunctivitis), and a lack of prior history with dry eye disease (DED). Otherwise healthy controls were included based on a comprehensive clinical examination. Participants were asked to specify the exact date of their official marriage and to indicate whether they were cohabiting in the same household.

Participants who had been married within the past six months and were in a cohabiting relationship were screened for the presence or absence of insomnia. Those selected were enrolled in the study along with their official spouses. Two hundred ninety-six couples were selected, comprising healthy spouses and insomniacs with a DA phenotype.

Three couples were excluded from the study because the women were either taking antibiotics known to affect the composition of oral microbiota or were pregnant. None of the female spouses were using medications known to interact with the hypothalamic-pituitary-adrenal axis. Seventeen participants were excluded due to low readings (n = 10) or missing data (n = 7). Additionally, one couple had divorced and had spent significant time living apart, while seven couples who had relocated to another city were also excluded.

The remaining 268 couples lived together in the same household. All spouses were instructed to maintain their baseline dietary habits, oral hygiene practices, and exercise routines. On Day 1 and Day 180, all couples participated in a study measuring oral microbiota composition and salivary cortisol levels. Data collected from the 268 couples were analyzed. A diagram illustrating the categorization and enrollment of participants is shown in Figure 1.

Psychometric assessments

The validated Persian versions of the BDI-II,[19] BAI,[21] and PSQI,[20] were utilized to assess depression, anxiety, and sleep quality, respectively. Participants were then categorized into two groups: (i) healthy spouses and (ii) insomniac spouses. Each group was further divided based on BDI-II and BAI scores into categories of "moderate depression" and "moderate anxiety". A combined DA phenotype was defined as having a BAI score between 16 and 25 and a BDI-II score of 14 or higher.[17]

The BDI-II is a widely used 21-item self-report inventory designed to assess depressive symptoms experienced over the past two weeks. Higher scores indicate more severe depressive symp-

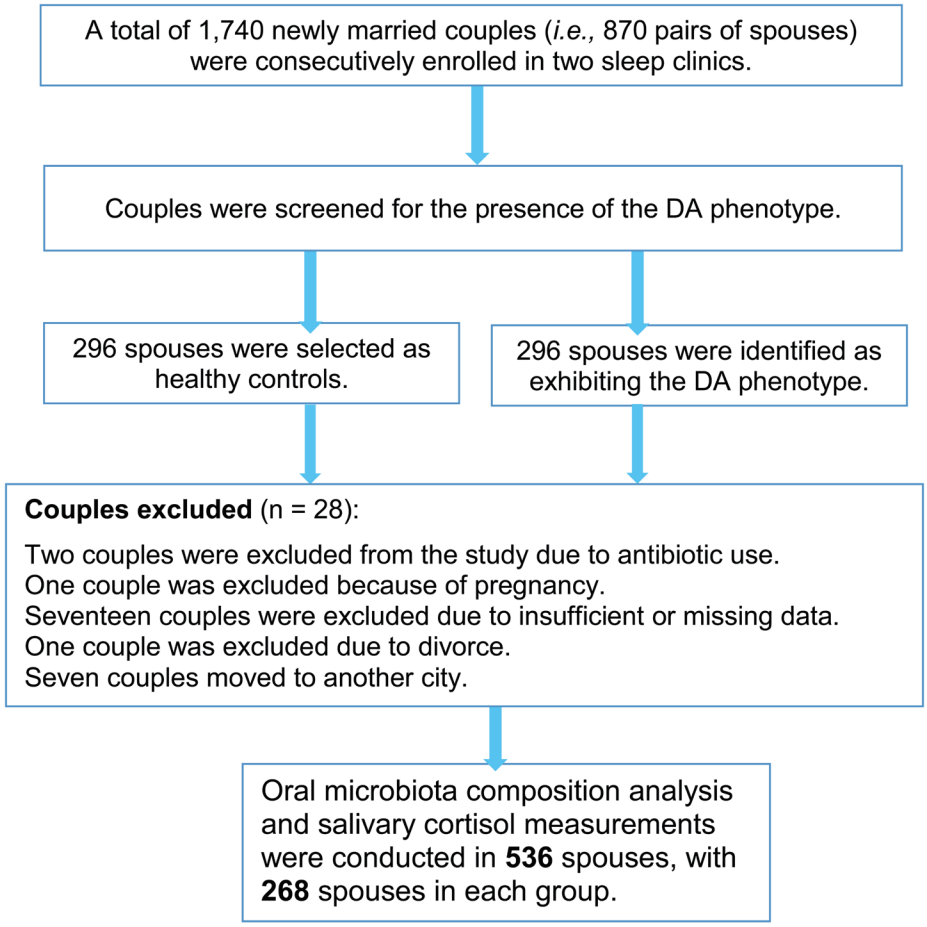


Fig. 1. Diagram showing the categorization of participants based on sleep disturbance status and depression-anxiety (DA) phenotype.

tomatology. In this study, depression was defined as a BDI-II score of 14 or higher.[22]

The self-administered PSQI questionnaire assesses sleep quality over the past month. Scores range from 0 to 21, with lower scores indicating better sleep quality. A score higher than 5 is considered indicative of poor sleep quality. Insomnia, defined as the coexistence of difficulty resuming sleep and daytime dysfunction, is referenced in the literature.[23]

The BAI questionnaire consists of 21 items, each scored on a scale from 0 to 3. A score of 0 indicates "not at all", 1 indicates "mildly, but it did not bother me much", 2 indicates "moderately, it was not pleasant at times", and 3 indicates "severely, it bothered me a lot" for all the items. The total score can range from 0 to 63, reflecting varying levels of anxiety. Scores from 0 to 7 indicate a minimal range (no anxiety), scores of 8–15 indicate mild anxiety, scores of 16–25 indicate moderate anxiety, and scores ≥26 represent severe anxiety. Furthermore, in comparison to the category of no anxiety, the three categories of mild, moderate, and severe anxiety are collectively considered as "yes".[24]

We did not include any spouses with severe symptoms who would require additional support.

Oral parameters

Oral samples were collected during clinic visits from enrollment until five to six months after marriage, as briefly described below.

Oral microbiota

We adhered to the guidelines established by Brzychczy-Sroka *et al*.[25] Accordingly, oral samples were collected from the palatine tonsils at the end of the examination, prior to the collection of posterior pharyngeal swabs. Swab samples were obtained from both the palatine tonsils and the pharynx. The samples were preserved in saline, placed in ziplock bags, and stored in portable freezer bags at −20 °C before being promptly delivered to the laboratory (within a maximum of 1–2 h). Samples were collected on three occasions: at baseline and again six months later.

The samples were then frozen at −80 °C until analysis. One of the researchers (RR) secured the collected materials in MoBio buffer and placed them in a small ice-filled cooler for transport, without specifying a precise transport temperature, as recommended by Brzychczy-Sroka *et al*.[25] The final materials were delivered to the laboratory within 3–4 h.

Bacterial DNA was extracted from each clinical sample. The subsequent step involved amplifying the bacterial DNA using polymerase chain reaction, specifically targeting the V3–V4 region of the 16S ribosomal RNA subunit. The resulting amplicons were utilized to create a genomic library through several stages: purification of the polymerase chain reaction products, indexing of the samples, and re-purification. Following this, the samples were quantified using fluorometry, and the genomic library was combined for next-generation sequencing on the MiSeq platform

Table 1. Baseline characteristics of participant spouses

	Healthy spouses (n = 268)	Insomniacs with depressive-anxiety phenotype* (n = 268)	*p*-value
No (%)	268 (50)	268 (50)	-
Age (years)	34.02 ± 8.1	34.70 ± 8.80	NS
Males	37.41 ± 7.96	38.02 ± 8.02	NS
Females	31.08 ± 8.5	32.11 ± 9.01	NS
Body mass index (kg/m^2)	23.33 ± 3.82	23.25 ± 3.60	NS
Males	24.98 ± 4.03	23.80 ± 4.24	NS
Females	23.10 ± 2.90	23.90 ± 3.51	NS
Beck depression inventory II	6.60 ± 2.20	15.60 ± 9.10	0.004**
Beck anxiety inventory	12.10 ± 4.90	24.80 ± 3.40	0.0001**
Global PSQI score	5.40 ± 2.80	8.10 ± 3.20	0.0001**
Salivary cortisol (ng/mL)	10.40 ± 13.20	39.10 ± 11.65	0.0001**
Socioeconomic status			
High	8 (2.70)	10 (3.37)	NS
Medium-high	291 (97.30)	282 (96.63)	NS

*A combined DA phenotype in this study was defined as the simultaneous presence of a Beck Anxiety Inventory score of 16–25 and a Beck Depression Inventory-II score of 14 or higher. Insomnia (sleeplessness) was defined as the coexistence of both difficulty resuming sleep and daytime dysfunction. **The Student's t-test revealed that insomniacs exhibiting a depressive-anxiety phenotype scored significantly higher on measures of depression (test statistic T = −15.7374), anxiety (test statistic T = −34.8601), and insomnia (test statistic T = −10.3952), and had notably elevated salivary cortisol levels (test statistic T = −26.6867) compared to their healthy spouses. DA, depression-anxiety; NS, non-significant; PSQI, Pittsburgh Sleep Quality Index.

(Illumina, San Diego, California, United States). The process of developing the genomic library for sequencing has been detailed in previous work.[26]

Salivary cortisol

Salivary samples were collected from participants using Oragene OG-500 kits (DNA Genotek, Ontario, Canada), which facilitate self-collection and stabilization of DNA at room temperature. Participants were instructed to refrain from consuming any food or drink, except water, for at least 30 m prior to sample collection. Salivary cortisol was measured using liquid chromatography-tandem mass spectrometry as previously described.[27]

All saliva specimens were stored at −80 °C for subsequent analysis. We utilized samples collected immediately upon awakening, prior to drinking, eating, or performing oral hygiene.

Covariates

Gender, age, body mass index (BMI), alcohol consumption and smoking status, total dietary sugar intake, and the presence of chronic kidney disease or hypertension were considered as covariates. Hypertension was defined as an average systolic blood pressure of ≥140 mmHg and an average diastolic blood pressure of ≥90 mmHg, or the use of antihypertensive medications, or a physician's diagnosis. Chronic kidney disease was defined as an estimated glomerular filtration rate of <60 mL/m/1.73 m^2.

Statistical analysis

Statistical analyses were conducted using SPSS software (version 17.0; SPSS, Chicago, IL). Differences between groups and subgroups were analyzed using Student's t-test for continuous parameters and the χ^2 test for categorical parameters. Where appropriate, the Bonferroni correction was applied to adjust for multiple testing. Intragroup changes were compared using a paired t-test. Primary and secondary endpoints were analyzed using analysis of covariance, with groups as fixed factors and baseline measurements as covariates. Furthermore, Pearson's and Spearman's correlation tests were employed to explore correlations between oral microbiota, anxiety, depression, and insomnia. Oral microbiota analysis was conducted using QIIME 2 version 2019.07.[28] Processed data were imported into phyloseq version 1.28.024 for further analysis.[29] Beta diversity was evaluated using Shannon's diversity index and Bray-Curtis dissimilarity. For a detailed description of the methodology used for bioinformatic processing, please refer to Wingfield B. *et al*.'s study.[2] To determine which taxa may be correlated with the DA phenotype, we performed L2-regularized logistic regression using the mikropml package in R,[30] a commonly applied methodology for conducting differential abundance analysis of microbiota data.[31]

Socioeconomic status was evaluated utilizing data on family size, parental educational qualifications, possession of a vehicle and particular household appliances, the square footage of the family's dwelling, and the average monthly income of the household.[32]

Results

Table 1 presents the baseline characteristics of the participants. Demographic factors, including gender, age, BMI, and socioeconomic status, were comparable between the healthy control group and the DA phenotype group. The mean ± standard deviation for age among male and female spouses was 37.20 ± 8.01 years and 31.02 ± 9.30 years, respectively. Couples had been married and cohabiting for an average of 5.91 ± 2.03 months. As expected, there were significant differences in salivary cortisol levels, global PSQI, BDI-II, and BAI scores between the healthy controls and the insomniacs in the DA phenotype group at baseline.

Table 2. The healthy spouses who married an insomniac with a depressive-anxiety phenotype had higher psychopathology and salivary cortisol compared to their own baseline

	Healthy spouses	Same healthy spouses married to an insomniac with depressive-anxiety phenotype*	*p*-value
	Baseline values (n = 268)	*Six-months values (n = 268)*	
Depression (BDI-II)	6.60 ± 2.20	9.40 ± 8.20	
Males	6.50 ± 2.40	9.00 ± 7.70	0.0001[a]
Females	8.20 ± 4.70	10.80 ± 1.20	0.0001[a]
Anxiety (BAI)	12.10 ± 4.90	17.60 ± 4.90	
Males	11.90 ± 5.30	15.10 ± 1.70	0.0001[a]
Females	14.20 ± 6.90	19.40 ± 5.30	0.0001[a]
Global PSQI score	5.40 ± 2.80	7.80 ± 5.70	
Males	5.20 ± 1.90	7.50 ± 5.30	0.0001[a]
Females	6.10 ± 3.20	8.10 ± 4.80	0.0001[a]
Salivary cortisol (ng/mL)	10.40 ± 13.20	17.50 ± 18.30	
Males	9.60 ± 14.10	12.10 ± 16.20	0.05[a]
Females	11.30 ± 8.40	20.80 ± 13.50	0.0001[a]

*A combined depression-anxiety phenotype in this study was defined as the simultaneous presence of a Beck Anxiety Inventory (BAI) score between 16 and 25 and a Beck Depression Inventory-II (BDI-II) score of 14 or higher. Moderate depression was characterized by a BDI-II score of 14 or higher, while moderate anxiety was indicated by a BAI score between 16 and 25. [a]The paired t-test for intra-group comparisons indicated that six months after marrying an insomniac with a DA-phenotype, healthy spouses exhibited significantly higher scores for depression, anxiety, and insomnia compared to their own baseline measurements. These differences were more pronounced in females than in males. DA, depression-anxiety; PSQI, Pittsburgh Sleep Quality Index.

Psychometric parameters

Table 2 shows that after six months, healthy spouses married to an insomniac with the DA phenotype scored significantly higher on PSQI, BDI-II, and BAI compared to their own baseline values. This indicates that sleep quality, depression, and anxiety scores changed and became more similar to those of their insomniac spouses, although they remained significantly lower than those of the insomniac group (all *p*-values <0.001). After six months, insomniacs with the DA phenotype exhibited a trend of increased salivary cortisol levels, as well as higher global PSQI, BDI-II, and BAI scores; however, these values did not reach statistical significance (non-significant differences are not shown).

In the gender subgroup analysis, the worsening of insomnia severity, an increase in salivary cortisol levels, and heightened depression and anxiety scores were more pronounced among female spouses after six months (Table 2).

In the crude model, insomnia severity was positively associated with anxiety and depression scores (odds ratio (OR): 1.62, 95% confidence interval (CI): 1.32–2.19, $p < 0.001$ for anxiety; OR: 2.30, 95% CI: 2.10–2.75, $p < 0.001$ for depression). After adjusting for age, gender, BMI, and education, insomnia continued to show a positive correlation with depression and anxiety scores, indicating a 1.8-fold and 2.1-fold increase, respectively, in odds compared to healthy spouses with normal sleep (OR: 1.8, 95% CI: 1.40–2.52, $p < 0.001$ for anxiety; OR: 2.1, 95% CI: 2.01–2.73, $p < 0.001$ for depression).

Salivary cortisol differences

Table 2 presents the results of a t-test analysis of salivary cortisol levels. In both male and female spouses with the DA phenotype, salivary cortisol was significantly higher in insomniac spouses at baseline compared to healthy control spouses ($p < 0.0001$). After six months, the salivary cortisol levels in spouses married to an insomniac with the DA phenotype were significantly elevated compared to baseline values ($p < 0.001$). This finding suggests that healthy spouses were likely to resemble their insomniac partners with the DA phenotype. Gender subgroup analysis indicated that the increase in salivary cortisol levels was more pronounced in female spouses than in male spouses.

Oral microbiota characteristics

From the perspective of taxa composition, a total of 33 bacterial phyla were identified. Linear discriminant analysis (LDA) of oral microbiota composition showed that the relative abundances of *Clostridia*, *Veillonella*, *Bacillus*, and *Lachnospiraceae* were significantly higher in the DA phenotype spouses than in healthy controls ($p < 0.001$, LDA scores >2, alpha error = 0.01).

A high-level analysis of phyla differences revealed that the composition of oral microbiota in healthy spouses with normal sleep patterns was significantly altered, becoming similar to that of their partner. Specifically, if one spouse exhibited a DA phenotype, the oral microbiota composition of the other spouse mirrored that of the DA phenotype partner ($p < 0.001$, LDA scores >2, alpha error = 0.01) (Table 3).

The relative abundance of *Fusobacteria* (r = +0.49–0.57), *Patescibacteria* (r = +0.38–0.42), *Campylobacterota* (r = +0.32–0.36), *Spirochaetota* (r = +0.42–0.52), and *Gracilibacteria* (r = +0.29–0.37) in the oral microbiota was positively correlated with the severity of insomnia in individuals with the DA phenotype (all *p*-values <0.01).

We applied an L2-regularized logistic regression model to next-generation sequencing data to investigate potential associations between the oral microbiome and the DA phenotype. This model indicated that *Fusobacteria*, *Patescibacteria*, *Campylobacterota*, *Spirochaetota*, and *Gracilibacteria* may be associated with the DA phenotype.

Table 3. Baseline and six-month comparison of oral microbiota in healthy spouses and spouses married to individuals with depression-anxiety phenotype*

Oral microbiota in healthy spouses at baseline		Oral microbiota in healthy spouses six months after marriage			*p*-value
Phyla	**% of abundance**	**Phyla**	**% of abundance**	**The absolute percent difference between the two groups**	
Order of rank abundance					
Firmicutes	36.10	*Firmicutes*	31.42	4.68	$0.005^{a,b}$ $NS^{c,d}$
Bacteroidetes	17.88	*Bacteroidetes*	27.81	9.93	$0.001^{a,b}$ $NS^{c,d}$
Proteobacteria	17.42	*Proteobacteria*	20.60	3.18	$0.01^{a,b}$ $NS^{c,d}$
Actinomycetota	11.33	*Fusobacteria*	8.03	-[e]	-[e]
Spirochaetes	7.67	*Actinomycetota*	5.30	-[e]	-[e]
Fusobacteria	5.40	*Patescibacteria*	3.47	-[e]	-[e]
Six above-mentioned phyla contain ~96% of the taxa. The rest of phyla contain approximately 4% of the taxa					
TM7 12	1.88	Campilobacterota	1.40	-[e]	-[e]
Synergistetes	1.62	Spirochaetota	1.02	-[e]	-[e]
Chlamydiae	0.20	Gracilibacteria	0.03	-[e]	-[e]
Other rare phyla	0.50	Other rare phyla	<0.92	0.42	$0.01^{a,b,c}$ 0.001^{d}

Oral microbiota in healthy spouses six months after marriage		Oral microbiota in insomniacs six months after marriage			*p*-value
Phyla	**% of abundance**	**Phyla**	**% of abundance**	**The absolute percent difference between the two groups**	
Order of rank abundance					
Firmicutes	30.20	*Firmicutes*	31.50	1.3	$0.005^{a,b}$ $NS^{c,d}$
Bacteroidetes	28.93	*Bacteroidetes*	27.51	1.42	$0.001^{a,b}$, $NS^{c,d}$
Proteobacteria	20.60	*Proteobacteria*	20.89	0.29	$0.01^{a,b}$, $NS^{c,d}$
Fusobacteria	7.98	*Fusobacteria*	8.14	0.16	$NS^{a,c,d}$, 0.01^{b}
Actinomycetota	5.69	*Actinomycetota*	5.12	0.57	$0.0001^{a,b}$, $NS^{c,d}$
Patescibacteria	3.40	*Patescibacteria*	3.23	0.17	$0.0005^{a,b}$, $NS^{c,d}$
Six above-mentioned phyla contain ~96% of the taxa. The rest of phyla contain approximately 4% of the taxa					
TM7 12	1.40	*Campilobacterota*	2.38	-[e]	-[e]
Spirochaetota	1.02	*Spirochaetota*	1.04	0.02	$0.0001^{a,b}$, $NS^{c,d}$
Gracilibacteria	0.03	*Gracilibacteria*	0.05	0.02	$0.01^{a,b,c,d}$
Other rare phyla	<0.75	*Other rare phyla*	<0.14	0.61	$0.01^{a,b}$, 0.001^{c}, 0.0005^{d}

*The DA phenotype is defined as the simultaneous presence of a Beck Anxiety Inventory score between 16 and 25 and a Beck Depression Inventory-II score of 14 or higher. [a]Comparison of the healthy group versus insomniacs at baseline. [b]Comparison of the healthy group versus itself after six months. [c]Comparison of healthy individuals versus insomniacs after six months. [d]Comparison of insomniacs versus insomniacs after six months.[e]The columns are left blank, indicating that the corresponding rankings for the phylum do not match. The composition of oral microbiota in the group of healthy spouses with normal sleep patterns was significantly altered, becoming similar to that of their respective partners. Specifically, if a spouse exhibited a DA phenotype, the oral microbiota composition of the other spouse also resembled that of the DA phenotype partner (all *p*-values <0.0001). At baseline, the three most abundant phyla in both healthy and insomniac spouses were *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, followed by *Actinomycetota*, *Spirochaetes*, and *Fusobacteria* in healthy spouses, and *Fusobacteria*, *Actinomycetota*, and *Patescibacteria* in insomniac spouses. Six months after marriage, the ranking of phyla changed; however, the top three phyla maintained their positions, albeit with some variations in their relative abundances. In healthy spouses, the phyla *Fusobacteria* and *Patescibacteria* were competitively displaced by *Actinomycetota* and *Spirochaetes*, respectively, after six months. The remaining ten phyla (i.e., *TM7*, *Synergistetes*, *Chlamydiae*, and other rare phyla) typically remained unchanged between the two groups of healthy spouses and insomniacs after six months. In terms of ranking, after six months of marriage to an insomniac, the most significant microbial shift was observed in the phyla *Fusobacteria*, *Patescibacteria*, and *Spirochaetes* among healthy spouses (*p*-value <0.01). In terms of percentage change, the most significant alterations were observed in the phyla *Bacteroidetes* (11.05% increase), *Proteobacteria* (3.18% increase), *Spirochaetes* (6.65% decrease), *Firmicutes* (5.9% decrease), *Actinomycetota* (5.64% decrease), and *Fusobacteria* (2.58% increase). In individuals with insomnia, after six months, both the ranking and percentage of the top ten phyla remained nearly unchanged (not significant). DA, depression-anxiety; NS, non-significant.

Gender differences

LDA and multivariable association analysis revealed multiple distinct bacterial genera that were significantly different in abundance between female and male spouses with the DA phenotype ($p < 0.001$, LDA scores >2, alpha error = 0.05). The phylum *Proteobacteria* was significantly more abundant in female spouses with the DA phenotype compared to their male counterparts. Additionally, certain members of the phyla *Firmicutes* and *Bacteroidetes* were also significantly more abundant in these female spouses. Interestingly, the genus *Dialister* (family *Firmicutes*) was found to be significantly more abundant in female spouses with the DA phenotype than in male spouses (all *p*-values <0.001).

Mediation analysis

To better understand the role of oral microbiota in salivary cortisol levels and DA phenotype status, we conducted an assessment of interaction effects and mediation analyses. We hypothesized that there are interaction effects among these three variables, with oral microbiota status serving as the potential mediator in the relationship between salivary cortisol and DA phenotype status. Indeed, the oral microbiota pattern is a predictor of DA phenotype status ($\beta = 0.37$, $p < 0.001$), which, in turn, is also a relevant predictor of salivary cortisol levels ($\beta = 0.15$, $p < 0.001$). The mediation analysis accounted for 35% of the variability in the data ($R^2 = 0.35$).

Discussion

Oral microbiota transfer between individuals in close contact, such as couples in the present study, may mediate depression and anxiety. Although there are no directly comparable human studies, there is substantial evidence of bacterial exchange between humans and dogs and livestock.[33,34] It is important to note that the results of studies investigating bacterial transmission from animals to humans may not be directly applicable to human studies. Nevertheless, there are reports of penile and genital microbiota exchange between partners.[35] These findings underscore the significance of bacterial exchange as a potential mediating mechanism for mood synchrony between spouses and partners. Various forms of physiological synchrony between couples have been documented, including cardiac synchrony,[13] diurnal cortisol pattern synchrony,[12] and sleep concordance.[4,14,36]

We found that changes in oral microbiota composition are associated with changes in the severity of insomnia, salivary cortisol levels, and depression and anxiety scores. Our findings align with previous studies on salivary cortisol levels as well as depression and anxiety scores.[2–7,12,37–41]

Recently, in a large cohort of couples who had been married and living together for an average of 5.91 months, we demonstrated that sleep disturbances can be partially attributed to changes in the gut microbiota.[15] Additionally, in another study, we identified a significant association between ocular microbiota and DED in individuals with insomnia, which may have been mediated through person-to-person contact.[16] In that study, we found that six months after marriage, spouses married to an insomniac exhibiting the DED phenotype were significantly more likely to develop DED during the six-month follow-up. Supporting our initial hypothesis, these changes occurred in parallel with alterations in ocular microbiota composition.[16]

The oral and gut microbiomes are interconnected,[42] exhibiting both distinct similarities and differences.[43] Additionally, established connections exist between oral microbiota and ocular microbiota.[44] Overall, these findings suggest that these networks are interrelated. Current literature supports this conclusion, indicating that the frequency and prevalence of DED in individuals with depression or anxiety are significantly higher than in healthy individuals, and vice versa.[45] Therefore, our findings have important implications for holistic medicine, family medicine, and personalized medicine. The practical and theoretical implications of this study encompass a wide range of areas, including sleep therapy and psychological states.

This research was a non-invasive, prospective observational study; however, there is compelling preclinical evidence supporting our hypothesis that transplantation of fecal microbiota from patients with depression to microbiota-depleted rats can induce physiological and behavioral characteristics typical of depression in the recipient animals.[46] Furthermore, Lee *et al*.[47] demonstrated that fecal microbiota-induced insomnia, immobilization stress, and depression-like behaviors in a mouse model can be alleviated by microbiota-modulating probiotics. These two reports strongly suggest a causal association between changes in microbiota and psychometric parameters such as depressive states and anxiety.[46,47] However, caution is necessary when translating findings from animal models to human studies. Taken together, our preliminary findings provide evidence that the association between changes in oral microbiota and mood alterations, or their synchrony in humans, is causally related.[47]

Future research should further evaluate this hypothesis.

Strength

Firstly, this study employed a robust methodology, utilizing a large sample size to ensure an effective follow-up program. Secondly, to our knowledge, this is the first study of its kind to investigate a wide range of variables simultaneously. Lastly, while the participants were not homogeneous, the focused choice of this specific group allowed us to examine the short- to medium-term impacts of bacterial transmission on depression and anxiety in a real-life setting.

Limitations

After six months, the worsening of insomnia severity, depression, and anxiety scores was more pronounced in female spouses. It is possible that we underestimated or underreported the frequency of insomnia, depression, and anxiety in male spouses, as we used PSQI, BDI-II, and BAI to estimate insomnia, depressive, and anxiety symptoms, respectively. It is important to acknowledge that our participant couples were aware of the study's purpose due to ethical constraints. Previous well-controlled and well-conducted research has identified a main effect of condition, indicating that both males and females reported significantly higher levels of insomnia severity,[48] depression,[22] and anxiety symptoms in the covert condition.[49] This suggests that we may have underestimated or underreported the frequency of insomnia, depression, and anxiety in male spouses, while potentially overestimating or overreporting these parameters in female spouses. Despite controlling for the most significant covariates, additional residual confounding may still exist. For instance, dietary intake is an important factor influencing the composition of the gut microbiome. Since couples were not randomly selected, the results may not be fully generalizable to all circumstances. We only measured morning salivary cortisol, and measuring salivary cortisol over three consecutive days could improve accuracy. While the primary sampling sites within the oral cavity include saliva, the supragingival region, the subgingival/submucosal area, infected root canals, and mucosal surfaces, our

assessment was limited to the palatine tonsils and pharynx due to financial constraints.

It can be argued that deriving a conclusive understanding from our preliminary findings is challenging without controlling for confounders (e.g., shared diet, stress exposure, and frequency of intimacy). Additionally, it is possible that the spouses may have underlying health issues that have not been verified through evaluations, which could affect the results. We acknowledge that the BDI-II has been criticized for its limitations in the absence of psychiatric assessments. However, the BDI-II is widely employed in non-clinical samples,[50] and in the present study, we did not include any spouses with severe symptoms requiring psychological care. Finally, we only employed the BDI-II and BAI, whereas clinical diagnoses of anxiety and depression based on the DSM would be a more precise method. We suggest that these important variables should be fully considered in future studies.

Future directions

Future research may involve the recruitment of clinical samples. Furthermore, since the body microbiota is implicated in many other diseases, it will be necessary to investigate the possibility of bacterial involvement in other seemingly non-communicable psychological and neurological conditions, as well as in other non-neurological conditions. Additionally, animal models will be instrumental in determining whether such relationships are causal. We also suggest that future research account for the possibility of bias in the study design due to the potential confounding effects created by bacterial transmission among individuals.

Conclusions

The transmission of oral microbiota plays a partial role in mediating depression and anxiety among couples. Since this study is associational, further research is needed to establish whether this association is causal. If it is determined that this association is indeed causal, it could have significant implications for contemporary research. We propose that, within the framework of diagnostic, predictive, preventive, and personalized medicine, the practical and theoretical implications of this study may enhance our understanding of various aspects of microbiota-host interactions.

Acknowledgments

We would like to thank the couples who participated in this study. We also wish to express our sincere gratitude to Dr. Javid Azizi for his generous financial support of this project and his careful involvement in each stage of the work. Finally, we would like to thank the managers of private sleep clinics for their excellent cooperation.

Funding

This work was generously funded by a private donation (Dr. Javid Azizi).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Study design (RR, CGI, ND), manuscript conception (BV, AS), coordination of research implementation, provision of medical data (RR, AS), writing of the manuscript (RR, BV), research analysis (RR, CGI). All authors have read and approved the final manuscript. RR is responsible for the overall content (as guarantor).

Ethical statement

The study was approved by the ethics committee of the Iran National Science Foundation (Research Ethical Code INSF: 98R026323-2024) and adhered to the tenets of the Declaration of Helsinki. Participants gave informed consent to participate in the study before taking part.

Data sharing statement

Data are available on reasonable request.

References

[1] Tao K, Yuan Y, Xie Q, Dong Z. Relationship between human oral microbiome dysbiosis and neuropsychiatric diseases: An updated overview. Behav Brain Res 2024;471:115111. doi:10.1016/j.bbr.2024.115111, PMID:38871130.

[2] Wingfield B, Lapsley C, McDowell A, Miliotis G, McLafferty M, O'Neill SM, *et al*. Variations in the oral microbiome are associated with depression in young adults. Sci Rep 2021;11(1):15009. doi:10.1038/s41598-021-94498-6, PMID:34294835.

[3] Simpson CA, Adler C, du Plessis MR, Landau ER, Dashper SG, Reynolds EC, *et al*. Oral microbiome composition, but not diversity, is associated with adolescent anxiety and depression symptoms. Physiol Behav 2020;226:113126. doi:10.1016/j.physbeh.2020.113126, PMID:32777312.

[4] Alex AM, Levendosky AA, Bogat GA, Muzik M, Nuttall AK, Knickmeyer RC, *et al*. Stress and mental health symptoms in early pregnancy are associated with the oral microbiome. BMJ Ment Health 2024;27(1):e301100. doi:10.1136/bmjment-2024-301100, PMID:39562142.

[5] Luo S, Lou F, Yan L, Dong Y, Zhang Y, Liu Y, *et al*. Comprehensive analysis of the oral microbiota and metabolome change in patients of burning mouth syndrome with psychiatric symptoms. J Oral Microbiol 2024;16(1):2362313. doi:10.1080/20002297.2024.2362313, PMID:38835338.

[6] Martínez M, Postolache TT, García-Bueno B, Leza JC, Figuero E, Lowry CA, *et al*. The Role of the Oral Microbiota Related to Periodontal Diseases in Anxiety, Mood and Trauma- and Stress-Related Disorders. Front Psychiatry 2021;12:814177. doi:10.3389/fpsyt.2021.814177, PMID:35153869.

[7] Simpson CA, Schwartz OS, Eliby D, Butler CA, Huang K, O'Brien-Simpson N, *et al*. Bugs and Brains, the Gut and Mental Health Study: a mixed-methods study investigating microbiota composition and function in anxiety, depression and irritable bowel syndrome. BMJ Open 2021;11(3):e043221. doi:10.1136/bmjopen-2020-043221, PMID:33722869.

[8] Cooper AN, May RW, Fincham FD. Stress spillover and crossover in couple relationships: Integrating religious beliefs and prayer. J Fam Theory Rev 2019;11(2):289–314. doi:10.1111/jftr.12330.

[9] Shrout MR. The health consequences of stress in couples: A review and new integrated Dyadic Biobehavioral Stress Model. Brain Behav Immun Health 2021;16:100328. doi:10.1016/j.bbih.2021.100328, PMID:34589814.

[10] Rastmanesh R. Does Person-to-person Contact Confound Microbiota Research? An Important Consideration in the Randomization of Study Arms. Explor Res Hypothesis Med 2024. doi:10.14218/

ERHM.2024.00030.
[11] Fang L, Yuen M, Zhang J, Ho EY, Chan S, Wu F, *et al*. A network analysis of positive developmental assets of Hong Kong school-age children during the Covid-19 pandemic. Curr Psychol 2023;43:12188–12197. doi:10.1007/s12144-023-04287-9, PMID:36718391.
[12] Liu S, Rovine MJ, Klein LC, Almeida DM. Synchrony of diurnal cortisol pattern in couples. J Fam Psychol 2013;27(4):579–588. doi:10.1037/a0033735, PMID:23978320.
[13] Coutinho J, Pereira A, Oliveira-Silva P, Meier D, Lourenço V, Tschacher W. When our hearts beat together: Cardiac synchrony as an entry point to understand dyadic co-regulation in couples. Psychophysiology 2021;58(3):e13739. doi:10.1111/psyp.13739, PMID:33355941.
[14] Gunn HE, Buysse DJ, Hasler BP, Begley A, Troxel WM. Sleep Concordance in Couples is Associated with Relationship Characteristics. Sleep 2015;38(6):933–939. doi:10.5665/sleep.4744, PMID:25581920.
[15] Rastmanesh R, Marotta F. Person-to-Person Bacterial Transmission Can Change the Sleep Pattern in Newly Married Couples. Microb Health Dis 2024;6:e1154.
[16] Rastmanesh R, Isacco CG, Vellingiri B, Marotta F, Sadeghinejad A, Skokrollahi S, *et al*. Person-to-Person Bacterial Transmission Can Influence Dry Eye Disease in Newly Married Couples: Six-Month Interim Results. Preprints 2024. doi:10.20944/preprints202411.0902.v1.
[17] Miskolczi C, Halász J, Mikics É. Changes in neuroplasticity following early-life social adversities: the possible role of brain-derived neurotrophic factor. Pediatr Res 2019;85(2):225–233. doi:10.1038/s41390-018-0205-7, PMID:30341412.
[18] Placzek M, Friede T. Clinical trials with nested subgroups: Analysis, sample size determination and internal pilot studies. Stat Methods Med Res 2018;27(11):3286–3303. doi:10.1177/0962280217696116, PMID:29298604.
[19] Ghassemzadeh H, Mojtabai R, Karamghadiri N, Ebrahimkhani N. Psychometric properties of a Persian-language version of the Beck Depression Inventory—Second edition: BDI-II-PERSIAN. Depress Anxiety 2005;21(4):185–192. doi:10.1002/da.20070, PMID:16075452.
[20] Chehri A, Goldaste N, Ahmadi S, Khazaie H, Jalali A. Psychometric properties of insomnia severity index in Iranian adolescents. Sleep Sci 2021;14(2):101–106. doi:10.5935/1984-0063.20200045, PMID:34381573.
[21] Hossein Kaviani H, Mousavi AS. Psychometric properties of the Persian version of Beck Anxiety Inventory (BAI). Tehran Univ Med J 2008;66(2):136–140.
[22] Hunt M, Auriemma J, Cashaw AC. Self-report bias and underreporting of depression on the BDI-II. J Pers Assess 2003;80(1):26–30. doi:10.1207/S15327752JPA8001_10, PMID:12584064.
[23] Itani O, Kaneita Y, Munezawa T, Mishima K, Jike M, Nakagome S, *et al*. Nationwide epidemiological study of insomnia in Japan. Sleep Med 2016;25:130–138. doi:10.1016/j.sleep.2016.05.013, PMID:27823706.
[24] Creamer M, Foran J, Bell R. The Beck Anxiety Inventory in a non-clinical sample. Behav Res Ther 1995;33(4):477–485. doi:10.1016/0005-7967(94)00082-u, PMID:7755538.
[25] Brzychczy-Sroka B, Talaga-Ćwiertnia K, Sroka-Oleksiak A, Gurgul A, Zarzecka-Francica E, Ostrowski W, *et al*. Standardization of the protocol for oral cavity examination and collecting of the biological samples for microbiome research using the next-generation sequencing (NGS): own experience with the COVID-19 patients. Sci Rep 2024;14(1):3717. doi:10.1038/s41598-024-53992-3, PMID:38355866.
[26] Brzychczy-Sroka B, Talaga-Ćwiertnia K, Sroka-Oleksiak A, Gurgul A, Zarzecka-Francica E, Ostrowski W, *et al*. Oral microbiota study of the patients after hospitalisation for COVID-19, considering selected dental indices and antibiotic therapy using the next generation sequencing method (NGS). J Oral Microbiol 2023;15(1):2264591. doi:10.1080/20002297.2023.2264591, PMID:37840855.
[27] El-Farhan N, Tennant S, Rees SE, Evans C, Rees DA. Salivary Cortisol Response to ACTH Stimulation Is a Reliable Alternative to Serum Cortisol in Evaluating Hypoadrenalism. J Clin Endocrinol Metab 2024;109(2):e579–e588. doi:10.1210/clinem/dgad576, PMID:37768151.
[28] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37(8):852–857. doi:10.1038/s41587-019-0209-9, PMID:31341288.
[29] McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013;8(4):e61217. doi:10.1371/journal.pone.0061217, PMID:23630581.
[30] Topçuoğlu BD, Lapp Z, Sovacool KL, Snitkin E, Wiens J, Schloss PD. mikropml: User-Friendly R Package for Supervised Machine Learning Pipelines. J Open Source Softw 2021;6(61):3073. doi:10.21105/joss.03073, PMID:34414351.
[31] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, *et al*. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12(6):R60. doi:10.1186/gb-2011-12-6-r60, PMID:21702898.
[32] Sobhani SR, Eini-Zinab H, Rezazadeh A. Socioeconomic Status and Changes in Iranian Household Food Basket Using National Household Budget and Expenditure Survey Data, 1991-2017. Iran J Public Health 2022;51(4):919–928. doi:10.18502/ijph.v51i4.9254, PMID:35936548.
[33] Gómez-Gallego C, Forsgren M, Selma-Royo M, Nermes M, Collado MC, Salminen S, *et al*. The Composition and Diversity of the Gut Microbiota in Children Is Modifiable by the Household Dogs: Impact of a Canine-Specific Probiotic. Microorganisms 2021;9(3):557. doi:10.3390/microorganisms9030557, PMID:33800493.
[34] Rwego IB, Isabirye-Basuta G, Gillespie TR, Goldberg TL. Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. Conserv Biol 2008;22(6):1600–1607. doi:10.1111/j.1523-1739.2008.01018.x, PMID:18717695.
[35] Jendraszak M, Skibińska I, Kotwicka M, Andrusiewicz M. The elusive male microbiome: revealing the link between the genital microbiota and fertility. Critical review and future perspectives. Crit Rev Clin Lab Sci 2024;61(7):559–587. doi:10.1080/10408363.2024.2331489, PMID:38523477.
[36] Hasler BP. Diurnal rhythms in co-sleeping couples: Does being "in sync" matter? The University of Arizona; 2009.
[37] Lin W, Yang Y, Zhu Y, Pan R, Liu C, Pan J. Linking Gut Microbiota, Oral Microbiota, and Serum Metabolites in Insomnia Disorder: A Preliminary Study. Nat Sci Sleep 2024;16:1959–1972. doi:10.2147/NSS.S472675, PMID:39664229.
[38] Liu M, Wang X, Wu F, Dai N, Chen M, Yu J, *et al*. Variations of Oral Microbiome in Chronic Insomnia Patients with Different Tongue Features. Am J Chin Med 2020;48(4):923–944. doi:10.1142/S0192415X20500445, PMID:32436424.
[39] Pesantes N, Barberá A, Pérez-Rocher B, Artacho A, Vargas SL, Moya A, *et al*. Influence of mental health medication on microbiota in the elderly population in the Valencian region. Front Microbiol 2023;14:1094071. doi:10.3389/fmicb.2023.1094071, PMID:37007475.
[40] Teles FRF, Chandrasekaran G, Martin L, Patel M, Kallan MJ, Furquim C, *et al*. Salivary and serum inflammatory biomarkers during periodontitis progression and after treatment. J Clin Periodontol 2024;51(12):1619–1631. doi:10.1111/jcpe.14048, PMID:39104016.
[41] Zilberstein NF, Engen PA, Swanson GR, Naqib A, Post Z, Alutto J, *et al*. The Bidirectional Effects of Periodontal Disease and Oral Dysbiosis on Gut Inflammation in Inflammatory Bowel Disease. J Crohns Colitis 2024;19(4):jjae162. doi:10.1093/ecco-jcc/jjae162, PMID:39447062.
[42] Kunath BJ, De Rudder C, Laczny CC, Letellier E, Wilmes P. The oral-gut microbiome axis in health and disease. Nat Rev Microbiol 2024;22:791–805. doi:10.1038/s41579-024-01075-5, PMID:39039286.
[43] Maki KA, Kazmi N, Barb JJ, Ames N. The Oral and Gut Bacterial Microbiomes: Similarities, Differences, and Connections. Biol Res Nurs 2021;23(1):7–20. doi:10.1177/1099800420941606, PMID:32691605.
[44] Aragona P, Baudouin C, Benitez Del Castillo JM, Messmer E, Barabino S, Merayo-Lloves J, *et al*. The ocular microbiome and microbiota and their effects on ocular surface pathophysiology and disorders. Surv Ophthalmol 2021;66(6):907–925. doi:10.1016/j.survophthal.2021.03.010, PMID:33819460.
[45] Li M, Gong L, Sun X, Chapin WJ. Anxiety and depression in patients with dry eye syndrome. Curr Eye Res 2011;36(1):1–7. doi:10.3109/02713683.2010.519850, PMID:21174591.
[46] Kelly JR, Borre Y, O'Brien C, Patterson E, El Aidy S, Deane J, *et al*. Transferring the blues: Depression-associated gut microbiota induces neu-

robehavioural changes in the rat. J Psychiatr Res 2016;82:109–118. doi:10.1016/j.jpsychires.2016.07.019, PMID:27491067.
[47] Lee DY, Baek JS, Shin YJ, Kim DH. Alleviation of Immobilization Stress or Fecal Microbiota-Induced Insomnia and Depression-like Behaviors in Mice by Lactobacillus plantarum and Its Supplement. Nutrients 2024;16(21):3711. doi:10.3390/nu16213711, PMID:39519543.
[48] Hong JK, Lee HJ, Chung S, Yoon IY. Differences in sleep measures and waking electroencephalography of patients with insomnia according to age and sex. J Clin Sleep Med 2021;17(6):1175–1182. doi:10.5664/jcsm.9156, PMID:33590824.
[49] Carney CE, Moss TG, Harris AL, Edinger JD, Krystal AD. Should we be anxious when assessing anxiety using the Beck Anxiety Inventory in clinical insomnia patients? J Psychiatr Res 2011;45(9):1243–1249. doi:10.1016/j.jpsychires.2011.03.011, PMID:21482427.
[50] Canals J, Bladé J, Carbajo G, Domenech-Llaberia E. The Beck Depression Inventory: psychometric characteristics and usefulness in nonclinical adolescents. Eur J Psychol Assess 2001;17(1):63–78. doi:10.1027/1015-5759.17.1.63.

Exploratory Research and Hypothesis in Medicine **2025** vol. 10(2) | 87–96
DOI: 10.14218/ERHM.2024.00042

Original Article

Prognostic and Clinicopathological Significance of circPVT1 in Solid Tumors: A Systematic Review and Meta-analysis

Menglan Li, Kai Qian, Zhixian Zhu, Yajing Deng and Pengfei Li*

Department of Clinical Laboratory, Affiliated Hospital of Nanjing University of Chinese Medicine, Jiangsu Province Hospital of Chinese Medicine, Nanjing, Jiangsu, China

Received: December 05, 2024 | **Revised:** February 27, 2025 | **Accepted:** March 24, 2025 | **Published online:** April 30, 2025

Abstract

Background and objectives: circPVT1 has emerged as a key regulator in disease progression and clinical outcomes. However, its prognostic relevance and association with clinicopathological parameters in solid malignancies remain to be fully elucidated. To address this, we conducted a meta-analysis to elucidate the clinical significance of circPVT1 in solid tumors.

Methods: A comprehensive literature search was conducted across multiple databases, including PubMed, Web of Science, Embase, the Cochrane Library, and CNKI, with a cutoff date of December 31, 2024. Statistical analyses were conducted using STATA 12.0 to calculate pooled hazard ratios (HRs) and odds ratios (ORs) with 95% confidence intervals (CIs), assessing the impact of circPVT1 expression on overall survival (OS) and its association with clinicopathological characteristics.

Results: This analysis included 27 clinical studies encompassing a total of 2,219 patients. Elevated circPVT1 expression was significantly associated with poorer OS in patients with solid tumors (HR = 1.68, 95% CI: 1.39–2.02, $P < 0.001$). This association was particularly notable in lung cancer (HR = 2.08, 95% CI: 1.51–2.88, $P < 0.001$) and osteosarcoma (HR = 1.65, 95% CI: 1.38–1.97, $P < 0.001$), with similar trends observed in hepatocellular carcinoma, colorectal cancer, and papillary thyroid carcinoma. Furthermore, the increased circPVT1 level was correlated with larger tumor size (OR = 1.36, 95% CI: 1.11–1.67, $P = 0.004$), lymph node metastasis (OR = 1.56, 95% CI: 1.22–2.00, $P < 0.001$), distant metastasis (OR = 1.80, 95% CI: 1.10–2.92, $P = 0.017$), and advanced tumor-node-metastasis stage (OR = 1.84, 95% CI: 1.50–2.25, $P < 0.001$).

Conclusions: Aberrant circPVT1 expression is associated with adverse OS and unfavorable clinicopathological features in solid tumors, underscoring its potential utility as a prognostic biomarker and indicator of tumor aggressiveness.

Introduction

Circular RNAs (circRNAs), characterized by their covalently closed-loop structure formed through back-splicing of introns or exons, represent a novel category of noncoding RNAs.[1] Increasing evidence indicates that circRNAs are more stable and abundantly expressed than their linear counterparts and possess potential as diagnostic and prognostic biomarkers for various diseases.[1,2] Among the extensively studied circRNAs, circPVT1 (circBase ID: hsa_circ_0001821) has emerged as a key player in cancer biology. Derived from an exon of the plasmacytoma variant translocation 1 (*PVT1*) gene, circPVT1 is located on chromosome 8q24—a genomic region widely recognized for its association with cancer susceptibility.[3] Unlike linear RNAs, circPVT1 forms a covalently closed loop, rendering it highly stable and resistant to RNA degradation. This structural integrity allows circPVT1 to function as a dynamic regulator of gene expression, primarily by acting as a microRNA (miRNA) sponge. By sequestering miRNAs, circPVT1 modulates the activity of downstream target genes, impacting essential cellular processes such as proliferation, apoptosis, and metastatic progression.[4]

Emerging studies have identified circPVT1 as a promising prognostic marker across multiple cancer types. For instance, in hepatocellular carcinoma, elevated circPVT1 expression is associated with worse overall survival (OS) and disease-free survival, supporting its potential as a clinical outcome predictor.[5] Additionally, Wang *et al*. revealed that circPVT1 is upregulated in breast

Keywords: circPVT1; Cancer; Prognostic; Clinicopathological; Overall survival; Solid tumors.

***Correspondence to**: Pengfei Li, Department of Clinical Laboratory, Affiliated Hospital of Nanjing University of Chinese Medicine, Jiangsu Province Hospital of Chinese Medicine, Nanjing 210029, Jiangsu, China. ORCID: https://orcid.org/0000-0002-5928-1578. Tel: +86-025-86617141, Fax: +86-025-86617141, E-mail: lipengfei@njucm.edu.cn

How to cite this article: Li M, Qian K, Zhu Z, Deng Y, Li P. Prognostic and Clinicopathological Significance of circPVT1 in Solid Tumors: A Systematic Review and Meta-analysis. *Explor Res Hypothesis Med* 2025;10(2):87–96. doi: 10.14218/ERHM.2024.00042.

cancer, where it enhances tumor cell invasion and metastasis by regulating the miR-29a-3p/AGR2/HIF-1α pathway.[6] However, conflicting findings have also been reported. Kong *et al*. discovered that circPVT1 expression is reduced in gastric cancer (GC), with lower levels correlating with deeper tumor invasion and lymph node metastasis.[7] These discrepancies highlight the complexity of circPVT1's role in cancer progression and underscore the necessity for a comprehensive evaluation of its prognostic and clinicopathological significance.

Given the growing interest in circPVT1 as a prospective marker and the inconsistent evidence regarding its clinical predictive value across different cancers, we executed a detailed meta-analysis of available clinical research. This analysis aimed to provide a clear and comprehensive assessment of circPVT1's prognostic value and its association with clinicopathological features in solid tumors, delivering valuable insights to guide future research and clinical strategies.

Materials and methods

Search strategy

This study was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[8] An exhaustive literature search was performed by two authors (Menglan Li and Kai Qian) across multiple databases, including PubMed, Web of Science, Embase, the Cochrane Library, and CNKI, with a cutoff date of December 31, 2024. The following search terms were utilized: ('circPVT1' or 'circular RNA PVT1' or 'hsa_circ_0001821') and ('prognosis' or 'prognostic' or 'survival' or 'characteristic') and ('tumor 'or 'cancer' or 'carcinoma'). During the evaluation process, the reference lists of the selected articles were also meticulously reviewed to identify additional relevant studies. This comprehensive approach ensured the inclusion of all pertinent literature examining the role of circPVT1 in tumor prognosis and clinicopathological features.

Study enrollment criteria

Inclusion criteria: (1) Clinical studies exploring the impact of circPVT1 expression on clinicopathological characteristics or survival outcomes in tumor patients; (2) Articles providing hazard ratios (HRs) and confidence intervals (CIs), or survival curves from which HRs could be indirectly calculated; (3) Full-text articles available for review; (4) Literature published in English or Chinese.

Exclusion criteria: (1) Studies lacking usable or sufficient data on cancer prognosis or clinicopathology; (2) Research focusing solely on the molecular mechanisms of circPVT1 without clinical data; (3) Duplicate publications, review articles, letters, comments, and conference abstracts, to avoid redundancy and ensure depth of analysis.

Data extraction and quality assessment

To ensure accuracy and comprehensiveness, two researchers (Menglan Li and Kai Qian) independently extracted data, with discrepancies resolved through consultation with a third party (Zhixian Zhu). The extracted data included: (1) The first author's name, publication year, country, cancer type, target microRNA, sample type and size, detection methods, cutoff value, circPVT1 expression status, source of HRs, survival outcomes, and follow-up duration; (2) Clinicopathological variables, including gender, tumor size, grade, lymph node metastasis, distant metastasis, tumor-node-metastasis (TNM) stage, and pathological T stage; (3) HRs with 95% CIs, either directly reported or calculated from survival curves.[9] The Newcastle-Ottawa Scale (NOS), a validated tool for evaluating the quality of non-randomized studies, was used for quality assessment.[10]

Statistical analysis

All statistical analyses were conducted using STATA 12.0 (StataCorp, College Station, TX, USA). To evaluate the prognostic and clinicopathological significance of circPVT1 across various cancer types, pooled HRs and ORs with corresponding 95% CIs were calculated. HRs were either directly extracted from the original studies or derived from Kaplan-Meier curves using Engauge Digitizer software (version 4.1). Statistical heterogeneity was examined using Cochrane's Q test. A fixed-effect model was applied when heterogeneity was low ($I^2 < 50\%$ or $P > 0.1$); otherwise, a random-effects model was used. Subgroup analyses were performed to explore potential sources of variation. Sensitivity analyses were conducted to assess the consistency of the results. For analyses involving six or more studies, Begg's and Egger's tests were used to detect potential publication bias.[11,12]

Results

Study selection and characteristics

A total of 312 articles from PubMed, Web of Science, Embase, Cochrane Library databases, and CNKI were initially recruited (Fig. 1). After applying the predetermined inclusion and exclusion criteria, 216 duplicate and irrelevant articles were excluded. Additionally, 14 reviews, three meta-analyses, four conference abstracts, and four comments were removed. Following a thorough review of titles and abstracts, an additional 35 studies not related to prognosis or clinicopathology aspects of circPVT1 were also excluded. The remaining 36 full-text articles were then meticulously reviewed for relevant data, resulting in the exclusion of nine articles due to inadequate information. Ultimately, 27 articles encompassing 2,219 individuals were included. Among these articles, 23 focused on prognosis[5,13–15] and 19 studies mentioned clinicopathology.[3,6,16,17] These articles were published between 2017 and 2024, with all but two originating from China. Key information extracted from these studies is summarized in Table 1,[3,5–7,13–35] providing a comprehensive overview of circPVT1's impact on solid tumors.

Associations between circPVT1 expression and OS

Due to the high degree of heterogeneity identified in the studies ($I^2 = 80.2\%$, $P < 0.001$), a random-effects model was employed to calculate the combined HR for overall survival OS. As shown in Figure 2, increased circPVT1 expression levels were strongly linked to poorer OS in solid tumor patients. The analysis revealed a combined HR of 1.68, with the 95% CI spanning from 1.39 to 2.02, indicating a statistically significant association ($P < 0.001$). Subsequently, we performed the analysis based on different cancer types. As shown in Figure 3, for lung cancer patients, high circPVT1 expression correlated with an HR of 2.08 (95% CI: 1.51–2.88, $P < 0.001$), indicating nearly a twofold increased risk of adverse OS in this patient population. Similarly, for osteosarcoma patients, high circPVT1 expression was linked to an HR of 1.65 (95% CI: 1.38–1.97, $P < 0.001$), indicating an approximately 1.7-fold increased risk of poor OS. Similar trends were also ob-

Table 1. Characteristics of included studies in this meta-analysis

Author	Year	Region	Cancer type	miRNA	Sample type	Sample size	Detection methods	Cutoff value	Expression		Follow-up (months)	HRs source	Expression tatus	Survival outcome	NOS
									low	high					
Wang *et al*.[6]	2020	China	BC	MiR-29a-3p	tissue	40	RT-PCR	median	20	20	NA	NA	Up	NA	6
Bian *et al*.[18]	2020	China	BC	miR-204-5p	tissue	99	RT-PCR	median	47	52	60	Direct	Up	OS	8
Lu *et al*.[19]	2020	China	NSCLC	NA	serum	96	RT-PCR	median	48	48	100	Direct	Up	OS	7
Yan *et al*.[16]	2020	China	osteosarcoma	miR-526b	tissue	48	RT-PCR	median	24	24	60	Curve	Up	OS	8
Zheng *et al*.[17]	2020	China	LAD	miR-145-5p	tissue	104	RT-PCR	mean	56	48	60	Direct	Up	OS	8
Zhu *et al*.[3]	2019	China	HCC	miR-203	tissue	70	RT-PCR	median	35	35	60	Curve	Up	OS	7
Wang *et al*.[20]	2019	China	CRC	miR-145	tissue	64	RT-PCR	median	32	32	60	Curve	Up	OS	7
Qin *et al*.[21]	2019	China	NSCLC	miR-497	tissue	70	RT-PCR	median	43	47	60	Curve	Up	OS	8
Zhu *et al*.[22]	2018	China	osteosarcoma	NA	tissue	80	RT-PCR	mean	50	30	60	Curve	Up	OS	7
Verduci *et al*.[23]	2017	Italy	HNSCC	NA	tissue	106	RT-PCR	median (X)-σ/2	35	71	70	Direct	Up	OS	8
Chen *et al*.[24]	2017	China	GC	NA	tissue	187	RT-PCR	Youdeng's index	80	107	100	Direct	Up	OS	7
Kong *et al*.[7]	2019	China	GC	NA	tissue	80	RT-PCR	mean	62	18	NA	NA	Down	NA	6
Tao *et al*.[25]	2019	China	PTC	miR-126	tissue	39	RT-PCR	mean	18	21	60	Curve	Up	OS	7
Zhou *et al*.[13]	2024	China	BLC	NA	tissue	162	RT-PCR	mean	50	112	97	Direct	Up	OS	7
Wang *et al*.[14]	2022	China	osteosarcoma	miR-24-3p	tissue	80	RT-PCR	median	40	40	60	Curve	Up	OS	8
Mo *et al*.[15]	2022	China	NPC	NA	tissue	159	ISH	mean	31	128	120	Direct	Up	OS	7
Shi *et al*.[26]	2021	China	LUSC	miR-30d/e	tissue	104	RT-PCR	median	50	54	60	Direct	Up	OS	8
Chen *et al*.[5]	2024	China	HCC	NA	tissue	96	RT-PCR	median	49	47	50	Direct	Up	OS	9
Lyu *et al*. [27]	2024	China	LaC	NA	tissue	65	RT-PCR	median	35	30	65	Curve	Up	OS	7
Zeng *et al*.[28]	2021	China	PTC	miR-195	tissue	50	RT-PCR	median	25	25	60	Curve	Up	OS	6
Wang *et al*.[29]	2021	China	GBC	miR-339-3p	tissue	36	RT-PCR	median	17	19	40	Direct	Up	OS	8
Hua *et al*.[30]	2022	China	PTC	miR-384	tissue	36	RT-PCR	median	18	18	NA	NA	Up	NA	7
Liu *et al*.[31]	2022	China	LC	miR-124-3p	tissue	60	RT-PCR	median	30	30	150	Curve	Up	OS	7
Wan *et al*.[32]	2020	China	osteosarcoma	miR-423-5p	tissue	36	RT-PCR	NA	NA	NA	50	Curve	Up	OS	7
Mai *et al*.[33]	2019	China	CRC	NA	plasma	148	RT-PCR	NA	62	86	60	Curve	Up	OS	7
Can *et al*.[34]	2023	Turkey	HNCs	NA	tissue	104	RT-PCR	median	53	51	NA	Direct	Up	OS	7
Qi *et al*.[35]	2022	China	ESCC	NA	tissue	40	RT-PCR	median	18	22	NA	NA	Up	NA	6

BC, breast cancer; BLC, bladder cancer; CRC, colorectal cancer; ESCC, esophageal squamous cell carcinoma; GBC, gallbladder cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; HR, hazard ratio; HNCs, head and neck cancer; HNSCC, head and neck squamous cell carcinoma; ISH, in situ hybridization; LaC, laryngeal cancer; LAD, lung adenocarcinoma; LC, lung cancer; LUSC, lung squamous cell carcinoma; NOS, Newcastle-Ottawa Scale; NPC, nasopharyngeal carcinoma; NSCLC, non-small cell lung cancer; PTC, papillary thyroid carcinoma; RT-PCR, Reverse Transcription Polymerase Chain Reaction.

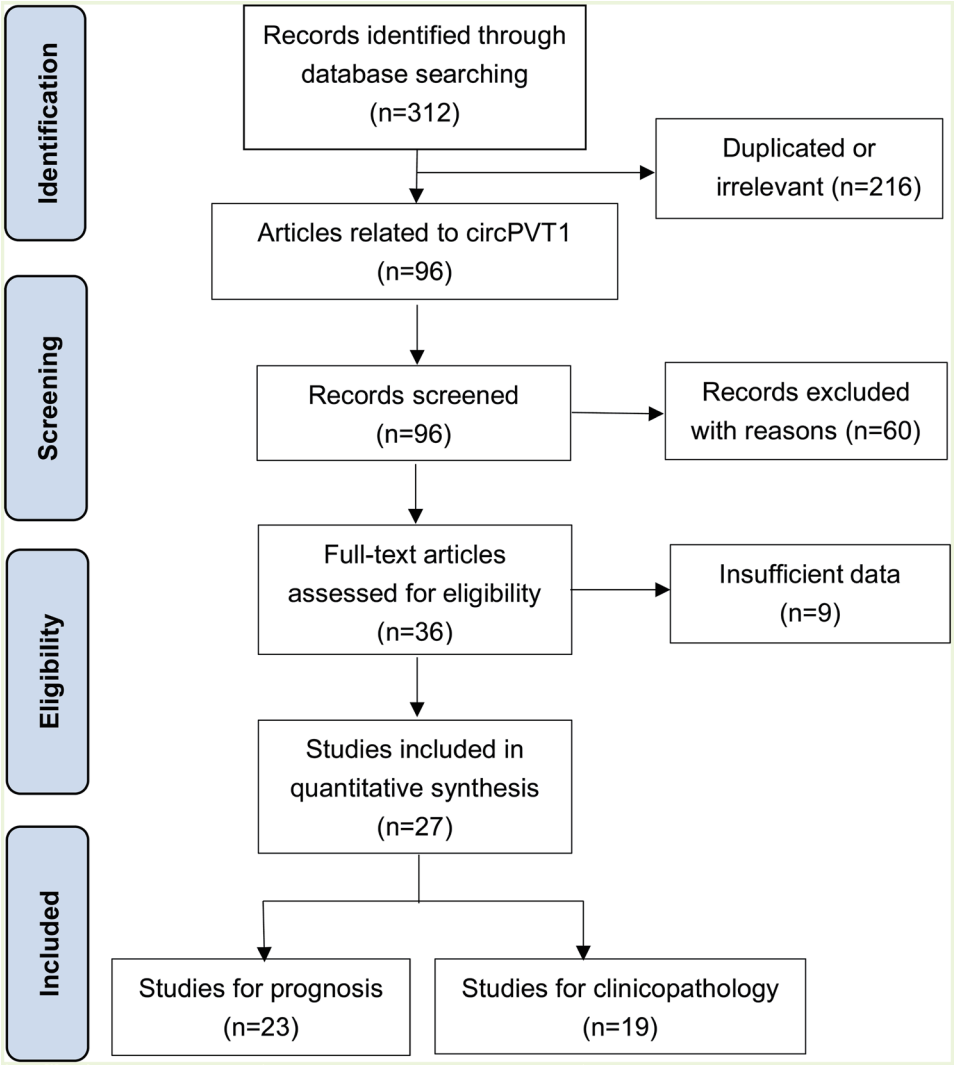


Fig. 1. The flowchart illustrating the study selection process.

served in hepatocellular carcinoma, colorectal cancer, and papillary thyroid carcinoma. These findings underscore the promising role of circPVT1 as a predictive biomarker across various types of solid tumors, highlighting its value in predicting unfavorable clinical outcomes.

Subgroup analysis

To investigate the source of heterogeneity in the OS outcome, we conducted subgroup analyses based on several factors, including sample size, interaction with miRNA, NOS score, and HR sources. As illustrated in Figure 4, significant heterogeneity was predominantly observed in subgroups with a sample size ≥ 100 (HR = 1.45, 95% CI: 0.98–2.14, I^2 = 87.3%), no interaction with miRNA (HR = 1.57, 95% CI: 1.08–2.28, I^2 = 89.0%), NOS score ≤ 7 (HR = 1.52, 95% CI: 1.19–1.95, I^2 = 85.3%), and OS data sourced directly from articles (HR = 1.63, 95% CI: 1.12–2.37, I^2 = 87.1%) (Table 2). In contrast, no heterogeneity was observed in groups with a sample size < 100 (HR = 1.78, 95% CI: 1.60–1.98, I^2 = 0.0%), interaction with miRNA (HR = 1.73, 95% CI: 1.54–1.94, I^2 = 0.0%), NOS score > 7 (HR = 1.88, 95% CI: 1.54–2.30, I^2 = 28.1%), and HRs derived from Kaplan-Meier curves (HR = 1.74, 95% CI: 1.56–1.95, I^2 = 0.0%) (Table 2).

Associations between circPVT1 and clinicopathological characteristics

This association analysis encompassed 19 articles (Table 3). The aggregated findings demonstrated that patients with increased circPVT1 expression levels had a higher risk of larger tumor dimensions (OR = 1.36, 95% CI: 1.11–1.67, P = 0.004), lymph node metastasis (OR = 1.56, 95% CI: 1.22–2.00, P < 0.001), distant metastasis (OR = 1.80, 95% CI: 1.10–2.92, P = 0.017), and advanced tumor TNM stage (OR = 1.84, 95% CI: 1.50–2.25, P < 0.001), indicating its potential as a marker for aggressive clinical pathological features. However, there was no significant evidence showing that abnormal circPVT1 expression was associated with gender, grade, or tumor stage.

Sensitivity analysis and assessment of publication bias

The sensitivity analysis indicated that there was no significant impact on the combined HR of OS after systematically eliminating

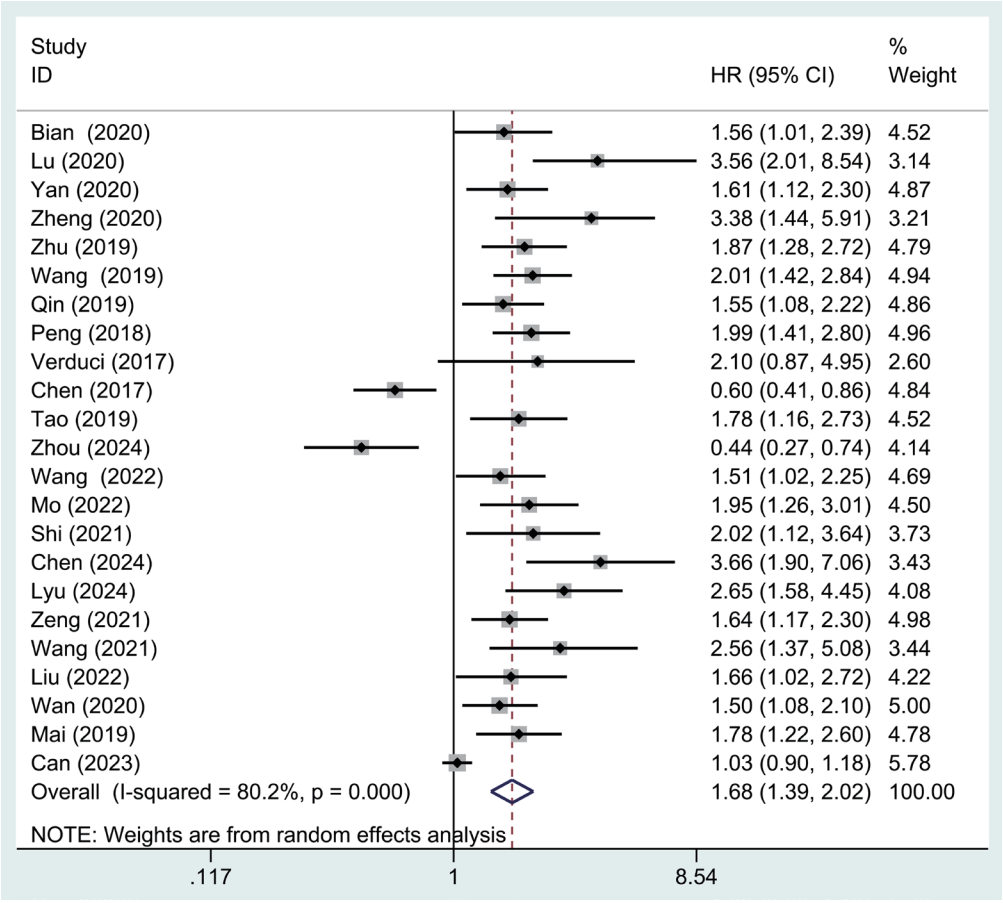


Fig. 2. Forest plot of the correlation between circPVT1 expression and OS in solid tumors. CI, confidence interval; HR, hazard ratio; OS, overall survival.

each article, suggesting that these studies were relatively reliable and stable (Fig. 5). To further assess the possibility of publication bias, Begg's and Egger's tests were conducted, yielding *P*-values of 0.082 and 0.063, respectively (Fig. 6). These non-significant *P*-values indicated that no substantial evidence of publication bias was detected in this meta-analysis, further supporting the validity and generalizability of our findings.

Discussion

Cancer continues to be the primary cause of global mortality, presenting a significant challenge to improving life expectancy worldwide. Given the substantial financial burden associated with cancer care, the identification of dependable prognostic biomarkers is crucial.[36] Circular RNAs, known for their circular configuration, constitute an intriguing category of noncoding RNAs. Plentiful investigations have confirmed that circRNAs are abundant, tissue-specific, evolutionarily conserved, and highly stable.[37,38] These properties position circRNAs as excellent candidates for cancer prognostic biomarkers.

circPVT1, an endogenous circular RNA derived from the *PVT1* gene within the cancer-associated genomic locus 8q24, has garnered significant attention. With a length of 410 nucleotides, circPVT1 is primarily synthesized through an exon circularization mechanism, which relies on complementary sequences in flanking intronic regions and the activity of RNA-binding proteins. Research has demonstrated that circPVT1 is highly expressed in various cancers and influences tumor initiation, progression, and metastasis through multiple mechanisms.[39] Its primary functions include acting as a miRNA sponge, regulating gene expression, and modulating cellular processes such as proliferation, apoptosis, migration, and invasion.[4] For instance, in non-small cell lung cancer, circPVT1 sequesters miR-124-3p, thereby modulating *EZH2* expression, which enhances lung cancer cell proliferation, invasion, and migration.[31] In gastric cancer, circPVT1 functions as a molecular sponge for the miR-125 family, restoring the expression of its downstream target gene *E2F2* and promoting cell proliferation.[24] In osteosarcoma, circPVT1 interacts with miR-423-5p, activating the Wnt5a/Ror2 signaling cascade to facilitate glycolysis and metastasis.[32] Additionally, Verduci *et al*. found that circPVT1 is highly expressed in head and neck squamous cell carcinoma harboring mutant p53 proteins and promotes tumorigenesis and progression through its interaction with the YAP/TEAD complex.[23] Studies have also revealed that circPVT1 expression is closely linked to chemoresistance of cancer cells. For example, in osteosarcoma, elevated levels of circPVT1 correlate with resistance to chemotherapy, as it regulates the expression of *ABCB1*, enhancing the resistance of tumor cells to doxorubicin and cisplatin.[22] circPVT1 also holds potential value in tumor diagnosis and prognosis. In gallbladder cancer, circPVT1 expression levels are linked to lymph node metastasis and advanced TNM stage, with higher circPVT1 expression associated with poor patient prognosis.[29]

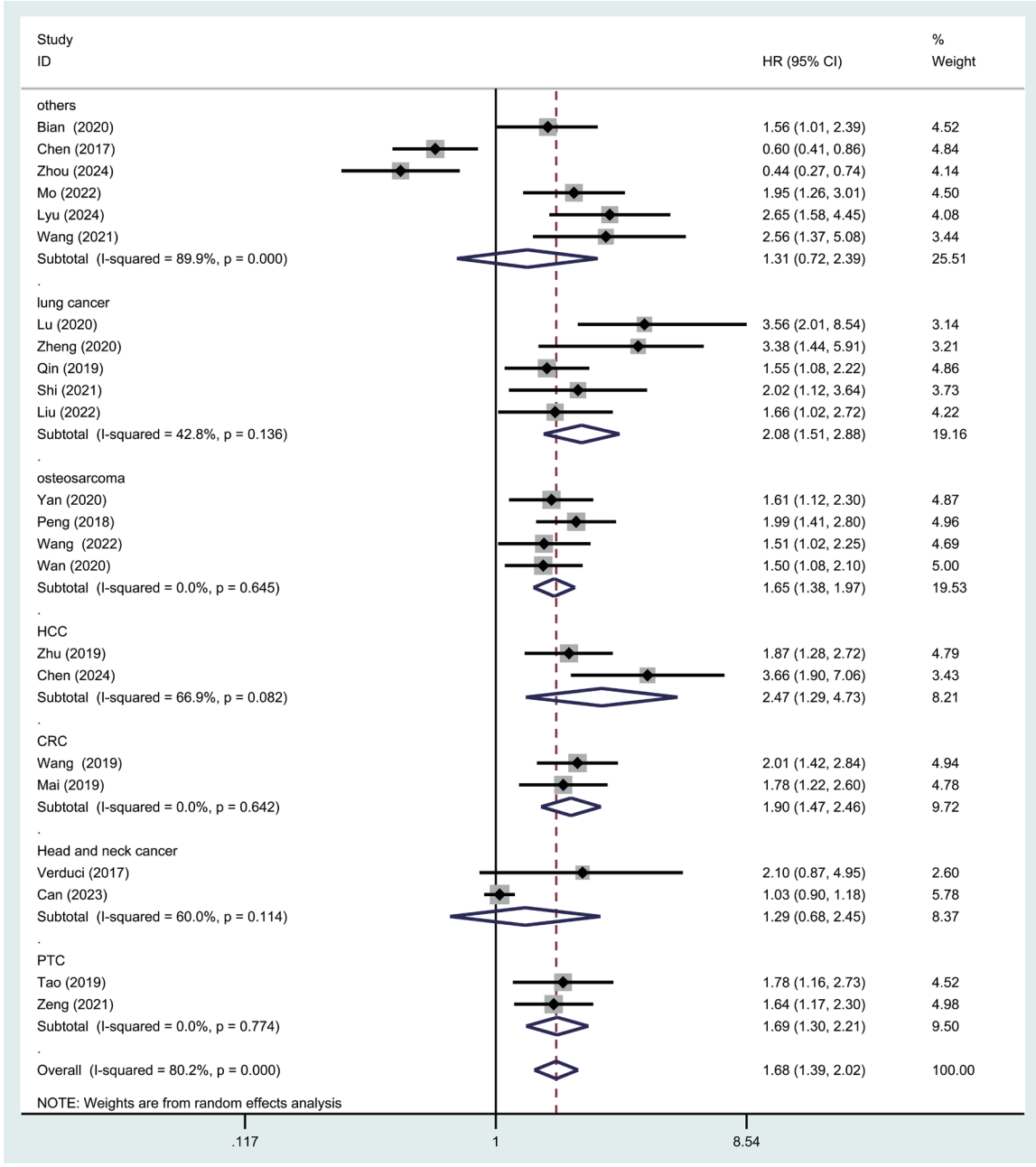


Fig. 3. Forest plots illustrating the correlation between circPVT1 expression and OS among various cancer types. CI, confidence interval; CRC, colorectal cancer; HCC, hepatocellular carcinoma; HR, hazard ratio; OS, overall survival; PTC, papillary thyroid carcinoma.

Therefore, circPVT1 expression levels might function as a predictive marker to categorize patients into groups at higher or lower risk, guiding more personalized treatment strategies. These findings underscore the critical function of circPVT1 in the advancement and prognostic assessment of various solid cancers. Subsequent investigations ought to center on evaluating the capabilities of circPVT1 as a non-invasive indicator in liquid biopsies for early-stage cancer identification, prognosis prediction, and treatment monitoring. Additionally, developing therapeutic strategies to target circPVT1, such as RNA interference or CRISPR-based approaches, could enhance the efficacy of existing treatments and provide new therapeutic options for cancer patients.

While circPVT1 has been studied extensively,[40–42] our systematic review and meta-analysis offer a thorough integration of the existing evidence, particularly focusing on its prognostic and clinicopathological significance across different cancer types. Based on current knowledge, this is the first investigation to quantitatively summarize the prognostic value of circPVT1 across diverse solid tumors, offering a broader perspective on its potential clinical utility. The results from the included studies indicated that upregulated circPVT1 expression was closely linked to unfavorable OS outcomes in cancers, with a pooled HR of 1.68. Moreover, can-

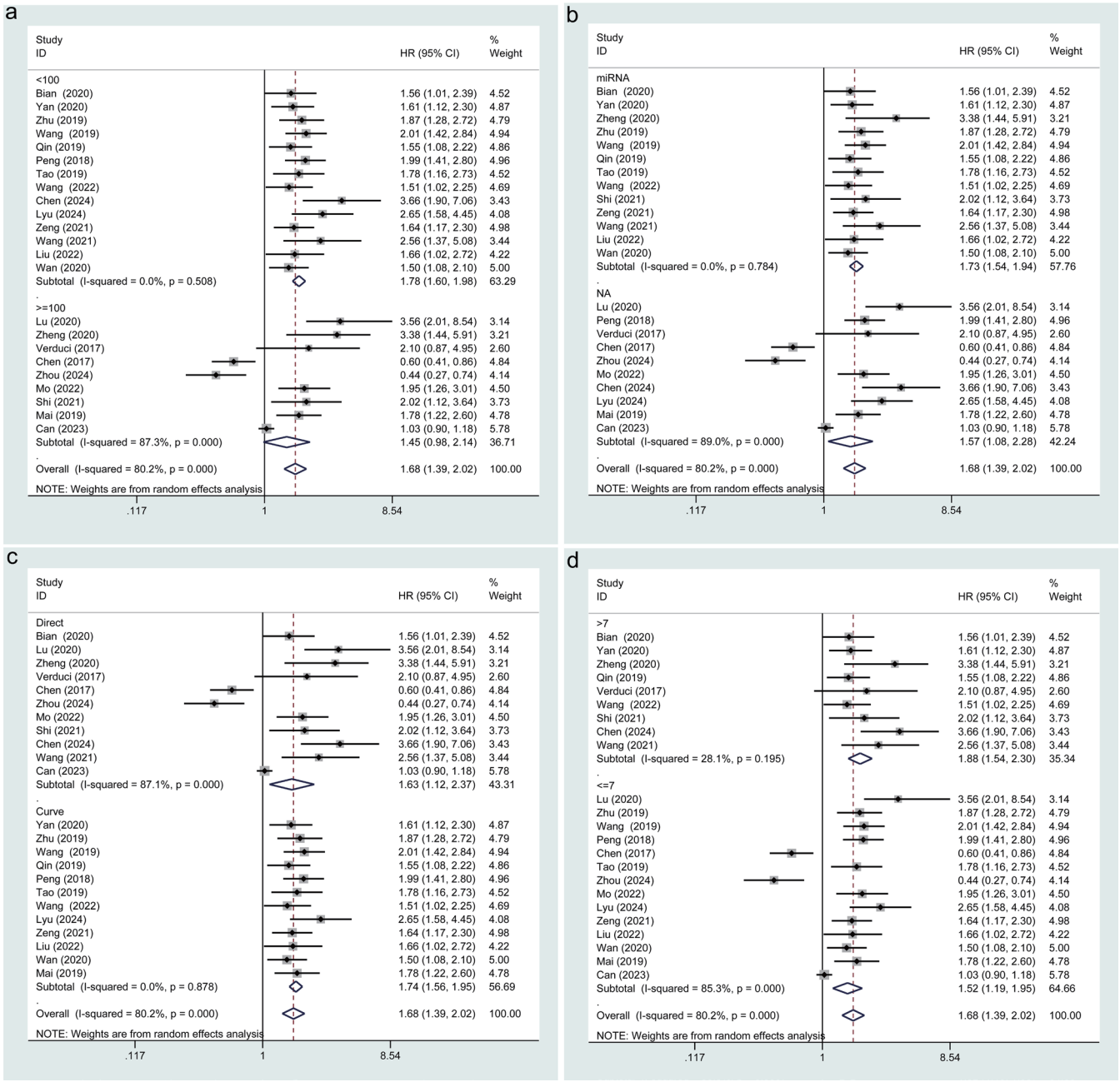


Fig. 4. Forest plots illustrating the correlation between circPVT1 expression levels and OS outcomes in stratified cohorts. (a) Sample size. (b) Interaction with miRNA. (c) NOS score. (d) HR source. CI, confidence interval; HR, hazard ratio; NOS, Newcastle-Ottawa Scale; OS, overall survival.

cers with higher levels of circPVT1 had a greater possibility of larger tumor size, lymph node metastasis, distant metastasis, and advanced tumor TNM stage, indicating that increased circPVT1 levels were a marker of aggressive clinicopathological features. However, there was significant heterogeneity in OS outcomes among the included articles. Although an appropriate effect model was employed during data merging, the origin of heterogeneity among the included studies remained ambiguous. Sensitivity analyses, which typically help in assessing the robustness of the findings and identifying potential outliers, were also inconclusive in explaining the observed heterogeneity. Given these challenges, we carried out subgroup analyses. The results indicated that heterogeneity might exist in the subgroups of sample size ≥ 100, not interacting with miRNA, NOS score ≤ 7, and OS data obtained directly from the articles.

Despite these findings, a number of limitations pertinent to this meta-analysis ought to be highlighted. Firstly, the insufficient number of enrolled studies and patients may have led to conflicting results. For example, Chen *et al*. showed circPVT1 was enhanced in 187 GC tissues compared with matched normal tissue and exhibited a tumor-promotive function,[24] while the results from Kong *et al*. showed that circPVT1 was signifi-

Table 2. Subgroup analyses of pooled HRs for OS

Subgroup	Studies (n)	OS		Heterogeneity	
		Pooled HR (95%CI)	*P*-value	I^2 (%)	*P*-value
Sample size					
<100	14	1.78 (1.60–1.98)	<0.001	0.00	0.508
≥100	9	1.45 (0.98–2.14)	0.062	83.7	<0.001
Interacted with miRNA					
miRNA	13	1.73 (1.54–1.94)	<0.001	0.00	0.784
NA	10	1.57 (1.08–2.28)	0.019	89.0	<0.001
NOS score					
<7	14	1.52 (1.19–1.95)	0.001	85.3	<0.001
≥7	9	1.88 (1.54–2.30)	<0.001	28.1	0.195
HRs source					
Direct	11	1.63 (1.55–2.09)	0.011	87.1	<0.001
Curve	12	1.74 (1.56–1.95)	<0.001	0.00	0.878

CI, confidence interval; HRs, hazard ratios; NOS, Newcastle-Ottawa Scale; OS, overall survival.

Table 3. Pooled analysis of circPVT1 expression and tumor clinicopathological characteristics

Clinicopathological parameters	Articles (n)	Cases (n)	Combined OR (95%CI)	Effects model	*P*-value	Heterogeneity	
						I^2 (%)	*P*-value
Gender (male vs female)	8	667	1.06 (0.93–1.21)	Fixed	0.353	3.10	0.406
Tumor size (≥3 vs <3)	14	1,230	1.36 (1.11–1.67)	Random	0.004	69.8	<0.001
Grade (high vs low)	9	812	0.97 (0.85–1.10)	Fixed	0.611	45.9	0.063
Lymph node metastasis (yes vs no)	16	1,345	1.56 (1.22–2.00)	Random	<0.001	74.8	<0.001
Distant metastasis (yes vs no)	6	645	1.80 (1.10–2.92)	Random	0.017	84.5	<0.001
TNM stage (III/IV vs I/II)	15	1,284	1.84 (1.50–2.25)	Random	<0.001	66.3	<0.001
Tumor stage (III/IV vs I/II)	6	636	1.05 (0.71–1.55)	Random	0.822	82.9	<0.001

CI, confidence interval; OR, odds ratios; TNM, tumor-node-metastasis.

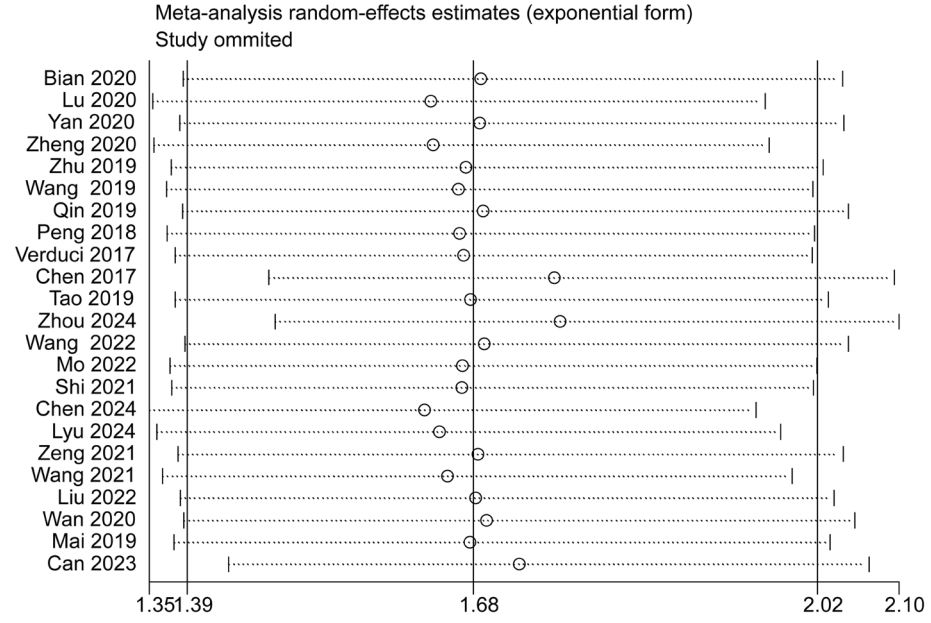


Fig. 5. Sensitivity analysis of included studies.

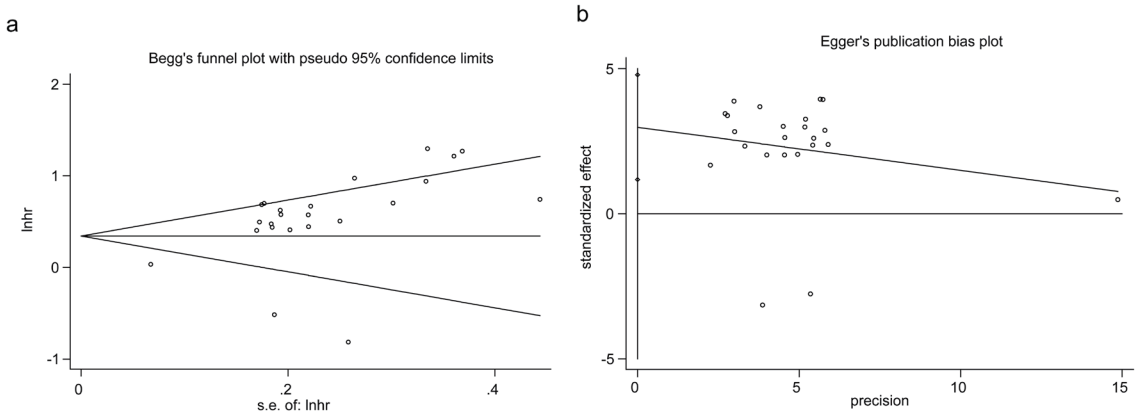


Fig. 6. Publication bias test. (a) Begg's funnel plot and (b) Egger's linear regression plot for bias evaluation.

cantly downregulated in 80 GC tissues.[7] Such discrepancies may be attributed to smaller sample sizes and individual variability. Secondly, the majority of the included studies were conducted in China, with only two papers coming from Italy and Turkey, respectively, which may limit the generalizability of the findings. Therefore, the conclusions should be cautiously interpreted in a broader context. Thirdly, some survival data were calculated from Kaplan-Meier survival curves instead of being directly extracted from the original studies, which might introduce minor discrepancies in the pooled results. Finally, all included studies were retrospective in design, potentially introducing selection bias. Consequently, additional prospective clinical trials are required to confirm the predictive significance of circPVT1 across various tumor types.

Future directions

To advance the clinical relevance of circPVT1, future studies should prioritize validating its prognostic utility through international, prospective cohorts encompassing diverse ethnicities and cancer subtypes, thereby mitigating current geographical biases. Concurrently, mechanistic investigations are essential to elucidate how circPVT1 drives chemoresistance and metastasis, particularly through its interplay with RNA-binding proteins or immune-modulatory pathways in tumor microenvironments. Translational efforts must focus on optimizing non-invasive circPVT1 detection in liquid biopsies for early diagnosis and real-time monitoring, coupled with developing targeted therapies such as CRISPR-based silencing or antisense oligonucleotides to counteract treatment resistance.

Conclusions

Our meta-analysis indicated that elevated circPVT1 expression levels are closely linked to unfavorable OS in tumor patients. circPVT1 has the potential to act as both a prognostic indicator and a molecular target for solid tumor therapy. Future research should focus on validating these findings through large-scale, multicenter prospective studies to further establish the clinical utility of circPVT1 in cancer management.

Acknowledgments

None.

Funding

This work was supported by Open Project Support for the National Clinical Research Base of Traditional Chinese Medicine (JD-2022SZ11), Developing Program for High-level Academic Talent in Jiangsu Hospital of Chinese Medicine (Grant No. y2018rc38), and Natural Science Foundation Project of Nanjing University of Chinese Medicine (No. XZR 2024010).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Contributed to study concept and design (ML, PL), acquisition of the data (ML, KQ), data analysis (ML, ZZ, KQ), provision of reagents, materials, and analysis tools (ZZ, KQ, YD), and drafting of the manuscript (ML, PL).

References

[1] Chen LL. The biogenesis and emerging roles of circular RNAs. Nat Rev Mol Cell Biol 2016;17(4):205–211. doi:10.1038/nrm.2015.32, PMID:26908011.
[2] Feng B, Zhou H, Wang T, Lin X, Lai Y, Chu X, *et al*. Insights Into circRNAs: Functional Roles in Lung Cancer Management and the Potential Mechanisms. Front Cell Dev Biol 2021;9:636913. doi:10.3389/fcell.2021.636913, PMID:33634138.
[3] Zhu Y, Liu Y, Xiao B, Cai H, Liu M, Ma L, *et al*. The circular RNA PVT1/miR-203/HOXD3 pathway promotes the progression of human hepatocellular carcinoma. Biol Open 2019;8(9):bio043687. doi:10.1242/bio.043687, PMID:31551242.
[4] Wu S, Hu Y, Lei X, Yang X. The Emerging Roles of CircPVT1 in Cancer Progression. Curr Pharm Biotechnol 2025;26(1):1–8. doi:10.2174/0113892010282141240226112253, PMID:38454774.
[5] Chen Y, Du C, Tang J, Zhao Y, Xie H, Zheng S, *et al*. Super-enhancer-associated circPVT1 promotes malignancy of hepatocellular carcinoma via YBX1-mediated RRM2 activation. Cancer Lett 2024;611:217395. doi:10.1016/j.canlet.2024.217395, PMID:39694225.
[6] Wang J, Huang K, Shi L, Zhang Q, Zhang S. CircPVT1 Promoted the Progression of Breast Cancer by Regulating MiR-29a-3p-Mediated AGR2-HIF-1alpha Pathway. Cancer Manag Res 2020;12:11477–11490. doi:10.2147/CMAR.S265579, PMID:33223849.
[7] Kong S, Yang Q, Tang C, Wang T, Shen X, Ju S. Identification of hsa_circ_0001821 as a Novel Diagnostic Biomarker in Gastric Cancer via Comprehensive Circular RNA Profiling. Front Genet 2019;10:878.

doi:10.3389/fgene.2019.00878, PMID:31616472.
[8] Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097, PMID:19621072.
[9] Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16. doi:10.1186/1745-6215-8-16, PMID:17555582.
[10] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25(9):603–605. doi:10.1007/s10654-010-9491-z, PMID:20652370.
[11] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315(7109):629–634. doi:10.1136/bmj.315.7109.629, PMID:9310563.
[12] Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50(4):1088–1101. PMID:7786990.
[13] Zhou H, Cui X, Zhu L, Xu Z, Wang Z, Shao J. circPVT1 Inhibits the Proliferation and Aids in Prediction of the Prognosis of Bladder Cancer. Pharmgenomics Pers Med 2024;17:1–11. doi:10.2147/PGPM.S427147, PMID:38204802.
[14] Wang B, Yan L, Shi W, Xie H, Chen R, Shao Y, *et al*. CircRNA PVT1 promotes proliferation and chemoresistance of osteosarcoma cells via the miR-24-3p/KLF8 axis. Int J Clin Oncol 2022;27(4):811–822. doi:10.1007/s10147-022-02122-y, PMID:35171359.
[15] Mo Y, Wang Y, Wang Y, Deng X, Yan Q, Fan C, *et al*. Circular RNA circPVT1 promotes nasopharyngeal carcinoma metastasis via the beta-TrCP/c-Myc/SRSF1 positive feedback loop. Mol Cancer 2022;21(1):192. doi:10.1186/s12943-022-01659-w, PMID:36199071.
[16] Yan M, Gao H, Lv Z, Liu Y, Zhao S, Gong W, *et al*. Circular RNA PVT1 promotes metastasis via regulating of miR-526b/FOXC2 signals in OS cells. J Cell Mol Med 2020;24(10):5593–5604. doi:10.1111/jcmm.15215, PMID:32249539.
[17] Zheng F, Xu R. CircPVT1 contributes to chemotherapy resistance of lung adenocarcinoma through miR-145-5p/ABCC1 axis. Biomed Pharmacother 2020;124:109828. doi:10.1016/j.biopha.2020.109828, PMID:31986409.
[18] Bian Q. Circular RNA PVT1 promotes the invasion and epithelial-mesenchymal transition of breast cancer cells through serving as a competing endogenous RNA for miR-204-5p. Onco Targets Ther 2019;12:11817–11826. doi:10.2147/OTT.S180850, PMID:32982273.
[19] Lu H, Xie X, Chen Q, Cai S, Liu S, Bao C, *et al*. Clinical significance of circPVT1 in patients with non-small cell lung cancer who received cisplatin combined with gemcitabine chemotherapy. Tumori 2021;107(3):204–208. doi:10.1177/0300891620941940, PMID:32734834.
[20] Wang Z, Su M, Xiang B, Zhao K, Qin B. Circular RNA PVT1 promotes metastasis via miR-145 sponging in CRC. Biochem Biophys Res Commun 2019;512(4):716–722. doi:10.1016/j.bbrc.2019.03.121, PMID:30922567.
[21] Qin S, Zhao Y, Lim G, Lin H, Zhang X, Zhang X. Circular RNA PVT1 acts as a competing endogenous RNA for miR-497 in promoting non-small cell lung cancer progression. Biomed Pharmacother 2019;111:244–250. doi:10.1016/j.biopha.2018.12.007, PMID:30590312.
[22] Kun-Peng Z, Xiao-Long M, Chun-Lin Z. Overexpressed circPVT1, a potential new circular RNA biomarker, contributes to doxorubicin and cisplatin resistance of osteosarcoma cells by regulating ABCB1. Int J Biol Sci 2018;14(3):321–330. doi:10.7150/ijbs.24360, PMID:29559849.
[23] Verduci L, Ferraiuolo M, Sacconi A, Ganci F, Vitale J, Colombo T, *et al*. The oncogenic role of circPVT1 in head and neck squamous cell carcinoma is mediated through the mutant p53/YAP/TEAD transcription-competent complex. Genome Biol 2017;18(1):237. doi:10.1186/s13059-017-1368-y, PMID:29262850.
[24] Chen J, Li Y, Zheng Q, Bao C, He J, Chen B, *et al*. Circular RNA profile identifies circPVT1 as a proliferative factor and prognostic marker in gastric cancer. Cancer Lett 2017;388:208–219. doi:10.1016/j.canlet.2016.12.006, PMID:27986464.
[25] Tao L, Yang L, Tian P, Guo X, Chen Y. Knockdown of circPVT1 inhibits progression of papillary thyroid carcinoma by sponging miR-126. RSC Advances 2019;9(23):13316–13324. doi:10.1039/c9ra01820d.
[26] Shi J, Lv X, Zeng L, Li W, Zhong Y, Yuan J, *et al*. CircPVT1 promotes proliferation of lung squamous cell carcinoma by binding to miR-30d/e. J Exp Clin Cancer Res 2021;40(1):193. doi:10.1186/s13046-021-01976-w, PMID:34112238.
[27] Lyu K, Tang B, Huang B, Xu Z, Liu T, Fang R, *et al*. Exosomal circPVT1 promotes angiogenesis in laryngeal cancer by activating the Rap1b-VEGFR2 signaling pathway. Carcinogenesis 2024;45(9):642–657. doi:10.1093/carcin/bgae030, PMID:38824399.
[28] Zeng L, Yuan S, Zhou P, Gong J, Kong X, Wu M. Circular RNA Pvt1 oncogene (CircPVT1) promotes the progression of papillary thyroid carcinoma by activating the Wnt/beta-catenin signaling pathway and modulating the ratio of microRNA-195 (miR-195) to vascular endothelial growth factor A (VEGFA) expression. Bioengineered 2021;12(2):11795–11810. doi:10.1080/21655979.2021.2008639, PMID:34927541.
[29] Wang S, Su TT, Tong H, Shi W, Ma F, Quan Z. CircPVT1 promotes gallbladder cancer growth by sponging miR-339-3p and regulates MCL-1 expression. Cell Death Discov 2021;7(1):191. doi:10.1038/s41420-021-00577-y, PMID:34312371.
[30] Hua T, Luo Y. Circular RNA PVT1 promotes progression of thyroid cancer by competitively binding miR-384. Exp Ther Med 2022;24(4):629. doi:10.3892/etm.2022.11566, PMID:36185502.
[31] Liu Y, Li L, Song X. Exosomal circPVT1 derived from lung cancer promotes the progression of lung cancer by targeting miR-124-3p/EZH2 axis and regulating macrophage polarization. Cell Cycle 2022;21(5):514–530. doi:10.1080/15384101.2021.2024997, PMID:35025697.
[32] Wan J, Liu Y, Long F, Tian J, Zhang C. circPVT1 promotes osteosarcoma glycolysis and metastasis by sponging miR-423-5p to activate Wnt5a/Ror2 signaling. Cancer Sci 2021;112(5):1707–1722. doi:10.1111/cas.14787, PMID:33369809.
[33] Mai S, Zhang Z, Mi W. Upregulation of circ_PVT1 and circ_001569 Indicate Unfavorable Prognosis in Colorectal Cancer. Ann Clin Lab Sci 2021;51(1):55–60. PMID:33653781.
[34] Muftuoglu C, Mert U, Akagunduz OO, Tavlayan E, Al-Omar A, Asadi M, *et al*. Profiling of circRNA expressions in radiation-treated head and neck cancer cells and the potential role of circPVT1. Arch Oral Biol 2023;150:105690. doi:10.1016/j.archoralbio.2023.105690, PMID:37027893.
[35] Qi HL, Yang Y, Shi XJ, Li YZ. Expression of circPVT1 in esophageal squamous cell carcinoma and its effect on cell proliferation and apoptosis. Zhejiang Medicine 2022;44 (07):690–694,701.
[36] Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer 2021;127(16):3029–3030. doi:10.1002/cncr.33587, PMID:34086348.
[37] Vo JN, Cieslik M, Zhang Y, Shukla S, Xiao L, Zhang Y, *et al*. The Landscape of Circular RNA in Cancer. Cell 2019;176(4):869–881.e813. doi:10.1016/j.cell.2018.12.021, PMID:30735636.
[38] Tang X, Ren H, Guo M, Qian J, Yang Y, Gu C. Review on circular RNAs and new insights into their roles in cancer. Comput Struct Biotechnol J 2021;19:910–928. doi:10.1016/j.csbj.2021.01.018, PMID:33598105.
[39] Zhou C, Balmer L, Song M, Wu K, Wang W, Wang H. CircPVT1 promotes migration and invasion by regulating miR-490-5p/HAVCR2 axis in osteosarcoma cells. J Cell Mol Med 2024;28(8):e18269. doi:10.1111/jcmm.18269, PMID:38568056.
[40] Nath M, Roy D, Choudhury Y. Circular RNAs are Potential Prognostic Markers of Head and Neck Squamous Cell Carcinoma: Findings of a Meta-Analysis Study. Front Oncol 2022;12:782439. doi:10.3389/fonc.2022.782439, PMID:35296016.
[41] Palcau AC, Canu V, Donzelli S, Strano S, Pulito C, Blandino G. CircPVT1: a pivotal circular node intersecting Long Non-Coding-PVT1 and c-MYC oncogenic signals. Mol Cancer 2022;21(1):33. doi:10.1186/s12943-022-01514-y, PMID:35090471.
[42] Wang Y, Li Z, Xu S, Guo J. Novel potential tumor biomarkers: Circular RNAs and exosomal circular RNAs in gastrointestinal malignancies. J Clin Lab Anal 2020;34(7):e23359. doi:10.1002/jcla.23359, PMID:32419229.

Exploratory Research and Hypothesis in Medicine **2025** vol. 10(2) | 97–105
DOI: 10.14218/ERHM.2024.00043

Review Article

Potential of Phytomedicine in Benefiting Both Long COVID and Acute Coronary Syndromes: A State-of-the-art Review

Xiao Jiang[1#], Yiran Lu[2#], Yu Ding[2#], Yuanyuan Liu[3], Zhen Zhao[1], Peizhong Liu[1], Chuangpeng Li[1], Song He[2], Qing Zhang[2], Rongyuan Yang[1*] and Qing Liu[1*]

[1]State Key Laboratory of Traditional Chinese Medicine Syndrome, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangdong Provincial Hospital of Traditional Chinese Medicine-Zhuhai Hospital, The Second Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, Guangzhou, Guangdong, China; [2]Department of Laboratory Animals, College of Animal Sciences, Jilin University, Changchun, Jilin, China; [3]Hubei University of Traditional Chinese Medicine, Wuhan, Hubei, China

Received: December 05, 2024 | **Revised:** April 02, 2025 | **Accepted:** April 10, 2025 | **Published online:** April 30, 2025

Abstract

Acute coronary syndrome (ACS) in patients with SARS-CoV-2 infection is primarily driven by inflammation-induced myocardial injury through both direct and indirect mechanisms. Effective clinical management requires a dual approach: addressing cardiovascular lesions while also mitigating virus-induced local and systemic inflammation. This comprehensive approach is essential for improving the diagnosis and treatment of SARS-CoV-2-associated ACS. Emerging evidence highlights the potential of myocardial protective agents, including angiotensin-converting enzyme 2-modulating drugs and traditional Chinese medicine, which not only stabilize plaques and improve endothelial function but also confer cardioprotective effects. Furthermore, advancements in nanotechnology offer promising strategies for targeted therapy—particularly through angiotensin-converting enzyme 2 receptor modulation—by enhancing the precision and efficacy of herbal medicine delivery. This review explores the complex interplay between SARS-CoV-2 infection and ACS pathogenesis, and evaluates the therapeutic potential of pharmacological, herbal, and nanotechnology-based interventions in managing this multifaceted condition.

Introduction

Myocardial injury represents a severe comorbidity of SARS-CoV-2 infection, particularly when infection-triggered coronary occlusion precipitates acute coronary syndromes (ACS).[1,2] As a critical cardiovascular manifestation, ACS substantially contributes to elevated mortality rates in affected populations.[1,2] Emerging evidence has demonstrated that impaired ventilatory efficiency is a characteristic functional impairment in COVID-19 survivors developing ACS, highlighting persistent cardiopulmonary sequelae post-infection.[3] However, the mechanisms by which viral infection triggers non-infectious ACS remain controversial. This mini-review aimed to highlight the potential association between long COVID and ACS while exploring the therapeutic potential of phytochemical compounds in alleviating SARS-CoV-2-related long-term symptoms in ACS patients.

Acute infection with SARS-CoV-2 triggers ACS

Acute infections, especially viral ones, may worsen ACS by triggering widespread inflammation that destabilizes plaques and impairs endothelial function.

In addition to the well-established view that ischemia in ACS is an aseptic inflammation process, acute infections by pathogens, including viruses and bacteria, are suggested to accelerate the pathological progression of ACS. Substantial elevations in serum inflammatory biomarkers—including acute-phase reactants (C-reactive protein and procalcitonin), neutrophil-derived enzymes (myeloperoxidase), and circulating leukocyte subsets (macrophages, T lymphocytes, and neutrophils)—are characteristically observed during ACS pathogenesis, with significantly higher levels than those detected in stable coronary artery disease patients.[4,5]

The inflammatory activity is not limited to the culprit lesion in the coronary artery but extends across the entire coronary tree,

Keywords: Long COVID; Acute coronary syndromes; ACS; Phytomedicin; Nanotechnology; ACE2 receptor; Lipid nanoparticles.

***Correspondence to:** Qing Liu and Rongyuan Yang, The Second Clinical School of Medicine, Guangzhou University of Chinese Medicine, Guangdong Provincial Hospital of Traditional Chinese Medicine-Zhuhai Hospital, Guangdong, China 510120. ORCID: https://orcid.org/0000-0002-2199-2999 (QL); https://orcid.org/0000-0002-8792-8017 (RYY). Tel: +86-13631223512 (QL); +8602081887233 (RYY), Fax: +86-0756-3325088 (QL); Fax: +8602081887233(RYY), E-mail: 851757626@qq.com (QL); yangrongyuan@163.com (RYY).

[#]These authors contributed equally to this work.

How to cite this article: Jiang X, Lu Y, Ding Y, Liu Y, Zhao Z, Liu P, *et al*. Potential of Phytomedicine in Benefiting Both Long COVID and Acute Coronary Syndromes: A State-of-the-art Review. *Explor Res Hypothesis Med* 2025;10(2):97–105. doi: 10.14218/ERHM.2024.00043.

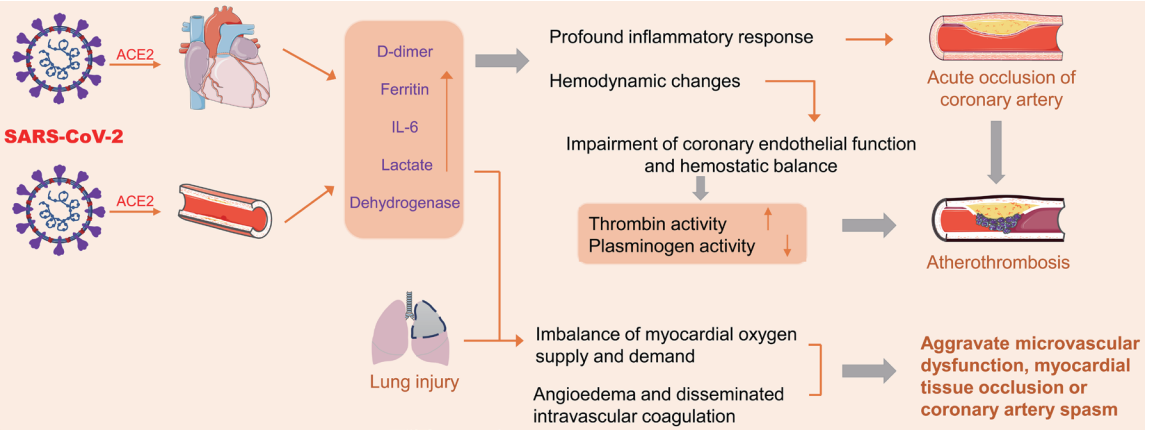


Fig. 1. COVID-19 invades the body through a large number of ACE2 receptors in the heart and blood vessels, affecting cytokine levels and triggering an inflammatory response. This leads to systemic cytokine storms, promotes thrombosis, and accelerates ACS. ACE2, angiotensin-converting enzyme 2; ACS, acute coronary syndrome; IL-6, interleukin-6.

indicating that this is a systemic inflammation. Growing evidence suggests that acute infection-induced inflammatory responses may directly influence atherosclerotic plaque stability, coronary arterial integrity, and peri-adventitial adipose tissue through cellular mediators and their molecular effectors, including cytokine networks, proteolytic enzymes, coagulation cascade components, oxidative metabolites, and vascular tone regulators.[5] These targeted inflammatory mechanisms potentiate endothelial dysfunction through vasa vasorum leakage, compromise fibrous cap integrity via necrotic core expansion, and propagate platelet-rich thrombus formation through coordinated P-selectin/GPIIb-IIIa interactions, collectively implicating sterile inflammation as a critical driver of both ACS initiation and progression.[6]

SARS-CoV-2 virus infection accelerates ACS through the angiotensin-converting enzyme (ACE) 2 receptor

SARS-CoV-2 induces heart damage via ACE2, causing inflammation, endothelial dysfunction, and clot formation that trigger myocardial injury and atherosclerosis through cytokine storms, plaque rupture, clotting changes, and oxygen supply issues, all of which increase the risk of ACS.

The acute SARS-CoV-2 infection led to a global pandemic, with acute myocardial injury (AMI) being prominently associated with comorbidities, alongside acute pulmonary injury.[7] AMI is pathologically defined by substantial elevations in high-sensitivity cardiac troponin and/or brain natriuretic peptide, biomarkers that reflect myocardial stress from ischemic insults (e.g., plaque rupture-induced coronary occlusion) or non-ischemic etiologies such as ACS, decompensated heart failure, inflammatory cardiomyopathies, and arrhythmogenic myocardial remodeling.[8]

For ischemic heart disease, elevated troponin levels can be driven not only by direct coronary artery occlusion but also by indirect myocardial injury, such as the cardiovascular inflammation process, which may accelerate ischemic myocardial injury.[9] Notably, the latter occurs during acute SARS-CoV-2 infection, when internalization of the SARS-CoV-2 virus requires the ACE2 receptor for transport.[10,11]

The extensive ACE2 expression in cardiovascular tissues predisposes them to SARS-CoV-2 infection, driving endothelial dysfunction and vascular inflammation. This cascade promotes thrombotic events, systemic inflammatory responses, and hypoxia, while adrenergic overactivation exacerbates myocardial stress during cytokine storm progression.[12–14] These effects may contribute to triggering ACS and accelerating atherosclerosis. The mechanisms are as follows (Fig. 1):

- Firstly, Elevated troponin levels correlate with inflammatory markers such as D-dimer (coagulation), ferritin (acute-phase response), interleukin (IL)-6 (cytokine activity), and lactate dehydrogenase (tissue injury), suggesting systemic immune dysregulation involving cytokine storms and reactive phagocytic/lymphocytic responses, rather than direct myocardial damage, during SARS-CoV-2 infection.[15–17]
- Secondly, marked inflammatory responses and hemodynamic instability predispose to atherosclerotic plaque rupture through mechanisms involving platelet activation/aggregation, ultimately precipitating acute coronary occlusion.[18,19]
- Moreover, the inflammatory response impairs coronary endothelial function and hemostatic balance, increasing thrombin activity while reducing plasminogen activity,[19] thus accelerating fibrin degradation products and D-dimer, leading to a pro-atherogenic and thrombogenic bias.[20,21]
- Last but not least, the myocardial oxygen supply-demand imbalance due to hypoxia and tachycardia caused by systemic cytokine storms and pulmonary injury,[22] probably becomes a synergistic factor that works with endothelial inflammation, vascular edema, and disseminated intravascular coagulation, ultimately exacerbating microvascular dysfunction, occlusion, or coronary spasm in myocardial tissue.[23–25]

Thus, it is deduced that myocardial injury in patients with SARS-CoV-2 infection could be due to inflammation-induced direct endothelial and vascular injury, plaque rupture with platelet activation and aggregation, microthrombi formation, microvascular dysfunction and occlusion, or coronary spasm.

Myocardial protective drugs can regulate the ACE2 receptor in SARS-CoV-2 virus infection

COVID-19, though primarily a respiratory illness, also affects the heart, raising questions about the use of ACE inhibitors (ACEI) and angiotensin receptor blockers (ARB) due to their effects on ACE2. Meanwhile, melatonin shows promise in protecting the

heart by improving blood vessel function and stabilizing plaques, potentially reducing ACS risk.

COVID-19 patients predominantly present with fever and cough, more frequently in adults than in children, along with dyspnea and myalgia, among other clinical features.[26] As SARS-CoV-2 virus entry into cells is ACE2-dependent, and ACEI and ARB are widely used in cardiovascular disorders, the rationale for the use of ACEI or ARB needs to be considered.[27,28] So far, there are conflicting data on whether these drugs increase or have minimal effects on ACE2 levels, which still require further investigation.

Nevertheless, reports on melatonin suggest a potential protective role against myocardial injury in SARS-CoV-2 infection. The oxygen supply-demand imbalance caused by infection-stimulated hemodynamic activation, hypoxemia, and inflammation-induced plaque instability with coronary hypoperfusion leads to ischemic heart damage. Melatonin could enhance the bioavailability of nitric oxide and improve coronary endothelial function, alleviating plaque instability by inhibiting intra-plaque inflammation. Thus, it exerts a myocardial protective function, potentially mitigating ACS risk and even myocardial ischemia-reperfusion injury.[29] Notably, this does not imply that melatonin is a specific medication targeting acute inflammation in AMI, but it inspires exploration of novel therapeutic approaches for alleviating ACS injury in patients with acute inflammation, such as those with SARS-CoV-2 infection.

Herb benefits both long COVID and myocardial diseases

The COVID-19 pandemic has accelerated research into traditional Chinese medicine and herbal therapies, highlighting their dual antiviral and cardioprotective benefits. These agents work through multiple mechanisms—blocking viral entry, suppressing replication, and reducing inflammation—potentially lowering ACS risk in COVID-19 patients.

Since the first novel coronavirus pneumonia case in 2019, COVID-19 has become a worldwide infectious disease. Following the outbreak, long COVID became a common symptom in most populations. In China, more than 85% of patients received traditional Chinese herbal medicine therapy.[30] Treatment with Chinese herbal medicine has been shown to relieve patients' symptoms.[31]

Accordingly, the most commonly used herb for patients is licorice. While the antiviral mechanisms of glycyrrhizin remain incompletely characterized, emerging evidence suggests its interaction with multiple signaling pathways—including protein kinase C, casein kinase II, AP-1, p38 MAPK, and NF-κB—modulates DNA repair mechanisms and transcriptional regulation.[32–34] Glycyrrhiza species exert cardioprotective effects via coordinated mechanisms, including attenuation of oxidative damage through Nrf2/ARE pathway activation, upregulation of endogenous antioxidant defenses, restoration of cardiac functional parameters, and preservation of myocardial structural integrity.[35] Moreover, glycyrrhizic acid and its bioactive metabolite glycyrrhetinic acid suppress inducible nitric oxide synthase expression in activated macrophages, reducing nitric oxide synthesis—a critical driver of oxidative and inflammatory cascades in acute lung injury pathogenesis. Agastache rugosa, Forsythia suspensa, Atractylodes macrocephala, and Scutellaria baicalensis are also widely used in the mild and moderate stages of COVID-19.[36] In clinical treatment, licorice is used in many traditional Chinese medicine prescriptions, such as QingFeiPaiDu decoction,[37] ShuFengJieDu granules,[38] JinHuaQingGan granules, and LianHua QingWen capsules.[39]

LianHua QingWen granules, a classic traditional Chinese medicine formula,[40,41] have been approved to treat fever, fatigue, and other symptoms caused by mild COVID-19. Regarding the effect of herbs in the composition of LianHua QingWen granules, Chen *et al*.[42] show that several anti-plague components from lotus play a potential role in inhibiting COVID-19 by significantly affecting the binding between ACE2 and S protein, which is a critical mechanism for preventing viral infection. Pan *et al*.[43] showed that puerarin and quercetin may also combat COVID-19 through a similar mechanism by affecting the interaction between S protein and ACE2.

Astragalus membranaceus has a variety of pharmacological activities, including anti-virus, anti-inflammatory, and immune system regulation. It is rich in Astragalus polysaccharides, which have antiviral effects.[44,45] Honeysuckle is also an effective anti-inflammatory Chinese herbal medicine, which is used for a variety of viral infections,[46] such as hepatitis B virus,[47] dengue virus,[48] and intestinal and respiratory viruses, etc. These two herbs have been used since ancient China. Yeh *et al*.[49] showed that honeysuckle-Astragalus preparation can not only improve the expression of a group of COVID-19-related miRNAs but also inhibit the expression of IL-6 and TNF-α, key inflammatory factors in cytokine storms, indicating that it has a certain inhibitory effect on cytokine storms. Furthermore, this preparation can inhibit the binding of COVID-19 protein to the ACE2 receptor, thus playing an antiviral role. In clinical practice, many Chinese patent medicines are in use, including Astragalus membranaceus or honeysuckle, such as Fuling Paidu decoction and Jinhua Qinggan capsules,[50] which can be used to treat patients at various stages.[51,52]

Herb-derived medicine is also under investigation for the treatment of SARS-CoV-2 virus infection. These compounds or phytomedicines include Coronil,[53] Cuphea ignea,[54] Reynoutria Rhizomes,[55] Cordycepin,[56–58] Glycyrrhizic Acid,[59–61] Perilla frutescens,[62,63] Cyperus rotundus Linn,[64] Thymoquinone,[65–67] and others. The underlying mechanisms for the antiviral effects involve preventing the replication of the virus (e.g., Reynoutria Rhizomes),[55] exhibiting inhibitory potential against the COVID-19 polymerase enzyme (RdRp) (e.g., Cordycepin),[68] effectively killing the SARS-CoV-2 virus by targeting key protein structures of the virus or through immune synergy with other antiviral drugs (e.g., Glycyrrhizic Acid),[59] blocking viral RNA and protein synthesis (e.g., Perilla frutescens leaf extract),[62] inhibiting SARS-CoV-2 virus proteases to reduce viral replication, and antagonizing angiotensin-converting enzyme 2 receptors (e.g., Thymoquinone).[69] Thus, these studies provide promising candidates for treating SARS-CoV-2 virus-induced ACS and, therefore, diminishing ACS injury (Table 1 and Fig. 2).[32,40–45,49,50,54,57,63–69]

Non-herbal medicine benefits both long COVID and myocardial diseases

The S proteins of SARS-CoV-1 and CoV-2 share 76% overall sequence identity, yet the receptor-binding domain of the latter has 10–20 times higher affinity for the human ACE2 receptor protein.[70] After the receptor-binding domain binds ACE2, two heptad repeat domains, HR1 and HR2, interact to form a six-helical bundle, bringing the viral and host membranes close to one another, resulting in fusion. Using a recently solved crystal structure of the HR1 and HR2 domains of the SARS-CoV-2 S protein, lipidated peptide fusion inhibitors have been designed to inhibit pseudovirus infection of cells, with IC50 values in the single-digit nanomolar range.[71] Due to its broad-spectrum anti-coronavirus activity, EK1C4 can be used for the treatment and prevention of infection

Table 1. Mechanism of traditional Chinese medicine in the modulation of COVID-19 and myocardial diseases

Name	Composition	Pharmacological action	Drug target/ related signal	Mechanism of action	Myocardial protection	Study type (sample size)	Population characteristics	Key findings (odds/ hazard ratio)	Reference
Honeysuckle Astragalus preparation	Astragalus membranaceus, Honeysuckle	Anti-virus, anti-inflammatory, regulating the immune system	IL-6, TNF-a	Suppressing cytokine storm, inhibiting the binding of COVID-19 protein and ACE2 receptor	Yes[65,66]	Review (one study); Preclinical (animal study); Pharmaceutical Analysis (two studies); Multiple Databases Analysis (one study)	N/A	N/A	40–45
Licorice	Glycyrrhizic Acid	Anti-inflammatory, antioxidant, antitumor	Protein kinase C, casein kinase, AP-1, MAPK-p38 and NF-κB, iNOS	Targeting important protein structures of the virus or immune synergy effect with other antivirus drugs, Inhibition of acute lung injury	Yes[67]	Review (two studies)	N/A	N/A	32,54
Nigella sativa	Thymoquinone	Antioxidant, anti-inflammatory, antiviral, antimicrobial, immunomodulatory, and anticoagulant	Cell surface heat shock protein (HSPA5), ACE2 receptor, IL-2, IL-4, IL-5, II-6, IL-12, IL-13	Inhibiting virus proteases to diminish viral replication and as antagonism to angiotensin-converting ACE2 receptors	Yes[68]	Review (one study)	N/A	N/A	64
Cuphea ignea	–	Antiulcerogenic, antitumor, antioxidant and antihypertensive	–	Inhibiting virus growth	No	Pharmaceutical Analysis (one study)	N/A	N/A	49
Reynoutria Rhizomes	–	Antioxidant, antitumor, anti-inflammatory, and antiviral	–	Preventing the replication of the virus, inhibiting virus growth	No	Pharmaceutical Analysis (one study)	N/A	N/A	50
Cordycepin	–	Anti-inflammatory, antiviral, antibacterial, etc.	–	Inhibiting RdRp	Yes[69]	Molecular Dynamic simulation(one study)	N/A	N/A	63
Perilla	Perilla frutescens leaf extract	Leaves are used to tonify stomach function, discharge heat, and improve healthy *qi*, and seeds decrease *qi*, resolve phlegm, relieve cough and asthma, and alleviate constipation	CXCL10, IL-6, TNF-α, IFN-γ, MCP1	Blocking viral RNA and protein synthesis, inhibiting proinflammatory factor expression	Unknown	Drug Extraction(one study)	N/A	N/A	57

ACE2 (angiotensin-converting enzyme 2) is a transmembrane glycoprotein with an extracellular catalytic domain that acts as a carboxypeptidase. It cleaves C-terminal residues from various substrates and shows 400-fold higher efficiency with angiotensin II than angiotensin I. ACE2 also serves as a functional receptor for the spike glycoproteins of coronaviruses like SARS-CoV, SARS-CoV-2, and HCoV-NL63. Interleukins (ILs) are key cytokines in immune regulation. IL-2, produced by activated T-lymphocytes, induces DNA synthesis in naïve lymphocytes and promotes MHC class II gene expression and B-cell proliferation. IL-4 drives eosinophil differentiation and stimulates activated B-lymphocytes to secrete immunoglobulins. IL-6 is crucial for maintaining homeostasis and is rapidly produced in response to infections or tissue injuries, contributing to host defense through acute-phase and immune responses. IP-10 (Interferon Gamma-Induced Protein 10), a small CXC chemokine (8.7 kDa), is secreted by monocytes, endothelial cells, and fibroblasts upon interferon-γ (IFN-γ) stimulation. TNF-α (Tumor Necrosis Factor-alpha), a serum glycoprotein from activated macrophages and other mammalian mononuclear leukocytes, exhibits antitumor activity and shares TNF receptors with TNF-β (lymphotoxin). Interferon-γ (IFN-γ), the primary interferon from stimulated lymphocytes, differs structurally from type I interferons and is involved in immunoregulation, including inducing class II MHC antigen expression, which can lead to autoimmune diseases. MCP-1, synthesized by vascular wall cells, is controlled by the NF-κB transcription factor. AP-1 (Activator Protein 1), a complex of c-jun and c-fos gene products, dimerizes to bind to the TPA-responsive element (TRE) and regulates gene transcription. p38 MAP kinases (mitogen-activated protein kinases) govern cell growth, differentiation, apoptosis, and inflammation, activated by cytokine receptors and pathogens. NF-κB (nuclear factor kappa B), a ubiquitously inducible transcription factor, is a heterodimer (NF-κB1/RelA) activated by pathogens. Finally, inducible nitric oxide synthase (iNOS), a calcium-independent enzyme, is transcriptionally regulated by cytokines and linked to immune function.

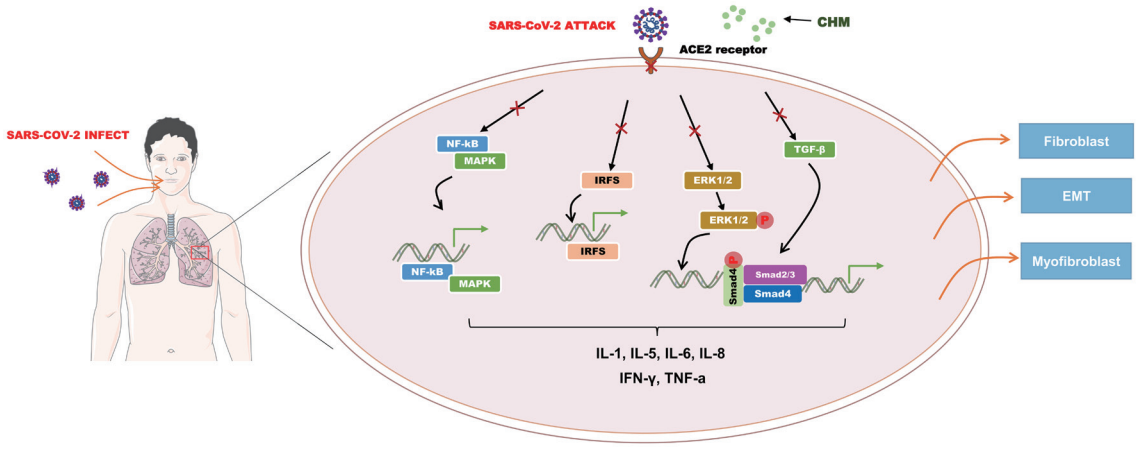


Fig. 2. Traditional Chinese medicine compounds inhibit viral invasion by acting on the ACE2 receptor, which is necessary for COVID-19 to enter the body. These compounds then affect downstream signaling pathways, such as ERK/MAPK, NF-κB, IRFs, and TGF-β, to reduce the expression of inflammatory factors, thereby aiding in the treatment of COVID-19. ACE2, angiotensin-converting enzyme 2; CHM, Chinese herbal medicine; EMT, Epithelial-Mesenchymal Transition; ERK, Extracellular Signal-Regulated Kinase; IFN-γ, interferon-γ; IL, interleukins; IRFS, Interferon regulatory factors; MAPK, Mitogen-Activated Protein Kinase; NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; TGF-β, Transforming Growth Factor Beta; TNF-α, Tumor Necrosis Factor alpha.

not only by SARS-CoV-2 but also by other HCoVs. However, its fundamental limitation is the substantial development costs. Anakinra is the first recombinant IL-1 receptor antagonist that binds to both IL-1α and IL-1β receptors and has received approval from the U.S. Food and Drug Administration (FDA) for treating rheumatoid arthritis. In survival analysis, the development of any thromboembolic event, pulmonary thromboembolism, and ACS was higher in the SoC group compared to the Anakinra group. The survival rate was also lower in the SoC group than in the Anakinra group for patients who experienced thromboembolic events and ACS.[72] However, its effectiveness in patients already suffering from respiratory failure has shown controversial results, and it is not recommended.[73]

Nanotechnology-based herbal medicine may benefit both SARS-CoV-2 virus infection and ACS by targeting the ACE2 receptor

Nanotechnology has revolutionized the COVID-19 response, enabling mRNA vaccine development and offering targeted therapies. Lipid nanoparticles have driven vaccine success, while nanoceria and silver nanoparticles show promise in fighting the virus and its complications through ACE2 modulation.

The U.S. FDA has granted Emergency Use Authorization for two mRNA vaccine candidates: Pfizer-BioNTech's BNT162b2 and Moderna's mRNA-1273.[74] Emerging studies demonstrate that comprehensive targeting of conserved immunodominant epitopes across the full-length spike glycoprotein in SARS-CoV-2 variants, while harnessing the anti-inflammatory potential of regulatory T cells, provides critical insights for developing pan-coronavirus vaccines with broad-spectrum efficacy.[75,76] The unprecedented efficacy of mRNA vaccines (BNT162b2 and mRNA-1273) has marked a turning point in pandemic containment. Nanoparticle platforms have proven instrumental in vaccine development, with lipid-based vectors shielding labile mRNA from ribonucleases while facilitating endosomal escape for cytosolic delivery. Phase III trials demonstrated 95% efficacy rates—a breakthrough stemming from innovative lipid nanoparticle delivery systems that optimize antigen presentation and enhance immunogenicity.[77]

Nanoceria (NC) is a rare-earth nano drug with catalase and superoxide dismutase mimic activity. In clinical studies, there is evidence that nanoceria can pass through TGF-β signaling pathways, potentially inhibiting the progression of fibrosis.[78] NC may effectively inhibit fibrosis and reduce collagen deposition.[79] Targeted transport of NC to the lungs may effectively alleviate acute respiratory distress syndrome.[80]

Targeted transportation may achieve better results. Silver nanoparticles (AgNPs) have also become one of the drugs that can inhibit SARS-CoV-2 virus infection due to their strong antiviral effect. AgNPs can attach to the virus genome to prevent viral replication and new virus release.[81] AgNPs are likely to inhibit severe inflammatory responses, cytokine storms, and pulmonary fibrosis in COVID-19.[82] More interestingly, reports have shown that cationic nanoparticles can directly bind ACE2, decrease its activity, and down-regulate its expression level in lung tissue, resulting in the deregulation of the renin-angiotensin system.[83] Binding to ACE2 by multivalent attachment of ligands to nanocarriers incorporating antiviral therapeutics could increase receptor avidity and impart specificity to these nanovectors for host cells in the pulmonary tract. These findings suggest that nanoparticle-carrying herbal medicine may exert both cardioprotective effects in ACS and anti-inflammatory effects in SARS-CoV-2 virus infection by targeting the ACE2 receptor.

Nanotechnology plays a role in COVID-19 detection,[84] diagnosis, treatment, and other stages.[85] Nanobodies demonstrate therapeutic potential for COVID-19 by attenuating pulmonary hyperinflammation through targeted viral neutralization and immunomodulatory mechanisms.[86] Nanodrugs have higher safety and biocompatibility and can provide more accurate drug targeting.[87] However, nanoparticles also have limitations due to their toxicity. Nanoparticles have low solubility and degradability, so they can persist in cells for a long time and are not easily degraded.[88] Furthermore, there is still a lack of a clear clearance mechanism.[89] Therefore, the application of nanotechnology still needs to be further explored.

Future directions

Current research progress in phytomedicine has established critical

directions for addressing SARS-CoV-2-associated cardiovascular complications, requiring systematic advancement across five key areas. First, large-scale, multicenter, randomized controlled trials should be prioritized to evaluate dose-response relationships of standardized phytochemical formulations (e.g., glycyrrhizic acid, astragaloside IV). These trials should be supported by longitudinal follow-up databases with a minimum three-year monitoring period to assess sustained efficacy and safety. Mechanistically, advanced structural biology techniques such as cryo-electron microscopy are needed to elucidate molecular interactions between bioactive plant compounds and ACE2 receptors. Concurrently, innovations in nanotechnology must address organ-targeting limitations through pharmacokinetic modeling to optimize nanoparticle delivery efficiency and biodegradability, alongside standardized toxicity assessments. Clinically, interdisciplinary frameworks should validate the synergistic effects between phytomedicine and conventional cardiovascular therapies across diverse ethnic populations, utilizing unified cardiovascular endpoints for efficacy evaluation. Furthermore, international consortia should integrate multi-omics platforms and leverage global COVID-19 cardiovascular sequelae registries to identify biomarkers predictive of phytotherapy responsiveness. These coordinated efforts will bridge gaps between preclinical research and clinical translation, ultimately informing evidence-based integrative cardiovascular care strategies.

Conclusions

Current research on SARS-CoV-2-associated ACS and herbal/nanotechnology therapies faces limitations, including reliance on observational data, unclear ACE2 mechanisms, and insufficient long-term safety evidence. Potential biases, such as overemphasis on positive results, may skew conclusions. SARS-CoV-2 triggers ACS through inflammation-induced myocardial injury, requiring therapies that target both cardiovascular damage and virus-driven inflammation. Promising approaches include ACE2-modulating drugs, traditional Chinese medicine, and nanotechnology, which stabilize plaques, protect endothelial function, and enable targeted delivery. However, challenges like nanoparticle toxicity must be addressed. A comprehensive, multi-modal strategy is essential for improving SARS-CoV-2-associated ACS outcomes. Compared to conventional therapeutic regimens, Chinese patent medicines demonstrate superior safety profiles characterized by a reduced incidence of adverse effects and lower treatment costs.

Acknowledgments

None.

Funding

This study was supported by the Zhuhai Social Development Field Science and Technology Plan-Key Project (No. 2320004000286, to RYY) and the Basic and Applied Basic Research of Guangzhou City-University Joint Funding Project (No. 202201020382, to RYY).

Conflict of interest

The authors declare that there is no conflict of interest in the authorship and publication of this contribution.

Author contributions

Contributed to study concept and design (QL, RYY, YD), drafting of the manuscript (XJ, YRL, HS,QZ), critical revision (YYL, ZZ), and finalization of the manuscript (QL). All authors read and approved the final manuscript (PZL, CPL).

References

[1] Timmis A, Vardas P, Townsend N, Torbica A, Katus H, De Smedt D, *et al*. European Society of Cardiology: cardiovascular disease statistics 2021: Executive Summary. European heart journal Quality of care & clinical outcomes 2022;8(4):377–382. doi:10.1093/ehjqcco/qcac014, PMID:35488372.

[2] GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392(10159):1736–1788. doi:10.1016/S0140-6736(18)32203-7, PMID:30496103.

[3] Provenzale G, Barbieri L, Tumminello G, Carugo S, Guazzi M. Exercise response in post-acute coronary syndrome patients survived to COVID-19 infection. Int J Cardiol 2024;411:132285. doi:10.1016/j.ijcard.2024.132285, PMID:38901570.

[4] Libby P, Hansson GK. Adaptive immunity in acute coronary syndromes: chicken or egg? Eur Heart J 2018;39(13):1098–1099. doi:10.1093/eurheartj/ehx743, PMID:29315374.

[5] Amsterdam EA, Wenger NK, Brindis RG, Casey DE Jr, Ganiats TG, Holmes DR Jr, *et al*. 2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2014;64(24):e139–e228. doi:10.1016/j.jacc.2014.09.017, PMID:25260718.

[6] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA 2020;323(11):1061–1069. doi:10.1001/jama.2020.1585, PMID:32031570.

[7] Ayoubkhani D, Khunti K, Nafilyan V, Maddox T, Humberstone B, Diamond I, *et al*. Post-covid syndrome in individuals admitted to hospital with covid-19: retrospective cohort study. BMJ 2021;372:n693. doi:10.1136/bmj.n693, PMID:33789877.

[8] Aye YN, Mai AS, Zhang A, Lim OZH, Lin N, Ng CH, *et al*. Acute myocardial infarction and myocarditis following COVID-19 vaccination. QJM 2023;116(4):279–283. doi:10.1093/qjmed/hcab252, PMID:34586408.

[9] Schmitz T, Meisinger C, Kirchberger I, Thilo C, Amann U, Baumeister SE, *et al*. Impact of COVID-19 pandemic lockdown on myocardial infarction care. Eur J Epidemiol 2021;36(6):619–627. doi:10.1007/s10654-021-00764-2, PMID:34091769.

[10] Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovasc Res 2020;116(6):1097–1100. doi:10.1093/cvr/cvaa078, PMID:32227090.

[11] Khan IH, Zahra SA, Zaim S, Harky A. At the heart of COVID-19. J Card Surg 2020;35(6):1287–1294. doi:10.1111/jocs.14596, PMID:32369872.

[12] Alhogbani T. Acute myocarditis associated with novel Middle east respiratory syndrome coronavirus. Ann Saudi Med 2016;36(1):78–80. doi:10.5144/0256-4947.2016.78, PMID:26922692.

[13] Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol 2020;17(5):259–260. doi:10.1038/s41569-020-0360-5, PMID:32139904.

[14] Imazio M, Klingel K, Kindermann I, Brucato A, De Rosa FG, Adler Y, *et al*. COVID-19 pandemic and troponin: indirect myocardial injury, myocardial inflammation or myocarditis? Heart 2020;106(15):1127–1131. doi:10.1136/heartjnl-2020-317186, PMID:32499236.

[15] Jaffe AS, Cleland JGF, Katus HA. Myocardial injury in severe COVID-19 infection. Eur Heart J 2020;41(22):2080–2082. doi:10.1093/eurheartj/ehaa447, PMID:32464642.

[16] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China.

Lancet 2020;395(10223):497–506. doi:10.1016/S0140-6736(20)30183-5, PMID:31986264.
[17] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395(10223):507–513. doi:10.1016/S0140-6736(20)30211-7, PMID:32007143.
[18] Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, *et al*. Cardiovascular Considerations for Patients, Health Care Workers, and Health Systems During the COVID-19 Pandemic. J Am Coll Cardiol 2020;75(18):2352–2371. doi:10.1016/j.jacc.2020.03.031, PMID:32201335.
[19] Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. N Engl J Med 2013;368(21):2004–2013. doi:10.1056/NEJMra1216063, PMID:23697515.
[20] Campello E, Bulato C, Spiezia L, Boscolo A, Poletto F, Cola M, *et al*. Thrombin generation in patients with COVID-19 with and without thromboprophylaxis. Clin Chem Lab Med 2021;59(7):1323–1330. doi:10.1515/cclm-2021-0108, PMID:33544518.
[21] Canzano P, Brambilla M, Porro B, Cosentino N, Tortorici E, Vicini S, *et al*. Platelet and Endothelial Activation as Potential Mechanisms Behind the Thrombotic Complications of COVID-19 Patients. JACC Basic Transl Sci 2021;6(3):202–218. doi:10.1016/j.jacbts.2020.12.009, PMID:33649738.
[22] Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. Cytokine 2018;104:8–13. doi:10.1016/j.cyto.2018.01.025, PMID:29414327.
[23] Wei ZY, Geng YJ, Huang J, Qian HY. Pathogenesis and management of myocardial injury in coronavirus disease 2019. Eur J Heart Fail 2020;22(11):1994–2006. doi:10.1002/ejhf.1967, PMID:32683753.
[24] Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, *et al*. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 2005;436(7047):112–116. doi:10.1038/nature03712, PMID:16001071.
[25] Fu Y, Cheng Y, Wu Y. Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools. Virol Sin 2020;35(3):266–271. doi:10.1007/s12250-020-00207-4, PMID:32125642.
[26] Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 2020;395(10223):470–473. doi:10.1016/S0140-6736(20)30185-9, PMID:31986257.
[27] Kaseb AO, Mohamed YI, Malek AE, Raad II, Altameemi L, Li D, *et al*. The Impact of Angiotensin-Converting Enzyme 2 (ACE2) Expression on the Incidence and Severity of COVID-19 Infection. Pathogens 2021;10(3):379. doi:10.3390/pathogens10030379, PMID:33809851.
[28] Bojadzic D, Alcazar O, Chen J, Chuang ST, Condor Capcha JM, Shehadeh LA, *et al*. Small-Molecule Inhibitors of the Coronavirus Spike: ACE2 Protein-Protein Interaction as Blockers of Viral Attachment and Entry for SARS-CoV-2. ACS Infect Dis 2021;7(6):1519–1534. doi:10.1021/acsinfecdis.1c00070, PMID:33979123.
[29] Fu Z, Jiao Y, Wang J, Zhang Y, Shen M, Reiter RJ, *et al*. Cardioprotective Role of Melatonin in Acute Myocardial Infarction. Front Physiol 2020;11:366. doi:10.3389/fphys.2020.00366, PMID:32411013.
[30] Ang L, Lee HW, Choi JY, Zhang J, Soo Lee M. Herbal medicine and pattern identification for treating COVID-19: a rapid review of guidelines. Integr Med Res 2020;9(2):100407. doi:10.1016/j.imr.2020.100407, PMID:32289016.
[31] Yang Y, Islam MS, Wang J, Li Y, Chen X. Traditional Chinese Medicine in the Treatment of Patients Infected with 2019-New Coronavirus (SARS-CoV-2): A Review and Perspective. Int J Biol Sci 2020;16(10):1708–1717. doi:10.7150/ijbs.45538, PMID:32226288.
[32] Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet 2003;361(9374):2045–2046. doi:10.1016/s0140-6736(03)13615-x, PMID:12814717.
[33] Chen F, Chan KH, Jiang Y, Kao RY, Lu HT, Fan KW, *et al*. In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. J Clin Virol 2004;31(1):69–75. doi:10.1016/j.jcv.2004.03.003, PMID:15288617.
[34] Dos Santos WG. Natural history of COVID-19 and current knowledge on treatment therapeutic options. Biomed Pharmacother 2020;129:110493. doi:10.1016/j.biopha.2020.110493, PMID:32768971.
[35] Ojha SK, Sharma C, Golechha MJ, Bhatia J, Kumari S, Arya DS. Licorice treatment prevents oxidative stress, restores cardiac function, and salvages myocardium in rat model of myocardial injury. Toxicol Ind Health 2015;31(2):140–152. doi:10.1177/0748233713491800, PMID:23771872.
[36] Gajewski A, Kośmider A, Nowacka A, Puk O, Wiciński M. Potential of herbal products in prevention and treatment of COVID-19. Literature review. Biomed Pharmacother 2021;143:112150. doi:10.1016/j.biopha.2021.112150, PMID:34507112.
[37] Yang R, Liu H, Bai C, Wang Y, Zhang X, Guo R, *et al*. Chemical composition and pharmacological mechanism of Qingfei Paidu Decoction and Ma Xing Shi Gan Decoction against Coronavirus Disease 2019 (COVID-19): In silico and experimental study. Pharmacol Res 2020;157:104820. doi:10.1016/j.phrs.2020.104820, PMID:32360484.
[38] Zhuang Z, Zhong X, Zhang H, Chen H, Huang B, Lin D, *et al*. Exploring the Potential Mechanism of Shufeng Jiedu Capsule for Treating COVID-19 by Comprehensive Network Pharmacological Approaches and Molecular Docking Validation. Comb Chem High Throughput Screen 2021;24(9):1377–1394. doi:10.2174/1386207323999201029122301, PMID:33135607.
[39] Xia QD, Xun Y, Lu JL, Lu YC, Yang YY, Zhou P, *et al*. Network pharmacology and molecular docking analyses on Lianhua Qingwen capsule indicate Akt1 is a potential target to treat and prevent COVID-19. Cell Prolif 2020;53(12):e12949. doi:10.1111/cpr.12949, PMID:33140889.
[40] Runfeng L, Yunlong H, Jicheng H, Weiqi P, Qinhai M, Yongxia S, *et al*. Lianhuaqingwen exerts anti-viral and anti-inflammatory activity against novel coronavirus (SARS-CoV-2). Pharmacol Res 2020;156:104761. doi:10.1016/j.phrs.2020.104761, PMID:32205232.
[41] Hu K, Guan WJ, Bi Y, Zhang W, Li L, Zhang B, *et al*. Efficacy and safety of Lianhuaqingwen capsules, a repurposed Chinese herb, in patients with coronavirus disease 2019: A multicenter, prospective, randomized controlled trial. Phytomedicine 2021;85:153242. doi:10.1016/j.phymed.2020.153242, PMID:33867046.
[42] Chen X, Wu Y, Chen C, Gu Y, Zhu C, Wang S, *et al*. Identifying potential anti-COVID-19 pharmacological components of traditional Chinese medicine Lianhuaqingwen capsule based on human exposure and ACE2 biochromatography screening. Acta Pharm Sin B 2021;11(1):222–236. doi:10.1016/j.apsb.2020.10.002, PMID:33072499.
[43] Pan B, Fang S, Zhang J, Pan Y, Liu H, Wang Y, *et al*. Chinese herbal compounds against SARS-CoV-2: Puerarin and quercetin impair the binding of viral S-protein to ACE2 receptor. Comput Struct Biotechnol J 2020;18:3518–3527. doi:10.1016/j.csbj.2020.11.010, PMID:33200026.
[44] Zheng Y, Ren W, Zhang L, Zhang Y, Liu D, Liu Y. A Review of the Pharmacological Action of Astragalus Polysaccharide. Front Pharmacol 2020;11:349. doi:10.3389/fphar.2020.00349, PMID:32265719.
[45] Xue H, Gan F, Zhang Z, Hu J, Chen X, Huang K. Astragalus polysaccharides inhibits PCV2 replication by inhibiting oxidative stress and blocking NF-κB pathway. Int J Biol Macromol 2015;81:22–30. doi:10.1016/j.ijbiomac.2015.07.050, PMID:26226456.
[46] Yu Y, Zhu C, Wang S, Song W, Yang Y, Shi J. Homosecoiridoid alkaloids with amino acid units from the flower buds of Lonicera japonica. J Nat Prod 2013;76(12):2226–2233. doi:10.1021/np4005773, PMID:24279769.
[47] Ge L, Xiao L, Wan H, Li J, Lv K, Peng S, *et al*. Chemical constituents from Lonicera japonica flower buds and their anti-hepatoma and anti-HBV activities. Bioorg Chem 2019;92:103198. doi:10.1016/j.bioorg.2019.103198, PMID:31446242.
[48] Lee YR, Yeh SF, Ruan XM, Zhang H, Hsu SD, Huang HD, *et al*. Honeysuckle aqueous extract and induced let-7a suppress dengue virus type 2 replication and pathogenesis. J Ethnopharmacol 2017;198:109–121. doi:10.1016/j.jep.2016.12.049, PMID:28052239.
[49] Yeh YC, Doan LH, Huang ZY, Chu LW, Shi TH, Lee YR, *et al*. Honeysuckle (Lonicera japonica) and Huangqi (Astragalus membranaceus) Suppress SARS-CoV-2 Entry and COVID-19 Related Cytokine Storm in Vitro. Front Pharmacol 2021;12:765553. doi:10.3389/fphar.2021.765553, PMID:35401158.
[50] Liu Z, Li X, Gou C, Li L, Luo X, Zhang C, *et al*. Effect of Jinhua Qinggan granules on novel coronavirus pneumonia in patients. J Tradit Chin Med 2020;40(3):467–472. doi:10.19852/j.cnki.jtcm.2020.03.016,

PMID:32506862.
[51] Lee DYW, Li QY, Liu J, Efferth T. Traditional Chinese herbal medicine at the forefront battle against COVID-19: Clinical experience and scientific basis. Phytomedicine 2021;80:153337. doi:10.1016/j.phymed.2020.153337, PMID:33221457.
[52] Zang Y, Wan J, Zhang Z, Huang S, Liu X, Zhang W. An updated role of astragaloside IV in heart failure. Biomed Pharmacother 2020;126:110012. doi:10.1016/j.biopha.2020.110012, PMID:32213428.
[53] Balkrishna A, Tomer M, Verma S, Joshi M, Sharma P, Srivastava J, *et al*. Phytometabolite profiling of Coronil, a herbal medicine for COVID-19, its identification by mass-spectroscopy and quality validation on liquid chromatographic platforms. J Sep Sci 2021;44(22):4064–4081. doi:10.1002/jssc.202100499, PMID:34510772.
[54] Mahmoud DB, Ismail WM, Moatasim Y, Kutkat O, ElMeshad AN, Ezzat SM, *et al*. Delineating a potent antiviral activity of Cuphea ignea extract loaded nano-formulation against SARS-CoV-2: In silico and in vitro studies. J Drug Deliv Sci Technol 2021;66:102845. doi:10.1016/j.jddst.2021.102845, PMID:34539819.
[55] Nawrot-Hadzik I, Zmudzinski M, Matkowski A, Preissner R, Kęsik-Brodacka M, Hadzik J, *et al*. Reynoutria Rhizomes as a Natural Source of SARS-CoV-2 Mpro Inhibitors-Molecular Docking and In Vitro Study. Pharmaceuticals (Basel) 2021;14(8):742. doi:10.3390/ph14080742, PMID:34451839.
[56] Verma AK. Cordycepin: a bioactive metabolite of Cordyceps militaris and polyadenylation inhibitor with therapeutic potential against COVID-19. J Biomol Struct Dyn 2022;40(8):3745–3752. doi:10.1080/07391102.2020.1850352, PMID:33225826.
[57] Verma AK, Aggarwal R. Repurposing potential of FDA-approved and investigational drugs for COVID-19 targeting SARS-CoV-2 spike and main protease and validation by machine learning algorithm. Chem Biol Drug Des 2021;97(4):836–853. doi:10.1111/cbdd.13812, PMID:33289334.
[58] Rabie AM. Potent Inhibitory Activities of the Adenosine Analogue Cordycepin on SARS-CoV-2 Replication. ACS Omega 2022;7(3):2960–2969. doi:10.1021/acsomega.1c05998, PMID:35071937.
[59] Sun Z, He G, Huang N, Thilakavathy K, Lim JCW, Kumar SS, *et al*. Glycyrrhizic Acid: A Natural Plant Ingredient as a Drug Candidate to Treat COVID-19. Front Pharmacol 2021;12:707205. doi:10.3389/fphar.2021.707205, PMID:34305613.
[60] Zheng W, Huang X, Lai Y, Liu X, Jiang Y, Zhan S. Glycyrrhizic Acid for COVID-19: Findings of Targeting Pivotal Inflammatory Pathways Triggered by SARS-CoV-2. Front Pharmacol 2021;12:631206. doi:10.3389/fphar.2021.631206, PMID:34177566.
[61] Yu S, Zhu Y, Xu J, Yao G, Zhang P, Wang M, *et al*. Glycyrrhizic acid exerts inhibitory activity against the spike protein of SARS-CoV-2. Phytomedicine 2021;85:153364. doi:10.1016/j.phymed.2020.153364, PMID:33041173.
[62] Tang WF, Tsai HP, Chang YH, Chang TY, Hsieh CF, Lin CY, *et al*. Perilla (Perilla frutescens) leaf extract inhibits SARS-CoV-2 via direct virus inactivation. Biomed J 2021;44(3):293–303. doi:10.1016/j.bj.2021.01.005, PMID:34119448.
[63] Jan JT, Cheng TR, Juang YP, Ma HH, Wu YT, Yang WB, *et al*. Identification of existing pharmaceuticals and herbal medicines as inhibitors of SARS-CoV-2 infection. Proc Natl Acad Sci U S A 2021;118(5):e2021579118. doi:10.1073/pnas.2021579118, PMID:33452205.
[64] Kumar SB, Krishna S, Pradeep S, Mathews DE, Pattabiraman R, Murahari M, *et al*. Screening of natural compounds from Cyperus rotundus Linn against SARS-CoV-2 main protease (M(pro)): An integrated computational approach. Comput Biol Med 2021;134:104524. doi:10.1016/j.compbiomed.2021.104524, PMID:34090015.
[65] Ahmad A, Raish M, Alkharfy KM. The potential role of thymoquinone in preventing the cardiovascular complications of COVID-19. Vascul Pharmacol 2021;141:106899. doi:10.1016/j.vph.2021.106899, PMID:34311073.
[66] Xu H, Liu B, Xiao Z, Zhou M, Ge L, Jia F, *et al*. Computational and Experimental Studies Reveal That Thymoquinone Blocks the Entry of Coronaviruses Into In Vitro Cells. Infect Dis Ther 2021;10(1):483–494. doi:10.1007/s40121-021-00400-2, PMID:33532909.
[67] Esharkawy ER, Almalki F, Hadda TB. In vitro potential antiviral SARS-CoV-19- activity of natural product thymohydroquinone and dithymoquinone from Nigella sativa. Bioorg Chem 2022;120:105587. doi:10.1016/j.bioorg.2021.105587, PMID:35026560.
[68] Bibi S, Hasan MM, Wang YB, Papadakos SP, Yu H. Cordycepin as a Promising Inhibitor of SARS-CoV-2 RNA Dependent RNA Polymerase (RdRp). Curr Med Chem 2022;29(1):152–162. doi:10.2174/0929867328666210820114025, PMID:34420502.
[69] Badary OA, Hamza MS, Tikamdas R. Thymoquinone: A Promising Natural Compound with Potential Benefits for COVID-19 Prevention and Cure. Drug Des Devel Ther 2021;15:1819–1833. doi:10.2147/DDDT.S308863, PMID:33976534.
[70] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, *et al*. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367(6483):1260–1263. doi:10.1126/science.abb2507, PMID:32075877.
[71] Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, *et al*. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res 2020;30(4):343–355. doi:10.1038/s41422-020-0305-x, PMID:32231345.
[72] Çakmak R, Yüce S, Ay M, Uyar MH, Kılıç Mİ, Bektaş M. Intravenous high-dose anakinra drops venous thrombosis and acute coronary syndrome in severe and critical COVID-19 patients: a propensity score matched study. Sci Rep 2024;14(1):12369. doi:10.1038/s41598-024-62079-y, PMID:38811592.
[73] Khani E, Shahrabi M, Rezaei H, Pourkarim F, Afsharirad H, Solduzian M. Current evidence on the use of anakinra in COVID-19. Int Immunopharmacol 2022;111:109075. doi:10.1016/j.intimp.2022.109075, PMID:35905562.
[74] Meo SA, Bukhari IA, Akram J, Meo AS, Klonoff DC. COVID-19 vaccines: comparison of biological, pharmacological characteristics and adverse effects of Pfizer/BioNTech and Moderna Vaccines. Eur Rev Med Pharmacol Sci 2021;25(3):1663–1669. doi:10.26355/eurrev_202102_24877, PMID:33629336.
[75] Kumar R, Srivastava Y, Muthuramalingam P, Singh SK, Verma G, Tiwari S, *et al*. Understanding Mutations in Human SARS-CoV-2 Spike Glycoprotein: A Systematic Review & Meta-Analysis. Viruses 2023;15(4):856. doi:10.3390/v15040856, PMID:37112836.
[76] Dhawan M, Rabaan AA, Alwarthan S, Alhajri M, Halwani MA, Alshengeti A, *et al*. Regulatory T Cells (Tregs) and COVID-19: Unveiling the Mechanisms, and Therapeutic Potentialities with a Special Focus on Long COVID. Vaccines (Basel) 2023;11(3):699. doi:10.3390/vaccines11030699, PMID:36992283.
[77] Khurana A, Allawadhi P, Khurana I, Allwadhi S, Weiskirchen R, Banothu AK, *et al*. Role of nanotechnology behind the success of mRNA vaccines for COVID-19. Nano Today 2021;38:101142. doi:10.1016/j.nantod.2021.101142, PMID:33815564.
[78] Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. Int J Antimicrob Agents 2020;55(3):105924. doi:10.1016/j.ijantimicag.2020.105924, PMID:32081636.
[79] Oró D, Yudina T, Fernández-Varo G, Casals E, Reichenbach V, Casals G, *et al*. Cerium oxide nanoparticles reduce steatosis, portal hypertension and display anti-inflammatory properties in rats with liver fibrosis. J Hepatol 2016;64(3):691–698. doi:10.1016/j.jhep.2015.10.020, PMID:26519601.
[80] Allawadhi P, Khurana A, Allwadhi S, Joshi K, Packirisamy G, Bharani KK. Nanoceria as a possible agent for the management of COVID-19. Nano Today 2020;35:100982. doi:10.1016/j.nantod.2020.100982, PMID:32952596.
[81] Salleh A, Naomi R, Utami ND, Mohammad AW, Mahmoudi E, Mustafa N, *et al*. The Potential of Silver Nanoparticles for Antiviral and Antibacterial Applications: A Mechanism of Action. Nanomaterials (Basel) 2020;10(8):1566. doi:10.3390/nano10081566, PMID:32784939.
[82] Allawadhi P, Singh V, Khurana A, Khurana I, Allwadhi S, Kumar P, *et al*. Silver nanoparticle based multifunctional approach for combating COVID-19. Sens Int 2021;2:100101. doi:10.1016/j.sintl.2021.100101, PMID:34766057.
[83] Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, *et al*. Large-scale association analysis identifies new risk loci for

coronary artery disease. Nat Genet 2013;45(1):25–33. doi:10.1038/ng.2480, PMID:23202125.
[84] Alhalaili B, Popescu IN, Kamoun O, Alzubi F, Alawadhia S, Vidu R. Nanobiosensors for the Detection of Novel Coronavirus 2019-nCoV and Other Pandemic/Epidemic Respiratory Viruses: A Review. Sensors (Basel) 2020;20(22):6591. doi:10.3390/s20226591, PMID:33218097.
[85] Rai M, Bonde S, Yadav A, Bhowmik A, Rathod S, Ingle P, *et al*. Nanotechnology as a Shield against COVID-19: Current Advancement and Limitations. Viruses 2021;13(7):1224. doi:10.3390/v13071224, PMID:34202815.
[86] Verma G, Dhawan M, Saied AA, Kaur G, Kumar R, Emran TB. Immunomodulatory approaches in managing lung inflammation in COVID-19: A double-edge sword. Immun Inflamm Dis 2023;11(9):e1020. doi:10.1002/iid3.1020, PMID:37773723.
[87] Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol 2010;10(11):787–796. doi:10.1038/nri2868, PMID:20948547.
[88] Kim HP, Kim JK, Jo MS, Park JD, Ahn K, Gulumian M, *et al*. Even lobar deposition of poorly soluble gold nanoparticles (AuNPs) is similar to that of soluble silver nanoparticles (AgNPs). Part Fibre Toxicol 2020;17(1):54. doi:10.1186/s12989-020-00384-w, PMID:33081787.
[89] Osman NM, Sexton DW, Saleem IY. Toxicological assessment of nanoparticle interactions with the pulmonary system. Nanotoxicology 2020;14(1):21–58. doi:10.1080/17435390.2019.1661043, PMID:31502904.

Exploratory Research and Hypothesis in Medicine **2025** vol. 10(2) | 106–121
DOI: 10.14218/ERHM.2024.00046

Review Article

Aging and DNA Damage: Investigating the Microbiome's Stealthy Impact – A Perspective

Swarup K. Chakrabarti[1*] and Dhrubajyoti Chattopadhyay[1,2]

[1]*H. P. Ghosh Research Center, New Town, Kolkata, West Bengal, India;* [2]*Sister Nivedita University, New Town, West Bengal, India*

Received: December 20, 2024 | **Revised:** January 28, 2025 | **Accepted:** February 21, 2025 | **Published online:** April 01, 2025

Abstract

This review explores how the gut microbiome influences aging, particularly examining the effects of microbiome imbalances (dysbiosis) on immune system function, inflammation, and the integrity of genetic material. As we age, there is a noticeable decline in cellular and physiological capabilities, which heightens the risk of diseases and diminishes the body's resilience to stress. A significant contributor to this decline is the change in the gut microbiome, which affects immune reactions, triggers chronic inflammation, and worsens DNA damage. The review is structured into several key areas: first, the connection between dysbiosis and age-related ailments such as rheumatoid arthritis, Crohn's disease, and systemic lupus erythematosus; second, how aging influences immune tolerance, especially regarding dendritic cells, and its link to autoimmune diseases; third, the acceleration of immunosenescence and the prolonged inflammatory responses associated with aging; and fourth, the impact of senescent cells and oxidative stress on increasing inflammation and damaging DNA. We also underscored the significance of short-chain fatty acids produced by beneficial gut bacteria in modulating immune responses and facilitating DNA repair. The discussion includes the potential use of probiotics and other microbiome-related interventions as treatment options to promote healthy aging. Ultimately, we stressed the necessity for additional research to deepen our comprehension of the microbiome's effect on DNA damage and to create personalized therapeutic strategies for fostering healthier aging and enhancing longevity.

Introduction

Aging is a multifaceted biological phenomenon characterized by a progressive decline in cellular and physiological functions, affecting all organisms universally.[1,2] This process undermines resilience to stress, the capacity for injury recovery, and the maintenance of internal stability, critical facets tightly intertwined with aging.[3–5] Recent scientific inquiries have illuminated an intriguing intersection between aging and the human microbiome—an intricate ecosystem of microorganisms cohabiting within our bodies.[6,7] In particular, the relationship between the microbiome, notably the gut microbiota, and aging has emerged as a focal point of research, unveiling profound implications for human health and longevity.[8–10]

DNA damage is widely considered one of the hypothesized hallmarks of aging.[11,12] It is influenced by various cumulative factors, including exposure to environmental agents such as ultraviolet radiation, pollution, and toxins.[13–15] Additionally, aging is associated with increased production of reactive oxygen species (ROS) due to declining mitochondrial function.[16–18] Furthermore, as cells divide, telomeres shorten, contributing to cellular senescence.[19,20] Moreover, age-related declines in DNA repair efficiency, epigenetic modifications affecting gene expression in DNA repair, and replication stress further exacerbate DNA damage.[21–23] Chronic inflammation, known as "inflammaging," which is associated with aging and induced by pro-inflammatory cytokines, exacerbates oxidative stress and the accumulation of DNA damage.[24–28] Genomic instability resulting from accumulated mutations and chromosomal abnormalities further contributes to age-related DNA damage.[29,30]

As individuals age, their microbiome undergoes substantial transformations characterized by diminished diversity and shifts in bacterial composition.[6,31] These changes involve an increase in opportunistic pathogens and a decline in beneficial bacteria.[32] Factors such as altered metabolic activity, which influences the production of essential short-chain fatty acids (SCFAs) crucial for gut health, contribute to these shifts.[8,33,34] Furthermore, aging-related immunosenescence, marked by diminished immune function, promotes chronic low-grade inflammation and compromises the integrity of the gut barrier, thereby disrupting the microbiome.[35–37] Oxidative stress, compounded by these changes, also influences

Keywords: Aging; Gut micorbiome; Dybiosis; Chronic inflammation; Immunosenescence; Short-chain fatty acids; DNA damage; Senescent cells; Probiotics.

***Correspondence to:** Swarup K. Chakrabarti, H. P. Ghosh Research Center, HIDCO (II), EK Tower, New Town, Kolkata, West Bengal 700161, India. ORCID: https://orcid.org/0000-0001-5666-7662. Tel: +91-9831643038, E-mail: swarupkchakrabarti@gmail.com

How to cite this article: Chakrabarti SK, Chattopadhyay D. Aging and DNA Damage: Investigating the Microbiome's Stealthy Impact – A Perspective. *Explor Res Hypothesis Med* 2025;10(2):106–121. doi: 10.14218/ERHM.2024.00046.

the microbiome's composition and functionality.[38–40] Additionally, dietary shifts, lifestyle modifications, increased antibiotic usage, and chronic diseases impact the microbiome's ability to synthesize vitamins, degrade complex polysaccharides, and regulate host metabolism.[41,42]

Importantly, this exploration of microbiota-mediated regulation of DNA damage and its implications for human health and aging reveals striking parallels in the mechanisms underlying age-related DNA damage and microbial dysregulation. An understanding of these complexities could pave the way for innovative therapeutic strategies aimed at fostering healthy aging and combating age-related diseases. Hence, this review aimed to critically examine the complex interactions between the microbiome, DNA damage, and aging, with a specific focus on their interrelationship and their broader implications for human health and disease. By exploring these interactions, we seek to deepen our understanding of the biological mechanisms that underlie human longevity and, more importantly, healthspan—the portion of life spent in optimal health.

An extensive literature search was performed to identify pertinent studies for this review. The search covered multiple academic databases, including PubMed, Scopus, Web of Science, and Google Scholar, with a focus on laboratory, *in vivo*, and clinical research. The search strategy was designed to align with the central themes of the review, utilizing targeted keywords and phrases relevant to the research focus. A total of 993 papers were identified through database searches. After removing 24 duplicates, 969 papers remained for screening. Of these, 600 papers were excluded based on title and abstract screening, primarily due to irrelevance to the narrative review, lack of methodological rigor, insufficient data or analysis, duplicate entries, or non-availability of full text. Ultimately, approximately 300 papers were included for full-text review. In this context, duplicates refer to instances where the same paper appeared multiple times across different database search results, either due to indexing in multiple databases or different versions of the same paper, as shown in the flowchart (Fig. 1).

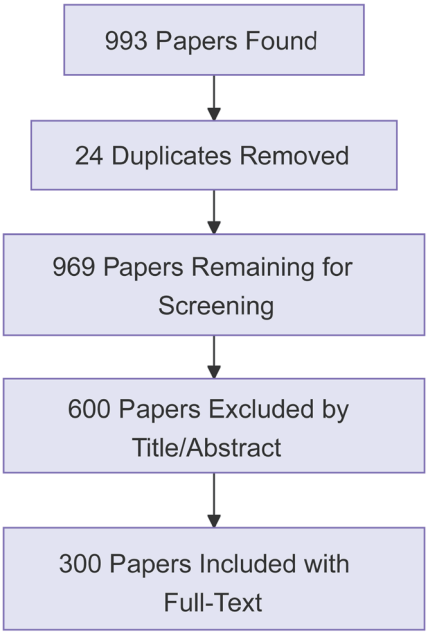


Fig. 1. Flowchart of the literature search and screening process.

The interplay between gut microbiota and immune function in health and disease

The human gut microbiome plays a crucial role in overall health, with its composition influenced by multiple factors such as genetics, immune system effectiveness, age, gender, and lifestyle choices, including diet, pregnancy, and stress management. Maintaining a balanced microbiome (eubiosis) is vital for optimal health, as it helps produce essential metabolites and boosts immune functions.[43,44] In contrast, dysbiosis occurs when there is an imbalance, resulting in the growth of harmful microorganisms while beneficial ones decline, which can lead to various health issues.[45,46]

An increasing number of studies are highlighting the link between gut bacteria and diverse health outcomes.[44,47,48] For instance, a research study conducted in Poland explored the impact of polyphenols, lignans, and herbal sterols on immune-modulating bacteria such as *Escherichia coli* and *Enterococcus spp*. in a group of 95 non-obese individuals. The findings suggested that a higher intake of these compounds might be linked to a lower risk of COVID-19, likely due to beneficial changes in gut microbiome composition.[49] This implies that altering the microbiome could be useful in preventing infections, particularly respiratory diseases like COVID-19. However, the authors stress the importance of conducting additional research to confirm these results and to investigate microbiome-centered approaches for tackling various health challenges.

Importantly, the gut microbiome plays a pivotal role in modulating immune responses, a factor of particular relevance in the context of COVID-19, where dysregulation of immune function is central to the pathophysiology and progression of the disease.[50,51] An excessively pro-inflammatory response can lead to severe complications. Proteins known as Toll-like receptors play a crucial role in detecting pathogens such as SARS-CoV-2 and initiating immune responses, including the release of type I interferons and inflammatory cytokines.[52,53] Nevertheless, an overly aggressive or delayed immune response during viral respiratory infections, including COVID-19, may worsen the severity of the disease. The gut microbiome is essential for regulating immune responses, including the activation of Toll-like receptors via the gut-lung axis, which links gut health to lung function.[54,55] A well-balanced microbiome enhances immune activation, ensuring an effective response to pathogens, whereas a disrupted microbiome may increase inflammation and the risk of severe disease.[43–46] Additionally, beneficial gut bacteria generate SCFAs, which help manage inflammation and maintain immune balance, potentially resulting in improved outcomes for various diseases.[8,33,34,56]

Recent studies also highlight the significant influence of the microbiome on overall well-being. Research linking intestinal bacteria to lung diseases such as asthma, chronic bronchitis, COPD (chronic obstructive pulmonary disease), and pulmonary arterial hypertension reveals distinct associations between certain gut bacteria and the risk of developing these diseases.[57–59] This emphasizes the microbiome's vital importance in both digestive and respiratory health. Furthermore, investigations into autoimmune diseases like juvenile idiopathic arthritis and uveitis indicate that changes in gut microbiota may play a role in these disorders.[60,61] Mendelian randomization studies provide new insights into their underlying mechanisms and possible treatments.[62] In addition to immune responses, the microbiota-gut-brain axis has gained interest for its influence on mental health.[63,64] Studies suggest that gut microbiota

can influence psychological issues such as post-traumatic stress disorder, highlighting notable differences in SCFAs and bacterial populations in those affected.[65,66] Research involving probiotics and fermented foods has shown promising results, indicating that modifying the gut microbiome might improve mental health treatments.[67,68] This connection underscores the essential role of gut health in our psychological state. Importantly, a significant factor linking gut microbes to overall well-being is systemic inflammation.[69,70] This relationship is becoming more important in the realm of predictive, preventive, and personalized medicine, as it unveils health risks and aids in effective care strategies. By using the microbiome and inflammation as indicators, we can develop early intervention strategies and tailored therapies to enhance health outcomes and individual treatments.

Research into the role of gut microbiota in zoonotic diseases like leptospirosis is currently ongoing.[71,72] This infection leads to approximately 1 million reported cases each year, with a mortality rate of 6.86%, translating to roughly 60,000 deaths globally.[73,74] The nature of the relationship between leptospirosis and gut microbiota is not yet fully understood; however, recent research has shown that a Leptospira infection alters the composition of gut microbes, notably raising the Firmicutes/Bacteroidetes ratio.[75,76] While antibiotics and steroids are frequently prescribed, there is increasing interest in probiotics as a potential treatment alternative.[77] Studies indicate that the use of antibiotics, which reduce gut microbiota, can worsen the infection's impact on various organs, whereas fecal microbiota transplantation may alleviate these effects.[77,78] This suggests that preserving or restoring a balanced gut microbiota could be vital for effectively managing leptospirosis. Therefore, probiotics, which help reestablish gut balance, may be advantageous in lessening the severity of the infection, improving immune responses, and aiding recovery.

Together, these studies highlight the crucial impact that gut microbiota has on various health issues, including immune response, respiratory illnesses, mental health, and infections. Growing evidence emphasizes the necessity for additional research into microbiome-focused therapies, which present exciting opportunities for disease prevention, health enhancement, and personalized treatment alternatives.

DNA repair, maintenance, and microbiome interconnections

DNA repair and maintenance are fundamental to genetic stability and represent pivotal elements within biological systems.[79] It is widely acknowledged that the accumulation of DNA damage, which is presumed to occur progressively over time, can potentially be mitigated through the microbiome's modulatory impact on DNA repair pathways.[80–82] Thus, fostering a robust microbiota capable of supporting optimal DNA repair mechanisms holds promise for potentially attenuating the aging process and enhancing genetic stability.[83–85]

Notably, cancer patients afflicted with erysipelas, a skin infection caused by *Streptococcus* pyogenes, experienced spontaneous regression of tumors due to the infection.[86–88] Additionally, *Escherichia coli* emerged as the first microorganism identified to demonstrate mutagenic properties.[89] Colibactin, a compound derived from *Escherichia coli*, has been found to induce double-strand DNA breaks through the alkylation of adenine residues, potentially leading to direct mutations associated with colorectal cancer.[90] Intriguingly, genotoxins secreted by the gut microbiota exhibit DNase activity.[91] Upon release in close proximity to gastrointestinal (GI) epithelial cells, these toxins induce double-strand DNA breaks, triggering a transient cell cycle arrest in host epithelial cells.[92,93] Alongside colibactin, a variety of other bacterial toxins that cause genetic damage can lead to considerable harm to the host's DNA. Cytolethal distending toxins (CDTs), secreted by Gram-negative bacteria such as *Escherichia coli*, *Shigella dysenteriae*, and *Campylobacter jejuni*, act as potent DNA-damaging agents.[94,95] These toxins cause genotoxic effects by interfering with the cell cycle and resulting in DNA fragmentation, which contributes to genomic instability. This process is crucial for the onset of colorectal cancer, where *E. coli* strains that produce CDTs play a role in DNA damage and the formation of tumors.[96] Another notable genotoxic toxin is typhoid toxin, which is generated by *Salmonella typhi*, *Salmonella paratyphi*, and other *Salmonella* subspecies (such as *arizonae and javiana*).[97] The toxin associated with typhoid harms DNA and plays a vital role in the development of typhoid fever, a widespread infection that causes serious digestive issues and other complications.[98] Moreover, shigellosis, which is caused by *Shigella dysenteriae*, along with campylobacteriosis from *Campylobacter jejuni*, also involves CDTs, which lead to severe GI inflammation and can sometimes result in complications outside the intestines, such as Guillain-Barré syndrome.[99,100] These observations underscore the crucial role of microbial toxins in perturbing the genomic stability of hosts, contributing to the emergence and progression of various infections and cancers.

On another front, the transcription factor tumor protein,[53] p53, well-known for its tumor-suppressing abilities, binds to distinct DNA sequences, activating transcription, regulating the cell cycle, and facilitating the repair of damaged genes.[101,102] Interestingly, a significant portion of p53 mutations commonly associated with cancer initiation are attributed to metabolites produced by the gut microbiota.[103,104] Furthermore, *Shigella flexneri*, using secretases like virulence gene A and inositol *phosphoinositide 4-phosphatase,* induces degradation of the host cell's p53, thereby elevating the incidence of DNA mutations.[105–107] Moreover, anaerobic Gram-negative bacteria such as *Fusobacterium nucleatum* are frequently observed in the microbiomes of individuals with colorectal cancer.[108,109] There is a general consensus that ROS and pro-inflammatory substances may contribute to the *Fusobacterium nucleatum* oncogenic process. ROS could potentially lead to alterations in 5′—C—phosphate—G—3′ site methylation, resulting in microsatellite instability and other epigenetic changes.[110,111] Concurrently, pro-inflammatory agents and ROS may induce DNA damage. Similarly, *Helicobacter pylori* triggers the production of hydrogen peroxide and ROS via spermine oxidase, potentially causing DNA mutation and promoting carcinogenesis.[112,113] Lastly, clinical investigations revealed a correlation between highly pathogenic mutations in the Adenomatous Polyposis Coli tumor suppressor gene in the intestinal cells of patients, an increase in *Fusobacterium mortiferum*, and a notable decrease in *Clostridium geniculatum* and *Bifidobacteria*.[114–116] These studies are among the numerous pieces of evidence linking the microbiome to DNA integrity. Additional pertinent studies are listed in Table 1.[117–121]

Dysbiosis, aging, and immunosenescence: mechanisms at the gut interface

As the body's ability to repair itself diminishes over time, chronic inflammation and immunosenescence create conditions conducive to increased DNA damage.[122,123] This convergence of factors sets the stage for a vicious cycle, where each element exacerbates the effects of the others.[124,125] Dysbiosis, an imbalance in the gut

Table 1. Studies on DNA repair, maintenance, and microbiome interconnections

Study title	Authors	Year	Main findings	Reference
Bacterial phenotypic heterogeneity in DNA repair and mutagenesis	Vincent *et al.*	2020	The article highlights how variation in DNA repair pathways can affect mutation rates and genome stability in bacteria, especially under antibiotic stress	117
Gut Microbiota as Important Mediator Between Diet and DNA Methylation and Histone Modifications in the Host	D'Aquila *et al.*	2020	The review highlights that gut microbiota, through its metabolites, plays a crucial role in shaping the host's epigenome by influencing DNA methylation and histone modifications, thereby affecting cellular activities	118
Bacterial DNA excision repair pathways	Wozniak *et al.*	2022	This article highlights newly discovered bacterial DNA repair pathways, including EndoMS and MrfAB, advancing our understanding of genome maintenance	119
DNA Damage and the Gut Microbiome: From Mechanisms to Disease Outcomes	Hsiao *et al.*	2023	This article explores how DNA damage impacts the gut microbiome, linking impaired microbial DNA repair to dysbiosis, disrupted host interactions, inflammation, and disease outcomes like gastrointestinal disorders, metabolic dysfunction, and cancer	120
Colibactin-induced damage in bacteria is cell contact-independent	Lowry *et al.*	2025	This study reveals that colibactin-induced DNA damage in bacterial cells occurs over long distances without direct contact. Using a fluorescent reporter system, researchers found that genotoxic effects saturated within 12 h and were detectable hundreds of microns away, challenging previous delivery assumptions	121

microbiota, accelerates immunosenescence, thereby accelerating DNA degradation associated with aging.[45,46,126,127] Alternatively, DNA damage escalates inflammation, perpetuating a self-reinforcing loop of decline.[128–130] Research indicates that dysbiosis may contribute to age-related immunosenescence through various mechanisms. One crucial aspect involves the modulation of the gut-associated lymphoid tissue (GALT), a pivotal component of the immune system residing in the GI tract, often regarded as the body's largest immune organ.[131–133] As people age, the functionality of GALT diminishes, leading to a weakened immune response and a higher risk of infections and diseases. Recent research has shown that aging results in a decrease in both the quantity and functionality of key immune cells found in GALT, such as naive T cells, B cells, and dendritic cells (DCs), which are vital for triggering immune responses.[134,135] This age-related reduction in immune cell populations is associated with an elevated vulnerability to GI cancers and systemic inflammation in older adults.[136,137] Within GALT, innate immune cells act as the frontline defenders of the gut mucosa, playing essential roles in pathogen recognition, initiating the innate immune response, and presenting antigens to activate the adaptive immune system downstream.[138–140] Moreover, GALT is integral in maintaining immunological tolerance to commensal bacteria, serving as a crucial link between the systemic immune response and the local immunological environment of the gut. Alterations in microbiota composition can profoundly influence the development and function of immune cells within GALT, thereby potentially impacting systemic immune responses.[141–143] This intricate interplay underscores the importance of gut microbiota in modulating immune function and highlights its relevance in the context of aging and immunosenescence (Fig. 2).

Additionally, there is compelling evidence connecting the loss of DC tolerance to gut dysbiosis.[144,145] Recent clinical studies have demonstrated that gut microbiota dysbiosis profoundly impacts DC-mediated immune tolerance, contributing to immune dysfunction.[144,146] In patients with inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, dysbiosis characterized by an overgrowth of pro-inflammatory bacteria impairs DC function, leading to a failure to induce tolerance to gut antigens and subsequent inflammation.[147,148] These patients also exhibit a reduction in T cells (Tregs) in the gut, further exacerbating immune dysregulation and promoting chronic inflammation. Similarly, individuals with autoimmune conditions like rheumatoid arthritis and systemic lupus erythematosus show altered gut microbiota profiles, which skew DC activation, promoting pro-inflammatory responses that drive autoimmune pathogenesis.[149,150] Furthermore, studies have demonstrated that microbiome-based interventions, such as probiotics, can restore DC function and enhance regulatory Treg induction, reducing inflammatory markers in elderly individuals with dysbiosis.[151,152] Collectively, these findings highlight the critical role of gut microbiota in modulating DC-mediated immune responses and underscore the potential of microbiome-targeted therapies to restore immune tolerance, reduce inflammation, and mitigate autoimmune diseases, offering promising strategies to counteract age-related immune dysfunction.

DCs also play a crucial role in maintaining immunological tolerance by orchestrating mechanisms such as inducing anergy, clonally deleting T cells, and promoting the generation of Tregs to suppress immune responses against self-antigens.[153–155] These processes collectively ensure that the immune system avoids harmful responses to its own tissues. Importantly, dysbiosis can disrupt the production of SCFAs, compromising the integrity of the gut barrier and facilitating the translocation of harmful microbial products.[156,157] Consequently, this triggers the activation of immune cells and contributes to systemic inflammation, ultimately culminating in immunosenescence. Notably, various signaling pathways, including SCFA synthesis by specific bacteria, mediate communication between the gut microbiota and the immune system.[158,159] SCFAs not only support intestinal barrier integrity but also possess anti-inflammatory properties, further underscoring their role in immune regulation.[160–162] Moreover, recent research has elucidated how dysbiosis influences T cell diversity, a crucial

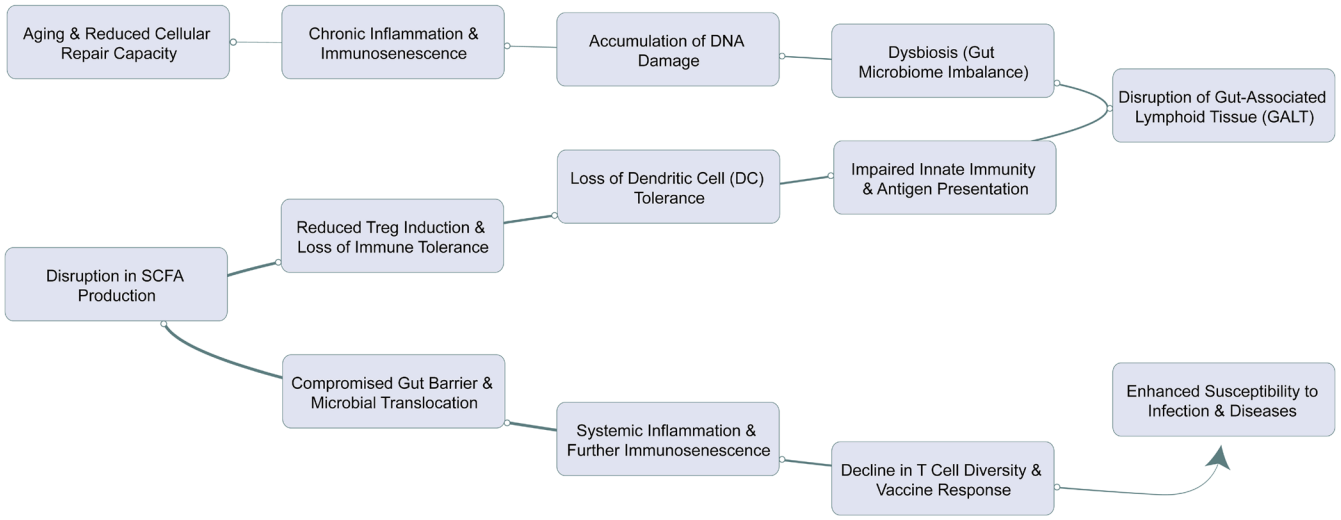


Fig. 2. A comprehensive schematic illustrating the interplay between gut dysbiosis, immunosenescence, and DNA damage in aging.

aspect of adaptive immune function.[163] With aging, individuals may experience a decline in the capacity of their T cells to respond effectively to new infections and vaccinations due to alterations in the composition of their gut flora.[164–166]

New research emphasizes the considerable role that imbalances in gut microbiota play in the processes associated with aging. Dysbiosis is linked to increased oxidative stress, mitochondrial dysfunction, and inadequate immune responses, all of which can accelerate the aging process.[167–169] Moreover, this imbalance in microbes heightens the likelihood of infections and weakens immune defenses in older adults, exacerbating the decline in immune function. Clinical trials indicate that probiotics such as *Lactiplantibacillus plantarum* may help alleviate inflammation and oxidative stress, which are significant factors in age-related deterioration.[170,171] These investigations encourage approaches to restore a balanced gut microbiota, which could enhance immune function and promote healthier aging by combating chronic inflammation. Overall, this research highlights the crucial importance of the gut microbiome for aging and immune health, pointing to the potential for microbiome-centric treatments to improve immunity and foster longevity among the elderly. Further evidence related to this is provided in Table 2.[172–176]

In essence, the complex interplay between immunosenescence and dysbiosis underscores the critical role of maintaining a diverse and balanced gut microbiota for optimal immune function as individuals age. However, the precise mechanisms linking age-related microbial dysbiosis to immunosenescence and inflammaging processes remain largely unclear. Therefore, further comprehensive research is essential to uncover these intricate connections and understand their broader implications for age-related immune dysfunction. By elucidating the relationship between dysbiosis, loss of immune tolerance, and inflammation, we can better understand the mechanisms underlying age-related immune decline. This understanding is vital for developing targeted interventions to mitigate the impact of immune dysfunction on health and aging. Continued research is crucial for identifying effective strategies to support older individuals in maintaining a healthy gut microbiome and a robust immune system. Comprehensive studies are essential to uncover these intricate connections and their broader implications for age-related immune dysfunction.

Understanding the dynamic relationship of immunosenescence and inflammation in aging

An intriguing aspect of the interplay between inflammation and immunosenescence is their mutually reinforcing relationship. While chronic inflammation is both a consequence and a contributor to immunosenescence, the sustained release of pro-inflammatory molecules, termed cytokines, can disrupt immune regulation and exacerbate age-related immune decline.[177,178] Although the immune system predominantly regulates the levels of pro- and anti-inflammatory cytokines, recent research in fibroblasts and epithelial cells has unveiled a correlation between cellular senescence and a significant increase in the secretion of 40–80 factors involved in intercellular signaling, collectively known as the "senescence-associated secretory phenotype".[179–181] Immune cells, like macrophages and natural killer cells, are typically recruited to eliminate senescent cells (SCs).[182,183] However, they may eventually lose their ability to do so, leading to an accumulation of SCs and heightened inflammation. Thus, within the intricate fabric of human health, the relationship between inflammation and immunosenescence emerges as a crucial component. This dynamic interaction underscores the importance of understanding how aging processes impact immune function and vice versa, offering potential insights into therapeutic strategies aimed at mitigating age-related immune dysfunction and chronic inflammation. Additional supporting evidence on this topic is presented in Table 3.[184–188]

As individuals age, various factors contribute to the complex interplay between inflammation and immunosenescence, impacting the functionality of the immune system. First, alterations in immune cell function occur, particularly in T cells, which play a pivotal role in directing immune responses. This decline in T cell efficacy may lead to diminished control over inflammation, resulting in prolonged and exaggerated inflammatory reactions.[189] Second, dysregulation of cytokines—crucial proteins for immune cell communication—ensues due to immunosenescence, favoring the production of pro-inflammatory cytokines and perpetuating a chronic inflammatory state associated with age-related disorders.[177–179] Third, the accumulation of SCs, known to release a mixture of pro-inflammatory molecules termed the SASP, further fuels both local and systemic inflammation. This process is facilitated by the persistence of these cells under conditions of immu-

Table 2. Microbiome-centric strategies for enhancing immunity and promoting longevity in the elderly

Study title	Authors	Year	Main findings	Reference
The Gut Microbiome, Aging, and Longevity: A Systematic Review	Badal *et al.*	2020	Aging is associated with distinct gut microbiota alterations, including reduced *Faecalibacterium*, *Bacteroidaceae,* and *Lachnospiraceae,* increased *Akkermansia*, and functional shifts in carbohydrate metabolism, amino acid synthesis, and short-chain fatty acid production, particularly in the oldest-old adults	172
Gut microbiota changes in the extreme decades of human life: a focus on centenarians	Sontoro *et al.*	2018	Centenarians provide a unique model for disentangling aging-related and non-aging-related gut microbiota changes, highlighting the influence of population, gender, and genetics, with implications for the gut-brain axis, neurodegenerative diseases, and microbiome-based therapies	173
Lactic Acid Bacteria and Aging: Unraveling the Interplay for Healthy Longevity	Liu *et al.*	2023	Lactic Acid Bacteria (LAB) may promote healthy aging by modulating key molecular pathways (e.g., IL-13, TNF-α, mTOR, Sirtuin-1, TLR2), enhancing gut balance, antioxidant potential, and cognitive health, though further human trials and mechanistic studies are needed to validate their anti-aging benefits	174
Microbiota medicine: towards clinical revolution	Gebrayal *et al.*	2022	The gut microbiota plays a crucial role in immunity, nutrient absorption, and disease prevention, with its dysbiosis linked to various systemic disorders, highlighting the need for targeted microbiome-based strategies in future medical treatments	175
The aging gut microbiome and its impact on host immunity	Bosco & Noti	2021	Aging disrupts the co-evolved gut microbiome-immune system axis, leading to *microb-aging*, *inflammaging*, and immunosenescence, increasing disease susceptibility and weakening vaccine responses, while emerging microbiome-targeted interventions like prebiotics and probiotics aim to restore microbial balance, reduce systemic inflammation, and rejuvenate immune function to enhance healthspan and longevity	176

Table 3. The link between inflammation and immunosenescence across aging

Study title	Authors	Year	Main findings	Reference
Immunosenescence and Inflamm-Aging: Clinical Interventions and the Potential for Reversal of Aging	Kumar *et al.*	2024	Immunosenescence, driven by inflammaging and lifelong pathogenic exposure, weakens immune resilience, while interventions like immunomodulation, vaccination, nutrition, microbiome therapy, stem cells, and exercise aim to slow or reverse age-related immune decline	184
Inflammation and aging: signaling pathways and intervention therapies	Li *et al.*	2023	Aging is driven by chronic inflammation (inflammaging), where the senescence-associated secretory phenotype (SASP) sustains a cycle of immune dysfunction, organ damage, and age-related diseases, highlighting the need for dimensionality reduction approaches, single-cell technologies, and targeted anti-inflammatory strategies to mitigate aging and enhance longevity	185
Immunosenescence: Aging and Immune System Decline	Goyani *et al.*	2024	Immunosenescence, marked by thymic involution, inflammaging, metabolic and hematopoietic changes, weakens immune responses in aging by impairing macrophages, neutrophils, T cells, dendritic cells, B cells, and NK cells, underscoring the need for strategies to counteract age-related immune decline	186
Immune-Inflammatory Response in Lifespan—What Role Does It Play in Extreme Longevity? A Sicilian Semi- and Supercentenarians Study	Accardi *et al.*	2024	Analysis of inflammatory scores (INFLA-score, SIRI) and ARIP in 249 individuals aged 19–111 years revealed age-related increases in inflammation but no significant differences in immune-inflammatory markers between semi- and supercentenarians and other age groups, suggesting that immune regulation may contribute to extreme longevity	187
The 3 I's of immunity and aging: immunosenescence, inflammaging, and immune resilience	Wrona *et al.*	2024	Immunosenescence, characterized by a decline in innate and adaptive immunity, chronic inflammation, and increased disease susceptibility, is influenced by aging hallmarks, sex, social determinants, and gut microbiota, with potential mitigation strategies including lifestyle interventions and gerotherapeutics to enhance immune resilience in the elderly	188

nosenescence.[179–181] Additionally, impaired immune surveillance, a consequence of immunosenescence, compromises the immune system's ability to detect and eliminate damaged cells. This compromise leads to inflammatory responses aimed at controlling potential threats. Finally, epigenetic changes induced by both inflammation and immunosenescence contribute to alterations in gene expression.[190–192] These changes exacerbate the pro-inflammatory condition and establish a reciprocal relationship between immunosenescence and epigenetic modifications.[193–195] Together, these interconnected processes underscore the intricate relationship between inflammation and immunosenescence, shaping the immune landscape in aging individuals.

Orchestrated insights into cellular interactions: gut microbiome, inflammation, and aging

The gut microbiome plays a crucial role in modulating immune and epithelial cell activities, significantly affecting the aging process through its involvement in inflammation—a key concept in cell biology. As we age, dysbiosis emerges, compromising the integrity of the gut epithelial barrier. Enterocytes (the cells that line the gut) typically maintain tight junctions that create a selective barrier against microbial products and toxins.[196] However, with age, oxidative stress and cellular senescence weaken these junctions, leading to increased gut permeability and allowing microbial endotoxins, such as lipopolysaccharides, to enter the bloodstream, initiating immune activation. Immune cells, such as macrophages, DCs, and T cells, play essential roles in this process.[197,198] In a healthy gut, macrophages maintain a tolerogenic state, preventing excessive immune responses to commensal bacteria.[199,200] However, with dysbiosis and aging, macrophages acquire a pro-inflammatory M1 phenotype, releasing cytokines like tumor necrosis factor (TNF)-α, interleukin-6, and interleukin-1β, which amplify systemic inflammation.[201,202] T cells, especially the balance between Tregs and pro-inflammatory T cells such as Th17, are crucial for immune homeostasis.[203,204] Aging and dysbiosis shift this balance toward pro-inflammatory states, exacerbating inflammation. DCs, responsible for antigen presentation and immune modulation, become less effective at distinguishing harmful from harmless microbes with aging, further impairing immune regulation and promoting inflammation.[36,37,40] Aging is also characterized by the presence of SCs that secrete pro-inflammatory molecules known as the SASP, which fuel ongoing inflammation and hinder cellular functions.[124,177,179–181] In the GI tract, senescence disrupts the repair of epithelial cells and enhances gut permeability, escalating inflammation further. Moreover, fibroblasts and myofibroblasts in the gut wall become activated due to inflammation, resulting in extracellular matrix remodeling that causes fibrosis and tissue scarring. This adversely affects tissue regeneration, nutrient absorption, and motility.[205,206] From a neurobiological standpoint, the enteric nervous system interacts with the brain through the gut-brain axis.[207] Dysbiosis initiates neuroinflammation by activating immune responses within the gut, affecting the enteric nervous system, and modifying gut motility and pain perception.[208] These alterations become more significant with aging, establishing a connection between gut dysbiosis and neurodegenerative diseases linked to age, such as Alzheimer's disease.[209,210] At the molecular level, beneficial bacteria produce microbial metabolites like SCFAs, which are essential for maintaining the health of the gut epithelium and modulating immune responses.[156,158] Conversely, dysbiosis leads to a decrease in SCFA production and an increase in microbial products like lipopolysaccharides, which compromise gut function and activate inflammatory pathways. Over the years, the reduction of beneficial microorganisms coupled with the increase of harmful bacteria creates a pro-inflammatory environment that exacerbates age-related issues such as cardiovascular diseases, diabetes, and neurodegenerative diseases.[211,212] The gut microbiome plays a crucial role in a complex interaction that includes immune system activation, cellular aging, remodeling of the extracellular matrix, and neuroinflammation, all of which accelerate the aging process.[213,214] This connection between dysbiosis, inflammation, and cellular dysfunction highlights the profound influence of the gut microbiome on inflammaging and diseases related to aging (Fig. 3).

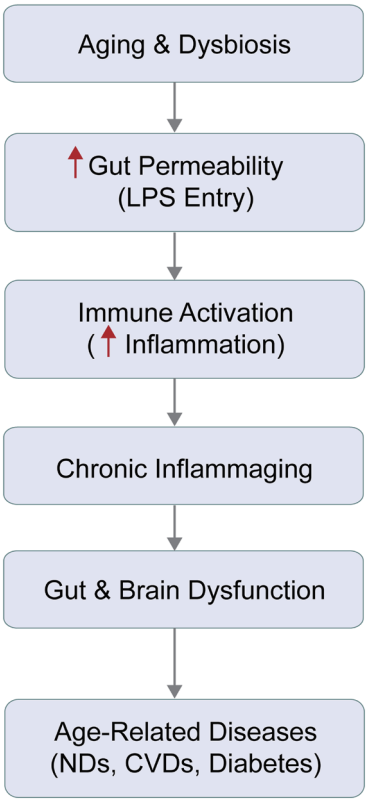


Fig. 3. The intricate interplay among the gut microbiome, inflammation, and aging significantly influences the development of age-related diseases. LPS, lipopolysaccharide; NDs, neurodegenerative diseases; CVDs, cardiovascular diseases.

The interplay of DNA damage and inflammation in aging dynamics

The intricate relationship between DNA damage and inflammation within the body signifies a close interconnection.[215,216] These processes engage in reciprocal signaling, exerting influence across a range of physiological and pathological contexts. Notably, chronic inflammation poses a significant threat to DNA stability. Inflammatory agents, like ROS, induce DNA damage by triggering oxidative stress, resulting in the formation of lesions such as base modifications and strand breaks.[217,218] Furthermore, specific inflammatory mediators, including TNF-α and interleukins, activate pathways that exacerbate DNA damage.[219,220] For instance, TNF-α stimulates the production of ROS, intensifying DNA damage.[221]

Conversely, DNA damage activates the DNA damage response, a multifaceted cellular mechanism involving signaling pathways

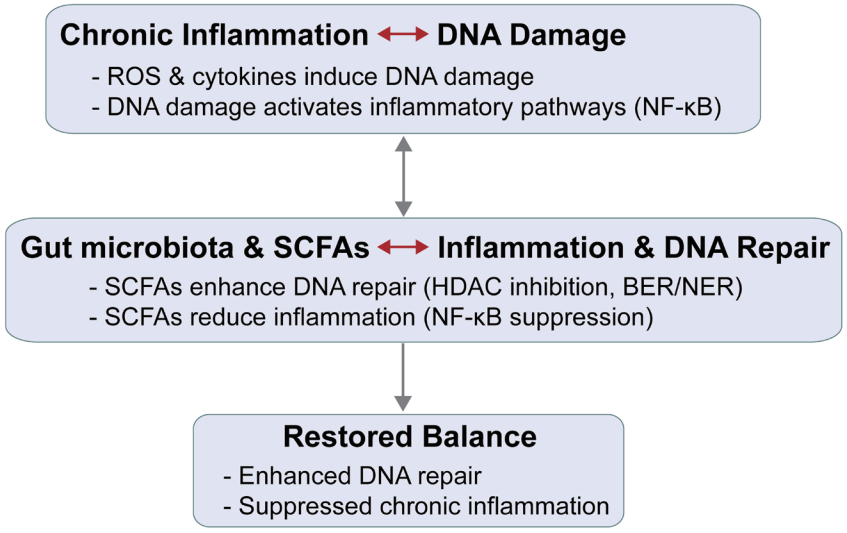


Fig. 4. Bidirectional interplay between DNA damage, inflammation, and gut microbiota-derived short-chain fatty acids (SCFAs). HDAC, histone deacetylase; BER, base excision repair; NER, nucleotide excision repair.

aimed at repairing damaged DNA and preserving genomic integrity.[222,223] Intriguingly, DNA damage response also influences inflammatory pathways. The activation of nuclear factor-kappa B by DNA damage leads to the generation of pro-inflammatory cytokines.[224,225] Additionally, immune cells, which are pivotal in both inflammation and DNA repair, significantly contribute to this bidirectional communication. Macrophages exemplify this dual role, participating in both inflammation and tissue repair. However, persistent inflammation may drive macrophages toward a phenotype that exacerbates DNA damage and disrupts repair mechanisms.[226–229]

Furthermore, SCFAs, such as butyrate, propionate, and acetate, produced by the gut microbiota through dietary fiber fermentation, play a crucial role in epigenetic modifications that affect DNA repair mechanisms.[230,231] SCFAs act as histone deacetylase inhibitors, increasing histone acetylation and thereby relaxing chromatin structure to facilitate DNA repair enzyme access to damaged sites. Additionally, SCFAs influence DNA methylation patterns by serving as substrates for enzymes involved in one-carbon metabolism, such as DNA methyltransferases.[232,233] This dual action of SCFAs enhances the expression of genes crucial for DNA repair pathways, like base excision repair and nucleotide excision repair, promoting efficient DNA damage repair and maintaining genomic stability (Fig. 4).[234,235] Moreover, SCFAs exert anti-inflammatory effects by inhibiting nuclear factor-kappa B activation and reducing pro-inflammatory cytokine production, indirectly supporting DNA repair mechanisms that might be impaired under inflammatory conditions.[236,237] Understanding these mechanisms holds promise for developing therapeutic interventions targeting chronic diseases and cancer by modulating SCFA levels through dietary adjustments or microbiome-targeted therapies to enhance epigenetic and DNA repair processes in clinical settings.

In summary, this article offers a focused perspective on key aspects, including the interplay between dysbiosis, immunosenescence-driven inflammation, and DNA damage in aging. Nevertheless, it acknowledges the complexity and breadth of this subject, aiming to spark further dialogue and deeper investigation into how the microbiome intricately links with aging. By discussing and emphasizing these mechanisms, the article highlights their interconnected nature, particularly how dysbiosis, inflammation, immunosenescence, and DNA repair collectively impact the health of elderly individuals (Fig. 5). This foundational understanding highlights the critical need for ongoing research to uncover precise interventions that can mitigate these multifaceted interactions, promoting better health outcomes for aging populations.

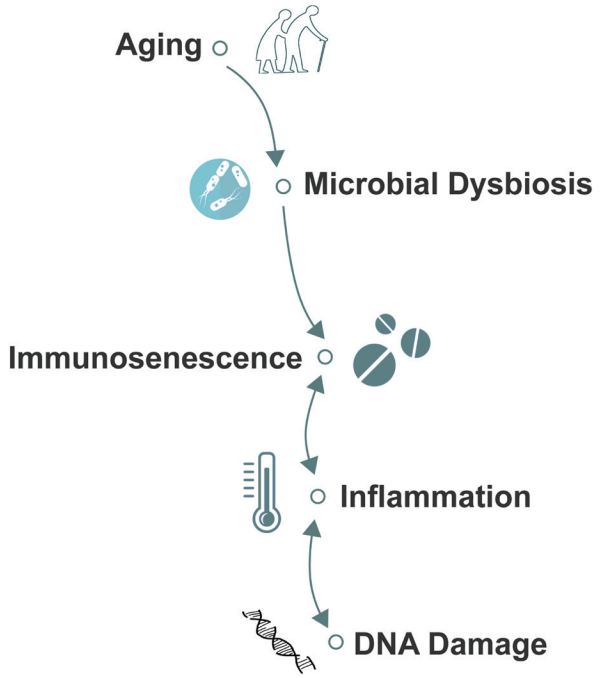


Fig. 5. Graphical overview of the underlying microbiome's impact on aging. Aging drives gut microbial dysbiosis, disrupting immune cell homeostasis and promoting immunosenescence, marked by the accumulation of senescent immune cells. These cells sustain chronic inflammation, accelerating immunosenescence in a self-reinforcing cycle. Inflammation also induces DNA damage, which further triggers inflammation and reinforces a bidirectional loop that exacerbates immune dysfunction.

Future directions

Further exploration of the intricate interplay between the microbiota and DNA damage offer potential strategies to support healthy aging and address microbiome-related disorders in later life stages. The burgeoning field of research on the connection between the human microbiome and genomic stability presents promising avenues for unraveling the complexities of aging. Recent studies emphasize the correlation between gut microbiota dysbiosis and heightened DNA damage, underscoring the significance of microbiome management in preserving genomic integrity. Accumulating evidence suggests that cultivating a robust and diverse microbiome may positively impact genomic stability and contribute to graceful aging. By expanding our understanding of how microbial dysbiosis influences genomic instability during aging, future investigations could focus on refining targeted interventions to restore and preserve a healthy gut microbiome and mitigate age-related DNA damage. This may involve identifying specific microbial strains or metabolites that enhance DNA repair mechanisms or mitigate oxidative stress-induced DNA damage.

Cutting-edge technologies like metagenomics, metatranscriptomics, and metabolomics can provide profound insights into the interactions between the host, microbiome, and DNA damage pathways during aging. Combining multi-omics approaches with long-term studies of aging populations could identify biomarkers of microbial health and DNA integrity, serving as early indicators of age-related disease risk or therapeutic intervention effectiveness. Recognizing the linkages between the gut microbiome, systemic inflammation, immune function, and age-related DNA damage, future research could explore synergistic approaches targeting inflammation, immunosenescence, and microbial dysbiosis comprehensively. This might involve developing lifestyle interventions, dietary strategies, or pharmacological agents that modulate both host and microbial factors implicated in aging. Promising intervention avenues are emerging from research on probiotic strains that reduce inflammation and promote DNA repair mechanisms.

However, several limitations should be acknowledged in the current body of research. While the exploration of the interplay between the microbiota and DNA damage holds great promise, the complexity and variability of the human microbiome across individuals presents challenges in drawing universal conclusions. Microbial composition can be influenced by a range of factors, including diet, lifestyle, genetics, geographic location, and environmental exposures, making it difficult to establish standardized biomarkers or treatments. Furthermore, most research on microbiome-DNA damage interactions has been conducted in preclinical models or under controlled conditions, which may not fully replicate the complexities of human aging. Translating these findings to humans requires careful consideration of confounding factors and the need for longitudinal studies to account for the long-term effects of microbiome alterations.

While advanced multi-omics technologies, such as metagenomics, metatranscriptomics, and metabolomics, provide powerful tools to study the microbiome and its role in aging, these approaches can be technically challenging and resource-intensive. The integration of large datasets across different omics layers also poses significant bioinformatics challenges, requiring sophisticated algorithms and computational models to discern meaningful relationships. Additionally, the precise mechanisms through which the microbiome influences DNA damage and repair remain underexplored. Despite promising correlations, causality has not yet been definitively established, and more mechanistic studies are needed to elucidate how microbial dysbiosis directly impacts genomic stability during aging.

Moreover, clinical trials investigating the role of the microbiome in DNA damage and aging are currently limited, and there is insufficient evidence to support therapeutic applications. Most studies to date have been preliminary or small-scale, and larger, well-designed clinical trials are necessary to substantiate the role of the microbiome in aging-related DNA damage. The lack of long-term, large-scale human studies and the challenge of controlling for confounding variables further complicate efforts to validate microbiome-based interventions for aging and associated diseases. Finally, the potential for therapeutic interventions—whether through probiotics, diet, or pharmacological agents—remains largely untested in the context of aging. While early studies suggest potential benefits, large-scale, long-term clinical trials are needed to validate these strategies and assess their safety and efficacy in diverse aging populations.

To this end, while this field holds significant promise for improving health during aging, further research, particularly through large-scale clinical trials, is needed to overcome these limitations and develop effective, personalized interventions that target the microbiome to preserve genetic integrity and promote healthy aging. As we continue to deepen our understanding of this intricate symbiotic relationship, new possibilities will emerge, presenting opportunities for innovative interventions aimed at improving health through precise adjustments to the microbiome. Drawing on insights from advancing research in this area, we are well-positioned to uncover strategies that enhance vitality and resilience throughout the aging process.

Conclusions

The microbiome's subtle yet profound influence on DNA damage and aging unveils a complex and dynamic interplay that significantly governs human health and longevity. As aging progresses, the accumulation of DNA damage, coupled with a decline in repair mechanisms, accelerates cellular senescence, while alterations in the microbiome drive persistent inflammation and immune dysregulation. Dysbiosis, through its modulation of immune responses and exacerbation of chronic low-grade inflammation, emerges as a critical instigator of the aging process. The microbiome's pivotal role in regulating DNA repair and inflammatory pathways—particularly through SCFAs and immune modulation—presents promising therapeutic avenues for mitigating age-related diseases. By fostering gut microbiome stability, we may enhance DNA repair mechanisms and attenuate the inflammatory cascade that accelerates aging. Targeted interventions, including microbiome-based therapies and dietary strategies, offer substantial potential to improve DNA repair, restore immune function, and ultimately promote healthier aging, thereby extending both healthspan and lifespan.

Acknowledgments

None.

Funding

This research is supported by Bandhan, Kolkata, India.

Conflict of interest

The authors state that they have no conflicts of interest regarding the publication of this research.

Author contributions

Conceptualization (SKC), formal analysis (SKC), original draft preparation (SKC), writing—review and editing (SKC, DC), supervision (SKC), project administration (SKC), and funding acquisition (SKC).

References

[1] Tenchov R, Sasso JM, Wang X, Zhou QA. Aging Hallmarks and Progression and Age-Related Diseases: A Landscape View of Research Advancement. ACS Chem Neurosci 2024;15(1):1–30. doi:10.1021/acschemneuro.3c00531, PMID:38095562.

[2] Li Z, Zhang Z, Ren Y, Wang Y, Fang J, Yue H, *et al*. Aging and age-related diseases: from mechanisms to therapeutic strategies. Biogerontology 2021;22(2):165–187. doi:10.1007/s10522-021-09910-5, PMID:33502634.

[3] Colon-Emeric C, Schmader K, Cohen HJ, Morey M, Whitson H. Ageing and physical resilience after health stressors. Stress Health 2023;39(S1):48–54. doi:10.1002/smi.3241, PMID:36879359.

[4] Ukraintseva S, Arbeev K, Duan M, Akushevich I, Kulminski A, Stallard E, *et al*. Decline in biological resilience as key manifestation of aging: Potential mechanisms and role in health and longevity. Mech Ageing Dev 2021;194:111418. doi:10.1016/j.mad.2020.111418, PMID:33340523.

[5] Walston J, Varadhan R, Xue QL, Buta B, Sieber F, Oni J, *et al*. A Study of Physical Resilience and Aging (SPRING): Conceptual framework, rationale, and study design. J Am Geriatr Soc 2023;71(8):2393–2405. doi:10.1111/jgs.18483, PMID:37386913.

[6] Bradley E, Haran J. The human gut microbiome and aging. Gut Microbes 2024;16(1):2359677. doi:10.1080/19490976.2024.2359677, PMID:38831607.

[7] O'Toole PW. Ageing, microbes and health. Microb Biotechnol 2024;17(5):e14477. doi:10.1111/1751-7915.14477, PMID:38801344.

[8] Ghosh TS, Shanahan F, O'Toole PW. The gut microbiome as a modulator of healthy ageing. Nat Rev Gastroenterol Hepatol 2022;19(9):565–584. doi:10.1038/s41575-022-00605-x, PMID:35468952.

[9] Donati Zeppa S, Agostini D, Ferrini F, Gervasi M, Barbieri E, Bartolacci A, *et al*. Interventions on Gut Microbiota for Healthy Aging. Cells 2022;12(1):34. doi:10.3390/cells12010034, PMID:36611827.

[10] Badal VD, Vaccariello ED, Murray ER, Yu KE, Knight R, Jeste DV, *et al*. The Gut Microbiome, Aging, and Longevity: A Systematic Review. Nutrients 2020;12(12):3759. doi:10.3390/nu12123759, PMID:33297486.

[11] Schumacher B, Pothof J, Vijg J, Hoeijmakers JHJ. The central role of DNA damage in the ageing process. Nature 2021;592(7856):695–703. doi:10.1038/s41586-021-03307-7, PMID:33911272.

[12] Baechle JJ, Chen N, Makhijani P, Winer S, Furman D, Winer DA. Chronic inflammation and the hallmarks of aging. Mol Metab 2023;74:101755. doi:10.1016/j.molmet.2023.101755, PMID:37329949.

[13] Płuciennik K, Sicińska P, Misztal W, Bukowska B. Important Factors Affecting Induction of Cell Death, Oxidative Stress and DNA Damage by Nano- and Microplastic Particles In Vitro. Cells 2024;13(9):768. doi:10.3390/cells13090768, PMID:38727304.

[14] Lee JW, Ratnakumar K, Hung KF, Rokunohe D, Kawasumi M. Deciphering UV-induced DNA Damage Responses to Prevent and Treat Skin Cancer. Photochem Photobiol 2020;96(3):478–499. doi:10.1111/php.13245, PMID:32119110.

[15] Vechtomova YL, Telegina TA, Buglak AA, Kritsky MS. UV Radiation in DNA Damage and Repair Involving DNA-Photolyases and Cryptochromes. Biomedicines 2021;9(11):1564. doi:10.3390/biomedicines9111564, PMID:34829793.

[16] Somasundaram I, Jain SM, Blot-Chabaud M, Pathak S, Banerjee A, Rawat S, *et al*. Mitochondrial dysfunction and its association with age-related disorders. Front Physiol 2024;15:1384966. doi:10.3389/fphys.2024.1384966, PMID:39015222.

[17] Craige SM, Mammel RK, Amiri N, Willoughby OS, Drake JC. Interplay of ROS, mitochondrial quality, and exercise in aging: Potential role of spatially discrete signaling. Redox Biol 2024;77:103371. doi:10.1016/j.redox.2024.103371, PMID:39357424.

[18] Giorgi C, Marchi S, Simoes ICM, Ren Z, Morciano G, Perrone M, *et al*. Mitochondria and Reactive Oxygen Species in Aging and Age-Related Diseases. Int Rev Cell Mol Biol 2018;340:209–344. doi:10.1016/bs.ircmb.2018.05.006, PMID:30072092.

[19] Eppard M, Passos JF, Victorelli S. Telomeres, cellular senescence, and aging: past and future. Biogerontology 2024;25(2):329–339. doi:10.1007/s10522-023-10085-4, PMID:38150087.

[20] Harley J, Santosa MM, Ng CY, Grinchuk OV, Hor JH, Liang Y, *et al*. Telomere shortening induces aging-associated phenotypes in hiPSC-derived neurons and astrocytes. Biogerontology 2024;25(2):341–360. doi:10.1007/s10522-023-10076-5, PMID:37987889.

[21] Soto-Palma C, Niedernhofer LJ, Faulk CD, Dong X. Epigenetics, DNA damage, and aging. J Clin Invest 2022;132(16):e158446. doi:10.1172/JCI158446, PMID:35968782.

[22] Herr LM, Schaffer ED, Fuchs KF, Datta A, Brosh RM Jr. Replication stress as a driver of cellular senescence and aging. Commun Biol 2024;7(1):616. doi:10.1038/s42003-024-06263-w, PMID:38777831.

[23] Huang W, Hickson LJ, Eirin A, Kirkland JL, Lerman LO. Cellular senescence: the good, the bad and the unknown. Nat Rev Nephrol 2022;18(10):611–627. doi:10.1038/s41581-022-00601-z, PMID:35922662.

[24] Fulop T, Larbi A, Pawelec G, Khalil A, Cohen AA, Hirokawa K, *et al*. Immunology of Aging: the Birth of Inflammaging. Clin Rev Allergy Immunol 2023;64(2):109–122. doi:10.1007/s12016-021-08899-6, PMID:34536213.

[25] Caldarelli M, Rio P, Marrone A, Giambra V, Gasbarrini A, Gambassi G, *et al*. Inflammaging: The Next Challenge-Exploring the Role of Gut Microbiota, Environmental Factors, and Sex Differences. Biomedicines 2024;12(8):1716. doi:10.3390/biomedicines12081716, PMID:39200181.

[26] Han Z, Wang K, Ding S, Zhang M. Cross-talk of inflammation and cellular senescence: a new insight into the occurrence and progression of osteoarthritis. Bone Res 2024;12(1):69. doi:10.1038/s41413-024-00375-z, PMID:39627227.

[27] MohanKumar SMJ, Murugan A, Palaniyappan A, MohanKumar PS. Role of cytokines and reactive oxygen species in brain aging. Mech Ageing Dev 2023;214:111855. doi:10.1016/j.mad.2023.111855, PMID:37541628.

[28] Yang J, Luo J, Tian X, Zhao Y, Li Y, Wu X. Progress in Understanding Oxidative Stress, Aging, and Aging-Related Diseases. Antioxidants (Basel) 2024;13(4):394. doi:10.3390/antiox13040394, PMID:38671842.

[29] López-Gil L, Pascual-Ahuir A, Proft M. Genomic Instability and Epigenetic Changes during Aging. Int J Mol Sci 2023;24(18):14279. doi:10.3390/ijms241814279, PMID:37762580.

[30] Herrick J. DNA Damage, Genome Stability, and Adaptation: A Question of Chance or Necessity? Genes (Basel) 2024;15(4):520. doi:10.3390/genes15040520, PMID:38674454.

[31] Kim M, Benayoun BA. The microbiome: an emerging key player in aging and longevity. Transl Med Aging 2020;4:103–116. PMID:32832742.

[32] Haran JP, McCormick BA. Aging, Frailty, and the Microbiome-How Dysbiosis Influences Human Aging and Disease. Gastroenterology 2021;160(2):507–523. doi:10.1053/j.gastro.2020.09.060, PMID:33307030.

[33] Thriene K, Michels KB. Human Gut Microbiota Plasticity throughout the Life Course. Int J Environ Res Public Health 2023;20(2):1463. doi:10.3390/ijerph20021463, PMID:36674218.

[34] Fusco W, Lorenzo MB, Cintoni M, Porcari S, Rinninella E, Kaitsas F, *et al*. Short-Chain Fatty-Acid-Producing Bacteria: Key Components of the Human Gut Microbiota. Nutrients 2023;15(9):2211. doi:10.3390/nu15092211, PMID:37432351.

[35] Theodorakis N, Feretzakis G, Hitas C, Kreouzi M, Kalantzi S, Spyridaki A, *et al*. Immunosenescence: How Aging Increases Susceptibility to Bacterial Infections and Virulence Factors. Microorganisms 2024;12(10):2052. doi:10.3390/microorganisms12102052, PMID:39458361.

[36] Lee TH, Chen JJ, Wu CY, Lin TY, Hung SC, Yang HY. Immunosenescence, gut dysbiosis, and chronic kidney disease: Interplay and implications for clinical management. Biomed J 2024;47(2):100638. doi:10.1016/j.bj.2023.100638, PMID:37524304.

[37] Gao H, Nepovimova E, Adam V, Heger Z, Valko M, Wu Q, *et al*. Age-associated changes in innate and adaptive immunity: role of the gut microbiota. Front Immunol 2024;15:1421062. doi:10.3389/fim-

mu.2024.1421062, PMID:39351234.
[38] Rusu ME, Fizeşan I, Vlase L, Popa DS. Antioxidants in Age-Related Diseases and Anti-Aging Strategies. Antioxidants (Basel) 2022;11(10):1868. doi:10.3390/antiox11101868, PMID:36290589.
[39] Semenova N, Garashchenko N, Kolesnikov S, Darenskaya M, Kolesnikova L. Gut Microbiome Interactions with Oxidative Stress: Mechanisms and Consequences for Health. Pathophysiology 2024;31(3):309–330. doi:10.3390/pathophysiology31030023, PMID:39051221.
[40] Li XJ, Shan QY, Wu X, Miao H, Zhao YY. Gut microbiota regulates oxidative stress and inflammation: a double-edged sword in renal fibrosis. Cell Mol Life Sci 2024;81(1):480. doi:10.1007/s00018-024-05532-5, PMID:39636415.
[41] Aziz T, Hussain N, Hameed Z, Lin L. Elucidating the role of diet in maintaining gut health to reduce the risk of obesity, cardiovascular and other age-related inflammatory diseases: recent challenges and future recommendations. Gut Microbes 2024;16(1):2297864. doi:10.1080/19490976.2023.2297864, PMID:38174551.
[42] Salvadori M, Rosso G. Update on the gut microbiome in health and diseases. World J Methodol 2024;14(1):89196. doi:10.5662/wjm.v14.i1.89196, PMID:38577200.
[43] Lee J-Y, Bays DJ, Savage HP, Bäumler AJ. The human gut microbiome in health and disease: time for a new chapter? Infect Immun 2024;92(11):e0030224. doi:10.1128/iai.00302-24, PMID:39347570.
[44] Afzaal M, Saeed F, Shah YA, Hussain M, Rabail R, Socol CT, *et al*. Human gut microbiota in health and disease: Unveiling the relationship. Front Microbiol 2022;13:999001. doi:10.3389/fmicb.2022.999001, PMID:36225386.
[45] Acevedo-Román A, Pagán-Zayas N, Velázquez-Rivera LI, Torres-Ventura AC, Godoy-Vitorino F. Insights into Gut Dysbiosis: Inflammatory Diseases, Obesity, and Restoration Approaches. Int J Mol Sci 2024;25(17):9715. doi:10.3390/ijms25179715, PMID:39273662.
[46] Alagiakrishnan K, Morgadinho J, Halverson T. Approach to the diagnosis and management of dysbiosis. Front Nutr 2024;11:1330903. doi:10.3389/fnut.2024.1330903, PMID:38706561.
[47] Jangi S, Hecht G. Microbiome 2.0: lessons from the 2024 Gut Microbiota for Health World Summit. Gut Microbes 2024;16(1):2400579. doi:10.1080/19490976.2024.2400579, PMID:39253878.
[48] Sung J, Rajendraprasad SS, Philbrick KL, Bauer BA, Gajic O, Shah A, *et al*. The human gut microbiome in critical illness: disruptions, consequences, and therapeutic frontiers. J Crit Care 2024;79:154436. doi:10.1016/j.jcrc.2023.154436, PMID:37769422.
[49] Micek A, Bolesławska I, Jagielski P, Konopka K, Waśkiewicz A, Witkowska AM, *et al*. Association of dietary intake of polyphenols, lignans, and phytosterols with immune-stimulating microbiota and COVID-19 risk in a group of Polish men and women. Front Nutr 2023;10:1241016. doi:10.3389/fnut.2023.1241016, PMID:37599696.
[50] Hussain I, Cher GLY, Abid MA, Abid MB. Role of Gut Microbiome in COVID-19: An Insight Into Pathogenesis and Therapeutic Potential. Front Immunol 2021;12:765965. doi:10.3389/fimmu.2021.765965, PMID:34721437.
[51] Tian S, Huang W. The causal relationship between gut microbiota and COVID-19: A two-sample Mendelian randomization analysis. Medicine (Baltimore) 2024;103(5):e36493. doi:10.1097/MD.0000000000036493, PMID:38306556.
[52] Kircheis R, Planz O. Special Issue "The Role of Toll-Like Receptors (TLRs) in Infection and Inflammation 2.0". Int J Mol Sci 2024;25(17):9709. doi:10.3390/ijms25179709, PMID:39273656.
[53] Mantovani S, Oliviero B, Varchetta S, Renieri A, Mondelli MU. TLRs: Innate Immune Sentries against SARS-CoV-2 Infection. Int J Mol Sci 2023;24(9):8065. doi:10.3390/ijms24098065, PMID:37175768.
[54] Song XL, Liang J, Lin SZ, Xie YW, Ke CH, Ao D, *et al*. Gut-lung axis and asthma: A historical review on mechanism and future perspective. Clin Transl Allergy 2024;14(5):e12356. doi:10.1002/clt2.12356, PMID:38687096.
[55] Druszczynska M, Sadowska B, Kulesza J, Gąsienica-Gliwa N, Kulesza E, Fol M. The Intriguing Connection Between the Gut and Lung Microbiomes. Pathogens 2024;13(11):1005. doi:10.3390/pathogens13111005, PMID:39599558.
[56] Du Y, He C, An Y, Huang Y, Zhang H, Fu W, *et al*. The Role of Short Chain Fatty Acids in Inflammation and Body Health. Int J Mol Sci 2024;25(13):7379. doi:10.3390/ijms25137379, PMID:39000498.
[57] Li R, Li J, Zhou X. Lung microbiome: new insights into the pathogenesis of respiratory diseases. Signal Transduct Target Ther 2024;9(1):19. doi:10.1038/s41392-023-01722-y, PMID:38228603.
[58] Wang M, Song J, Yang H, Wu X, Zhang J, Wang S. Gut microbiota was highly related to the immune status in chronic obstructive pulmonary disease patients. Aging (Albany NY) 2024;16(4):3241–3256. doi:10.18632/aging.205532, PMID:38349864.
[59] Sun M, Lu F, Yu D, Wang Y, Chen P, Liu S. Respiratory diseases and gut microbiota: relevance, pathogenesis, and treatment. Front Microbiol 2024;15:1358597. doi:10.3389/fmicb.2024.1358597, PMID:39081882.
[60] Wang X, Yuan W, Yang C, Wang Z, Zhang J, Xu D, *et al*. Emerging role of gut microbiota in autoimmune diseases. Front Immunol 2024;15:1365554. doi:10.3389/fimmu.2024.1365554, PMID:38765017.
[61] Horai R, Caspi RR. Microbiome and Autoimmune Uveitis. Front Immunol 2019;10:232. doi:10.3389/fimmu.2019.00232, PMID:30837991.
[62] Hong JB, Chen YX, Su ZY, Chen XY, Lai YN, Yang JH. Causal association of juvenile idiopathic arthritis or JIA-associated uveitis and gut microbiota: a bidirectional two-sample Mendelian randomisation study. Front Immunol 2024;15:1356414. doi:10.3389/fimmu.2024.1356414, PMID:39114654.
[63] Toader C, Dobrin N, Costea D, Glavan LA, Covache-Busuioc RA, Dumitrascu DI, *et al*. Mind, Mood and Microbiota-Gut-Brain Axis in Psychiatric Disorders. Int J Mol Sci 2024;25(6):3340. doi:10.3390/ijms25063340, PMID:38542314.
[64] You M, Chen N, Yang Y, Cheng L, He H, Cai Y, *et al*. The gut microbiota-brain axis in neurological disorders. MedComm (2020) 2024;5(8):e656. doi:10.1002/mco2.656, PMID:39036341.
[65] Ke S, Hartmann J, Ressler KJ, Liu YY, Koenen KC. The emerging role of the gut microbiome in posttraumatic stress disorder. Brain Behav Immun 2023;114:360–370. doi:10.1016/j.bbi.2023.09.005, PMID:37689277.
[66] Xiong RG, Li J, Cheng J, Zhou DD, Wu SX, Huang SY, *et al*. The Role of Gut Microbiota in Anxiety, Depression, and Other Mental Disorders as Well as the Protective Effects of Dietary Components. Nutrients 2023;15(14):3258. doi:10.3390/nu15143258, PMID:37513676.
[67] Ansari F, Neshat M, Pourjafar H, Jafari SM, Samakkhah SA, Mirzakhani E. The role of probiotics and prebiotics in modulating of the gut-brain axis. Front Nutr 2023;10:1173660. doi:10.3389/fnut.2023.1173660, PMID:37565035.
[68] Valentino V, Magliulo R, Farsi D, Cotter PD, O'Sullivan O, Ercolini D, *et al*. Fermented foods, their microbiome and its potential in boosting human health. Microb Biotechnol 2024;17(2):e14428. doi:10.1111/1751-7915.14428, PMID:38393607.
[69] Al Bander Z, Nitert MD, Mousa A, Naderpoor N. The Gut Microbiota and Inflammation: An Overview. Int J Environ Res Public Health 2020;17(20):7618. doi:10.3390/ijerph17207618, PMID:33086688.
[70] Cano-Ortiz A, Laborda-Illanes A, Plaza-Andrades I, Membrillo Del Pozo A, Villarrubia Cuadrado A, Rodríguez Calvo de Mora M, *et al*. Connection between the Gut Microbiome, Systemic Inflammation, Gut Permeability and FOXP3 Expression in Patients with Primary Sjögren's Syndrome. Int J Mol Sci 2020;21(22):8733. doi:10.3390/ijms21228733, PMID:33228011.
[71] Petakh P, Oksenych V, Kamyshna I, Boisak I, Lyubomirskaya K, Kamyshnyi O. Exploring the complex interplay: gut microbiome, stress, and leptospirosis. Front Microbiol 2024;15:1345684. doi:10.3389/fmicb.2024.1345684, PMID:38476949.
[72] Chen X, Xie X, Sun N, Liu X, Liu J, Zhang W, *et al*. Gut microbiota-derived butyrate improved acute leptospirosis in hamster via promoting macrophage ROS mediated by HDAC3 inhibition. mBio 2024;15(10):e0190624. doi:10.1128/mbio.01906-24, PMID:39287437.
[73] Bradley EA, Lockaby G. Leptospirosis and the Environment: A Review and Future Directions. Pathogens 2023;12(9):1167. doi:10.3390/pathogens12091167, PMID:37764975.
[74] Krishnan BK, Balasubramanian G, Kumar PP. Leptospirosis in India: insights on circulating serovars, research lacunae and proposed strategies to control through one health approach. One Health Outlook 2024;6(1):11. doi:10.1186/s42522-024-00098-5, PMID:38849946.
[75] Xie X, Liu J, Chen X, Zhang S, Tang R, Wu X, *et al*. Gut microbiota involved in leptospiral infections. ISME J 2022;16(3):764–773. doi:10.1038/s41396-021-01122-6, PMID:34588617.

[76] Petakh P, Isevych V. Gut microbiota status in leptospirosis: An unrecognized player? New Microbes New Infect 2022;49-50:101038. doi:10.1016/j.nmni.2022.101038, PMID:36385749.
[77] Petakh P, Behzadi P, Oksenych V, Kamyshnyi O. Current treatment options for leptospirosis: a mini-review. Front Microbiol 2024;15:1403765. doi:10.3389/fmicb.2024.1403765, PMID:38725681.
[78] Yadegar A, Bar-Yoseph H, Monaghan TM, Pakpour S, Severino A, Kuijper EJ, *et al*. Fecal microbiota transplantation: current challenges and future landscapes. Clin Microbiol Rev 2024;37(2):e0006022. doi:10.1128/cmr.00060-22, PMID:38717124.
[79] Chen J, Potlapalli R, Quan H, Chen L, Xie Y, Pouriyeh S, *et al*. Exploring DNA Damage and Repair Mechanisms: A Review with Computational Insights. BioTech (Basel) 2024;13(1):3. doi:10.3390/biotech13010003, PMID:38247733.
[80] Delint-Ramirez I, Madabhushi R. DNA damage and its links to neuronal aging and degeneration. Neuron 2025;113(1):7–28. doi:10.1016/j.neuron.2024.12.001, PMID:39788088.
[81] Ahmad A, Braden A, Khan S, Xiao J, Khan MM. Crosstalk between the DNA damage response and cellular senescence drives aging and age-related diseases. Semin Immunopathol 2024;46(3-4):10. doi:10.1007/s00281-024-01016-7, PMID:39095660.
[82] Lucca C, Ferrari E, Shubassi G, Ajazi A, Choudhary R, Bruhn C, *et al*. Sch9(S6K) controls DNA repair and DNA damage response efficiency in aging cells. Cell Rep 2024;43(6):114281. doi:10.1016/j.celrep.2024.114281, PMID:38805395.
[83] Ji S, Xiong M, Chen H, Liu Y, Zhou L, Hong Y, *et al*. Cellular rejuvenation: molecular mechanisms and potential therapeutic interventions for diseases. Signal Transduct Target Ther 2023;8(1):116. doi:10.1038/s41392-023-01343-5, PMID:36918530.
[84] Sun Y, Wang X, Li L, Zhong C, Zhang Y, Yang X, *et al*. The role of gut microbiota in intestinal disease: from an oxidative stress perspective. Front Microbiol 2024;15:1328324. doi:10.3389/fmicb.2024.1328324, PMID:38419631.
[85] Feng P, Xue X, Bukhari I, Qiu C, Li Y, Zheng P, *et al*. Gut microbiota and its therapeutic implications in tumor microenvironment interactions. Front Microbiol 2024;15:1287077. doi:10.3389/fmicb.2024.1287077, PMID:38322318.
[86] Oelschlaeger TA. Bacteria as tumor therapeutics? Bioeng Bugs 2010;1(2):146–147. doi:10.4161/bbug.1.2.11248, PMID:21326940.
[87] Kucerova P, Cervinkova M. Spontaneous regression of tumour and the role of microbial infection—possibilities for cancer treatment. Anticancer Drugs 2016;27(4):269–277. doi:10.1097/CAD.0000000000000337, PMID:26813865.
[88] Radha G, Lopus M. The spontaneous remission of cancer: Current insights and therapeutic significance. Transl Oncol 2021;14(9):101166. doi:10.1016/j.tranon.2021.101166, PMID:34242964.
[89] Ruiz N, Silhavy TJ. How Escherichia coli Became the Flagship Bacterium of Molecular Biology. J Bacteriol 2022;204(9):e0023022. doi:10.1128/jb.00230-22, PMID:35916528.
[90] Zhang G, Sun D. The synthesis of the novel Escherichia coli toxin-colibactin and its mechanisms of tumorigenesis of colorectal cancer. Front Microbiol 2024;15:1501973. doi:10.3389/fmicb.2024.1501973, PMID:39744397.
[91] Cao Y, Oh J, Xue M, Huh WJ, Wang J, Gonzalez-Hernandez JA, *et al*. Commensal microbiota from patients with inflammatory bowel disease produce genotoxic metabolites. Science 2022;378(6618):eabm3233. doi:10.1126/science.abm3233, PMID:36302024.
[92] Martin OCB, Frisan T. Bacterial Genotoxin-Induced DNA Damage and Modulation of the Host Immune Microenvironment. Toxins (Basel) 2020;12(2):63. doi:10.3390/toxins12020063, PMID:31973033.
[93] Cuevas-Ramos G, Petit CR, Marcq I, Boury M, Oswald E, Nougayrède JP. Escherichia coli induces DNA damage in vivo and triggers genomic instability in mammalian cells. Proc Natl Acad Sci U S A 2010;107(25):11537–11542. doi:10.1073/pnas.1001261107, PMID:20534522.
[94] Le LHM, Elgamoudi B, Colon N, Cramond A, Poly F, Ying L, *et al*. Campylobacter jejuni extracellular vesicles harboring cytolethal distending toxin bind host cell glycans and induce cell cycle arrest in host cells. Microbiol Spectr 2024;12(3):e0323223. doi:10.1128/spectrum.03232-23, PMID:38319111.
[95] Huerta-Cantillo J, Chavez-Dueñas L, Zaidi MB, Estrada-García T, Navarro-Garcia F. Cytolethal distending toxin-producing Escherichia coli clinical isolates from Mexican children harbor different cdt types causing CDT-induced epithelial pathological phenotypes. Med Microbiol Immunol 2025;214(1):7. doi:10.1007/s00430-025-00816-4, PMID:39894890.
[96] Jian C, Yinhang W, Jing Z, Zhanbo Q, Zefeng W, Shuwen H. Escherichia coli on colorectal cancer: A two-edged sword. Microb Biotechnol 2024;17(10):e70029. doi:10.1111/1751-7915.70029, PMID:39400440.
[97] Chang SJ, Hsu YT, Chen Y, Lin YY, Lara-Tejero M, Galan JE. Typhoid toxin sorting and exocytic transport from Salmonella Typhi-infected cells. Elife 2022;11:e78561. doi:10.7554/eLife.78561, PMID:35579416.
[98] Gibani MM, Jones E, Barton A, Jin C, Meek J, Camara S, *et al*. Investigation of the role of typhoid toxin in acute typhoid fever in a human challenge model. Nat Med 2019;25(7):1082–1088. doi:10.1038/s41591-019-0505-4, PMID:31270506.
[99] Metreveli M, Bulia S, Shalamberidze I, Tevzadze L, Tsanava S, Goenaga JC, *et al*. Campylobacteriosis, Shigellosis and Salmonellosis in Hospitalized Children with Acute Inflammatory Diarrhea in Georgia. Pathogens 2022;11(2):232. doi:10.3390/pathogens11020232, PMID:35215176.
[100] Finsterer J. Triggers of Guillain-Barré Syndrome: Campylobacter jejuni Predominates. Int J Mol Sci 2022;23(22):14222. doi:10.3390/ijms232214222, PMID:36430700.
[101] Sammons MA, Nguyen TT, McDade SS, Fischer M. Tumor suppressor p53: from engaging DNA to target gene regulation. Nucleic Acids Res 2020;48(16):8848–8869. doi:10.1093/nar/gkaa666, PMID:32797160.
[102] Hernández Borrero LJ, El-Deiry WS. Tumor suppressor p53: Biology, signaling pathways, and therapeutic targeting. Biochim Biophys Acta Rev Cancer 2021;1876(1):188556. doi:10.1016/j.bbcan.2021.188556, PMID:33932560.
[103] Song B, Yang P, Zhang S. Cell fate regulation governed by p53: Friends or reversible foes in cancer therapy. Cancer Commun (Lond) 2024;44(3):297–360. doi:10.1002/cac2.12520, PMID:38311377.
[104] Kadosh E, Snir-Alkalay I, Venkatachalam A, May S, Lasry A, Elyada E, *et al*. The gut microbiome switches mutant p53 from tumour-suppressive to oncogenic. Nature 2020;586(7827):133–138. doi:10.1038/s41586-020-2541-0, PMID:32728212.
[105] Tran Van Nhieu G, Latour-Lambert P, Enninga J. Modification of phosphoinositides by the Shigella effector IpgD during host cell infection. Front Cell Infect Microbiol 2022;12:1012533. doi:10.3389/fcimb.2022.1012533, PMID:36389142.
[106] Köseoğlu VK, Jones MK, Agaisse H. The type 3 secretion effector IpgD promotes S. flexneri dissemination. PLoS Pathog 2022;18(2):e1010324. doi:10.1371/journal.ppat.1010324, PMID:35130324.
[107] Roncaioli JL, Babirye JP, Chavez RA, Liu FL, Turcotte EA, Lee AY, *et al*. A hierarchy of cell death pathways confers layered resistance to shigellosis in mice. Elife 2023;12:e83639. doi:10.7554/eLife.83639, PMID:36645406.
[108] Chiscuzzu F, Crescio C, Varrucciu S, Rizzo D, Sali M, Delogu G, *et al*. Current Evidence on the Relation Between Microbiota and Oral Cancer-The Role of Fusobacterium nucleatum-A Narrative Review. Cancers (Basel) 2025;17(2):171. doi:10.3390/cancers17020171, PMID:39857953.
[109] Ibeanu GC, Rowaiye AB, Okoli JC, Eze DU. Microbiome Differences in Colorectal Cancer Patients and Healthy Individuals: Implications for Vaccine Antigen Discovery. Immunotargets Ther 2024;13:749–774. doi:10.2147/ITT.S486731, PMID:39698218.
[110] Yamamoto H, Watanabe Y, Arai H, Umemoto K, Tateishi K, Sunakawa Y. Microsatellite instability: A 2024 update. Cancer Sci 2024;115(6):1738–1748. doi:10.1111/cas.16160, PMID:38528657.
[111] Chen Y, Shen YQ. Role of reactive oxygen species in regulating epigenetic modifications. Cell Signal 2025;125:111502. doi:10.1016/j.cellsig.2024.111502, PMID:39521028.
[112] Duan Y, Xu Y, Dou Y, Xu D. Helicobacter pylori and gastric cancer: mechanisms and new perspectives. J Hematol Oncol 2025;18(1):10. doi:10.1186/s13045-024-01654-2, PMID:39849657.
[113] McNamara KM, Sierra JC, Latour YL, Hawkins CV, Asim M, Williams KJ, *et al*. Spermine oxidase promotes Helicobacter pylori-mediated gastric carcinogenesis through acrolein production. Oncogene

2025;44(5):296–306. doi:10.1038/s41388-024-03218-7, PMID:39523394.
[114] Noe O, Filipiak L, Royfman R, Campbell A, Lin L, Hamouda D, *et al*. Adenomatous polyposis coli in cancer and therapeutic implications. Oncol Rev 2021;15(1):534. doi:10.4081/oncol.2021.534, PMID:34267890.
[115] Rubinstein MR, Baik JE, Lagana SM, Han RP, Raab WJ, Sahoo D, *et al*. Fusobacterium nucleatum promotes colorectal cancer by inducing Wnt/β-catenin modulator Annexin A1. EMBO Rep 2019;20(4):e47638. doi:10.15252/embr.201847638, PMID:30833345.
[116] Sun J, Chen F, Wu G. Potential effects of gut microbiota on host cancers: focus on immunity, DNA damage, cellular pathways, and anticancer therapy. ISME J 2023;17(10):1535–1551. doi:10.1038/s41396-023-01483-0, PMID:37553473.
[117] Vincent MS, Uphoff S. Bacterial phenotypic heterogeneity in DNA repair and mutagenesis. Biochem Soc Trans 2020;48(2):451–462. doi:10.1042/BST20190364, PMID:32196548.
[118] D'Aquila P, Carelli LL, De Rango F, Passarino G, Bellizzi D. Gut Microbiota as Important Mediator Between Diet and DNA Methylation and Histone Modifications in the Host. Nutrients 2020;12(3):597. doi:10.3390/nu12030597, PMID:32106534.
[119] Wozniak KJ, Simmons LA. Bacterial DNA excision repair pathways. Nat Rev Microbiol 2022;20(8):465–477. doi:10.1038/s41579-022-00694-0, PMID:35210609.
[120] Hsiao Y-C, Liu C-W, Yang Y, Feng J, Zhao H, Lu K. DNA Damage and the Gut Microbiome: From Mechanisms to Disease Outcomes. DNA 2023;3(1):13–32. doi:10.3390/dna3010002.
[121] Lowry E, Mitchell A. Colibactin-induced damage in bacteria is cell contact independent. mBio 2025;16(1):e0187524. doi:10.1128/mbio.01875-24, PMID:39576109.
[122] Kell L, Simon AK, Alsaleh G, Cox LS. The central role of DNA damage in immunosenescence. Front Aging 2023;4:1202152. doi:10.3389/fragi.2023.1202152, PMID:37465119.
[123] Wang Y, Dong C, Han Y, Gu Z, Sun C. Immunosenescence, aging and successful aging. Front Immunol 2022;13:942796. doi:10.3389/fimmu.2022.942796, PMID:35983061.
[124] Teissier T, Boulanger E, Cox LS. Interconnections between Inflammageing and Immunosenescence during Ageing. Cells 2022;11(3):359. doi:10.3390/cells11030359, PMID:35159168.
[125] Müller L, Di Benedetto S. Inflammaging, immunosenescence, and cardiovascular aging: insights into long COVID implications. Front Cardiovasc Med 2024;11:1384996. doi:10.3389/fcvm.2024.1384996, PMID:38988667.
[126] Mostafavi Abdolmaleky H, Zhou JR. Gut Microbiota Dysbiosis, Oxidative Stress, Inflammation, and Epigenetic Alterations in Metabolic Diseases. Antioxidants (Basel) 2024;13(8):985. doi:10.3390/antiox13080985, PMID:39199231.
[127] Holmes A, Finger C, Morales-Scheihing D, Lee J, McCullough LD. Gut dysbiosis and age-related neurological diseases; an innovative approach for therapeutic interventions. Transl Res 2020;226:39–56. doi:10.1016/j.trsl.2020.07.012, PMID:32755639.
[128] Tong J, Song J, Zhang W, Zhai J, Guan Q, Wang H, *et al*. When DNA-damage responses meet innate and adaptive immunity. Cell Mol Life Sci 2024;81(1):185. doi:10.1007/s00018-024-05214-2, PMID:38630271.
[129] Zhao Y, Simon M, Seluanov A, Gorbunova V. DNA damage and repair in age-related inflammation. Nat Rev Immunol 2023;23(2):75–89. doi:10.1038/s41577-022-00751-y, PMID:35831609.
[130] Nastasi C, Mannarino L, D'Incalci M. DNA Damage Response and Immune Defense. Int J Mol Sci 2020;21(20):7504. doi:10.3390/ijms21207504, PMID:33053746.
[131] Bemark M, Pitcher MJ, Dionisi C, Spencer J. Gut-associated lymphoid tissue: a microbiota-driven hub of B cell immunity. Trends Immunol 2024;45(3):211–223. doi:10.1016/j.it.2024.01.006, PMID:38402045.
[132] Mörbe UM, Jørgensen PB, Fenton TM, von Burg N, Riis LB, Spencer J, *et al*. Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. Mucosal Immunol 2021;14(4):793–802. doi:10.1038/s41385-021-00389-4, PMID:33753873.
[133] Jain S, Bemark M, Spencer J. Human gut-associated lymphoid tissue: A dynamic hub propagating modulators of inflammation. Clin Transl Med 2023;13(9):e1417. doi:10.1002/ctm2.1417, PMID:37735775.
[134] Malik JA, Zafar MA, Lamba T, Nanda S, Khan MA, Agrewala JN. The impact of aging-induced gut microbiome dysbiosis on dendritic cells and lung diseases. Gut Microbes 2023;15(2):2290643. doi:10.1080/19490976.2023.2290643, PMID:38087439.
[135] Zheng H, Zhang C, Wang Q, Feng S, Fang Y, Zhang S. The impact of aging on intestinal mucosal immune function and clinical applications. Front Immunol 2022;13:1029948. doi:10.3389/fimmu.2022.1029948, PMID:36524122.
[136] Kim ME, Lee JS. Immune Diseases Associated with Aging: Molecular Mechanisms and Treatment Strategies. Int J Mol Sci 2023;24(21):15584. doi:10.3390/ijms242115584, PMID:37958564.
[137] Hong H, Wang Q, Li J, Liu H, Meng X, Zhang H. Aging, Cancer and Immunity. J Cancer 2019;10(13):3021–3027. doi:10.7150/jca.30723, PMID:31281479.
[138] Jiao Y, Wu L, Huntington ND, Zhang X. Crosstalk Between Gut Microbiota and Innate Immunity and Its Implication in Autoimmune Diseases. Front Immunol 2020;11:282. doi:10.3389/fimmu.2020.00282, PMID:32153586.
[139] Silva-Sanchez A, Randall TD. Anatomical Uniqueness of the Mucosal Immune System (GALT, NALT, iBALT) for the Induction and Regulation of Mucosal Immunity and Tolerance. In: Kiyono H, Pascual DW (eds). Mucosal Vaccines. Academic Press: 2020:21–54. doi:10.1016/B978-0-12-811924-2.00002-X, PMID:31974759.
[140] Wang R, Lan C, Benlagha K, Camara NOS, Miller H, Kubo M, *et al*. The interaction of innate immune and adaptive immune system. MedComm (2020) 2024;5(10):e714. doi:10.1002/mco2.714, PMID:39286776.
[141]Heidari M, Maleki Vareki S, Yaghobi R, Karimi MH. Microbiota activation and regulation of adaptive immunity. Front Immunol 2024;15:1429436. doi:10.3389/fimmu.2024.1429436, PMID:39445008.
[142] Zhang R, Ding N, Feng X, Liao W. The gut microbiome, immune modulation, and cognitive decline: insights on the gut-brain axis. Front Immunol 2025;16:1529958. doi:10.3389/fimmu.2025.1529958, PMID:39911400.
[143] Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI. Correction: Yoo, J.Y., *et al*. Gut Microbiota and Immune System Interactions. Microorganisms 2020, 8, 1587. Microorganisms 2020;8(12):2046. doi:10.3390/microorganisms8122046, PMID:33371530.
[144] Bashir H, Singh S, Singh RP, Agrewala JN, Kumar R. Age-mediated gut microbiota dysbiosis promotes the loss of dendritic cells tolerance. Aging Cell 2023;22(6):e13838. doi:10.1111/acel.13838, PMID:37161603.
[145] Stagg AJ. Intestinal Dendritic Cells in Health and Gut Inflammation. Front Immunol 2018;9:2883. doi:10.3389/fimmu.2018.02883, PMID:30574151.
[146] Hrncir T. Gut Microbiota Dysbiosis: Triggers, Consequences, Diagnostic and Therapeutic Options. Microorganisms 2022;10(3):578. doi:10.3390/microorganisms10030578, PMID:35336153.
[147] Khan I, Ullah N, Zha L, Bai Y, Khan A, Zhao T, *et al*. Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD Treatment Targeting the Gut Microbiome. Pathogens 2019;8(3):126. doi:10.3390/pathogens8030126, PMID:31412603.
[148] Yue N, Hu P, Tian C, Kong C, Zhao H, Zhang Y, *et al*. Dissecting Innate and Adaptive Immunity in Inflammatory Bowel Disease: Immune Compartmentalization, Microbiota Crosstalk, and Emerging Therapies. J Inflamm Res 2024;17:9987–10014. doi:10.2147/JIR.S492079, PMID:39634289.
[149]Pan Q, Guo F, Huang Y, Li A, Chen S, Chen J, *et al*. Gut Microbiota Dysbiosis in Systemic Lupus Erythematosus: Novel Insights into Mechanisms and Promising Therapeutic Strategies. Front Immunol 2021;12:799788. doi:10.3389/fimmu.2021.799788, PMID:34925385.
[150] Mu F, Rusip G, Florenly F. Gut microbiota and autoimmune diseases: Insights from Mendelian randomization. FASEB Bioadv 2024;6(11):467–476. doi:10.1096/fba.2024-00037, PMID:39512840.
[151] Hitch TCA, Hall LJ, Walsh SK, Leventhal GE, Slack E, de Wouters T, *et al*. Microbiome-based interventions to modulate gut ecology and the immune system. Mucosal Immunol 2022;15(6):1095–1113. doi:10.1038/s41385-022-00564-1, PMID:36180583.
[152] Loh JS, Mak WQ, Tan LKS, Ng CX, Chan HH, Yeow SH, *et al*. Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. Signal Transduct Target Ther 2024;9(1):37.

doi:10.1038/s41392-024-01743-1, PMID:38360862.
[153] Kenison JE, Stevens NA, Quintana FJ. Therapeutic induction of antigen-specific immune tolerance. Nat Rev Immunol 2024;24(5):338–357. doi:10.1038/s41577-023-00970-x, PMID:38086932.
[154] Fucikova J, Palova-Jelinkova L, Bartunkova J, Spisek R. Induction of Tolerance and Immunity by Dendritic Cells: Mechanisms and Clinical Applications. Front Immunol 2019;10:2393. doi:10.3389/fimmu.2019.02393, PMID:31736936.
[155] Hasegawa H, Matsumoto T. Mechanisms of Tolerance Induction by Dendritic Cells In Vivo. Front Immunol 2018;9:350. doi:10.3389/fimmu.2018.00350, PMID:29535726.
[156] Cheng J, Hu H, Ju Y, Liu J, Wang M, Liu B, *et al*. Gut microbiota-derived short-chain fatty acids and depression: deep insight into biological mechanisms and potential applications. Gen Psychiatr 2024;37(1):e101374. doi:10.1136/gpsych-2023-101374, PMID:38390241.
[157] Pi Y, Fang M, Li Y, Cai L, Han R, Sun W, *et al*. Interactions between Gut Microbiota and Natural Bioactive Polysaccharides in Metabolic Diseases: Review. Nutrients 2024;16(17):2838. doi:10.3390/nu16172838, PMID:39275156.
[158] Hays KE, Pfaffinger JM, Ryznar R. The interplay between gut microbiota, short-chain fatty acids, and implications for host health and disease. Gut Microbes 2024;16(1):2393270. doi:10.1080/19490976.2024.2393270, PMID:39284033.
[159] Ullah H, Arbab S, Tian Y, Chen Y, Liu CQ, Li Q, *et al*. Crosstalk between gut microbiota and host immune system and its response to traumatic injury. Front Immunol 2024;15:1413485. doi:10.3389/fimmu.2024.1413485, PMID:39144142.
[160] Lange O, Proczko-Stepaniak M, Mika A. Short-Chain Fatty Acids-A Product of the Microbiome and Its Participation in Two-Way Communication on the Microbiome-Host Mammal Line. Curr Obes Rep 2023;12(2):108–126. doi:10.1007/s13679-023-00503-6, PMID:37208544.
[161] Ventura I, Chomon-García M, Tomás-Aguirre F, Palau-Ferré A, Legidos-García ME, Murillo-Llorente MT, *et al*. Therapeutic and Immunologic Effects of Short-Chain Fatty Acids in Inflammatory Bowel Disease: A Systematic Review. Int J Mol Sci 2024;25(20):10879. doi:10.3390/ijms252010879, PMID:39456661.
[162] Albaladejo-Riad N, El Qendouci M, Cuesta A, Esteban MÁ. Ability of short-chain fatty acids to reduce inflammation and attract leucocytes to the inflamed skin of gilthead seabream (Sparus aurata L.). Sci Rep 2024;14(1):31404. doi:10.1038/s41598-024-83033-y, PMID:39732927.
[163] DiPalma MP, Blattman JN. The impact of microbiome dysbiosis on T cell function within the tumor microenvironment (TME). Front Cell Dev Biol 2023;11:1141215. doi:10.3389/fcell.2023.1141215, PMID:37009485.
[164] Quiros-Roldan E, Sottini A, Natali PG, Imberti L. The Impact of Immune System Aging on Infectious Diseases. Microorganisms 2024;12(4):775. doi:10.3390/microorganisms12040775, PMID:38674719.
[165] Hou Y, Chen M, Bian Y, Hu Y, Chuan J, Zhong L, *et al*. Insights into vaccines for elderly individuals: from the impacts of immunosenescence to delivery strategies. NPJ Vaccines 2024;9(1):77. doi:10.1038/s41541-024-00874-4, PMID:38600250.
[166] Caetano-Silva ME, Shrestha A, Duff AF, Kontic D, Brewster PC, Kasperek MC, *et al*. Aging amplifies a gut microbiota immunogenic signature linked to heightened inflammation. Aging Cell 2024;23(8):e14190. doi:10.1111/acel.14190, PMID:38725282.
[167] Gupta N, El-Gawaad NSA, Mallasiy LO, Gupta H, Yadav VK, Alghamdi S, *et al*. Microbial dysbiosis and the aging process: a review on the potential age-deceleration role of Lactiplantibacillus plantarum. Front Microbiol 2024;15:1260793. doi:10.3389/fmicb.2024.1260793, PMID:38440135.
[168] Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, *et al*. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. Signal Transduct Target Ther 2022;7(1):391. doi:10.1038/s41392-022-01251-0, PMID:36522308.
[169] Li Y, Tian X, Luo J, Bao T, Wang S, Wu X. Molecular mechanisms of aging and anti-aging strategies. Cell Commun Signal 2024;22(1):285. doi:10.1186/s12964-024-01663-1, PMID:38790068.
[170] Beltrán-Velasco AI, Reiriz M, Uceda S, Echeverry-Alzate V. Lactiplantibacillus (Lactobacillus) plantarum as a Complementary Treatment to Improve Symptomatology in Neurodegenerative Disease: A Systematic Review of Open Access Literature. Int J Mol Sci 2024;25(5):3010. doi:10.3390/ijms25053010, PMID:38474254.
[171] Zhao W, Peng C, Sakandar HA, Kwok LY, Zhang W. Meta-Analysis: Randomized Trials of Lactobacillus plantarum on Immune Regulation Over the Last Decades. Front Immunol 2021;12:643420. doi:10.3389/fimmu.2021.643420, PMID:33828554.
[172] Beaver LM, Jamieson PE, Wong CP, Hosseinikia M, Stevens JF, Ho E. Promotion of Healthy Aging Through the Nexus of Gut Microbiota and Dietary Phytochemicals. Adv Nutr 2025;16(3):100376. doi:10.1016/j.advnut.2025.100376, PMID:39832641.
[173] Santoro A, Ostan R, Candela M, Biagi E, Brigidi P, Capri M, *et al*. Gut microbiota changes in the extreme decades of human life: a focus on centenarians. Cell Mol Life Sci 2018;75(1):129–148. doi:10.1007/s00018-017-2674-y, PMID:29032502.
[174] Liu R, Sun B. Lactic Acid Bacteria and Aging: Unraveling the Interplay for Healthy Longevity. Aging Dis 2023;15(4):1487–1498. doi:10.14336/AD.2023.0926, PMID:37962461.
[175] Gebrayel P, Nicco C, Al Khodor S, Bilinski J, Caselli E, Comelli EM, *et al*. Microbiota medicine: towards clinical revolution. J Transl Med 2022;20(1):111. doi:10.1186/s12967-022-03296-9, PMID:35255932.
[176] Bosco N, Noti M. The aging gut microbiome and its impact on host immunity. Genes Immun 2021;22(5-6):289–303. doi:10.1038/s41435-021-00126-8, PMID:33875817.
[177] Pająk J, Nowicka D, Szepietowski JC. Inflammaging and Immunosenescence as Part of Skin Aging-A Narrative Review. Int J Mol Sci 2023;24(9):7784. doi:10.3390/ijms24097784, PMID:37175491.
[178] Müller L, Di Benedetto S. From aging to long COVID: exploring the convergence of immunosenescence, inflammaging, and autoimmunity. Front Immunol 2023;14:1298004. doi:10.3389/fimmu.2023.1298004, PMID:37942323.
[179] Saito Y, Yamamoto S, Chikenji TS. Role of cellular senescence in inflammation and regeneration. Inflamm Regen 2024;44(1):28. doi:10.1186/s41232-024-00342-5, PMID:38831382.
[180] Yang H, Zhang X, Xue B. New insights into the role of cellular senescence and chronic wounds. Front Endocrinol (Lausanne) 2024;15:1400462. doi:10.3389/fendo.2024.1400462, PMID:39558972.
[181] Malaquin N, Rodier F. Dynamic and scalable assessment of the senescence-associated secretory phenotype (SASP). Methods Cell Biol 2024;181:181–195. doi:10.1016/bs.mcb.2022.10.005, PMID:38302239.
[182] Deng X, Terunuma H. Adoptive NK cell therapy: a potential revolutionary approach in longevity therapeutics. Immun Ageing 2024;21(1):43. doi:10.1186/s12979-024-00451-2, PMID:38926847.
[183] Song P, An J, Zou MH. Immune Clearance of Senescent Cells to Combat Ageing and Chronic Diseases. Cells 2020;9(3):671. doi:10.3390/cells9030671, PMID:32164335.
[184] Kumar SJ, Shukla S, Kumar S, Mishra P. Immunosenescence and Inflamm-Aging: Clinical Interventions and the Potential for Reversal of Aging. Cureus 2024;16(1):e53297. doi:10.7759/cureus.53297, PMID:38435871.
[185] Li X, Li C, Zhang W, Wang Y, Qian P, Huang H. Inflammation and aging: signaling pathways and intervention therapies. Signal Transduct Target Ther 2023;8(1):239. doi:10.1038/s41392-023-01502-8, PMID:37291105.
[186] Goyani P, Christodoulou R, Vassiliou E. Immunosenescence: Aging and Immune System Decline. Vaccines (Basel) 2024;12(12):1314. doi:10.3390/vaccines12121314, PMID:39771976.
[187] Accardi G, Calabrò A, Caldarella R, Caruso C, Ciaccio M, Di Simone M, *et al*. Immune-Inflammatory Response in Lifespan-What Role Does It Play in Extreme Longevity? A Sicilian Semi- and Supercentenarians Study. Biology (Basel) 2024;13(12):1010. doi:10.3390/biology13121010, PMID:39765677.
[188] Wrona MV, Ghosh R, Coll K, Chun C, Yousefzadeh MJ. The 3 I's of immunity and aging: immunosenescence, inflammaging, and immune resilience. Front Aging 2024;5:1490302. doi:10.3389/fragi.2024.1490302, PMID:39478807.
[189] Gao Z, Feng Y, Xu J, Liang J. T-cell exhaustion in immune-mediated inflammatory diseases: New implications for immunotherapy. Front Immunol 2022;13:977394. doi:10.3389/fimmu.2022.977394, PMID:36211414.
[190] Caldwell BA, Li L. Epigenetic regulation of innate immune dy-

namics during inflammation. J Leukoc Biol 2024;115(4):589–606. doi:10.1093/jleuko/qiae026, PMID:38301269.
[191] Lefkowitz RB, Miller CM, Martinez-Caballero JD, Ramos I. Epigenetic Control of Innate Immunity: Consequences of Acute Respiratory Virus Infection. Viruses 2024;16(2):197. doi:10.3390/v16020197, PMID:38399974.
[192] Amedei A, Parolini C. Editorial: Epigenetics of inflammatory reactions and pharmacological modulation. Front Pharmacol 2024;15:1505196. doi:10.3389/fphar.2024.1505196, PMID:39529877.
[193] Ray D, Yung R. Immune senescence, epigenetics and autoimmunity. Clin Immunol 2018;196:59–63. doi:10.1016/j.clim.2018.04.002, PMID:29654845.
[194] Vezzani B, Carinci M, Previati M, Giacovazzi S, Della Sala M, Gafà R, *et al*. Epigenetic Regulation: A Link between Inflammation and Carcinogenesis. Cancers (Basel) 2022;14(5):1221. doi:10.3390/cancers14051221, PMID:35267528.
[195] Hu Y, Liu Y, Zheng H, Liu L. Risk Factors for Long COVID in Older Adults. Biomedicines 2023;11(11):3002. doi:10.3390/biomedicines 11113002, PMID:38002002.
[196] Dmytriv TR, Storey KB, Lushchak VI. Intestinal barrier permeability: the influence of gut microbiota, nutrition, and exercise. Front Physiol 2024;15:1380713. doi:10.3389/fphys.2024.1380713, PMID:39040079.
[197] Wu YL, Xu J, Rong XY, Wang F, Wang HJ, Zhao C. Gut microbiota alterations and health status in aging adults: From correlation to causation. Aging Med (Milton) 2021;4(3):206–213. doi:10.1002/agm2.12167, PMID:34553118.
[198] Li L, Peng P, Ding N, Jia W, Huang C, Tang Y. Oxidative Stress, Inflammation, Gut Dysbiosis: What Can Polyphenols Do in Inflammatory Bowel Disease? Antioxidants (Basel) 2023;12(4):967. doi:10.3390/antiox12040967, PMID:37107341.
[199] Ning S, Zhang Z, Zhou C, Wang B, Liu Z, Feng B. Cross-talk between macrophages and gut microbiota in inflammatory bowel disease: a dynamic interplay influencing pathogenesis and therapy. Front Med (Lausanne) 2024;11:1457218. doi:10.3389/fmed.2024.1457218, PMID:39355844.
[200] Yang Y, Cadwell K. Beyond antiviral: Interferon induced by bacteria maintains tolerance in the gut. J Exp Med 2024;221(1):e20232011. doi:10.1084/jem.20232011, PMID:38091026.
[201] Pinitchun C, Panpetch W, Bhunyakarnjanarat T, Udompornpitak K, Do HT, Visitchanakun P, *et al*. Aging-induced dysbiosis worsens sepsis severity but is attenuated by probiotics in D-galactose-administered mice with cecal ligation and puncture model. PLoS One 2024;19(10):e0311774. doi:10.1371/journal.pone.0311774, PMID:39423218.
[202] Di Vincenzo F, Del Gaudio A, Petito V, Lopetuso LR, Scaldaferri F. Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. Intern Emerg Med 2024;19(2):275–293. doi:10.1007/s11739-023-03374-w, PMID:37505311.
[203] Ma R, Su H, Jiao K, Liu J. Role of Th17 cells, Treg cells, and Th17/Treg imbalance in immune homeostasis disorders in patients with chronic obstructive pulmonary disease. Immun Inflamm Dis 2023;11(2):e784. doi:10.1002/iid3.784, PMID:36840492.
[204] Zhang S, Gang X, Yang S, Cui M, Sun L, Li Z, *et al*. The Alterations in and the Role of the Th17/Treg Balance in Metabolic Diseases. Front Immunol 2021;12:678355. doi:10.3389/fimmu.2021.678355, PMID:34322117.
[205] Moretti L, Stalfort J, Barker TH, Abebayehu D. The interplay of fibroblasts, the extracellular matrix, and inflammation in scar formation. J Biol Chem 2022;298(2):101530. doi:10.1016/j.jbc.2021.101530, PMID:34953859.
[206] Xin S, Liu X, He C, Gao H, Wang B, Hua R, *et al*. Inflammation accelerating intestinal fibrosis: from mechanism to clinic. Eur J Med Res 2024;29(1):335. doi:10.1186/s40001-024-01932-2, PMID:38890719.
[207] Chaudhry TS, Senapati SG, Gadam S, Mannam HPSS, Voruganti HV, Abbasi Z, *et al*. The Impact of Microbiota on the Gut-Brain Axis: Examining the Complex Interplay and Implications. J Clin Med 2023;12(16):5231. doi:10.3390/jcm12165231, PMID:37629273.
[208] Ullah H, Arbab S, Tian Y, Liu CQ, Chen Y, Qijie L, *et al*. The gut microbiota-brain axis in neurological disorder. Front Neurosci 2023;17:1225875. doi:10.3389/fnins.2023.1225875, PMID:37600019.
[209] Wei W, Wang S, Xu C, Zhou X, Lian X, He L, *et al*. Gut microbiota, pathogenic proteins and neurodegenerative diseases. Front Microbiol 2022;13:959856. doi:10.3389/fmicb.2022.959856, PMID:36466655.
[210] Khatoon S, Kalam N, Rashid S, Bano G. Effects of gut microbiota on neurodegenerative diseases. Front Aging Neurosci 2023;15:1145241. doi:10.3389/fnagi.2023.1145241, PMID:37323141.
[211] Jin L, Shi X, Yang J, Zhao Y, Xue L, Xu L, *et al*. Gut microbes in cardiovascular diseases and their potential therapeutic applications. Protein Cell 2021;12(5):346–359. doi:10.1007/s13238-020-00785-9, PMID:32989686.
[212] Koutsokostas C, Merkouris E, Goulas A, Aidinopoulou K, Sini N, Dimaras T, *et al*. Gut Microbes Associated with Neurodegenerative Disorders: A Comprehensive Review of the Literature. Microorganisms 2024;12(8):1735. doi:10.3390/microorganisms12081735, PMID:39203576.
[213] Mou Y, Du Y, Zhou L, Yue J, Hu X, Liu Y, *et al*. Gut Microbiota Interact With the Brain Through Systemic Chronic Inflammation: Implications on Neuroinflammation, Neurodegeneration, and Aging. Front Immunol 2022;13:796288. doi:10.3389/fimmu.2022.796288, PMID:35464431.
[214] Parker A, Fonseca S, Carding SR. Gut microbes and metabolites as modulators of blood-brain barrier integrity and brain health. Gut Microbes 2020;11(2):135–157. doi:10.1080/19490976.2019.1638722, PMID:31368397.
[215] Mecca M, Picerno S, Cortellino S. The Killer's Web: Interconnection between Inflammation, Epigenetics and Nutrition in Cancer. Int J Mol Sci 2024;25(5):2750. doi:10.3390/ijms25052750, PMID:38473997.
[216] Boccardi V, Marano L. Aging, Cancer, and Inflammation: The Telomerase Connection. Int J Mol Sci 2024;25(15):8542. doi:10.3390/ijms25158542, PMID:39126110.
[217] Azzouz D, Palaniyar N. How Do ROS Induce NETosis? Oxidative DNA Damage, DNA Repair, and Chromatin Decondensation. Biomolecules 2024;14(10):1307. doi:10.3390/biom14101307, PMID:39456240.
[218] Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, *et al*. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch Toxicol 2023;97(10):2499–2574. doi:10.1007/s00204-023-03562-9, PMID:37597078.
[219] Kadry MO, Ali HM. Inflammatory mediators-induced DNA damage in liver and brain injury: Therapeutic approach of 5-Methoy-N-acetyltryptamine. Toxicol Rep 2024;13:101816. doi:10.1016/j.toxrep.2024.101816, PMID:39624222.
[220] Alotaibi AG, Li JV, Gooderham NJ. Tumour necrosis factor-α (TNF-α) enhances dietary carcinogen-induced DNA damage in colorectal cancer epithelial cells through activation of JNK signaling pathway. Toxicology 2021;457:152806. doi:10.1016/j.tox.2021.152806, PMID:33961948.
[221] Hong Y, Boiti A, Vallone D, Foulkes NS. Reactive Oxygen Species Signaling and Oxidative Stress: Transcriptional Regulation and Evolution. Antioxidants (Basel) 2024;13(3):312. doi:10.3390/antiox13030312, PMID:38539845.
[222] Nikfarjam S, Singh KK. DNA damage response signaling: A common link between cancer and cardiovascular diseases. Cancer Med 2023;12(4):4380–4404. doi:10.1002/cam4.5274, PMID:36156462.
[223] Malamos P, Papanikolaou C, Gavriatopoulou M, Dimopoulos MA, Terpos E, Souliotis VL. The Interplay between the DNA Damage Response (DDR) Network and the Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway in Multiple Myeloma. Int J Mol Sci 2024;25(13):6991. doi:10.3390/ijms25136991, PMID:39000097.
[224] Anilkumar S, Wright-Jin E. NF-κB as an Inducible Regulator of Inflammation in the Central Nervous System. Cells 2024;13(6):485. doi:10.3390/cells13060485, PMID:38534329.
[225] Wang W, Mani AM, Wu ZH. DNA damage-induced nuclear factor-kappa B activation and its roles in cancer progression. J Cancer Metastasis Treat 2017;3:45–59. doi:10.20517/2394-4722.2017.03, PMID:28626800.
[226] Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018;9(6):7204–7218. doi:10.18632/oncotarget.23208, PMID:29467962.
[227] Caballero-Sánchez N, Alonso-Alonso S, Nagy L. Regenerative inflammation: When immune cells help to re-build tissues. FEBS J

2024;291(8):1597–1614. doi:10.1111/febs.16693, PMID:36440547.
[228] Yan L, Wang J, Cai X, Liou YC, Shen HM, Hao J, *et al*. Macrophage plasticity: signaling pathways, tissue repair, and regeneration. MedComm (2020) 2024;5(8):e658. doi:10.1002/mco2.658, PMID:39092292.
[229] Rodríguez-Morales P, Franklin RA. Macrophage phenotypes and functions: resolving inflammation and restoring homeostasis. Trends Immunol 2023;44(12):986–998. doi:10.1016/j.it.2023.10.004, PMID:37940394.
[230] Kopczyńska J, Kowalczyk M. The potential of short-chain fatty acid epigenetic regulation in chronic low-grade inflammation and obesity. Front Immunol 2024;15:1380476. doi:10.3389/fimmu.2024.1380476, PMID:38605957.
[231] Kim B, Song A, Son A, Shin Y. Gut microbiota and epigenetic choreography: Implications for human health: A review. Medicine (Baltimore) 2024;103(29):e39051. doi:10.1097/MD.0000000000039051, PMID:39029010.
[232] Martínez-Montoro JI, Martín-Núñez GM, González-Jiménez A, Garrido-Sánchez L, Moreno-Indias I, Tinahones FJ. Interactions between the gut microbiome and DNA methylation patterns in blood and visceral adipose tissue in subjects with different metabolic characteristics. J Transl Med 2024;22(1):1089. doi:10.1186/s12967-024-05746-y, PMID:39617894.
[233] Choi SW, Friso S. Modulation of DNA methylation by one-carbon metabolism: a milestone for healthy aging. Nutr Res Pract 2023;17(4):597–615. doi:10.4162/nrp.2023.17.4.597, PMID:37529262.
[234] Kumar N, Moreno NC, Feltes BC, Menck CF, Houten BV. Cooperation and interplay between base and nucleotide excision repair pathways: From DNA lesions to proteins. Genet Mol Biol 2020;43(1 suppl. 1):e20190104. doi:10.1590/1678-4685-GMB-2019-0104, PMID:32141475.
[235] Zhang X, Yin M, Hu J. Nucleotide excision repair: a versatile and smart toolkit. Acta Biochim Biophys Sin (Shanghai) 2022;54(6):807–819. doi:10.3724/abbs.2022054, PMID:35975604.
[236] Liu XF, Shao JH, Liao YT, Wang LN, Jia Y, Dong PJ, *et al*. Regulation of short-chain fatty acids in the immune system. Front Immunol 2023;14:1186892. doi:10.3389/fimmu.2023.1186892, PMID:37215145.
[237] Facchin S, Bertin L, Bonazzi E, Lorenzon G, De Barba C, Barberio B, *et al*. Short-Chain Fatty Acids and Human Health: From Metabolic Pathways to Current Therapeutic Implications. Life (Basel) 2024;14(5):559. doi:10.3390/life14050559, PMID:38792581.

Exploratory Research and Hypothesis in Medicine **2025** vol. 10(2) | 122–134
DOI: 10.14218/ERHM.2024.00045

Review Article

The Hidden Drivers of Aging: Microbial Influence on Genomic Stability and Telomere Dynamics

Swarup K. Chakrabarti[1*] and Dhrubajyoti Chattopadhyay[1,2]

[1]*H. P. Ghosh Research Center, New Town, Kolkata, West Bengal, India;* [2]*Sister Nivedita University, New Town, West Bengal, India*

Received: December 20, 2024 | **Revised:** February 19, 2025 | **Accepted:** February 25, 2025 | **Published online:** April 17, 2025

Abstract

This review explores the complex interplay between the microbiome and human aging, highlighting how dysbiosis impacts host physiology and health, particularly in relation to genomic stability and telomere attrition. Recent advances in cellular and molecular biology have underscored the role of both intrinsic and extrinsic factors in human aging, with the microbiome emerging as a key determinant of host physiology and health. Dysbiosis—disruptions in microbiome composition—is linked to various age-related diseases and impacts genomic stability and telomere attrition, the progressive shortening of telomeres that limits cell division and contributes to aging. This review examines how microbiome dynamics influence aging by triggering inflammation, oxidative stress, immune dysregulation, and metabolic dysfunction, all of which affect two primary hallmarks of aging: genomic instability and telomere attrition. Understanding these interactions is essential for developing targeted interventions to restore microbiome balance and promote healthy aging, offering potential treatments to extend healthspan and alleviate aging-related diseases. The convergence of microbiome and aging research promises transformative insights and new avenues for improving global population well-being.

Introduction

In recent years, research on human aging has placed greater emphasis on the biological processes that underlie it.[1] This shift has been driven by technological advances and a deeper understanding of the intricate cellular and molecular interactions that regulate aging.[2,3] Central to this exploration is the recognition that aging is not a singular event but a complex interaction of numerous inherent and external factors that together shape the timing and nature of the process.[4,5] Among these factors, the human microbiome has emerged as an important influence on host physiology and health outcomes.[6–8] Consisting of a diverse array of microorganisms that inhabit various areas of the body, the microbiome significantly impacts metabolic functions, immune responses, and even brain health.[9] Dysbiosis, or imbalances in the microbiome, is linked to age-related conditions such as cardiovascular diseases (CVDs), neurodegenerative diseases (NDs), and metabolic syndromes.[10] DNA repair mechanisms and cell cycle checkpoints protect genetic material, ensuring its stability across cell generations.[11] However, internal and external factors—such as reactive oxygen species (ROS), radiation, and environmental toxins—continuously threaten this stability by causing DNA damage.[12] As DNA damage accumulates over time, it can result in mutations, chromosomal abnormalities, and, eventually, cellular dysfunction and aging.[13–15] An important factor in cellular aging is the progressive shortening of telomeres, repetitive DNA sequences found at the ends of chromosomes.[16] Telomeres protect chromosomal ends, preventing them from being mistaken for DNA breaks and maintaining genomic stability.[17] However, with each cell division, telomeres shorten because DNA polymerase cannot fully replicate the lagging strand. As a result, telomeres act as a molecular timer, restricting the ability of cells to proliferate and leading to replicative senescence.[18,19] Understanding how the human microbiome, genomic stability, and telomere shortening are interconnected is crucial to uncovering the mechanisms of aging and developing strategies for healthy aging.[20–25]

Thus, this review aimed to integrate current studies and provide a comprehensive understanding of the complex interactions among various factors that contribute to the aging process. In particular, we emphasize genomic instability and telomere attrition—two crucial primary hallmarks of aging outlined by López-Otín C and colleagues.[26] These hallmarks are essential to the biology of aging and are closely linked to cellular dysfunction and the emergence of age-related diseases. Genomic instability, resulting from the buildup of DNA damage and mutations, accelerates aging at the cellular level, whereas telomere attrition, which limits

Keywords: Microbiome; Aging; Dysbiosis; Genomic instability; Telomere attrition; Inflammation; Oxidative stress; Immune dysregulation; Metabolic dysfunction; Healthspan; Age-related disease.

***Correspondence to:** Swarup K. Chakrabarti, H. P. Ghosh Research Center, HIDCO (II), EK Tower, New Town, Kolkata, West Bengal 700161, India. ORCID: https://orcid.org/0000-0001-5666-7662. Tel: +91-9831643038, E-mail: swarupkchakrabarti@gmail.com

How to cite this article: Chakrabarti SK, Chattopadhyay D. The Hidden Drivers of Aging: Microbial Influence on Genomic Stability and Telomere Dynamics. *Explor Res Hypothesis Med* 2025;10(2):122–134. doi: 10.14218/ERHM.2024.00045.

cellular replication, plays a key role in cellular senescence.[20–25,27] While epigenetic changes and the loss of proteostasis also qualify as primary hallmarks of aging, their inclusion would significantly expand the scope of this review.[27] Considering the complexity of these processes and their evolving connections with the microbiome, we have chosen to focus specifically on genomic instability and telomere attrition. This targeted approach allows for a more detailed and precise investigation of these well-established aging mechanisms, which are directly affected by the microbiome, without delving into the complex interactions with other hallmarks. Through this strategy, we aimed to provide valuable insights into the evolving relationship between aging and the microbiome, a growing area of interest in gerontological studies.

Key hallmarks that shape our journey through life

The phenomenon of biological aging is complex and gradual, marked by a steady decline in both physiological and cellular functions, resulting in reduced resilience to stress, slower healing, and disrupted homeostasis in the organism.[1–5,28] Scientists are actively exploring the molecular and cellular foundations of aging, identifying twelve distinct hallmarks associated with this process.[26,28–31] This growing body of knowledge highlights the intricate nature of aging and drives ongoing efforts to unravel its essential mechanisms and develop strategies that promote healthier aging trajectories. The proposed hallmarks of aging involve various cellular and molecular mechanisms that are crucial in the aging process of diverse organisms. These features include genomic instability, telomere shortening, epigenetic changes, a decline in proteostasis, disrupted nutrient sensing, mitochondrial dysfunction (MD), cellular senescence, stem cell depletion, altered cell communication, dysbiosis, chronic inflammation, and impaired macroautophagy.[28–31] Genomic instability refers to the buildup of DNA damage, whereas telomere attrition involves the progressive shortening of the protective ends of chromosomes.[20–25] In contrast, epigenetic modifications change gene expression without altering the DNA sequence, while loss of proteostasis disrupts the structure and function of proteins.[27,32,33] Disruption of nutrient sensing negatively impacts metabolic pathways, and MD results in diminished energy output and increased production of ROS.[34,35] Cellular senescence halts cell growth permanently, stem cell decline reduces tissue regeneration, and altered intercellular communication disrupts tissue balance.[36–38] Dysbiosis disrupts gut microbe balance, chronic inflammation keeps the immune system overactive, and impaired macroautophagy leads to the accumulation of cellular waste, exacerbating age-related decline.[39–41] These hallmarks reveal the complex interactions of cellular, molecular, and microbial processes in aging, highlighting the potential for targeted interventions to promote healthier aging.[26] It is important to emphasize that each hallmark should be present during normal aging, with their exacerbation potentially speeding up aging and their alleviation possibly extending a healthy lifespan.[28–31]

Microbiome and hallmarks of aging

The human microbiome, especially the gut microbiota, develops in tandem with its host, and changes within it are significantly linked to the aging process. The microbiome consists of a wide range of microorganisms inhabiting various regions of the body, and it undergoes ongoing transformations in harmony with its host— a trend supported by many scientific investigations exploring its complex relationship with aging.[21–25] Research has revealed that alterations in gut microbiota composition can affect how individuals respond to stress and their overall health as they age. Recent empirical studies suggest an innovative method focused on gut microbiota to alleviate symptoms associated with brain aging and improve cognitive health.[42] Experimental data shows that gut microbiota metabolism changes with age, suggesting a possible link to age-related metabolic disorders.[23,43,44] Studies tracking specific groups over time have shown that the composition of gut microbiota evolves gradually as a person ages, and these changes are linked to the onset of age-related diseases.[45,46] These findings highlight the strong impact of the gut microbiota on aging and the need for further study into the mechanisms behind this connection. Research also shows that maintaining a balanced gut microbiota is crucial for encouraging optimal physical and mental development during infancy and childhood.[47,48] Aging causes physiological changes that alter gut microbiota composition and function, highlighting their role in dysbiosis and the aging process.[49,50] Furthermore, dysbiosis-related changes in gut microbiota can impact various aspects of aging through the body's interconnected ecosystem.[51–53]

Thus, this review article takes an important initial step in exploring the complex connection between the microbiome and critical aspects of aging, including genomic instability and telomere shortening. Acknowledging the difficulties inherent in this subject, the following sections aim to clarify the nuanced interaction between these elements. By initiating this journey of exploration, this review paves the way for further research and advancements in this vibrant and rapidly evolving field.

Evolving interaction between the microbiota and genomic instability

In the intricate realm of human biology, the relationship between the human microbiome and the stability of our genes is a fascinating topic that warrants further exploration. Recent pioneering research has illuminated the considerable impact that the microbial populations residing within us have on various health and disease factors. As our understanding grows, an engaging narrative unfolds: the evolving connection between genetic stability and these microorganisms.[21–24,42–44] One common characteristic observed in numerous diseases is genomic instability, which refers to the increased frequency of genetic alterations, including DNA mutations, chromosomal rearrangements, and aberrant recombination events. In biomedical research, understanding the causes and factors contributing to this instability has been essential.[54] However, new findings are revealing a crucial, yet previously overlooked, element affecting genetic stability: the complex microbial communities within both our internal and external environments.[11–15]

Central to this investigation is a pivotal shift in perspective: microbiota are now recognized not merely as passive bystanders but as active contributors to the development of host physiology, including the optimal maintenance of the host genome, a process intricately linked to aging. The dynamic interplay between the microbiota and the genome introduces additional complexity, as environmental factors, diet, antibiotic use, and host genetics combine to shape microbial communities, influencing genomic stability in diverse ways.[55–58] This convergence of genetic and microbiological research represents a transformative shift in our understanding, emphasizing the microbiota's crucial role in human health and disease. Embracing this comprehensive perspective not only enhances our insight into the origins of diseases but also opens avenues for new treatments and personalized medicine aimed at

preserving genomic balance. For instance, studies have demonstrated the profound effects of *Helicobacter pylori* infection on the gastrointestinal (GI) system, unveiling DNA-damaging processes triggered by this pathogenic bacterium.[59,60] These studies revealed the detrimental impacts of *H. pylori* on host cells and provided preliminary evidence linking microbial infections to the activation of DNA damage responses (DDR).[59–61] Moreover, they highlighted the disruption of the host's DNA repair mechanisms in the presence of *H. pylori*, underscoring the potential threat to genomic stability posed by pathogenic bacteria and the urgent need to explore targeted therapeutic strategies to mitigate microbial-induced DNA damage. Similarly, *Fusobacterium nucleatum*, a bacterium frequently found in the human digestive system, causes persistent inflammation, leading to the production of ROS, which damage cellular components, including DNA.[62,63] Over time, this accumulation of DNA damage may hasten the aging process by promoting cellular senescence, weakening DNA repair functions, and contributing to the hallmarks of aging.[64,65] Additionally, *Enterococcus faecalis* produces superoxide anions and hydrogen peroxide during metabolism, both of which can cause oxidative damage to DNA, resulting in base modifications, strand breaks, and cross-linking.[66] Over time, the continuous cycle of DNA damage and repair leads to the accumulation of mutations and chromosomal instability, indicators of aging that reduce cellular functionality and increase the risk of degenerative diseases, including cognitive deterioration.[64,65] Gut microbiota also play a critical role in processing bile acids, which is vital for preserving DNA integrity.[67] Gut microbiota convert primary bile acids into secondary forms, including deoxycholic acid, which can produce ROS and directly interact with DNA, causing strand breaks and genetic mutations.[67–69] This DNA damage is particularly significant as we age, as the accumulation of damage can overwhelm the cells' repair capabilities, impairing function in critical organs like the colon and liver. As a result, chronic exposure to dysbiosis-induced altered bile acid compositions may contribute to inflammation related to aging and tissue degeneration.[70,71] Moreover, the role of *Clostridium difficile* further exemplifies how gut bacteria contribute to DNA damage. This bacterium secretes powerful toxins, such as Toxin A and Toxin B, which enzymatically modify Rho GTPases, impairing the actin cytoskeleton and cellular signaling.[72,73] The resulting disruption of cellular integrity activates DDR pathways, initiating repair or inducing cell death when the damage is irreparable. Prolonged exposure to these toxins induces inflammation, exacerbating cellular stress and accelerating the accumulation of DNA damage. Over time, these chronic assaults can overwhelm DNA repair mechanisms, causing genomic instability and mutations that hinder tissue function.[64,65] The intestinal epithelium, with its rapid turnover rate, is particularly vulnerable to accelerated aging and degeneration as DNA damage disrupts regeneration and organ function.[74,75] Furthermore, the synthesis of genotoxins, such as colibactin and *Bacteroides fragilis* toxin, by commensal bacterial strains like *Escherichia coli* and *Bacteroides fragilis* presents a significant concern for cellular health.[76,77] Interestingly, the healthy GI tract is predominantly inhabited by obligate anaerobic bacteria, including *Bacteroides*, *Bifidobacterium*, *Clostridium*, and *Ruminococcus*,[78–80] which help maintain gut health and support essential metabolic functions. However, dysbiosis—an imbalance in the microbiota—leads to an increase in facultative anaerobes such as *Escherichia*, *Enterobacter*, *Enterococcus*, and *Klebsiella*.[81,82] Unlike obligate anaerobes, facultative anaerobes can thrive in both oxygen-rich and oxygen-poor environments, and their proliferation triggers gut inflammation, resulting in ROS production and DNA damage. This cascade of events accelerates genomic instability, particularly in the GI tract, and increases the risk of age-related disorders.[83–85] Furthermore, older individuals exhibit elevated oxidative stress markers, such as 8-oxoguanine and γ-H2AX, which indicate DNA damage that worsens with dysbiosis, contributing to neurodegeneration and systemic aging.[86,87] Dysbiosis also promotes MD, further exacerbating mtDNA damage and cellular aging. This process is characterized by impaired energy production and immune responses—hallmarks of aging.[88,89]

Recent research has reinforced the connection between gut microbiota, genomic instability, and aging, emphasizing the pivotal role of inflammation in this process. Studies have found that aging impairs DNA double-strand break repair in mouse livers following diethylnitrosamine exposure, with gut microbiota-induced inflammation playing a crucial role.[90,91] Age-related microbiota changes lead to dysregulated innate immunity, heightened inflammatory cytokine levels, and reduced DNA repair efficiency.[92,93] Notably, interventions such as antibiotic treatment, MyD88 gene (myeloid differentiation primary response gene 88) deletion, or germ-free conditions have been shown to reduce inflammation and enhance DNA repair in older mice.[94–96] Conversely, pro-inflammatory factors, such as a high-fat diet, exacerbate DNA repair deficiencies; however, antibiotic treatment mitigates this effect, highlighting the microbiota's significant influence.[97,98] These findings suggest that modifying inflammatory responses, rather than directly targeting DNA repair mechanisms, may help prevent genomic instability during aging.[99,100]

Clinical trials investigating the microbiota-genomic instability link are also exploring potential therapeutic avenues. For example, the Canakinumab trial, which targets interleukin-1β to reduce inflammation, demonstrated benefits in managing age-related diseases like hypertension and diabetes, further confirming inflammation's role in genomic instability.[101–103] Similarly, the Metformin Aging Study suggests that metformin, a diabetes drug, may enhance genomic stability and slow cellular aging, positioning it as a potential anti-aging therapy.[104,105] Additionally, fecal microbiota transplantation trials aim to restore a healthy microbiome, potentially improving genomic stability and mitigating aging-related issues.[106,107] Trials examining rapamycin, a drug believed to delay menopause, further suggest that modulating immune responses and cellular aging mechanisms can help combat genomic instability.[108–110] Collectively, these studies underscore the significant role of microbiota and inflammation in aging, offering insights into novel therapies that could enhance healthspan and reduce the impact of genomic instability. In summary, microbial metabolites and toxins contribute to DNA damage through oxidative stress, genotoxic by-products, and direct interactions with DNA. The accumulation of this damage over time drives genomic instability. While the relationship between the microbiome and aging remains complex, growing evidence suggests that microbial effects on DNA integrity play a pivotal role in the aging process. This underscores the importance of maintaining a balanced microbiome for healthy aging and minimizing the risk of age-related diseases.

Intricate interplay between microbiome dynamics and telomere attrition

At the ends of each chromosome, there is a protective structure called a telomere, which consists of repeating DNA sequences of the motif TTAGGG.[17,18] Telomeres play an essential role in protecting genetic information during cell replication, as the processes responsible for copying DNA encounter challenges in fully repli-

cating the ends of chromosomes.[17–20] Additionally, telomeres are composed of repetitive DNA sequences and are capped by a unique protein complex known as shelterin.[111–114] This shelterin assembly includes six specific proteins: telomeric-repeat binding factor (TRF) 1, TRF2, tripeptidyl peptidase 1, protection of telomerase 1, TRF1-interacting nuclear factor 2, and repressor activator protein 1. The shelterin complex is essential for protecting telomeres and executing several critical functions. First, it acts as a shield, preventing the identification of telomeres as sites of DNA damage. This blockage of DDR pathways helps prevent undesirable biological reactions, such as cell cycle arrest or apoptosis. Second, shelterin plays a vital role in preserving the integrity of telomeres, stopping the cellular machinery responsible for DNA repair from mistakenly identifying them as damaged. This action helps prevent the fusion of telomeres with the ends of other chromosomes, ultimately reducing the risk of genomic instability or rearrangement. Lastly, shelterin modulates the telomerase enzyme, which restores lost telomeric DNA during cellular division by managing its availability and function. This regulation ensures that telomerase is active only when needed, avoiding excessive elongation of telomeres that could result in age-related disorders. Together, the shelterin complex plays a crucial role in maintaining telomere integrity by striking a balance between shielding them from damage and ensuring they function correctly in essential biological activities like replication and repair.[111–117] Although telomerase activation is typically inhibited in mature somatic cells, it peaks during the developmental phases of humans.[118] Throughout embryonic growth, telomerase remains highly functional to promote effective cell division and tissue development.[119] However, as cells specialize and mature, the activity of telomerase is generally reduced in most somatic cells.[120] Nevertheless, specific cell types, such as stem cells and immune cells, retain their telomerase activity throughout an individual's life.[121–123] This persistent activity allows stem cells to continually divide and differentiate into different cell types, repairing damaged or aging cells. Similarly, immune cells rely on telomerase to aid in their rapid growth and effective function when responding to pathogens and ensuring ongoing immune monitoring.[124,125] The persistence of telomerase activity within these essential cell types enables the body to maintain tissue balance, healing, and regeneration over time. This highlights the importance of telomerase not just for enhancing lifespan but also for safeguarding tissue quality and functionality as aging occurs.[126,127]

Research indicates that dysbiosis initiates a cascade of events leading to shortened telomeres in host cells through various pathways.[128–130] Initially, dysbiosis triggers chronic, low-grade inflammation, which produces ROS and other damaging substances that accelerate telomere shortening.[131,132] This inflammation also increases cellular turnover and oxidative stress, further hastening telomere loss. Additionally, dysbiotic microbiota produce metabolites and byproducts that contribute to oxidative stress in host cells.[133] Interestingly, certain beneficial microorganisms, such as those generating short-chain fatty acids (SCFAs), have been identified as potential protectors of telomeres.[133–135] SCFAs influence both oxidative stress and inflammation, which are closely linked to telomere shortening.[136,137] Thus, microbial metabolites like SCFAs may mitigate telomere loss, creating a favorable environment for maintaining telomere length and cellular vitality. Additionally, an imbalanced microbiota disrupts the host immune system, leading to irregular immune responses, including impaired T cell activity and increased production of pro-inflammatory cytokines. These changes amplify inflammation and oxidative stress, which, in turn, accelerate telomere shortening.[138–140] Moreover, dysbiosis impacts host metabolism, leading to complications such as insulin resistance and dyslipidemia.[141,142] These metabolic disturbances exacerbate systemic inflammation and oxidative stress, contributing to accelerated telomere attrition. Furthermore, microbial imbalances could influence the host's epigenetic processes, including changes in DNA methylation and histone modifications.[143–145] These epigenetic alterations can disrupt telomere regulation, accelerating telomere shortening. For instance, modifications in DNA methylation at genes associated with telomeres might impair telomerase function or affect telomere structure, exacerbating telomere loss.[146,147]

Numerous studies reinforce the link between dysbiosis, telomere shortening, and the onset of diseases. Research shows that metabolic diseases, like type 2 diabetes, are associated with accelerated aging due to shortened telomeres, which may result from disrupted gut microbiota.[148,149] Changes in diet or weight-loss surgery can alter gut microbiota composition, reducing inflammation caused by gut-derived Gram-negative bacterial fragments known as endotoxins.[150,151] This shift may similarly influence the gradual shortening of telomeres over time. A distinct study further demonstrated that enhancing telomerase activity, particularly in the GI tract, could counteract aging processes. This intervention extended the lifespan of telomerase-deficient *zebrafish* and alleviated age-related symptoms in wild-type counterparts.[152,153] This finding underscores the potential of strategic interventions to mitigate the impacts of aging and improve health outcomes. Additionally, research into the gut microbiome's role in mental health, particularly depression, reveals a connection between telomere shortening and an imbalance of beneficial gut bacteria.[154,155] These studies contribute to the growing body of evidence suggesting that changes in gut microbiota composition play a crucial role in the acceleration of telomere shortening, especially in mood disorders like depression. Further research highlights the gut microbiome's critical role in regulating telomere length and the development of age-related diseases, primarily through inflammation and oxidative stress. Studies show that a diverse microbiota helps preserve telomere length, while imbalances or dysbiosis accelerate telomere loss and exacerbate aging-related issues.[156,157] For instance, individuals with less diverse microbiota exhibit significantly shorter telomeres, indicating that microbial imbalances increase the risk of age-related conditions. Animal models support these findings, with germ-free mice showing notably shortened telomeres due to abnormal immune responses and increased oxidative stress.[158,159] In humans, an imbalanced microbiome is correlated with higher inflammatory markers, such as C-reactive protein, which contribute to telomere shortening.[160] Conversely, correcting dysbiosis in animal models has been shown to reverse telomere shortening, likely by reducing inflammation and oxidative stress through beneficial SCFAs.[161,162] A balanced microbiome, supported by dietary interventions or probiotics, has also been associated with longer telomeres, suggesting that such strategies could slow telomere loss and delay aging.[163,164] These studies strongly suggest that maintaining a balanced microbiome is essential for preserving telomere length and preventing age-related diseases.

Recent human studies have further established a link between gut microbiome changes and telomere shortening, emphasizing the importance of gut health in aging. Research indicates that individuals with a more diverse microbiome tend to have longer telomeres, suggesting that a balanced microbiota can protect against aging.[165,166] Diet, particularly fiber-rich foods, plays a key role by promoting beneficial bacteria that produce SCFAs, such as butyrate.[163,164] These SCFAs reduce inflammation and oxidative stress—major contributors to telomere shortening—and enhance immune func-

tion. The microbiome also significantly influences immune cells like T-cells, which in turn affect telomere length. Imbalances in microbiota can weaken immune responses and accelerate telomere loss. Overall, these findings demonstrate that the microbiome's regulation of inflammation, oxidative stress, and immune function is vital for preserving telomere length and slowing aging.[167,168]

In summary, imbalances in the microbiota trigger a series of physiological changes, including inflammation, oxidative stress, immune dysfunction, metabolic issues, and genetic modifications. Together, these factors lead to shorter telomeres and genomic instability, accelerating cellular aging and increasing the risk of aging-related diseases. However, a comprehensive analysis of how disruptions in microbial balance influence these interconnected factors goes beyond the scope of this article. Nonetheless, this section aims to shed light on the intricate relationship between microbiota, telomere shortening, and aging, emphasizing the need for continued research in this evolving field.

Microbiome and aging: interplay between genome stability and telomere attrition

In the complex field of human health and aging, an important molecular-level topic emerges: the relationship between genome stability and the gradual deterioration of telomeres. This section explores this connection comprehensively by summarizing findings from key studies in the literature, with a focus on how genome stability and telomere attrition impact human health and aging, particularly in relation to the microbiome. For example, Werner syndrome (WS) is an inherited disorder characterized by the early onset of aging symptoms.[169] At the molecular level, WS results from a mutation in the Werner protein, which is a crucial member of the RecQ helicase family involved in DNA replication, repair, and recombination.[170,171] Consequently, WS is associated with heightened genomic instability, accelerating the development of aging and age-related diseases. Cells obtained from WS patients exhibit limited growth in culture, entering a senescent state after a certain number of cell divisions, indicating telomere dysfunction.[172] Nevertheless, bypassing p53- and retinoblastoma protein-dependent tumor-suppressing mechanisms allows for enhanced cell division and an extended replicative lifespan, further emphasizing the link between telomere dysfunction and the accelerated aging observed in WS.[173,174] Critically short telomeres in humans initiate signaling through the p53 and retinoblastoma protein tumor suppressor pathways, exacerbating genomic instability.[175] This relationship highlights why individuals with WS face a higher risk of age-related diseases, given the close connection between genomic instability and aging, likely stemming from telomere dysfunction. It underscores the increasing recognition of the interconnections among the hallmarks of aging, particularly in the context of accelerated aging in WS. Although direct links between microbial dysbiosis and WS are still emerging, numerous studies suggest that dysbiosis may exacerbate aging-related conditions, potentially impacting WS.[176,177] For instance, research has shown that an imbalance in the microbiome can lead to increased levels of pro-inflammatory cytokines and ROS, both of which can negatively affect cell health and intensify concerns tied to premature aging, such as in WS.[178,179] Impaired DNA repair mechanisms are a hallmark of WS, and factors triggered by dysbiosis, such as oxidative stress and inflammation, might indirectly impede these repair processes, potentially worsening genomic instability in WS patients. Additional studies present intriguing results regarding the efficacy of telomeric DNA repair across different cell types.[180,181] Comparisons indicate that as individuals age, their cells exhibit diminished repair efficiency, particularly in those from older adults.[182] This reduction is notably severe in cells from individuals with WS. While repair efficiency in cells from Alzheimer's patients meets expectations, there is a slight drop in efficiency observed in WS cells.[183,184] These findings prompt inquiries into the possible functional ramifications of compromised telomeric repair mechanisms in relation to the genomic instability linked to aging. Furthermore, the connection between reduced DNA repair capacity and age-associated alterations in the microbiome underscores a complex interplay between cellular aging mechanisms and microbial dynamics, highlighting how these factors may collectively influence the aging process and age-related diseases.[185–189]

The role of the gut microbiome in longevity: insights from centenarians

Centenarians, those who live to be 100 years old or more, demonstrate remarkable longevity and resilience to age-related health challenges.[190,191] Recent research has highlighted how their gut microbiome composition may play a critical role in sustaining their long lives.[192,193] Studies suggest that the microbiomes of centenarians differ significantly from those of younger individuals, exhibiting greater diversity of beneficial microbes.[192,194,195] Notably, centenarians tend to have increased levels of gut bacteria such as *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae*, which are linked to anti-inflammatory effects and improved gut health. These microbial compositions likely play a pivotal role in maintaining immune homeostasis, reducing systemic inflammation, and modulating metabolic pathways, all of which are crucial for promoting healthy aging and extending lifespan. Moreover, centenarians possess a broader variety of microbes that produce beneficial compounds like SCFAs, which support both gut health and overall physiological balance.[196,197] While the evidence remains limited, primarily due to small sample sizes and observational studies, current findings suggest significant correlations between specific gut microbiota profiles in centenarians and factors such as SCFA production and gut health. However, these studies do not yet establish definitive causal relationships, and further research is needed to clarify how the microbiome connects with the hallmarks of aging. Understanding these microbial patterns could offer valuable insights for developing strategies to promote healthy aging and potentially extend lifespan in broader populations. This exploration of the microbiome's influence on aging intersects with broader biological processes like telomere shortening and genetic stability. Telomeres, the protective caps at the ends of chromosomes, naturally shorten as we age, and this genetic instability is a key driver of age-related decline. The relationship between the microbiome, telomere preservation, and genome stability is a fascinating area of study. Researchers are increasingly focused on understanding how the microbiome may help protect telomeres, which could open new avenues for therapies aimed at promoting healthy aging. These studies highlight that the gut microbiome plays a crucial role in protecting overall well-being, offering a pathway to improve longevity and health through its potential interactions with genome stability and telomere length.[198–200]

Studies on centenarians from around the world reinforce these concepts, highlighting specific microbial features linked to longevity. For instance, research on Okinawan centenarians has found that their microbiomes are enriched with *Akkermansia muciniphila*, a bacterium known for enhancing gut barrier function and reducing inflammation.[201,202] This microbe, along with others like

Bifidobacterium, is associated with improved metabolic health, a balanced immune system, and lower levels of inflammation—factors crucial for aging well.[203,204] Similarly, Italian centenarians possess a microbiome with greater diversity, including strains like *Faecalibacterium prausnitzii* and *Bifidobacterium*, which are recognized for their anti-inflammatory properties.[205] These microbes may help reduce chronic inflammation, a key driver of age-related diseases such as CVDs and type 2 diabetes.[206] The microbial diversity in these populations suggests that a varied and balanced gut microbiome may be protective against these diseases and contribute to healthy aging. In Sardinia, a region known for its significant number of centenarians, studies have revealed that these individuals possess microbiomes that generate higher levels of SCFAs, such as butyrate, which are crucial for preserving gut health, immune responses, and metabolic balance.[205,207] The microbiome of Sardinian centenarians strengthens the gut barrier and aids in diminishing inflammation, further supporting the notion that a robust gut microbiome is a vital component of healthy aging.[208] Additional research indicates that centenarians exhibit greater microbial diversity compared to younger people and older individuals who do not live to 100.[209] This diversity, along with a prevalence of bacteria such as *Bifidobacterium* and *Prevotella*, is linked to enhanced metabolic health and reduced inflammation, implying that these microbial characteristics shield centenarians from the typical diseases related to aging, thus bolstering their overall well-being.[210,211] A recent cross-sectional study involving 1,575 participants ranging from 20 to 117 years of age in Guangxi province, China, which included 297 centenarians, further examined the relationship between the gut microbiome and increased longevity.[198] This research found that centenarians have microbiomes commonly found in younger people, dominated by *Bacteroides* species, showing increased species diversity and enrichment with potentially beneficial *Bacteroidetes*. Additionally, the microbiomes of centenarians demonstrated a reduction in potential pathobionts, which are thought to help lower systemic inflammation and improve metabolic health—key elements in preserving genomic stability and reducing telomere shortening.[212,213] Although current findings indicate that the microbiome plays a role in promoting conditions favorable for genomic stability and telomere maintenance, conclusive studies directly comparing telomere length and genomic stability in centenarians with younger or similarly aged non-centenarians are still required. The results show a strong link but do not yet confirm that enhanced genomic stability or longer telomeres are defining traits of centenarians. Across these studies, one clear theme emerges: centenarians tend to have gut microbiomes rich in specific, beneficial microbes that support immune function, reduce inflammation, and regulate metabolic processes. These factors are critical for promoting healthy aging and may help extend lifespan. While much of the evidence remains correlational rather than causal, the research underscores the significant role the microbiome plays in aging. It may hold the key to understanding longevity at a microbial level, offering a promising path forward in efforts to improve health and extend longevity (Fig. 1).

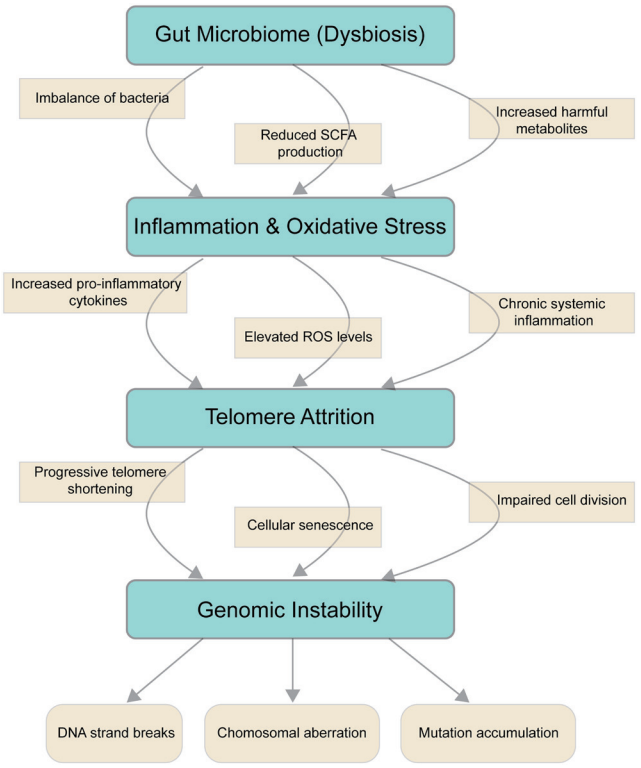


Fig. 1. This figure depicts the pathways linking gut microbiome dysbiosis, telomere attrition, and genomic instability. In dysbiosis, an imbalance between beneficial and harmful gut bacteria reduces protective metabolites like short-chain fatty acids (SCFAs) and increases harmful substances, leading to systemic inflammation and oxidative stress. These conditions accelerate telomere shortening, which contributes to cellular senescence and DNA damage. As telomeres shorten, chromosomal instability increases, elevating the risk of age-related diseases such as neurodegeneration and metabolic disorders. Chronic inflammation further disrupts the microbiome, creating a feedback loop that accelerates telomere attrition and genomic instability.

Future directions

Future investigations into how the microbiome influences aging have the potential to significantly enhance our comprehension of personal health while also delivering substantial benefits to society. By elucidating the molecular mechanisms involved in aging, we can reduce the financial burden that age-related diseases place on healthcare systems. This research could lay the foundation for innovative treatments aimed at prolonging both lifespan and healthspan. Such advancements could transform healthcare systems worldwide, alleviating the pressure of aging-related challenges and promoting a healthier, more active aging population. Furthermore, expanding our understanding of genome stability and telomere health is essential for understanding the complex relationships between these elements, aging, and the microbiome. Exploring these relationships could reveal new avenues for interventions, ultimately improving public health. Importantly, the close anatomical connection between the gut microbiota and immune cells in the intestine underscores the potential effects of telomere shortening and genomic instability in these immune cells on the fragile balance of the gut microbiome. Disruptions to this balance may lead to microbial imbalances, further complicating the ongoing interactions between the microbiome, telomere attrition, and genomic instability. This interconnectedness highlights the critical need for thorough studies to enhance our understanding of how these elements influence overall health. The convergence of microbiome research and aging biology presents an exciting new realm in biomedical science, offering the potential for significant advancements in aging studies. However, the field must also account for the complex interactions between genomic stability,

telomere attrition, and the other hallmarks of aging to better understand the independent role these factors play in the aging process. Grasping the microbiome's impact on each hallmark of aging requires comprehensive research to unravel the intricate network of interactions that drive aging processes. In essence, this review has focused on two primary hallmarks of aging—genomic instability and telomere degradation—and their potential interactions, elucidating their roles within the broader framework of aging biology. While these hallmarks are inherently interrelated, their connections with other aging mechanisms remain inadequately explored, indicating the need for further inquiry. Future studies should strive to unravel the individual contributions of each hallmark to longevity, providing deeper insights into their singular and synergistic impacts on the aging process. Furthermore, the meta-hallmark framework, which examines the interconnections between various aging mechanisms, offers a crucial perspective for enhancing our comprehension of the aging process.[214,215] This approach acknowledges that aging arises not from isolated biological events but from intricate interactions among diverse cellular and systemic systems. By focusing on meta-hallmarks, scientists can gain deeper insights into how distinct hallmarks—such as genomic instability, telomere shortening, epigenetic changes, and loss of proteostasis—interrelate, affecting each other in ways that can either accelerate or decelerate the aging process. This comprehensive framework is crucial for pinpointing potential therapeutic targets capable of addressing multiple dimensions of aging concurrently, rather than isolating a single factor. In addition, the meta-hallmark framework has substantial implications for the research of age-related diseases. Many chronic conditions linked to aging, including NDs and CVDs, arise from complex, multifaceted disturbances in cellular and systemic processes. Gaining insights into the interconnections of these disruptions through the lens of meta-hallmarks could facilitate the development of more comprehensive and effective strategies for disease prevention, intervention, and treatment.[216] By recognizing that aging is a systemic process shaped by the interactions of multiple biological pathways, we can more accurately pinpoint the root causes of age-related diseases and formulate therapies targeting these fundamental mechanisms. Moreover, the meta-hallmark perspective encourages a more integrated approach to understanding aging, healthspan, and lifespan. By reframing these aspects as interconnected rather than isolated phenomena, this perspective creates a cohesive framework in which the interactions among biological processes influence not only the aging trajectory but also the overall quality of life during aging. This approach underscores the importance of a multi-faceted understanding of aging that encompasses not just cellular health but also the systemic balance and resilience required to promote healthy aging and extend lifespan.

This review synthesizes findings from existing literature, forming a foundation for future studies aimed at refining this framework and investigating the interrelationships among aging mechanisms. As the field evolves, this comprehensive approach will be critical for devising targeted strategies that promote human health, enhance healthspan, and ultimately extend longevity. Considering the complex nature of aging and the intricate interplay of biological factors, ongoing research is imperative for deepening our understanding and developing interventions that foster healthier aging and improved quality of life across various populations.

Conclusions

The hallmarks of aging—genome stability and telomere shortening—serve as crucial indicators of the complex aging process, underscoring the intricate relationship between cellular health and the passage of time. Their close connection to a variety of human diseases reveals the significant complexity of aging. Within this complexity, the host microbiome plays a critical role, creating a dynamic and reciprocal relationship that influences the course of aging and longevity. The interdependent relationship between the microbiome and the hallmarks of aging is not merely coincidental; it forms a central axis around which the aging process unfolds. This two-way communication emphasizes the interconnectedness of various physiological functions and presents an exciting opportunity for therapeutic strategies. Understanding and manipulating this interplay could hold significant potential for preventing and managing age-related diseases. By altering the composition and activity of the microbiome through dietary modifications, probiotics, or targeted microbial approaches, it may be possible to mitigate the adverse effects of genome instability and telomere attrition. Additionally, combining these microbiome-focused strategies with methods aimed at directly improving genome stability and preserving telomere length could provide a holistic approach to addressing the fundamental causes of age-associated conditions.

Acknowledgments

This research is supported by Bandhan, Kolkata, India.

Funding

None.

Conflict of interest

The authors affirm that they have no conflicts of interest to disclose in relation to the publication of this research.

Author contributions

Conceptualization (SKC), formal analysis (SKC), original draft preparation (SKC), writing—review and editing (SKC, DC), supervision (SKC), project administration (SKC), and funding acquisition (SKC).

References

[1] Ferrucci L, Gonzalez-Freire M, Fabbri E, Simonsick E, Tanaka T, Moore Z, *et al*. Measuring biological aging in humans: A quest. Aging Cell 2020;19(2):e13080. doi:10.1111/acel.13080, PMID:31833194.

[2] Mc Auley MT, Guimera AM, Hodgson D, Mcdonald N, Mooney KM, Morgan AE, *et al*. Modelling the molecular mechanisms of aging. Biosci Rep 2017;37(1):BSR20160177. doi:10.1042/BSR20160177, PMID:28096317.

[3] Li Z, Zhang W, Duan Y, Niu Y, Chen Y, Liu X, *et al*. Progress in biological age research. Front Public Health 2023;11:1074274. doi:10.3389/fpubh.2023.1074274, PMID:37124811.

[4] Melzer D, Pilling LC, Ferrucci L. The genetics of human ageing. Nat Rev Genet 2020;21(2):88–101. doi:10.1038/s41576-019-0183-6, PMID:31690828.

[5] Fraser HC, Kuan V, Johnen R, Zwierzyna M, Hingorani AD, Beyer A, *et al*. Biological mechanisms of aging predict age-related disease co-occurrence in patients. Aging Cell 2022;21(4):e13524. doi:10.1111/acel.13524, PMID:35259281.

[6] Ogunrinola GA, Oyewale JO, Oshamika OO, Olasehinde GI. The Human Microbiome and Its Impacts on Health. Int J Microbiol

2020;2020:8045646. doi:10.1155/2020/8045646, PMID:32612660.
[7] Aggarwal N, Kitano S, Puah GRY, Kittelmann S, Hwang IY, Chang MW. Microbiome and Human Health: Current Understanding, Engineering, and Enabling Technologies. Chem Rev 2023;123(1):31–72. doi:10.1021/acs.chemrev.2c00431, PMID:36317983.
[8] Martinez FD. The human microbiome. Early life determinant of health outcomes. Ann Am Thorac Soc 2014;11(Suppl 1):S7–12. doi:10.1513/AnnalsATS.201306-186MG, PMID:24437411.
[9] Afzaal M, Saeed F, Shah YA, Hussain M, Rabail R, Socol CT, *et al*. Human gut microbiota in health and disease: Unveiling the relationship. Front Microbiol 2022;13:999001. doi:10.3389/fmicb.2022.999001, PMID:36225386.
[10] Durack J, Lynch SV. The gut microbiome: Relationships with disease and opportunities for therapy. J Exp Med 2019;216(1):20–40. doi:10.1084/jem.20180448, PMID:30322864.
[11] Choi EH, Yoon S, Koh YE, Seo YJ, Kim KP. Maintenance of genome integrity and active homologous recombination in embryonic stem cells. Exp Mol Med 2020;52(8):1220–1229. doi:10.1038/s12276-020-0481-2, PMID:32770082.
[12] Jensen RB, Rothenberg E. Preserving genome integrity in human cells via DNA double-strand break repair. Mol Biol Cell 2020;31(9):859–865. doi:10.1091/mbc.E18-10-0668, PMID:32286930.
[13] Ferrari S, Gentili C. Maintaining Genome Stability in Defiance of Mitotic DNA Damage. Front Genet 2016;7:128. doi:10.3389/fgene.2016.00128, PMID:27493659.
[14] Schumacher B, Pothof J, Vijg J, Hoeijmakers JHJ. The central role of DNA damage in the ageing process. Nature 2021;592(7856):695–703. doi:10.1038/s41586-021-03307-7, PMID:33911272.
[15] Chen JH, Hales CN, Ozanne SE. DNA damage, cellular senescence and organismal ageing: causal or correlative? Nucleic Acids Res 2007;35(22):7417–7428. doi:10.1093/nar/gkm681, PMID:17913751.
[16] Shammas MA. Telomeres, lifestyle, cancer, and aging. Curr Opin Clin Nutr Metab Care 2011;14(1):28–34. doi:10.1097/MCO.0b013e32834121b1, PMID:21102320.
[17] Srinivas N, Rachakonda S, Kumar R. Telomeres and Telomere Length: A General Overview. Cancers (Basel) 2020;12(3):558. doi:10.3390/cancers12030558, PMID:32121056.
[18] Muraki K, Nyhan K, Han L, Murnane JP. Mechanisms of telomere loss and their consequences for chromosome instability. Front Oncol 2012;2:135. doi:10.3389/fonc.2012.00135, PMID:23061048.
[19] Victorelli S, Passos JF. Telomeres and Cell Senescence - Size Matters Not. EBioMedicine 2017;21:14–20. doi:10.1016/j.ebiom.2017.03.027, PMID:28347656.
[20] Razgonova MP, Zakharenko AM, Golokhvast KS, Thanasoula M, Sarandi E, Nikolouzakis K, *et al*. Telomerase and telomeres in aging theory and chronographic aging theory (Review). Mol Med Rep 2020;22(3):1679–1694. doi:10.3892/mmr.2020.11274, PMID:32705188.
[21] Jang DH, Shin JW, Shim E, Ohtani N, Jeon OH. The connection between aging, cellular senescence and gut microbiome alterations: A comprehensive review. Aging Cell 2024;23(10):e14315. doi:10.1111/acel.14315, PMID:39148278.
[22] Assis V, de Sousa Neto IV, Ribeiro FM, de Cassia Marqueti R, Franco OL, da Silva Aguiar S, *et al*. The Emerging Role of the Aging Process and Exercise Training on the Crosstalk between Gut Microbiota and Telomere Length. Int J Environ Res Public Health 2022;19(13):7810. doi:10.3390/ijerph19137810, PMID:35805470.
[23] Al-Daghri NM, Abdi S, Sabico S, Alnaami AM, Wani KA, Ansari MGA, *et al*. Gut-Derived Endotoxin and Telomere Length Attrition in Adults with and without Type 2 Diabetes. Biomolecules 2021;11(11):1693. doi:10.3390/biom11111693, PMID:34827691.
[24] Malik JA, Zafar MA, Lamba T, Nanda S, Khan MA, Agrewala JN. The impact of aging-induced gut microbiome dysbiosis on dendritic cells and lung diseases. Gut Microbes 2023;15(2):2290643. doi:10.1080/19490976.2023.2290643, PMID:38087439.
[25] Salazar J, Durán P, Díaz MP, Chacín M, Santeliz R, Mengual E, *et al*. Exploring the Relationship between the Gut Microbiota and Ageing: A Possible Age Modulator. Int J Environ Res Public Health 2023;20(10):5845. doi:10.3390/ijerph20105845, PMID:37239571.
[26] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. Cell 2023;186(2):243–278. doi:10.1016/j.cell.2022.11.001, PMID:36599349.
[27] López-Gil L, Pascual-Ahuir A, Proft M. Genomic Instability and Epigenetic Changes during Aging. Int J Mol Sci 2023;24(18):14279. doi:10.3390/ijms241814279, PMID:37762580.
[28] Tenchov R, Sasso JM, Wang X, Zhou QA. Aging Hallmarks and Progression and Age-Related Diseases: A Landscape View of Research Advancement. ACS Chem Neurosci 2024;15(1):1–30. doi:10.1021/acschemneuro.3c00531, PMID:38095562.
[29] Tartiere AG, Freije JMP, López-Otín C. The hallmarks of aging as a conceptual framework for health and longevity research. Front Aging 2024;5:1334261. doi:10.3389/fragi.2024.1334261, PMID:38292053.
[30] Keshavarz M, Xie K, Schaaf K, Bano D, Ehninger D. Targeting the "hallmarks of aging" to slow aging and treat age-related disease: fact or fiction? Mol Psychiatry 2023;28(1):242–255. doi:10.1038/s41380-022-01680-x, PMID:35840801.
[31] Skowronska-Krawczyk D. Hallmarks of Aging: Causes and Consequences. Aging Biol 2023;1(1):20230011. doi:10.59368/agingbio.20230011, PMID:38274125.
[32] Hamilton JP. Epigenetics: principles and practice. Dig Dis 2011;29(2):130–135. doi:10.1159/000323874, PMID:21734376.
[33] Labbadia J, Morimoto RI. The biology of proteostasis in aging and disease. Annu Rev Biochem 2015;84:435–464. doi:10.1146/annurev-biochem-060614-033955, PMID:25784053.
[34] Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. Nature 2015;517(7534):302–310. doi:10.1038/nature14190, PMID:25592535.
[35] Srivastava S. The Mitochondrial Basis of Aging and Age-Related Disorders. Genes (Basel) 2017;8(12):398. doi:10.3390/genes8120398, PMID:29257072.
[36] Di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol 2021;22(2):75–95. doi:10.1038/s41580-020-00314-w, PMID:33328614.
[37] Mancinelli L, Intini G. Age-associated declining of the regeneration potential of skeletal stem/progenitor cells. Front Physiol 2023;14:1087254. doi:10.3389/fphys.2023.1087254, PMID:36818437.
[38] Larson A, Codden CJ, Huggins GS, Rastegar H, Chen FY, Maron BJ, *et al*. Altered intercellular communication and extracellular matrix signaling as a potential disease mechanism in human hypertrophic cardiomyopathy. Sci Rep 2022;12(1):5211. doi:10.1038/s41598-022-08561-x, PMID:35338173.
[39] Talapko J, Včev A, Meštrović T, Pustijanac E, Jukić M, Škrlec I. Homeostasis and Dysbiosis of the Intestinal Microbiota: Comparing Hallmarks of a Healthy State with Changes in Inflammatory Bowel Disease. Microorganisms 2022;10(12):2405. doi:10.3390/microorganisms10122405, PMID:36557658.
[40] Nieto-Torres JL, Hansen M. Macroautophagy and aging: The impact of cellular recycling on health and longevity. Mol Aspects Med 2021;82:101020. doi:10.1016/j.mam.2021.101020, PMID:34507801.
[41] Cheon SY, Kim H, Rubinsztein DC, Lee JE. Autophagy, Cellular Aging and Age-related Human Diseases. Exp Neurobiol 2019;28(6):643–657. doi:10.5607/en.2019.28.6.643, PMID:31902153.
[42] Boehme M, Guzzetta KE, Wasén C, Cox LM. The gut microbiota is an emerging target for improving brain health during ageing. Gut Microbiome (Camb) 2023;4:E2. doi:10.1017/gmb.2022.11, PMID:37179659.
[43] Juárez-Fernández M, Porras D, García-Mediavilla MV, Román-Sagüillo S, González-Gallego J, Nistal E, *et al*. Aging, Gut Microbiota and Metabolic Diseases: Management through Physical Exercise and Nutritional Interventions. Nutrients 2020;13(1):16. doi:10.3390/nu13010016, PMID:33374578.
[44] Bradley E, Haran J. The human gut microbiome and aging. Gut Microbes 2024;16(1):2359677. doi:10.1080/19490976.2024.2359677, PMID:38831607.
[45] Grieneisen L, Blekhman R, Archie E. How longitudinal data can contribute to our understanding of host genetic effects on the gut microbiome. Gut Microbes 2023;15(1):2178797. doi:10.1080/19490976.2023.2178797, PMID:36794811.
[46] Andreani NA, Sharma A, Dahmen B, Specht HE, Mannig N, Ruan V, *et al*. Longitudinal analysis of the gut microbiome in adolescent patients with anorexia nervosa: microbiome-related factors associated with clinical outcome. Gut Microbes 2024;16(1):2304158. doi:10.10

80/19490976.2024.2304158, PMID:38294867.
[47] Pantazi AC, Balasa AL, Mihai CM, Chisnoiu T, Lupu VV, Kassim MAK, *et al*. Development of Gut Microbiota in the First 1000 Days after Birth and Potential Interventions. Nutrients 2023;15(16):3647. doi:10.3390/nu15163647, PMID:37630837.
[48] Park H, Park NY, Koh A. Scarring the early-life microbiome: its potential life-long effects on human health and diseases. BMB Rep 2023;56(9):469–481. doi:10.5483/BMBRep.2023-0114, PMID:37605613.
[49] Hrncir T. Gut Microbiota Dysbiosis: Triggers, Consequences, Diagnostic and Therapeutic Options. Microorganisms 2022;10(3):578. doi:10.3390/microorganisms10030578, PMID:35336153.
[50] Acevedo-Román A, Pagán-Zayas N, Velázquez-Rivera LI, Torres-Ventura AC, Godoy-Vitorino F. Insights into Gut Dysbiosis: Inflammatory Diseases, Obesity, and Restoration Approaches. Int J Mol Sci 2024;25(17):9715. doi:10.3390/ijms25179715, PMID:39273662.
[51] Kontizas E, Tastsoglou S, Karamitros T, Karayiannis Y, Kollia P, Hatzigeorgiou AG, *et al*. Impact of Helicobacter pylori Infection and Its Major Virulence Factor CagA on DNA Damage Repair. Microorganisms 2020;8(12):2007. doi:10.3390/microorganisms8122007, PMID:33339161.
[52] Haran JP, McCormick BA. Aging, Frailty, and the Microbiome-How Dysbiosis Influences Human Aging and Disease. Gastroenterology 2021;160(2):507–523. doi:10.1053/j.gastro.2020.09.060, PMID:33307030.
[53] Farah A, Paul P, Khan AS, Sarkar A, Laws S, Chaari A. Targeting gut microbiota dysbiosis in inflammatory bowel disease: a systematic review of current evidence. Front Med (Lausanne) 2025;12:1435030. doi:10.3389/fmed.2025.1435030, PMID:40041456.
[54] Veschetti L, Treccani M, De Tomi E, Malerba G. Genomic Instability Evolutionary Footprints on Human Health: Driving Forces or Side Effects? Int J Mol Sci 2023;24(14):11437. doi:10.3390/ijms241411437, PMID:37511197.
[55] Ussar S, Fujisaka S, Kahn CR. Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. Mol Metab 2016;5(9):795–803. doi:10.1016/j.molmet.2016.07.004, PMID:27617202.
[56] Abdul-Aziz MA, Cooper A, Weyrich LS. Exploring Relationships between Host Genome and Microbiome: New Insights from Genome-Wide Association Studies. Front Microbiol 2016;7:1611. doi:10.3389/fmicb.2016.01611, PMID:27785127.
[57] Ahn J, Hayes RB. Environmental Influences on the Human Microbiome and Implications for Noncommunicable Disease. Annu Rev Public Health 2021;42:277–292. doi:10.1146/annurev-publhealth-012420-105020, PMID:33798404.
[58] Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, *et al*. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms 2019;7(1):14. doi:10.3390/microorganisms7010014, PMID:30634578.
[59] Kalisperati P, Spanou E, Pateras IS, Korkolopoulou P, Varvarigou A, Karavokyros I, *et al*. Inflammation, DNA Damage, Helicobacter pylori and Gastric Tumorigenesis. Front Genet 2017;8:20. doi:10.3389/fgene.2017.00020, PMID:28289428.
[60] Cai Q, Shi P, Yuan Y, Peng J, Ou X, Zhou W, *et al*. Inflammation-Associated Senescence Promotes Helicobacter pylori-Induced Atrophic Gastritis. Cell Mol Gastroenterol Hepatol 2021;11(3):857–880. doi:10.1016/j.jcmgh.2020.10.015, PMID:33161156.
[61] Murata-Kamiya N, Hatakeyama M. Helicobacter pylori-induced DNA double-stranded break in the development of gastric cancer. Cancer Sci 2022;113(6):1909–1918. doi:10.1111/cas.15357, PMID:35359025.
[62] Fan Z, Tang P, Li C, Yang Q, Xu Y, Su C, *et al*. Fusobacterium nucleatum and its associated systemic diseases: epidemiologic studies and possible mechanisms. J Oral Microbiol 2023;15(1):2145729. doi:10.1080/20002297.2022.2145729, PMID:36407281.
[63] Groeger S, Zhou Y, Ruf S, Meyle J. Pathogenic Mechanisms of Fusobacterium nucleatum on Oral Epithelial Cells. Front Oral Health 2022;3:831607. doi:10.3389/froh.2022.831607, PMID:35478496.
[64] Delint-Ramirez I, Madabhushi R. DNA damage and its links to neuronal aging and degeneration. Neuron 2025;113(1):7–28. doi:10.1016/j.neuron.2024.12.001, PMID:39788088.
[65] Qin Y, Liu H, Wu H. Cellular Senescence in Health, Disease, and Lens Aging. Pharmaceuticals (Basel) 2025;18(2):244. doi:10.3390/ph18020244, PMID:40006057.
[66] Djorić D, Kristich CJ. Oxidative stress enhances cephalosporin resistance of Enterococcus faecalis through activation of a two-component signaling system. Antimicrob Agents Chemother 2015;59(1):159–169. doi:10.1128/AAC.03984-14, PMID:25331701.
[67] Larabi AB, Masson HLP, Bäumler AJ. Bile acids as modulators of gut microbiota composition and function. Gut Microbes 2023;15(1):2172671. doi:10.1080/19490976.2023.2172671, PMID:36740850.
[68] Qu Y, Su C, Zhao Q, Shi A, Zhao F, Tang L, *et al*. Gut Microbiota-Mediated Elevated Production of Secondary Bile Acids in Chronic Unpredictable Mild Stress. Front Pharmacol 2022;13:837543. doi:10.3389/fphar.2022.837543, PMID:35321330.
[69] Matei E, Ionescu AC, Enciu M, Popovici V, Mitroi AF, Aschie M, *et al*. Cell death and DNA damage via ROS mechanisms after applied antibiotics and antioxidants doses in prostate hyperplasia primary cell cultures. Medicine (Baltimore) 2024;103(37):e39450. doi:10.1097/MD.0000000000039450, PMID:39287312.
[70] Jena PK, Sheng L, Di Lucente J, Jin LW, Maezawa I, Wan YY. Dysregulated bile acid synthesis and dysbiosis are implicated in Western diet-induced systemic inflammation, microglial activation, and reduced neuroplasticity. FASEB J 2018;32(5):2866–2877. doi:10.1096/fj.201700984RR, PMID:29401580.
[71] Ma J, Hong Y, Zheng N, Xie G, Lyu Y, Gu Y, *et al*. Gut microbiota remodeling reverses aging-associated inflammation and dysregulation of systemic bile acid homeostasis in mice sex-specifically. Gut Microbes 2020;11(5):1450–1474. doi:10.1080/19490976.2020.1763770, PMID:32515683.
[72] Chandrasekaran R, Lacy DB. The role of toxins in Clostridium difficile infection. FEMS Microbiol Rev 2017;41(6):723–750. doi:10.1093/femsre/fux048, PMID:29048477.
[73] Aktories K, Schwan C, Jank T. Clostridium difficile Toxin Biology. Annu Rev Microbiol 2017;71:281–307. doi:10.1146/annurev-micro-090816-093458, PMID:28657883.
[74] Li X, Liu J, Zhou Y, Wang L, Wen Y, Ding K, *et al*. Jwa participates the maintenance of intestinal epithelial homeostasis via ERK/FBXW7-mediated NOTCH1/PPARγ/STAT5 axis and acts as a novel putative aging related gene. Int J Biol Sci 2022;18(14):5503–5521. doi:10.7150/ijbs.72751, PMID:36147468.
[75] Wang Q, Qi Y, Shen W, Xu J, Wang L, Chen S, *et al*. The Aged Intestine: Performance and Rejuvenation. Aging Dis 2021;12(7):1693–1712. doi:10.14336/AD.2021.0202, PMID:34631215.
[76] Li S, Liu J, Zheng X, Ren L, Yang Y, Li W, *et al*. Tumorigenic bacteria in colorectal cancer: mechanisms and treatments. Cancer Biol Med 2021;19(2):147–162. doi:10.20892/j.issn.2095-3941.2020.0651, PMID:34586760.
[77] Zhang G, Sun D. The synthesis of the novel Escherichia coli toxin-colibactin and its mechanisms of tumorigenesis of colorectal cancer. Front Microbiol 2024;15:1501973. doi:10.3389/fmicb.2024.1501973, PMID:39744397.
[78] Rezazadegan M, Forootani B, Hoveyda Y, Rezazadegan N, Amani R. Major heavy metals and human gut microbiota composition: a systematic review with nutritional approach. J Health Popul Nutr 2025;44(1):21. doi:10.1186/s41043-025-00750-4, PMID:39871318.
[79] Maier E, Anderson RC, Roy NC. Understanding how commensal obligate anaerobic bacteria regulate immune functions in the large intestine. Nutrients 2014;7(1):45–73. doi:10.3390/nu7010045, PMID:25545102.
[80] Browne HP, Neville BA, Forster SC, Lawley TD. Transmission of the gut microbiota: spreading of health. Nat Rev Microbiol 2017;15(9):531–543. doi:10.1038/nrmicro.2017.50, PMID:28603278.
[81] Baldelli V, Scaldaferri F, Putignani L, Del Chierico F. The Role of Enterobacteriaceae in Gut Microbiota Dysbiosis in Inflammatory Bowel Diseases. Microorganisms 2021;9(4):697. doi:10.3390/microorganisms9040697, PMID:33801755.
[82] Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 2015;26:26191. doi:10.3402/mehd.v26.26191, PMID:25651997.
[83] Lu Z, Imlay JA. When anaerobes encounter oxygen: mechanisms

of oxygen toxicity, tolerance and defence. Nat Rev Microbiol 2021;19(12):774–785. doi:10.1038/s41579-021-00583-y, PMID:34183820.
[84] Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal 2014;20(7):1126–1167. doi:10.1089/ars.2012.5149, PMID:23991888.
[85] Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev 2014;94(2):329–354. doi:10.1152/physrev.00040.2012, PMID:24692350.
[86] Zhang X, Li L. The Significance of 8-oxoGsn in Aging-Related Diseases. Aging Dis 2020;11(5):1329–1338. doi:10.14336/AD.2019.1021, PMID:33014540.
[87] Radak Z, Bori Z, Koltai E, Fatouros IG, Jamurtas AZ, Douroudos II, *et al*. Age-dependent changes in 8-oxoguanine-DNA glycosylase activity are modulated by adaptive responses to physical exercise in human skeletal muscle. Free Radic Biol Med 2011;51(2):417–423. doi:10.1016/j.freeradbiomed.2011.04.018, PMID:21569841.
[88] Somasundaram I, Jain SM, Blot-Chabaud M, Pathak S, Banerjee A, Rawat S, *et al*. Mitochondrial dysfunction and its association with age-related disorders. Front Physiol 2024;15:1384966. doi:10.3389/fphys.2024.1384966.
[89] Bartman S, Coppotelli G, Ross JM. Mitochondrial Dysfunction: A Key Player in Brain Aging and Diseases. Curr Issues Mol Biol 2024;46(3):1987–2026. doi:10.3390/cimb46030130, PMID:38534746.
[90] Kay J, Thadhani E, Samson L, Engelward B. Inflammation-induced DNA damage, mutations and cancer. DNA Repair (Amst) 2019;83:102673. doi:10.1016/j.dnarep.2019.102673, PMID:31387777.
[91] Gao H, Nepovimova E, Adam V, Heger Z, Valko M, Wu Q, *et al*. Age-associated changes in innate and adaptive immunity: role of the gut microbiota. Front Immunol 2024;15:1421062. doi:10.3389/fimmu.2024.1421062, PMID:39351234.
[92] Guedj A, Volman Y, Geiger-Maor A, Bolik J, Schumacher N, Künzel S, *et al*. Gut microbiota shape 'inflamm-ageing' cytokines and account for age-dependent decline in DNA damage repair. Gut 2020;69(6):1064–1075. doi:10.1136/gutjnl-2019-318491, PMID:31586932.
[93] Obonyo M, Rickman B, Guiney DG. Effects of myeloid differentiation primary response gene 88 (MyD88) activation on Helicobacter infection in vivo and induction of a Th17 response. Helicobacter 2011;16(5):398–404. doi:10.1111/j.1523-5378.2011.00861.x, PMID:21923686.
[94] Liu JC, Wang P, Zeng QX, Yang C, Lyu M, Li Y, *et al*. Myd88 Signaling Is Involved in the Inflammatory Response in LPS-Induced Mouse Epididymitis and Bone-Marrow-Derived Dendritic Cells. Int J Mol Sci 2023;24(9):7838. doi:10.3390/ijms24097838, PMID:37175545.
[95] Everard A, Geurts L, Caesar R, Van Hul M, Matamoros S, Duparc T, *et al*. Intestinal epithelial MyD88 is a sensor switching host metabolism towards obesity according to nutritional status. Nat Commun 2014;5:5648. doi:10.1038/ncomms6648, PMID:25476696.
[96] Lee JY, Cevallos SA, Byndloss MX, Tiffany CR, Olsan EE, Butler BP, *et al*. High-Fat Diet and Antibiotics Cooperatively Impair Mitochondrial Bioenergetics to Trigger Dysbiosis that Exacerbates Pre-inflammatory Bowel Disease. Cell Host Microbe 2020;28(2):273–284.e6. doi:10.1016/j.chom.2020.06.001, PMID:32668218.
[97] Dudek-Wicher RK, Junka A, Bartoszewicz M. The influence of antibiotics and dietary components on gut microbiota. Prz Gastroenterol 2018;13(2):85–92. doi:10.5114/pg.2018.76005, PMID:30002765.
[98] Zhao Y, Simon M, Seluanov A, Gorbunova V. DNA damage and repair in age-related inflammation. Nat Rev Immunol 2023;23(2):75–89. doi:10.1038/s41577-022-00751-y, PMID:35831609.
[99] Andarawi S, Vodickova L, Uttarilli A, Hanak P, Vodicka P. Defective DNA repair: a putative nexus linking immunological diseases, neurodegenerative disorders, and cancer. Mutagenesis 2025;40(1):4–19. doi:10.1093/mutage/geae029, PMID:39937585.
[100] Everett BM, Donath MY, Pradhan AD, Thuren T, Pais P, Nicolau JC, *et al*. Anti-Inflammatory Therapy With Canakinumab for the Prevention and Management of Diabetes. J Am Coll Cardiol 2018;71(21):2392–2401. doi:10.1016/j.jacc.2018.03.002, PMID:29544870.
[101] Rothman AM, MacFadyen J, Thuren T, Webb A, Harrison DG, Guzik TJ, *et al*. Effects of Interleukin-1β Inhibition on Blood Pressure, Incident Hypertension, and Residual Inflammatory Risk: A Secondary Analysis of CANTOS. Hypertension 2020;75(2):477–482. doi:10.1161/HYPERTENSIONAHA.119.13642, PMID:31884854.
[102] Ridker PM, MacFadyen JG, Glynn RJ, Koenig W, Libby P, Everett BM, *et al*. Inhibition of Interleukin-1β by Canakinumab and Cardiovascular Outcomes in Patients With Chronic Kidney Disease. J Am Coll Cardiol 2018;71(21):2405–2414. doi:10.1016/j.jacc.2018.03.490, PMID:29793629.
[103] Soukas AA, Hao H, Wu L. Metformin as Anti-Aging Therapy: Is It for Everyone? Trends Endocrinol Metab 2019;30(10):745–755. doi:10.1016/j.tem.2019.07.015, PMID:31405774.
[104] Chen S, Gan D, Lin S, Zhong Y, Chen M, Zou X, *et al*. Metformin in aging and aging-related diseases: clinical applications and relevant mechanisms. Theranostics 2022;12(6):2722–2740. doi:10.7150/thno.71360, PMID:35401820.
[105] Zikou E, Koliaki C, Makrilakis K. The Role of Fecal Microbiota Transplantation (FMT) in the Management of Metabolic Diseases in Humans: A Narrative Review. Biomedicines 2024;12(8):1871. doi:10.3390/biomedicines12081871, PMID:39200335.
[106] Biazzo M, Deidda G. Fecal Microbiota Transplantation as New Therapeutic Avenue for Human Diseases. J Clin Med 2022;11(14):4119. doi:10.3390/jcm11144119, PMID:35887883.
[107] Selvarani R, Mohammed S, Richardson A. Effect of rapamycin on aging and age-related diseases-past and future. Geroscience 2021;43(3):1135–1158. doi:10.1007/s11357-020-00274-1, PMID:33037985.
[108] Sharp ZD, Strong R. Rapamycin, the only drug that has been consistently demonstrated to increase mammalian longevity. An update. Exp Gerontol 2023;176:112166. doi:10.1016/j.exger.2023.112166, PMID:37011714.
[109] Blagosklonny MV. Rapamycin for longevity: opinion article. Aging (Albany NY) 2019;11(19):8048–8067. doi:10.18632/aging.102355, PMID:31586989.
[110] Lim CJ, Cech TR. Shaping human telomeres: from shelterin and CST complexes to telomeric chromatin organization. Nat Rev Mol Cell Biol 2021;22(4):283–298. doi:10.1038/s41580-021-00328-y, PMID:33564154.
[111] Mir SM, Samavarchi Tehrani S, Goodarzi G, Jamalpoor Z, Asadi J, Khelghati N, *et al*. Shelterin Complex at Telomeres: Implications in Ageing. Clin Interv Aging 2020;15:827–839. doi:10.2147/CIA.S256425, PMID:32581523.
[112] Ghilain C, Gilson E, Giraud-Panis MJ. Multifunctionality of the Telomere-Capping Shelterin Complex Explained by Variations in Its Protein Composition. Cells 2021;10(7):1753. doi:10.3390/cells10071753, PMID:34359923.
[113] Diotti R, Loayza D. Shelterin complex and associated factors at human telomeres. Nucleus 2011;2(2):119–135. doi:10.4161/nucl.2.2.15135, PMID:21738835.
[114] Shiekh S, Feldt D, Jack A, Kodikara SG, Alfehaid J, Pasha S, Yildiz A, Balci H. Protection of the Telomeric Junction by the Shelterin Complex. J Am Chem Soc 2024;146(36):25158–25165. doi:10.1021/jacs.4c08649, PMID:39207958.
[115] Romero-Zamora D, Rogers S, Low RRJ, Page SG, Lane BJE, Kosaka S, *et al*. A CPC-shelterin-BTR axis regulates mitotic telomere deprotection. Nat Commun 2025;16(1):2277. doi:10.1038/s41467-025-57456-8, PMID:40097392.
[116]Amir M, Khan P, Queen A, Dohare R, Alajmi MF, Hussain A, *et al*. Structural Features of Nucleoprotein CST/Shelterin Complex Involved in the Telomere Maintenance and Its Association with Disease Mutations. Cells 2020;9(2):359. doi:10.3390/cells9020359, PMID:32033110.
[117] Kalmykova A. Telomere checkpoint in aging and development. Int J Mol Sci 2023;24(21):15979. doi:10.3390/ijms242115979, PMID:37958962.
[118] Anifandis G, Samara M, Simopoulou M, Messini CI, Chatzimeletiou K, Thodou E, *et al*. Insights into the Role of Telomeres in Human Embryological Parameters. Opinions Regarding IVF. J Dev Biol 2021;9(4):49. doi:10.3390/jdb9040049, PMID:34842724.
[119] Huang X, Huang L, Lu J, Cheng L, Wu D, Li L, *et al*. The Relationship between telomere length and aging-related diseases. Clin Exp Med 2025;25(1):72. doi:10.1007/s10238-025-01608-z, PMID:40044947.
[120] Lupatov AY, Yarygin KN. Telomeres and Telomerase in the Control of

Stem Cells. Biomedicines 2022;10(10):2335. doi:10.3390/biomedicines10102335, PMID:36289597.
[121] Li JSZ, Denchi EL. How stem cells keep telomeres in check. Differentiation 2018;100:21–25. doi:10.1016/j.diff.2018.01.004, PMID:29413749.
[122] Penev A, Markiewicz-Potoczny M, Sfeir A, Lazzerini Denchi E. Stem cells at odds with telomere maintenance and protection. Trends Cell Biol 2022;32(6):527–536. doi:10.1016/j.tcb.2021.12.007, PMID:35063336.
[123] Effros RB. Telomere/telomerase dynamics within the human immune system: effect of chronic infection and stress. Exp Gerontol 2011;46(2-3):135–140. doi:10.1016/j.exger.2010.08.027, PMID:20833238.
[124] Nassour J, Przetocka S, Karlseder J. Telomeres as hotspots for innate immunity and inflammation. DNA Repair (Amst) 2024;133:103591. doi:10.1016/j.dnarep.2023.103591, PMID:37951043.
[125] Schellnegger M, Hofmann E, Carnieletto M, Kamolz LP. Unlocking longevity: the role of telomeres and its targeting interventions. Front Aging 2024;5:1339317. doi:10.3389/fragi.2024.1339317, PMID:38333665.
[126] Bernardes de Jesus B, Blasco MA. Potential of telomerase activation in extending health span and longevity. Curr Opin Cell Biol 2012;24(6):739–743. doi:10.1016/j.ceb.2012.09.004, PMID:23085234.
[127] El Maï M, Bird M, Allouche A, Targen S, Şerifoğlu N, Lopes-Bastos B, *et al*. Gut-specific telomerase expression counteracts systemic aging in telomerase-deficient zebrafish. Nat Aging 2023;3(5):567–584. doi:10.1038/s43587-023-00401-5, PMID:37142828.
[128] Li R, Li J, Zhou X. Lung microbiome: new insights into the pathogenesis of respiratory diseases. Signal Transduct Target Ther 2024;9(1):19. doi:10.1038/s41392-023-01722-y, PMID:38228603.
[129] Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, Zhu D, Koya JB, Wei L, Li J, Chen ZS. Microbiota in health and diseases. Signal Transduct Target Ther 2022;7(1):135. doi:10.1038/s41392-022-00974-4.
[130] Barnes RP, Fouquerel E, Opresko PL. The impact of oxidative DNA damage and stress on telomere homeostasis. Mech Ageing Dev 2019;177:37–45. doi:10.1016/j.mad.2018.03.013, PMID:29604323.
[131] Ruiz A, Flores-Gonzalez J, Buendia-Roldan I, Chavez-Galan L. Telomere Shortening and Its Association with Cell Dysfunction in Lung Diseases. Int J Mol Sci 2021;23(1):425. doi:10.3390/ijms23010425, PMID:35008850.
[132] Tan J, Taitz J, Nanan R, Grau G, Macia L. Dysbiotic Gut Microbiota-Derived Metabolites and Their Role in Non-Communicable Diseases. Int J Mol Sci 2023;24(20):15256. doi:10.3390/ijms242015256, PMID:37894934.
[133] Fusco W, Lorenzo MB, Cintoni M, Porcari S, Rinninella E, Kaitsas F, *et al*. Short-Chain Fatty-Acid-Producing Bacteria: Key Components of the Human Gut Microbiota. Nutrients 2023;15(9):2211. doi:10.3390/nu15092211, PMID:37432351.
[134] Galiè S, Canudas S, Muralidharan J, García-Gavilán J, Bulló M, Salas-Salvadó J. Impact of Nutrition on Telomere Health: Systematic Review of Observational Cohort Studies and Randomized Clinical Trials. Adv Nutr 2020;11(3):576–601. doi:10.1093/advances/nmz107, PMID:31688893.
[135] Du Y, He C, An Y, Huang Y, Zhang H, Fu W, *et al*. The Role of Short Chain Fatty Acids in Inflammation and Body Health. Int J Mol Sci 2024;25(13):7379. doi:10.3390/ijms25137379, PMID:39000498.
[136] Ogłuszka M, Lipiński P, Starzyński RR. Effect of Omega-3 Fatty Acids on Telomeres-Are They the Elixir of Youth? Nutrients 2022;14(18):3723. doi:10.3390/nu14183723, PMID:36145097.
[137] Shim JA, Ryu JH, Jo Y, Hong C. The role of gut microbiota in T cell immunity and immune mediated disorders. Int J Biol Sci 2023;19(4):1178–1191. doi:10.7150/ijbs.79430, PMID:36923929.
[138] Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI. Correction: Yoo, J.Y., *et al*. Gut Microbiota and Immune System Interactions. Microorganisms 2020, 8, 1587. Microorganisms 2020;8(12):2046. doi:10.3390/microorganisms8122046, PMID:33371530.
[139] Calder PC, Ortega EF, Meydani SN, Adkins Y, Stephensen CB, Thompson B, *et al*. Nutrition, Immunosenescence, and Infectious Disease: An Overview of the Scientific Evidence on Micronutrients and on Modulation of the Gut Microbiota. Adv Nutr 2022;13(5):S1–S26. doi:10.1093/advances/nmac052, PMID:36183242.
[140] Scheithauer TPM, Rampanelli E, Nieuwdorp M, Vallance BA, Verchere CB, van Raalte DH, *et al*. Gut Microbiota as a Trigger for Metabolic Inflammation in Obesity and Type 2 Diabetes. Front Immunol 2020;11:571731. doi:10.3389/fimmu.2020.571731, PMID:33178196.
[141] Dabke K, Hendrick G, Devkota S. The gut microbiome and metabolic syndrome. J Clin Invest 2019;129(10):4050–4057. doi:10.1172/JCI129194, PMID:31573550.
[142] Reva K, Laranjinha J, Rocha BS. Epigenetic Modifications Induced by the Gut Microbiota May Result from What We Eat: Should We Talk about Precision Diet in Health and Disease? Metabolites 2023;13(3):375. doi:10.3390/metabo13030375, PMID:36984815.
[143] Woo V, Alenghat T. Epigenetic regulation by gut microbiota. Gut Microbes 2022;14(1):2022407. doi:10.1080/19490976.2021.2022407, PMID:35000562.
[144] Wu Y, Wang CZ, Wan JY, Yao H, Yuan CS. Dissecting the Interplay Mechanism between Epigenetics and Gut Microbiota: Health Maintenance and Disease Prevention. Int J Mol Sci 2021;22(13):6933. doi:10.3390/ijms22136933, PMID:34203243.
[145] Carlund O, Norberg A, Osterman P, Landfors M, Degerman S, Hultdin M. DNA methylation variations and epigenetic aging in telomere biology disorders. Sci Rep 2023;13(1):7955. doi:10.1038/s41598-023-34922-1, PMID:37193737.
[146] Kmiołek T, Filipowicz G, Bogucka D, Wajda A, Ejma-Multański A, Stypińska B, *et al*. Aging and the impact of global DNA methylation, telomere shortening, and total oxidative status on sarcopenia and frailty syndrome. Immun Ageing 2023;20(1):61. doi:10.1186/s12979-023-00384-2, PMID:37964387.
[147] Wang J, Dong X, Cao L, Sun Y, Qiu Y, Zhang Y, *et al*. Association between telomere length and diabetes mellitus: A meta-analysis. J Int Med Res 2016;44(6):1156–1173. doi:10.1177/0300060516667132, PMID:28322101.
[148] Khalangot M, Krasnienkov D, Vaiserman A. Telomere length in different metabolic categories: Clinical associations and modification potential. Exp Biol Med (Maywood) 2020;245(13):1115–1121. doi:10.1177/1535370220931509, PMID:32515222.
[149] Davis CD. The Gut Microbiome and Its Role in Obesity. Nutr Today 2016;51(4):167–174. doi:10.1097/NT.0000000000000167, PMID:27795585.
[150] Hamamah S, Hajnal A, Covasa M. Influence of Bariatric Surgery on Gut Microbiota Composition and Its Implication on Brain and Peripheral Targets. Nutrients 2024;16(7):1071. doi:10.3390/nu16071071, PMID:38613104.
[151] Şerifoğlu N, Lopes-Bastos B, Ferreira MG. Lack of telomerase reduces cancer incidence and increases lifespan of zebrafish tp53M214K mutants. Sci Rep 2024;14(1):5382. doi:10.1038/s41598-024-56153-8, PMID:38443436.
[152] Kishi S, Slack BE, Uchiyama J, Zhdanova IV. Zebrafish as a genetic model in biological and behavioral gerontology: where development meets aging in vertebrates—a mini-review. Gerontology 2009;55(4):430–441. doi:10.1159/000228892, PMID:19654474.
[153] Xiong RG, Li J, Cheng J, Zhou DD, Wu SX, Huang SY, *et al*. The Role of Gut Microbiota in Anxiety, Depression, and Other Mental Disorders as Well as the Protective Effects of Dietary Components. Nutrients 2023;15(14):3258. doi:10.3390/nu15143258, PMID:37513676.
[154] Limbana T, Khan F, Eskander N. Gut Microbiome and Depression: How Microbes Affect the Way We Think. Cureus 2020;12(8):e9966. doi:10.7759/cureus.9966.10.7759/cureus.9966.
[155] Donati Zeppa S, Agostini D, Ferrini F, Gervasi M, Barbieri E, Bartolacci A, *et al*. Interventions on Gut Microbiota for Healthy Aging. Cells 2022;12(1):34. doi:10.3390/cells12010034, PMID:36611827.
[156] Yan Z, Guan G, Jia H, Li H, Zhuoga S, Zheng S. The association between gut microbiota and accelerated aging and frailty: a Mendelian randomization study. Aging Clin Exp Res 2025;37(1):82. doi:10.1007/s40520-025-02971-3, PMID:40074999.
[157] Luczynski P, McVey Neufeld KA, Oriach CS, Clarke G, Dinan TG, Cryan JF. Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. Int J Neuropsychopharmacol 2016;19(8):pyw020. doi:10.1093/ijnp/pyw020, PMID:26912607.
[158] Aghighi F, Salami M. What we need to know about the germ-free animal models. AIMS Microbiol 2024;10(1):107–147. doi:10.3934/microbiol.2024007, PMID:38525038.
[159] Hu J, Cheng S, Yao J, Lin X, Li Y, Wang W, *et al*. Correlation between al-

tered gut microbiota and elevated inflammation markers in patients with Crohn's disease. Front Immunol 2022;13:947313. doi:10.3389/fimmu.2022.947313, PMID:36045690.
[160] Li L, Peng P, Ding N, Jia W, Huang C, Tang Y. Oxidative Stress, Inflammation, Gut Dysbiosis: What Can Polyphenols Do in Inflammatory Bowel Disease? Antioxidants (Basel) 2023;12(4):967. doi:10.3390/antiox12040967, PMID:37107341.
[161] Mostafavi Abdolmaleky H, Zhou JR. Gut Microbiota Dysbiosis, Oxidative Stress, Inflammation, and Epigenetic Alterations in Metabolic Diseases. Antioxidants (Basel) 2024;13(8):985. doi:10.3390/antiox13080985, PMID:39199231.
[162] Boyajian JL, Ghebretatios M, Schaly S, Islam P, Prakash S. Microbiome and Human Aging: Probiotic and Prebiotic Potentials in Longevity, Skin Health and Cellular Senescence. Nutrients 2021;13(12):4550. doi:10.3390/nu13124550, PMID:34960102.
[163] Landete JM, Gaya P, Rodríguez E, Langa S, Peirotén Á, Medina M, *et al*. Probiotic Bacteria for Healthier Aging: Immunomodulation and Metabolism of Phytoestrogens. Biomed Res Int 2017;2017:5939818. doi:10.1155/2017/5939818, PMID:29109959.
[164] Badal VD, Vaccariello ED, Murray ER, Yu KE, Knight R, Jeste DV, *et al*. The Gut Microbiome, Aging, and Longevity: A Systematic Review. Nutrients 2020;12(12):3759. doi:10.3390/nu12123759, PMID:33297486.
[165] Coman V, Vodnar DC. Gut microbiota and old age: Modulating factors and interventions for healthy longevity. Exp Gerontol 2020;141:111095. doi:10.1016/j.exger.2020.111095, PMID:32979504.
[166] Xu X, Li G, Zhang D, Zhu H, Liu GH, Zhang Z. Gut Microbiota is Associated with Aging-Related Processes of a Small Mammal Species under High-Density Crowding Stress. Adv Sci (Weinh) 2023;10(14):e2205346. doi:10.1002/advs.202205346, PMID:36965140.
[167] Kunst C, Schmid S, Michalski M, Tümen D, Buttenschön J, Müller M, *et al*. The Influence of Gut Microbiota on Oxidative Stress and the Immune System. Biomedicines 2023;11(5):1388. doi:10.3390/biomedicines11051388, PMID:37239059.
[168] Oshima J, Sidorova JM, Monnat RJ Jr. Werner syndrome: Clinical features, pathogenesis and potential therapeutic interventions. Ageing Res Rev 2017;33:105–114. doi:10.1016/j.arr.2016.03.002, PMID:26993153.
[169] Lebel M, Monnat RJ Jr. Werner syndrome (WRN) gene variants and their association with altered function and age-associated diseases. Ageing Res Rev 2018;41:82–97. doi:10.1016/j.arr.2017.11.003, PMID:29146545.
[170] Mukherjee S, Sinha D, Bhattacharya S, Srinivasan K, Abdisalaam S, Asaithamby A. Werner Syndrome Protein and DNA Replication. Int J Mol Sci 2018;19(11):3442. doi:10.3390/ijms19113442, PMID:30400178.
[171] Shamanna RA, Croteau DL, Lee JH, Bohr VA. Recent Advances in Understanding Werner Syndrome. F1000Res 2017;6:1779. doi:10.12688/f1000research.12110.1, PMID:29043077.
[172] Flores M, Goodrich DW. Retinoblastoma Protein Paralogs and Tumor Suppression. Front Genet 2022;13:818719. doi:10.3389/fgene.2022.818719, PMID:35368709.
[173] Zhu L, Lu Z, Zhao H. Antitumor mechanisms when pRb and p53 are genetically inactivated. Oncogene 2015;34(35):4547–4557. doi:10.1038/onc.2014.399, PMID:25486431.
[174] Wang H, Guo M, Wei H, Chen Y. Targeting p53 pathways: mechanisms, structures, and advances in therapy. Signal Transduct Target Ther 2023;8(1):92. doi:10.1038/s41392-023-01347-1, PMID:36859359.
[175] Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The Central Nervous System and the Gut Microbiome. Cell 2016;167(4):915–932. doi:10.1016/j.cell.2016.10.027, PMID:27814521.
[176] Tsuge K, Shimamoto A. Research on Werner Syndrome: Trends from Past to Present and Future Prospects. Genes (Basel) 2022;13(10):1802. doi:10.3390/genes13101802, PMID:36292687.
[177] Ren J, Li H, Zeng G, Pang B, Wang Q, Wei J. Gut microbiome-mediated mechanisms in aging-related diseases: are probiotics ready for prime time? Front Pharmacol 2023;14:1178596. doi:10.3389/fphar.2023.1178596, PMID:37324466.
[178] Giorgi C, Marchi S, Simoes ICM, Ren Z, Morciano G, Perrone M, *et al*. Mitochondria and Reactive Oxygen Species in Aging and Age-Related Diseases. Int Rev Cell Mol Biol 2018;340:209–344. doi:10.1016/bs.ircmb.2018.05.006, PMID:30072092.
[179] Nelson CB, Alturki TM, Luxton JJ, Taylor LE, Maranon DG, Muraki K, *et al*. Telomeric Double Strand Breaks in G1 Human Cells Facilitate Formation of 5′ C-Rich Overhangs and Recruitment of TERRA. Front Genet 2021;12:644803. doi:10.3389/fgene.2021.644803, PMID:33841503.
[180] Fouquerel E, Parikh D, Opresko P. DNA damage processing at telomeres: The ends justify the means. DNA Repair (Amst) 2016;44:159–168. doi:10.1016/j.dnarep.2016.05.022, PMID:27233113.
[181] Yousefzadeh M, Henpita C, Vyas R, Soto-Palma C, Robbins P, Niedernhofer L. DNA damage-how and why we age? Elife 2021;10:e62852. doi:10.7554/eLife.62852, PMID:33512317.
[182] Golde TE. Disease-Modifying Therapies for Alzheimer's Disease: More Questions than Answers. Neurotherapeutics 2022;19(1):209–227. doi:10.1007/s13311-022-01201-2, PMID:35229269.
[183] Wang H, Lautrup S, Caponio D, Zhang J, Fang EF. DNA Damage-Induced Neurodegeneration in Accelerated Ageing and Alzheimer's Disease. Int J Mol Sci 2021;22(13):6748. doi:10.3390/ijms22136748, PMID:34201700.
[184] Abavisani M, Faraji S, Ebadpour N, Karav S, Sahebkar A. Beyond the Hayflick limit: How microbes influence cellular aging. Ageing Res Rev 2025;104:102657. doi:10.1016/j.arr.2025, PMID:39788433.
[185] Ghosh TS, Shanahan F, O'Toole PW. The gut microbiome as a modulator of healthy ageing. Nat Rev Gastroenterol Hepatol 2022;19(9):565–584. doi:10.1038/s41575-022-00605-x, PMID:35468952.
[186] Rampelli S, Candela M, Turroni S, Biagi E, Collino S, Franceschi C, *et al*. Functional metagenomic profiling of intestinal microbiome in extreme ageing. Aging (Albany NY) 2013;5(12):902–912. doi:10.18632/aging.100623, PMID:24334635.
[187] Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, *et al*. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012;488(7410):178–184. doi:10.1038/nature11319, PMID:22797518.
[188] Hohman LS, Osborne LC. A gut-centric view of aging: Do intestinal epithelial cells contribute to age-associated microbiota changes, inflammaging, and immunosenescence? Aging Cell 2022;21(9):e13700. doi:10.1111/acel.13700, PMID:36000805.
[189] Andersen SL. Centenarians as Models of Resistance and Resilience to Alzheimer's Disease and Related Dementias. Adv Geriatr Med Res 2020;2(3):e200018. doi:10.20900/agmr20200018.10.20900/agmr20200018.
[190] Holstege H, Beker N, Dijkstra T, Pieterse K, Wemmenhove E, Schouten K, *et al*. The 100-plus Study of cognitively healthy centenarians: rationale, design and cohort description. Eur J Epidemiol 2018;33(12):1229–1249. doi:10.1007/s10654-018-0451-3, PMID:30362018.
[191] Sepp E, Smidt I, Rööp T, Štšepetova J, Kõljalg S, Mikelsaar M, *et al*. Comparative Analysis of Gut Microbiota in Centenarians and Young People: Impact of Eating Habits and Childhood Living Environment. Front Cell Infect Microbiol 2022;12:851404. doi:10.3389/fcimb.2022.851404, PMID:35372105.
[192] Santoro A, Ostan R, Candela M, Biagi E, Brigidi P, Capri M, *et al*. Gut microbiota changes in the extreme decades of human life: a focus on centenarians. Cell Mol Life Sci 2018;75(1):129–148. doi:10.1007/s00018-017-2674-y, PMID:29032502.
[193] Wang J, Qie J, Zhu D, Zhang X, Zhang Q, Xu Y, *et al*. The landscape in the gut microbiome of long-lived families reveals new insights on longevity and aging - relevant neural and immune function. Gut Microbes 2022;14(1):2107288. doi:10.1080/19490976.2022.2107288, PMID:35939616.
[194] Kim BS, Choi CW, Shin H, Jin SP, Bae JS, Han M, *et al*. Comparison of the Gut Microbiota of Centenarians in Longevity Villages of South Korea with Those of Other Age Groups. J Microbiol Biotechnol 2019;29(3):429–440. doi:10.4014/jmb.1811.11023, PMID:30661321.
[195] Wu L, Xie X, Li Y, Liang T, Zhong H, Yang L, *et al*. Gut microbiota as an antioxidant system in centenarians associated with high antioxidant activities of gut-resident Lactobacillus. NPJ Biofilms Microbiomes 2022;8(1):102. doi:10.1038/s41522-022-00366-0, PMID:36564415.
[196] Chulenbayeva L, Issilbayeva A, Sailybayeva A, Bekbossynova M, Kozhakhmetov S, Kushugulova A. Short-Chain Fatty Acids and Their Metabolic Interactions in Heart Failure. Biomedicines 2025;13(2):343. doi:10.3390/biomedicines13020343, PMID:40002756.

[197] Pang S, Chen X, Lu Z, Meng L, Huang Y, Yu X, *et al*. Longevity of centenarians is reflected by the gut microbiome with youth-associated signatures. Nat Aging 2023;3(4):436–449. doi:10.1038/s43587-023-00389-y, PMID:37117794.
[198] Lozada-Martinez ID, Lozada-Martinez LM, Anaya JM. Gut microbiota in centenarians: A potential metabolic and aging regulator in the study of extreme longevity. Aging Med (Milton) 2024;7(3):406–413. doi:10.1002/agm2.12336, PMID:38975304.
[199] Cătoi AF, Corina A, Katsiki N, Vodnar DC, Andreicuț AD, Stoian AP, *et al*. Gut microbiota and aging-A focus on centenarians. Biochim Biophys Acta Mol Basis Dis 2020;1866(7):165765. doi:10.1016/j.bbadis.2020.165765, PMID:32169505.
[200] Morita H, Ichishima M, Tada I, Shiroma H, Miyagi M, Nakamura T, *et al*. Gut microbial composition of elderly women born in the Japanese longevity village Ogimi. Biosci Microbiota Food Health 2021;40(1):75–79. doi:10.12938/bmfh.2019-055, PMID:33520572.
[201] Rodrigues VF, Elias-Oliveira J, Pereira ÍS, Pereira JA, Barbosa SC, Machado MSG, *et al*. Akkermansia muciniphila and Gut Immune System: A Good Friendship That Attenuates Inflammatory Bowel Disease, Obesity, and Diabetes. Front Immunol 2022;13:934695. doi:10.3389/fimmu.2022.934695, PMID:35874661.
[202] Gavzy SJ, Kensiski A, Lee ZL, Mongodin EF, Ma B, Bromberg JS. Bifidobacterium mechanisms of immune modulation and tolerance. Gut Microbes 2023;15(2):2291164. doi:10.1080/19490976.2023.2291164, PMID:38055306.
[203] Ruiz L, Delgado S, Ruas-Madiedo P, Sánchez B, Margolles A. Bifidobacteria and Their Molecular Communication with the Immune System. Front Microbiol 2017;8:2345. doi:10.3389/fmicb.2017.02345, PMID:29255450.
[204] Wu L, Zeng T, Zinellu A, Rubino S, Kelvin DJ, Carru C. A Cross-Sectional Study of Compositional and Functional Profiles of Gut Microbiota in Sardinian Centenarians. mSystems 2019;4(4):e00325–19. doi:10.1128/mSystems.00325-19, PMID:31289141.
[205] Tang J, Wei Y, Pi C, Zheng W, Zuo Y, Shi P, *et al*. The therapeutic value of bifidobacteria in cardiovascular disease. NPJ Biofilms Microbiomes 2023;9(1):82. doi:10.1038/s41522-023-00448-7, PMID:37903770.
[206] Palmas V, Pisanu S, Madau V, Casula E, Deledda A, Cusano R, *et al*. Gut Microbiota Markers and Dietary Habits Associated with Extreme Longevity in Healthy Sardinian Centenarians. Nutrients 2022;14(12):2436. doi:10.3390/nu14122436, PMID:35745166.
[207] Palmas V, Pisanu S, Madau V, Casula E, Deledda A, Cusano R, *et al*. Gut Microbiota Markers and Dietary Habits Associated with Extreme Longevity in Healthy Sardinian Centenarians. Nutrients 2022;14(12):2436. doi:10.3390/nu14122436, PMID:35745166.
[208] Beaver LM, Jamieson PE, Wong CP, Hosseinikia M, Stevens JF, Ho E. Promotion of Healthy Aging Through the Nexus of Gut Microbiota and Dietary Phytochemicals. Adv Nutr 2025;16(3):100376. doi:10.1016/j.advnut.2025.100376, PMID:39832641.
[209] Ku S, Haque MA, Jang MJ, Ahn J, Choe D, Jeon JI, *et al*. The role of Bifidobacterium in longevity and the future of probiotics. Food Sci Biotechnol 2024;33(9):2097–2110. doi:10.1007/s10068-024-01631-y, PMID:39130652.
[210] Brooks CN, Wight ME, Azeez OE, Bleich RM, Zwetsloot KA. Growing old together: What we know about the influence of diet and exercise on the aging host's gut microbiome. Front Sports Act Living 2023;5:1168731. doi:10.3389/fspor.2023.1168731, PMID:37139301.
[211] Piggott DA, Tuddenham S. The gut microbiome and frailty. Transl Res 2020;221:23–43. doi:10.1016/j.trsl.2020.03.012, PMID:32360945.
[212] Galkin F, Mamoshina P, Aliper A, Putin E, Moskalev V, Gladyshev VN, *et al*. Human Gut Microbiome Aging Clock Based on Taxonomic Profiling and Deep Learning. iScience 2020;23(6):101199. doi:10.1016/j.isci.2020.101199, PMID:32534441.
[213] Biga PR, Duan JE, Young TE, Marks JR, Bronikowski A, Decena LP, *et al*. Hallmarks of aging: A user's guide for comparative biologists. Ageing Res Rev 2025;104:102616. doi:10.1016/j.arr.2024.102616, PMID:39643212.
[214] López-Otín C, Pietrocola F, Roiz-Valle D, Galluzzi L, Kroemer G. Meta-hallmarks of aging and cancer. Cell Metab 2023;35(1):12–35. doi:10.1016/j.cmet.2022.11.001, PMID:36599298.
[215] Tenchov R, Sasso JM, Wang X, Zhou QA. Aging Hallmarks and Progression and Age-Related Diseases: A Landscape View of Research Advancement. ACS Chem Neurosci 2024;15(1):1–30. doi:10.1021/acschemneuro.3c00531.10.1021/acschemneuro.3c00531.
[216] Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, Li J. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. Signal Transduct Target Ther 2022;7(1):391. doi:10.1038/s41392-022-01251-0, PMID:36522308.

Exploratory Research and Hypothesis in Medicine **2025** vol. 10(2) | 135–139
DOI: 10.14218/ERHM.2024.00038

Mini Review

Circadian Rhythms in Tumor Regulation: Impacts on Tumor Progression and the Immune Microenvironment

Jinxin Li[1#], Peng Luo[2#], Ying Liu[1#], Yu Fang[1], Linhui Wang[1*] and Aimin Jiang[1*]

[1]*Department of Urology, Changhai Hospital, Naval Medical University (Second Military Medical University), Shanghai, China;* [2]*The Department of Oncology, Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China*

Received: November 07, 2024 | **Revised:** November 16, 2024 | **Accepted:** December 25, 2024 | **Published online:** February 24, 2025

Abstract

The spatial heterogeneity of tumors has long been a subject of significant interest in oncology. Recent research has revealed that tumors and their microenvironments undergo dynamic changes over time, particularly in the form of periodic circadian rhythms. Disruptions to these rhythms have been recognized as a pivotal factor in the advancement of tumorigenesis. Such disruptions not only induce dysregulation of gene expression within tumor cells, influencing tumor growth, metabolism, the cell cycle, and vascular homeostasis but also facilitate metastasis. Furthermore, they mediate the remodeling of the tumor immune microenvironment, fostering the development of an immunosuppressive milieu. Additionally, the *in vivo* metabolism and therapeutic responsiveness of tumor treatments—including chemotherapy, targeted therapy, and immunotherapy—have been shown to be modulated by circadian rhythms. This suggests that time-specific drug administration may enhance treatment efficacy, offering novel insights for precision cancer therapy. In this review, we systematically update contemporary research on the impact of circadian rhythms on tumor biology, encompassing both tumor progression and the efficacy of drug therapies. Building upon these insights, we explore the potential for a synergistic approach that integrates the targeting of rhythmic genes with current tumor treatment modalities. We also discuss the feasibility of tailoring tumor therapy to the rhythmic alterations that define *in vivo* metabolism and the efficacy of specific therapeutic agents, highlighting the significance of rhythm-based strategies in the personalized treatment of tumors and the prevention of associated diseases.

Introduction

The alternation of day and night in nature has driven living organisms to evolve strict time-maintenance mechanisms, known as circadian rhythms, to better adapt to external changes.[1] In humans, circadian rhythms are complex processes mediated by a regulatory center located in the suprachiasmatic nuclei of the hypothalamus and are regulated by a variety of rhythm-associated genes, which play a role in regulating a wide range of life activities, such as sleep/wake cycles, feeding/fasting, endocrine function, immunity, and metabolism.[2]

With increasing understanding and research, it has become clear in recent years that circadian dysregulation is closely linked to the development of tumors. Although a variety of factors and pathways are involved, this effect is generally manifested in two main aspects: through the regulation of the tumor itself and through the modulation of the tumor immune microenvironment.

The effect of circadian rhythms on the biological function of tumors

The regulation of tumor biological characteristics by circadian rhythm disruption is an important mechanism promoting tumorigenesis and progression, including tumor initiation, stemness, metabolic reprogramming, and immune microenvironment remodeling (Fig. 1). Enhanced fatty acid oxidation-mediated oncogenic metabolic signaling promoting tumorigenesis has been observed in sleep deprivation-induced circadian rhythm disorders. This effect is mainly mediated by the dysregulated CLOCK gene, which overactivates long-chain fatty acyl coenzyme A synthetase 1, leading to increased production of palmitoyl coenzyme A. This, in turn, promotes CLOCK-Cys194 S-palmitoylation. This approach elimi-

Keywords: Circadian rhythm; Tumor therapy; Tumor immune microenvironment; Time-specific drug administration; Precision cancer therapy; Rhythm gene-targeted therapy; Personalized tumor treatment strategies.

***Correspondence to:** Linhui Wang and Aimin Jiang, Department of Urology, Changhai Hospital, Naval Medical University (Second Military Medical University), Shanghai 200433, China. ORCID: https://orcid.org/0000-0002-4056-0462 (LW); https://orcid.org/0000-0002-9563-983X (AJ). Tel: +86-13101635510 (LW); +86-18301935531 (AJ), E-mail: wanglinhui@smmu.edu.cn (LW); czjiangaimin@smmu.edu.cn (AJ)

[#]These authors contributed equally to this work.

How to cite this article: Li J, Luo P, Liu Y, Fang Y, Wang L, Jiang A. Circadian Rhythms in Tumor Regulation: Impacts on Tumor Progression and the Immune Microenvironment. *Explor Res Hypothesis Med* 2025;10(2):135–139. doi: 10.14218/ERHM.2024.00038.

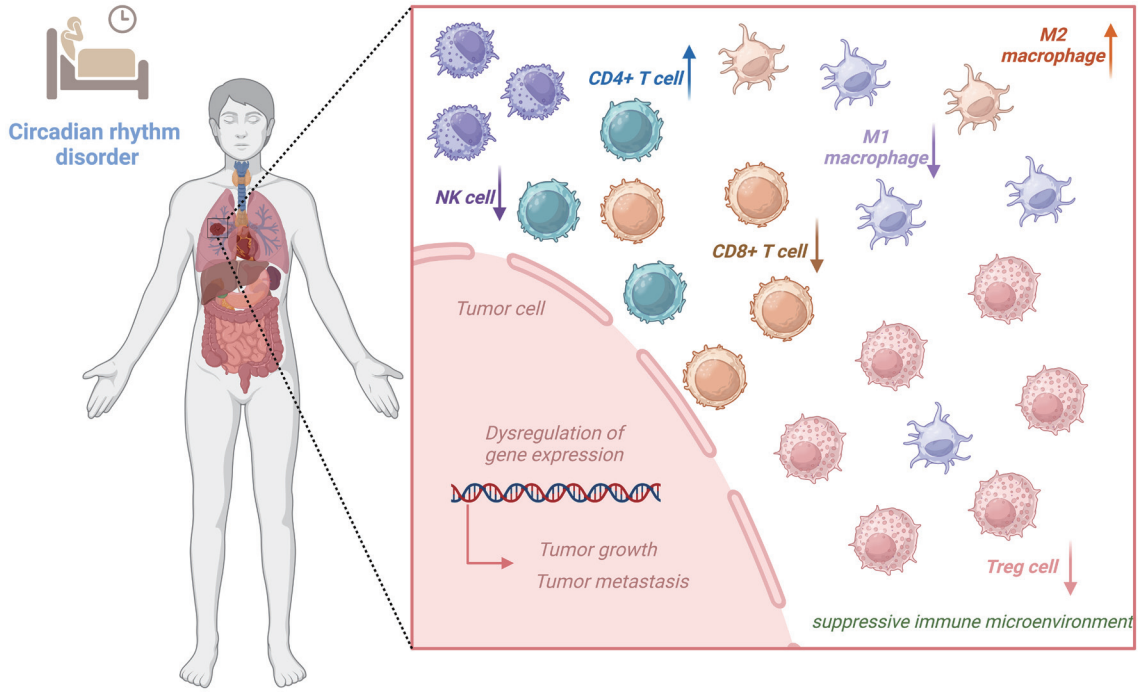


Fig. 1. Impact of circadian rhythm disorder on tumor progression and immune microenvironment. On the one hand, circadian rhythm disorder contributes to tumor growth and metastasis by influencing the dysregulation of gene expression affecting the tumor itself. On the other hand, circadian rhythm disorder can also promote the formation of a suppressive immune microenvironment, reduce the number of natural killer (NK) cells and the M1/M2 ratio, and increase the CD4/CD8 ratio and the number of regulatory T (Treg) cells, thus indirectly promoting tumor progression. This figure was created using tools provided by Biorender (www.biorender.com). CD, cluster of differentiation; M1, pro-inflammatory; M2, anti-inflammatory.

nates the CLOCK gene's ubiquitination degradation, disrupting circadian rhythms and promoting cancer stemness.[3] Abnormally expressed CLOCK genes can also alter the secretion patterns of tumor cell chemokines and cytokines, promoting tumor inflammation and angiogenesis. Dysregulation of rhythm-associated genes is both a cause and an effect, serving as a link between circadian rhythm disruption and tumor development. Rhythmic genes, such as BMAL1 and CLOCK, play crucial roles in regulating signaling pathways and molecular expression related to various cellular processes, including the cell cycle, epithelial-mesenchymal transition, apoptosis, ferroptosis, cellular metabolism, and immunity. These genes exert their influence either individually or through the formation of complexes, impacting cellular vascularity, growth, metastasis, immune response, and other functions (Fig. 2). Essentially, the coordinated expression of CLOCK genes regulates the timing and order of various cellular processes, while circadian rhythm disruption induces dysregulation of CLOCK gene expression, leading to a loss of normal cellular function regulation.

The impact of rhythm-related genes on tumors is broad and direct, including tumor metastasis. Often, more attention is given to spatial variations in tumor metastasis to specify the affected tissues and organs for more targeted therapeutic decisions. However, tumor metastasis also exhibits temporal heterogeneity, as demonstrated by the significant influence of the sleep/wake cycle on tumor metastasis. Given that hematogenous dissemination due to circulating tumor cells (CTCs) is the primary mode of metastasis for most tumors, a study tracked the dynamics of CTCs and revealed the significant impact of circadian rhythms on tumor metastasis.[4] Specifically, the production of CTCs in patients was found to be greatly disturbed by circadian rhythms, with the majority of CTCs (78.3%) found in samples obtained during resting periods. This finding was further validated in animal experiments, where mice in the resting phase showed a six- to eight-fold increase in CTCs compared to the active phase, with a maximum increase of 278-fold. By interfering with the sleep cycle and administering rhythm-related hormones (e.g., melatonin), it was found that CTCs produced during the rest phase were not only more numerous but also exhibited a stronger metastatic capacity compared to those produced during the active phase. These results suggest that the production of CTCs is not constant but exhibits significant time-dependence and temporal heterogeneity, regulated by circadian rhythms. This also implies that dynamic regulation of drug administration schedules could be feasible during tumor therapy, with intensive resting-phase-focused therapy potentially achieving promising therapeutic outcomes.

In addition to affecting tumor function, circadian rhythms also play a crucial regulatory role in the tumor-immune microenvironment. On one hand, rhythm genes directly regulate the expression of immune checkpoints in tumor cells, indirectly affecting immune cell activation. For example, Period2 competitively binds to heat shock protein 90 via the PAS1 structural domain, reducing its interaction with inhibitors of kappa B kinase (IKKs). This leads to increased ubiquitination degradation of IKK-α/β and facilitates nuclear translocation of p65, inhibiting the IKK/NF-κB pathway and reducing PD-L1 expression.[5] On the other hand, the composition and percentage of immune cells fluctuate dynamically, and these fluctuations are regulated by circadian rhythms. Taking macrophages as an example, M1 and M2 macrophages are the main types in the tumor immune microenvironment. These macrophages not only play opposing roles but also exhibit opposite states

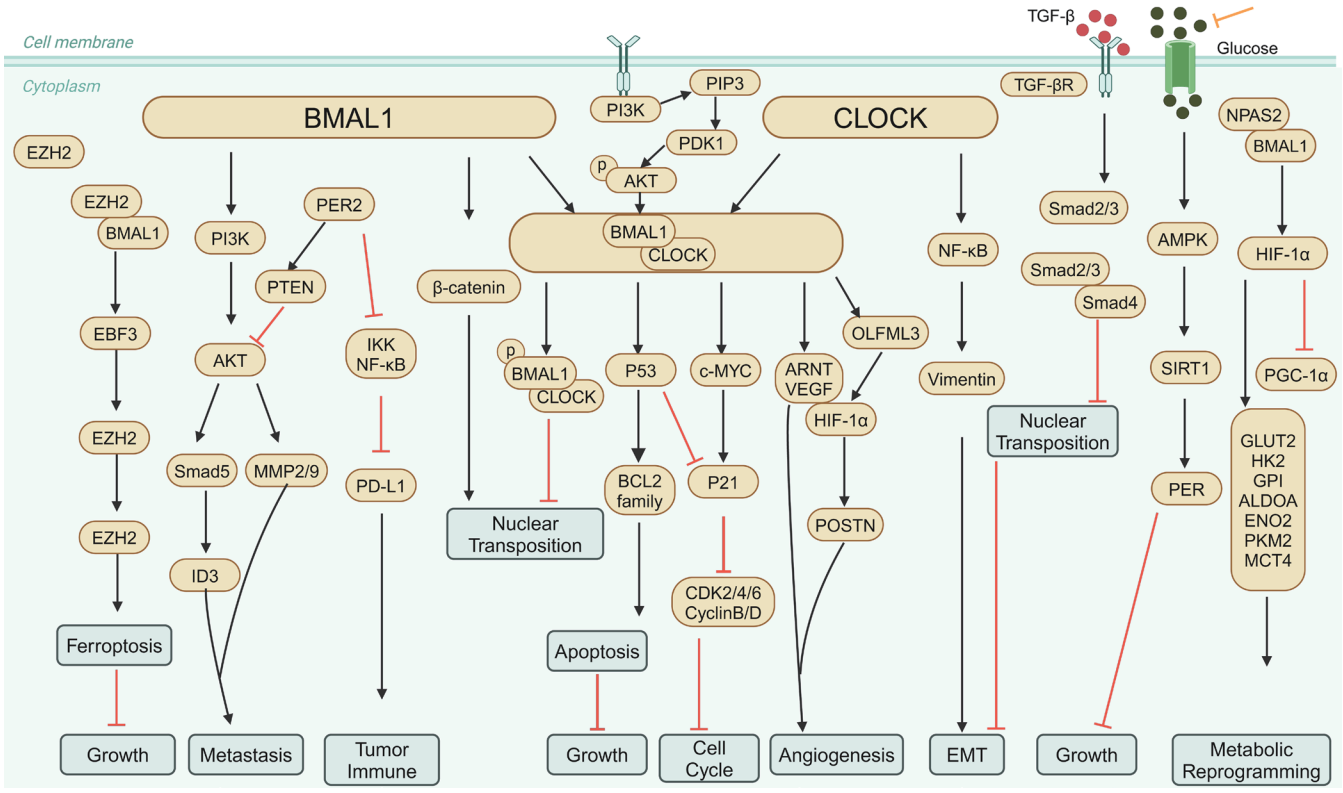


Fig. 2. Pathways and molecular regulatory networks of circadian rhythm-related genes in tumor cells. Rhythmic genes such as BMAL1, CLOCK, and others, either individually or through the formation of complexes, regulate signaling pathways and molecular expression associated with various cellular processes such as the cell cycle, epithelial-mesenchymal transition (EMT), apoptosis, ferroptosis, cellular metabolism, and immunity. These genes influence cellular angiogenesis, growth, metastasis, immune responses, and other functions. This figure was created using tools provided by Biorender (www.biorender.com).

of change during circadian fluctuations. In the normal light-dark cycle, M1 macrophages predominate at night, while M2 macrophages are more prevalent during the day, which is considered the normal macrophage daily pattern. However, circadian rhythm disruption disrupts the daily pattern of both, significantly reducing the M1/M2 ratio and resulting in the development of a suppressive immune microenvironment.[6] In addition to macrophages, a decrease in the number of $CD8^+$ T cells, an increase in the CD4/CD8 ratio, and an increase in $FoxP3^+$ Treg cells, along with greater infiltration of immunosuppressive microglial cells in neurological tumors, also contribute to remodeling the tumor immune microenvironment. These changes promote the formation of metastatic niches and tumor immune escape.[7] These studies have deepened our understanding of the relationship between circadian rhythms and tumor immunity. More importantly, by identifying the major differential genes and their mechanisms of action, we can pinpoint target genes that can inhibit the adverse effects of rhythm disruption on tumor immunity, aiding in the development of new tumor immunomodulatory drugs targeting rhythm-related genes.

The effect of circadian rhythms on the efficacy of antitumor drug therapy

Given the important role that rhythm genes play in tumor progression, it is reasonable to believe that targeting these genes will provide new options for tumor therapy. Indeed, studies have already revealed the unique role of rhythm gene modulators in controlling circadian rhythm disorders and enhancing antitumor therapy. For example, CLK8 increases circadian rhythm amplitude by inhibiting CLOCK dimerization with BMAL1.[8] KL001 and its derivative SHP656 can inhibit the growth of glioblastoma stem cells through the simultaneous activation of CRY1 and CRY2.[9] Retinoic acid-related orphan receptor α agonists inhibit the growth of gastric cancer cells both *in vitro* and *in vivo*, making them promising antitumor agents.[10] However, current research on these rhythm gene-modulating drugs is still mainly in the basic research phase, and clinical studies are needed to further confirm their effectiveness and potential for clinical application.

Moreover, the activity and antitumor effects of many antitumor agents, including chemotherapeutic agents, targeted therapies, and immunotherapies, are strongly influenced by circadian rhythms, particularly in terms of fluctuating pharmacokinetics, efficacy over time, and the mediation of drug resistance events. For example, one study evaluated the effect of circadian rhythms on the pharmacokinetics of linifanib, a novel tyrosine kinase inhibitor selective for vascular endothelial growth factor and platelet-derived growth factor receptors. The results showed that evening dosing significantly affected the oral bioavailability of linifanib, with a dose-normalized Cmax that was 64% of that observed after morning dosing.[11] At specific times of the day, antitumor drugs can exert a tumor-killing effect, but their efficacy is greatly disturbed when the circadian clock is defective, suggesting that drug efficacy fluctuates with the rhythm.[12] Mechanistically, this rhythm-dependent feature is regulated by the cell cycle and is dependent on the cyclic expression of target proteins. Furthermore, given the serious chal-

lenge of drug tolerance in tumor therapy, new studies have found that the development of tolerance to some drugs is also influenced by circadian rhythms.[13] Periodic circadian expression patterns of key genes are important contributors to this phenomenon.

Chronomodulated chemotherapy regimens targeting cytotoxic drugs have also demonstrated promising results.[14] A systematic review that included 18 randomized trials and 2,547 cancer patients found that the majority of studies (14/18) supported the ability of chronomodulated chemotherapy to improve outcomes while reducing drug toxicity, with potential gender differences.[15] Although a small number of studies suggested that chronomodulated chemotherapy could lead to a shift in toxicity response and one study reported a worse toxicity response, no study reported a clear reduction in efficacy. This provides new insights into how tumor drug resistance arises and demonstrates that temporal therapies targeting rhythmic regulation have great potential to overcome antitumor drug resistance. Therefore, emphasizing the temporal dimension in the development of antitumor therapeutic strategies may enhance the precision and targeting of drugs while reducing the development of drug resistance, or even reversing drug resistance that has already occurred.

The strong circadian rhythmicity of the immune system also has a significant impact on the effectiveness of immunotherapy.[16] Studies have shown that circadian rhythm changes drive cyclic oscillations of T cells. At the highest abundance of suppressive immune cells, $CD8^+$ T cell function is severely suppressed. However, since the expression of immune checkpoints, such as PD-L1, peaks in immunosuppressive cells, administering anti-PD-L1 therapy at this time can be more effective.[17] In addition, the rhythmic changes in $CD8^+$ T cells were also reflected by dendritic cells, showing amplified efficacy by synchronizing tumor immunotherapy with dendritic cell function.[18] Tumor immunotherapy targeting immune cells is benefiting cancer patients, but low sensitivity and high drug resistance still limit its clinical application. Immunotherapy that incorporates the circadian characteristics of immune cells may be a viable solution, whether for immune checkpoint inhibitors, tumor vaccines, or chimeric antigen receptor T-cell therapies.

In light of these findings, an increasing number of studies have focused on the impact of modulating circadian rhythms on tumor therapy. Evidence suggests that circadian rhythms significantly affect the sensitivity of tumor radiotherapy. Meanwhile, adjusting the timing of drug administration has been shown to affect its bioavailability. Additionally, adjusting circadian rhythms through phototherapy and other means can improve symptoms such as tumor-related fatigue. Several ongoing studies are also evaluating the effects of circadian rhythm disruption on tumor progression as well as tumor-associated adverse effects, which will provide valuable insights for improving tumor therapy (Table S1).

Future directions

A thorough understanding of the dynamic alterations in tumor circadian rhythms is essential for refining precision cancer therapy, shifting the focus from specific targets to a more nuanced appreciation of the therapeutic window. As research evolves, an increasing number of rhythm-associated tumor therapeutic targets are being elucidated, promising to diversify therapeutic strategies. Consequently, interventions aimed at circadian rhythm genes may emerge as a novel modality in cancer treatment, potentially synergizing with established approaches such as chemotherapy, targeted therapies, and immunotherapies. However, current research in this domain remains limited and predominantly confined to the basic science stage; further studies are imperative to substantiate the viability of this concept.

Moreover, elucidating the cyclical expression patterns of target genes and the dynamic spatial and temporal interplay between tumor cells and immune cells is crucial for the development of more targeted cancer therapies. Tailoring drug dosing according to the metabolic and efficacy profiles of specific drugs can optimize therapeutic outcomes while minimizing adverse effects.

Given that circadian rhythm regulation is predominantly governed by central neural and hormonal mechanisms, therapies targeting these rhythms may be subject to variability among individual patients.[19,20] Occupational and lifestyle factors that can induce circadian rhythm disorders or even inversions necessitate a more personalized approach to such treatments, requiring selection based on each patient's unique rhythmic characteristics.[21]

Considering the influence of circadian fluctuations on tumor growth, metastasis, drug resistance, and immune microenvironment formation, there is a pressing need to focus on the deleterious effects of circadian disruption on the organism, potentially exacerbating oncogenesis. This awareness is vital for prompting individuals to recognize the risks associated with unhealthy habits and to encourage behavioral changes. Future research should also consider disease prevention strategies for occupations that may contribute to circadian rhythm disruptions.

Conclusions

Disruptions in circadian rhythms have been implicated in the initiation and progression of tumorigenesis through a variety of molecular pathways. Targeting circadian rhythm-associated genes represents a potentially efficacious therapeutic strategy for cancer treatment, particularly when employed in conjunction with established tumor therapies. However, further research is warranted to substantiate this hypothesis. A deeper understanding of the temporal metabolic profiles and efficacy fluctuations of tumor therapeutics is essential for the enhancement of precision cancer therapy. This knowledge will facilitate the optimization of treatment timing and dosing, ultimately aiming to maximize therapeutic benefits while minimizing side effects.

While this review provides a comprehensive look at the effects of circadian rhythms on tumors, it still has some limitations. The findings presented are predominantly derived from the current scientific literature and thus are subject to the constraints of the quality, scope, and depth of the published research. Furthermore, the practical application of our proposed rhythm-based strategies in personalized tumor therapy and disease prevention necessitates additional empirical validation through experimental and clinical trials to ascertain their efficacy and feasibility. Nevertheless, we have delineated critical issues and challenges that warrant attention, which will serve as a roadmap for future investigative endeavors.

Acknowledgments

Not applicable.

Funding

This work was sponsored by the National Natural Science Foundation of China (grant numbers: 81872074 and 82372883) and the Changfeng Talent Development Programme (grant number: 2023CF001).

Conflict of interest

The authors have declared that no competing interests exist.

Author contributions

Analyzed the literature and wrote the manuscript (JL, PL, YL), drafted the figure (JL), conceived the idea (LW, AJ), and reviewed and revised the manuscript (YF, LW, AJ). All authors gave the final approval of the submitted version.

References

[1] Rosenwasser AM, Turek FW. Neurobiology of Circadian Rhythm Regulation. Sleep Med Clin 2022;17(2):141–150. doi:10.1016/j.jsmc.2022.02.006, PMID:35659069.

[2] Crislip GR, Johnston JG, Douma LG, Costello HM, Juffre A, Boyd K, *et al*. Circadian Rhythm Effects on the Molecular Regulation of Physiological Systems. Compr Physiol 2021;12(1):2769–2798. doi:10.1002/cphy.c210011, PMID:34964116.

[3] Peng F, Lu J, Su K, Liu X, Luo H, He B, *et al*. Oncogenic fatty acid oxidation senses circadian disruption in sleep-deficiency-enhanced tumorigenesis. Cell Metab 2024;36(7):1598–1618.e11. doi:10.1016/j.cmet.2024.04.018, PMID:38772364.

[4] Diamantopoulou Z, Castro-Giner F, Schwab FD, Foerster C, Saini M, Budinjas S, *et al*. The metastatic spread of breast cancer accelerates during sleep. Nature 2022;607(7917):156–162. PMID:35732738.

[5] Zhang Z, Sun D, Tang H, Ren J, Yin S, Yang K. PER2 binding to HSP90 enhances immune response against oral squamous cell carcinoma by inhibiting IKK/NF-kappaB pathway and PD-L1 expression. J Immunother Cancer 2023;11(11):e007627. PMID:37914384.

[6] Aiello I, Fedele MLM, Roman F, Marpegan L, Caldart C, Chiesa JJ, *et al*. Circadian disruption promotes tumor-immune microenvironment remodeling favoring tumor cell proliferation. Sci Adv 2020;6(42):eaaz4530. doi:10.1126/sciadv.aaz4530, PMID:33055171.

[7] Liu JL, Xu X, Rixiati Y, Wang CY, Ni HL, Chen WS, *et al*. Dysfunctional circadian clock accelerates cancer metastasis by intestinal microbiota triggering accumulation of myeloid-derived suppressor cells. Cell Metab 2024;36(6):1320–1334.e9. doi:10.1016/j.cmet.2024.04.019, PMID:38838643.

[8] Luna-Marco C, Devos D, Cacace J, Fernandez-Reyes M, Diaz-Pozo P, Salazar JD, *et al*. Molecular circadian clock disruption in the leukocytes of individuals with type 2 diabetes and overweight, and its relationship with leukocyte-endothelial interactions. Diabetologia 2024;67(10):2316–2328. doi:10.1007/s00125-024-06219-z, PMID:38981930.

[9] Miller S, Kesherwani M, Chan P, Nagai Y, Yagi M, Cope J, *et al*. CRY2 isoform selectivity of a circadian clock modulator with antiglioblastoma efficacy. Proc Natl Acad Sci USA 2022;119(40):e2203936119. doi:10.1073/pnas.2203936119, PMID:36161947.

[10] Wang X, Zhang J, Wu Y, Zhang Y, Zhang S, Li A, *et al*. RORalpha inhibits gastric cancer proliferation through attenuating G6PD and PFKFB3 induced glycolytic activity. Cancer Cell Int 2024;24(1):12. doi:10.1186/s12935-023-03201-4, PMID:38184549.

[11] Xiong H, Chiu YL, Ricker JL, LoRusso P. Results of a phase 1, randomized study evaluating the effects of food and diurnal variation on the pharmacokinetics of linifanib. Cancer Chemother Pharmacol 2014;74(1):55–61. doi:10.1007/s00280-014-2475-z, PMID:24810181.

[12] Lee Y, Fong SY, Shon J, Zhang SL, Brooks R, Lahens NF, *et al*. Time-of-day specificity of anticancer drugs may be mediated by circadian regulation of the cell cycle. Sci Adv 2021;7(7):eabd2645. doi:10.1126/sciadv.abd2645, PMID:33579708.

[13] Wang J, Huang Q, Hu X, Zhang S, Jiang Y, Yao G, *et al*. Disrupting Circadian Rhythm via the PER1-HK2 Axis Reverses Trastuzumab Resistance in Gastric Cancer. Cancer Res 2022;82(8):1503–1517. doi:10.1158/0008-5472.CAN-21-1820, PMID:35255118.

[14] Marcu LG. Developments on tumour site-specific chrono-oncology towards personalised treatment. Crit Rev Oncol Hematol 2022;179:103803. doi:10.1016/j.critrevonc.2022.103803, PMID:36058443.

[15] Printezi MI, Kilgallen AB, Bond MJG, Stibler U, Putker M, Teske AJ, *et al*. Toxicity and efficacy of chronomodulated chemotherapy: a systematic review. Lancet Oncol 2022;23(3):e129–e143. doi:10.1016/S1470-2045(21)00639-2, PMID:35240088.

[16] Zeng Y, Guo Z, Wu M, Chen F, Chen L. Circadian rhythm regulates the function of immune cells and participates in the development of tumors. Cell Death Discov 2024;10(1):199. doi:10.1038/s41420-024-01960-1, PMID:38678017.

[17] Fortin BM, Pfeiffer SM, Insua-Rodriguez J, Alshetaiwi H, Moshensky A, Song WA, *et al*. Circadian control of tumor immunosuppression affects efficacy of immune checkpoint blockade. Nat Immunol 2024;25(7):1257–1269. doi:10.1038/s41590-024-01859-0, PMID:38806707.

[18] Wang C, Barnoud C, Cenerenti M, Sun M, Caffa I, Kizil B, *et al*. Dendritic cells direct circadian anti-tumour immune responses. Nature 2023;614(7946):136–143. doi:10.1038/s41586-022-05605-0, PMID:36470303.

[19] Vasey C, McBride J, Penta K. Circadian Rhythm Dysregulation and Restoration: The Role of Melatonin. Nutrients 2021;13(10):3480. doi:10.3390/nu13103480, PMID:34684482.

[20] Montaruli A, Castelli L, Mule A, Scurati R, Esposito F, Galasso L, *et al*. Biological Rhythm and Chronotype: New Perspectives in Health. Biomolecules 2021;11(4):487. doi:10.3390/biom11040487, PMID:33804974.

[21] Gentry NW, Ashbrook LH, Fu YH, Ptáček LJ. Human circadian variations. J Clin Invest 2021;131(16):e148282. doi:10.1172/JCI148282, PMID:34396981.

Exploratory Research and Hypothesis in Medicine **2025** vol. 10(2) | 140–142
DOI: 10.14218/ERHM.2024.00030

Opinion

Does Person-to-person Contact Confound Microbiota Research? An Important Consideration in the Randomization of Study Arms

Reza Rastmanesh*

Independent Researcher, Private Clinic, Tehran, Iran

Received: August 25, 2024 | **Revised:** September 24, 2024 | **Accepted:** September 26, 2024 | **Published online:** November 14, 2024

The current paradigm assumes that randomization eliminates all confounding factors.[1] In this opinion, I challenge this view. For instance, changes in parent-child relationships and/or fraternal relationships represent a new phenomenon that did not exist before.[2,3] A curious question arises: Is it possible that changes in person-to-person contact due to pandemics, social distancing, and other factors could alter the microbiota composition in individuals?

A recent paper by Valles-Colomer, published in Nature, on person-to-person transmission of the gut and oral microbiomes has significant implications for medical/biomedical research, medical practice, study design, and data analysis.[4] However, these implications have not received much attention, particularly in contemporary probiotic and antimicrobial research. The study detected astonishing patterns of extensive bacterial strain sharing among individuals, with marked and recognizable intra-household, mother-to-infant, and intra-population transmission patterns. This finding, along with similar studies,[5,6] will likely impact medical and biomedical sciences in many ways. In this opinion, I attempted to connect these pioneering works with recent probiotic supplementation studies conducted during the COVID-19 pandemic,[4–10] just to mention a few. There is no doubt that these studies followed standard procedures. However, I argue that there is a "possibility" of hidden bias that might have arisen due to altered social dynamics, closeness, and person-to-person microbial transmission during the COVID-19 pandemic, particularly in non-randomized clinical trials, and potentially even in small-sample randomized clinical trials.

Compelling evidence shows that changes in parent-child relationships and/or fraternal relationships due to COVID-19-imposed social distancing may introduce bias, leading to inaccurate estimates of results. In particular, publication and expectation biases could lead to significantly higher estimates of efficacy in studies on oral and gut microbiota.[11,12] Here, we must take a closer look at "closeness", defined as the average distance from one node to all others.[13] Recent studies conducted during the COVID-19 pandemic show that the "closeness" between parents and their children/infants was highly dynamic among families.[13] This suggests that behaviors such as kissing and other forms of bodily contact, which can lead to microbiota transmission, varied significantly and were not necessarily consistent across all families.

Methodologically, randomization ensures that potential confounding factors are evenly distributed among treatment groups.[1] However, in short-duration studies, uncertainties may arise from factors such as the nature of oral ecology, microbiome transmissibility, microbial population dynamics, and the varying time courses of interactions and medication effects.[14,15] In such cases, it is unlikely that randomization alone can be considered a reliable method—especially in studies using single-dose interventions. These interventions may preclude the exploration of optimal dose-response relationships for microbiota strains and sub-strains in treatment.

Supporting this argument, there is evidence that even after randomization, significant differences in calorie, carbohydrate, fat, and protein intake may exist between two arms of the clinical trial,[16,17] all of which can significantly influence baseline microbiota levels. Even more interestingly, some randomized clinical trials have shown that participants' baseline gut microbiota (confirmed through beta diversity analyses) differed significantly from controls, though not from each other.[18] Intriguingly, in other medical disciplines like pulmonary medicine, differences in baseline microbiomes have been reported between groups in trials comparing sputum microbiota in adults with cystic fibrosis.[19] This direct and indirect evidence suggests that the possibility of non-normality in baseline microbiota in clinical trials may not be easily dismissed.

The most direct supporting evidence comes from a recent paper by Griffen *et al.*,[5] which enrolled 55 biological and 50 adoptive mother-child dyads to determine the effect of genetic relatedness on the fidelity of oral bacterial transmission. Adoptive mother-child dyads were recruited through adoption agencies. To match the adoptive group by parents' socioeconomic status and children's age, a biological group was also enrolled. To minimize bacterial transmission from biological mothers, only children adopted at birth and unrelated to the adoptive family were included. In the biological group, only genetic birth mothers were included, and fathers and siblings were also sampled when available. To allow for the establishment of an oral bacterial community, children in both the biological and adoptive groups were between three months

*Correspondence to: Reza Rastmanesh, Independent Researcher, Private Clinic, Tehran 196183555, Iran. ORCID: https://orcid.org/0000-0002-6221-9062. Tel: +98-2122750414, Fax: +98-2122735950, E-mail: r.rastmanesh@gmail.com

How to cite this article: Rastmanesh R. Does Person-to-person Contact Confound Microbiota Research? An Important Consideration in the Randomization of Study Arms. *Explor Res Hypothesis Med* 2025;10(2):140–142. doi: 10.14218/ERHM.2024.00030.

and 12 years of age. Exclusion criteria for all participants included chronic diseases affecting the immune system, oral cavity, or early onset periodontitis. For all three niches sampled—supragingival plaque, saliva/soft tissue, and subgingival plaque—the microbial profiles of adopted and biological children were equally similar to their mothers at both the species and strain levels. No genetic influence was found on the acquisition of oral bacteria. At the strain level, all mothers and their children, regardless of genetic relationship, were significantly more similar to each other than unrelated mother-child pairs. This relationship was less pronounced at the lower resolution species-level approach. Similar results were observed for comparisons between adoptive and biological groups (ISR soft tissue/saliva) when using relative abundance measures instead of presence/absence measures. For instance, one study investigated the effect of fecal microbiomes on mother-infant dyads, especially during the early postpartum period.[6] Based on this study, there is a complex microbial interaction between breastfeeding mothers and their infants, which indirectly supports the idea that changes in the milk microbiome may influence the infant's gastrointestinal microbiome. These two findings provide the most direct evidence for our argument that altered "closeness" during the COVID-19 pandemic has the potential to introduce uncertainties in bacterial transmission.[5,6]

Considering the complex network of correlations between parent-infant relationships and microbiome transmission, the results of probiotic supplementation studies would inevitably be affected, especially when sample sizes are small. It would be prudent to consider these issues when designing future studies.[20–22]

Altered oral and gut microbiota are implicated in the development and progression of many medical conditions.[23] On the other hand, clinical trials typically enroll a minimum sample size based on alpha statistics.[24,25] With these considerations, it seems highly unlikely that randomization alone accounts for the confounding effect of inter-individual microbiota variation and differences in closeness. This implies that many clinical trials conducted during the COVID-19 pandemic may have been subject to hidden bias. This bias is not confined to clinical trials but spans a wide range of diseases influenced by differential oral and gut microbiota. It also affects daily clinical practice. Heterogeneous results in clinical trials might be partially explained by the lack of standardized methodologies to match participants (i.e., cases and controls) in terms of oral and gut microbiota dynamics at each step of the study process, highlighting the need for clear guidelines.[26–29]

Moreover, the perspective of these novel studies on person-to-person microbial transmission creates a unique opportunity to test a myriad of hypotheses.[4–6] For instance, if microbiome composition contributes to a particular disease or condition, sharing a household with someone who has a distinct gut or oral microbiota pattern could influence study results and potentially predict the outcome of interest, at least to some extent.

Consider this hypothesis: if microbiome composition contributes to glucose intolerance, sharing a household with a shift worker who is already known to have a higher risk of metabolic disturbances would theoretically increase the risk of metabolic disturbances, again, at least to some extent.[30] Most readers would agree that conducting a clinical trial under such conditions would be methodologically, practically, and economically challenging. However, a researcher could easily test this hypothesis by co-housing host mice with a mouse exposed to the variable of interest and then measuring the microbiota and glucose homeostasis of the host to gather preliminary data. Similar experiments based on studies of person-to-person microbial transmission would represent a major advance in microbiota research.[4–6]

This argument can be criticized in several ways:

- Firstly, clinical trials should account for this bias in future research. However, there is currently very little insight or perspective on how to address this issue in real clinical trial settings. Many factors, some of which are still unknown, can impact microbiome composition and, consequently, disease outcomes. The real question is how to incorporate this consideration. I call for suggestions on the best methods to account for this potential bias. One lesson for the next pandemic is the need to develop tools to measure "closeness" as a confounding factor, both qualitatively and quantitatively.
- Secondly, it might be argued that the level of closeness between parents and children was different during the pandemic, such as through behaviors like kissing. Do we know for certain if parents' behavior changed during the pandemic? Yes, we spent more time with our families, but outside the pandemic, children would have been exposed to other kids at school or kindergarten, which would have introduced them to a broader array of people and children, potentially affecting their microbiomes differently.
- Thirdly, applying proper randomization and using an appropriate sample size should balance out any effects that social distancing might have had on the microbiome. Additionally, the period of social distancing was relatively short, and normal life has resumed since the end of the pandemic. It could be argued that we are uncertain whether there will be any lasting effects.
- Lastly, many other factors, such as genetics, nutrition, lifestyle, and access to healthcare systems, would likely play a more significant role in this context.

In conclusion, if this argument proves valid, we could extrapolate that all prognostic, diagnostic, cross-sectional, and interventional studies should account for the potential confounding effect of closeness differences when designing studies that involve body microbiota. This possible confounding variable would also impact allocation methods and sample size determination formulas used in clinical trials. This opinion has important implications for pharmacological, microbial, and infection studies, both clinically and epidemiologically. Furthermore, it underscores the need to develop practical tools for measuring closeness as a confounding factor, both qualitatively and quantitatively, for future preparedness.

Aesthetically, I like to refer to this confounding phenomenon as "French Kiss Bias", even though we know oral and gut microbiota are transmitted via multiple routes.

Acknowledgments

None.

Funding

This research received no specific grant from any funding agency, whether commercial or not-for-profit.

Conflict of interest

The author declares no competing interests.

Author contributions

RR is the sole author of this manuscript.

References

[1] Colnet B, Mayer I, Chen G, Dieng A, Li R, Varoquaux G, *et al*. Causal inference methods for combining randomized trials and observational studies: a review. Statistical Sci 2024;39(1):165–191. doi:10.1214/23-STS889.

[2] Cassinat JR, Whiteman SD, Serang S, Dotterer AM, Mustillo SA, Maggs JL, *et al*. Changes in family chaos and family relationships during the COVID-19 pandemic: Evidence from a longitudinal study. Dev Psychol 2021;57(10):1597–1610. doi:10.1037/dev0001217, PMID:34807683.

[3] Elgormus N, Senyigit A, Okuyan O, Bozkurt F, Aydin DS, Uzun H. Evaluation of the Effect of Early-Onset Steroid Treatment in the COVID-19-Positive Pregnant Women on Pregnancy Outcomes. Viruses 2024;16(9):1453. doi:10.3390/v16091453, PMID:39339929.

[4] Valles-Colomer M, Blanco-Míguez A, Manghi P, Asnicar F, Dubois L, Golzato D, *et al*. The person-to-person transmission landscape of the gut and oral microbiomes. Nature 2023;614(7946):125–135. doi:10.1038/s41586-022-05620-1, PMID:36653448.

[5] Mukherjee C, Moyer CO, Steinkamp HM, Hashmi SB, Beall CJ, Guo X, *et al*. Acquisition of oral microbiota is driven by environment, not host genetics. Microbiome 2021;9(1):54. doi:10.1186/s40168-020-00986-8, PMID:33622378.

[6] Williams JE, Carrothers JM, Lackey KA, Beatty NF, Brooker SL, Peterson HK, *et al*. Strong Multivariate Relations Exist Among Milk, Oral, and Fecal Microbiomes in Mother-Infant Dyads During the First Six Months Postpartum. J Nutr 2019;149(6):902–914. doi:10.1093/jn/nxy299, PMID:31063198.

[7] Cabrera SM, Coren AT, Pant T, Ciecko AE, Jia S, Roethle MF, *et al*. Probiotic normalization of systemic inflammation in siblings of type 1 diabetes patients: an open-label pilot study. Sci Rep 2022;12(1):3306. doi:10.1038/s41598-022-07203-6, PMID:35228584.

[8] Chen K, Jin S, Ma Y, Cai L, Xu P, Nie Y, *et al*. Adjunctive efficacy of Lactis XLTG11 for Acute diarrhea in children: A randomized, blinded, placebo-controlled study. Nutrition 2023;111:112052. doi:10.1016/j.nut.2023.112052, PMID:37172455.

[9] DeMuri GP, Lehtoranta LM, Eickhoff JC, Lehtinen MJ, Wald ER. Ex vivo peripheral blood mononuclear cell response to R848 in children after supplementation with the probiotic Lactobacillus acidophilus NCFM/Bifidobacterium lactis Bi-07. Benef Microbes 2021;12(1):85–93. doi:10.3920/BM2020.0068, PMID:33550937.

[10] Marcelo TLP, Pellicciari CR, Artioli TO, Leiderman DBD, Gradinar ALT, Mimica M, *et al*. Probiotic therapy outcomes in body composition of children and adolescent with obesity, a nonrandomized controlled trial. Arch Endocrinol Metab 2022;66(6):815–822. doi:10.20945/2359-3997000000526, PMID:36219201.

[11] Bardosh KL. How did the Covid pandemic response harm society? A global evaluation and state of knowledge review (2020-21). SSRN Electronic Journal 2023. doi:10.2139/ssrn.4447806.

[12] Busa F, Bardanzellu F, Pintus MC, Fanos V, Marcialis MA. COVID-19 and School: To Open or Not to Open, That Is the Question. The First Review on Current Knowledge. Pediatr Rep 2021;13(2):257–278. doi:10.3390/pediatric13020035, PMID:34205837.

[13] Fang L, Yuen M, Zhang J, Ho EY, Chan S, Wu F, *et al*. A network analysis of positive developmental assets of Hong Kong school-age children during the Covid-19 pandemic. Curr Psychol 2024;43:12188–12197. doi:10.1007/s12144-023-04287-9, PMID:36718391.

[14] Hasain Z, Che Roos NA, Rahmat F, Mustapa M, Raja Ali RA, Mokhtar NM. Diet and Pre-Intervention Washout Modifies the Effects of Probiotics on Gestational Diabetes Mellitus: A Comprehensive Systematic Review and Meta-Analysis of Randomized Controlled Trials. Nutrients 2021;13(9):3045. doi:10.3390/nu13093045, PMID:34578921.

[15] Martín-Peláez S, Cano-Ibáñez N, Pinto-Gallardo M, Amezcua-Prieto C. The Impact of Probiotics, Prebiotics, and Synbiotics during Pregnancy or Lactation on the Intestinal Microbiota of Children Born by Cesarean Section: A Systematic Review. Nutrients 2022;14(2):341. doi:10.3390/nu14020341, PMID:35057522.

[16] Nilholm C, Larsson E, Sonestedt E, Roth B, Ohlsson B. Assessment of a 4-Week Starch- and Sucrose-Reduced Diet and Its Effects on Gastrointestinal Symptoms and Inflammatory Parameters among Patients with Irritable Bowel Syndrome. Nutrients 2021;13(2):416. doi:10.3390/nu13020416, PMID:33525489.

[17] Ramezani N, Moafi A, Nadjarzadeh A, Yousefian S, Reisi N, Salehi-Abargouei A. The Effect of Soy Nut Compared to Cowpea Nut on Body Weight, Blood Cells, Inflammatory Markers and Chemotherapy Complications in Children with Acute Lymphoblastic Leukemia: A Randomized Controlled Clinical Trial. Nutr Cancer 2018;70(7):1017–1025. doi:10.1080/01635581.2018.1495240, PMID:30198779.

[18] Thorpe CM, Kane AV, Chang J, Tai A, Vickers RJ, Snydman DR. Enhanced preservation of the human intestinal microbiota by ridinilazole, a novel Clostridium difficile-targeting antibacterial, compared to vancomycin. PLoS One 2018;13(8):e0199810. doi:10.1371/journal.pone.0199810, PMID:30071046.

[19] Heirali A, Thornton C, Acosta N, Somayaji R, Laforest Lapointe I, Storey D, *et al*. Sputum microbiota in adults with CF associates with response to inhaled tobramycin. Thorax 2020;75(12):1058–1064. doi:10.1136/thoraxjnl-2019-214191, PMID:33139451.

[20] Carozza S, Leong V. The Role of Affectionate Caregiver Touch in Early Neurodevelopment and Parent-Infant Interactional Synchrony. Front Neurosci 2021;14:613378. doi:10.3389/fnins.2020.613378, PMID:33584178.

[21] Dubois L, Valles-Colomer M, Ponsero A, Helve O, Andersson S, Kolho KL, *et al*. Paternal and induced gut microbiota seeding complement mother-to-infant transmission. Cell Host Microbe 2024;32(6):1011–1024.e4. doi:10.1016/j.chom.2024.05.004, PMID:38870892.

[22] Hermes GDA, Eckermann HA, de Vos WM, de Weerth C. Does entry to center-based childcare affect gut microbial colonization in young infants? Sci Rep 2020;10(1):10235. doi:10.1038/s41598-020-66404-z, PMID:32581284.

[23] Kunath BJ, De Rudder C, Laczny CC, Letellier E, Wilmes P. The oral-gut microbiome axis in health and disease. Nat Rev Microbiol 2024. doi:10.1038/s41579-024-01075-5, PMID:39039286.

[24] Althubaiti A. Sample size determination: A practical guide for health researchers. J Gen Fam Med 2023;24(2):72–78. doi:10.1002/jgf2.600, PMID:36909790.

[25] Granholm A, Alhazzani W, Derde LPG, Angus DC, Zampieri FG, Hammond NE, *et al*. Randomised clinical trials in critical care: past, present and future. Intensive Care Med 2022;48(2):164–178. doi:10.1007/s00134-021-06587-9, PMID:34853905.

[26] Giordano-Kelhoffer B, Lorca C, March Llanes J, Rábano A, Del Ser T, Serra A, *et al*. Oral Microbiota, Its Equilibrium and Implications in the Pathophysiology of Human Diseases: A Systematic Review. Biomedicines 2022;10(8):1803. doi:10.3390/biomedicines10081803, PMID:36009350.

[27] Maki KA, Kazmi N, Barb JJ, Ames N. The Oral and Gut Bacterial Microbiomes: Similarities, Differences, and Connections. Biol Res Nurs 2021;23(1):7–20. doi:10.1177/1099800420941606, PMID:32691605.

[28] Tan X, Wang Y, Gong T. The interplay between oral microbiota, gut microbiota and systematic diseases. J Oral Microbiol 2023;15(1):2213112. doi:10.1080/20002297.2023.2213112, PMID:37200866.

[29] Chung The H, Le SH. Dynamic of the human gut microbiome under infectious diarrhea. Curr Opin Microbiol 2022;66:79–85. doi:10.1016/j.mib.2022.01.006, PMID:35121284.

[30] Wang D, Ruan W, Chen Z, Peng Y, Li W. Shift work and risk of cardiovascular disease morbidity and mortality: A dose-response meta-analysis of cohort studies. Eur J Prev Cardiol 2018;25(12):1293–1302. doi:10.1177/2047487318783892, PMID:29929393.

Exploratory Research and Hypothesis in Medicine **2025** vol. 10(2) | 143–145
DOI: 10.14218/ERHM.2024.00700

Commentary

The Application of GLP-1 Receptor Agonists and SGLT2 Inhibitors in Obstructive Sleep Apnea: Breakthrough or Overhyped?

Lei Huang[1] and Tong Feng[2*]

[1]*The Third People's Hospital of Nanbu County, Nanchong, Sichuan, China;* [2]*The Second School of Clinical Medicine, Southern Medical University, Guangzhou, Guangdong, China*

Received: August 19, 2024 | **Revised:** September 25, 2024 | **Accepted:** October 03, 2024 | **Published online:** January 02, 2025

In June 2024, *The New England Journal of Medicin*e (NEJM) published an article titled "Tirzepatide for the Treatment of Obstructive Sleep Apnea and Obesity", reporting the results of a Phase III clinical trial on the use of tirzepatide in patients with moderate to severe obstructive sleep apnea (OSA) and obesity.[1] The study demonstrated that tirzepatide significantly reduced the apnea-hypopnea index (AHI), a key indicator for assessing the severity of OSA. Over the course of the 52-week study, patients who did not receive continuous positive airway pressure (PAP) therapy experienced an average reduction in AHI of 25.3 events per hour, while those using PAP therapy saw a reduction of 29.3 events per hour. Additionally, tirzepatide significantly reduced patients' body weight and the nocturnal hypoxia burden associated with OSA. These findings suggest that tirzepatide not only effectively reduces body weight but also markedly improves OSA symptoms, potentially reducing the dependence on PAP therapy. Further research indicates that this drug may also reduce cardiovascular disease risk, offering new hope for personalized treatment approaches in the future.

For patients with OSA combined with metabolic syndrome, continuous positive airway pressure therapy and lifestyle-based metabolic interventions (such as dietary adjustments and weight loss) have been shown to significantly improve health outcomes. These interventions not only help alleviate OSA symptoms but also improve cardiometabolic health and reduce the risk of cardiovascular diseases. Treating OSA-related obesity, even with modest weight loss, can lead to significant cardiometabolic improvements, such as lowering blood pressure, improving insulin sensitivity, and reducing inflammatory markers.[2] However, despite the effectiveness of these interventions, long-term adherence remains a challenge. Many patients struggle with maintaining lifestyle changes and consistent use of PAP, leading to lower compliance. Therefore, current research is actively exploring new treatment methods to address the metabolic disturbances associated with OSA. Pharmacological therapies, such as glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose cotransporter 2 (SGLT2) inhibitors, have shown potential in improving OSA and related metabolic issues.[3,4] These treatments may not only directly affect metabolic pathways but also reduce body weight, lower systemic inflammation, and enhance cardiovascular health, providing new treatment options for patients who find it difficult to adhere to traditional therapies.

The emergence of GLP-1 receptor agonists (GLP-1RAs) marks a shift in the approach to treating metabolic diseases, particularly in managing type 2 diabetes and obesity. A substantial body of evidence suggests that these drugs play a critical role in the long-term control of these conditions, sparking widespread interest in their potential application in obesity-related conditions like OSA. GLP-1RAs function through various mechanisms, including the activation of GLP-1 receptors on pancreatic β-cells, enhancing glucose-dependent insulin synthesis and secretion, thereby reducing the risk of hypoglycemia.[5] Additionally, GLP-1RAs delay gastric emptying, increase satiety, reduce food intake, and support weight management. Furthermore, these drugs act on the hypothalamus, inhibiting orexigenic pathways that control appetite, thereby further supporting weight management.

Existing research indicates that GLP-1RAs have a positive impact on OSA treatment, particularly in significantly reducing AHI.[6,7] The improvements in daytime sleepiness scores and AHI resulting from GLP-1RA intervention are closely related to reductions in body weight, body mass index, and waist circumference.[8,9] Moreover, GLP-1RAs may improve respiratory control through mechanisms independent of weight management.[10] The hypothalamus contains GLP-1 receptors, which play a role in regulating the sleep-wake cycle and respiratory drive. GLP-1RAs can modulate neurotransmitter release when interacting with these receptors, which may enhance respiratory stability and reduce apnea episodes, independent of weight changes. GLP-1RAs have been shown to affect the sensitivity of chemoreceptors, particularly in the carotid bodies, which detect changes in blood oxygen and carbon dioxide levels. By altering chemoreceptor responsiveness, GLP-1RAs can improve respiratory drive, contributing to more stable breathing patterns during sleep. GLP-1RAs also possess anti-inflammatory properties that reduce systemic inflammation, which is often elevated in OSA patients. This reduction can im-

***Correspondence to:** Tong Feng, The Second School of Clinical Medicine, Southern Medical University, No. 1023 Shatai South Road, Baiyun District, Guangzhou, Guangdong 515000, China. ORCID: https://orcid.org/0009-0004-6324-8502. Tel: +86-185 83681025, E-mail: 543051181@qq.com

How to cite this article: Huang L, Feng T. The Application of GLP-1 Receptor Agonists and SGLT2 Inhibitors in Obstructive Sleep Apnea: Breakthrough or Overhyped? *Explor Res Hypothesis Med* 2025;10(2):143–145. doi: 10.14218/ERHM.2024.00700.

prove upper airway muscle function, lower airway resistance, and enhance respiratory control without being solely dependent on weight loss. There is evidence suggesting that GLP-1RAs have neuroprotective effects that may enhance brainstem function, which is critical in regulating breathing. This mechanism can stabilize respiratory patterns during sleep, independent of weight loss effects. GLP-1RAs may also reduce sympathetic nervous system activity while enhancing parasympathetic activity, helping to regulate airway tone and respiratory muscles. This effect can improve overall breathing control in OSA patients, even without changes in body weight. The hypothalamus, as the primary regulator of the sleep-wake cycle, contains GLP-1 receptors, allowing GLP-1RAs to directly modulate this process. By acting on these receptors in the hypothalamus, GLP-1RAs regulate neurotransmitter release, influencing sleep quality and wakefulness.[11] This mechanism may explain the potential of GLP-1RAs to improve OSA-related symptoms of excessive daytime sleepiness.

The bioactivity of GLP-1RAs primarily depends on their interaction with GLP-1 receptors. However, due to individual differences in GLP-1 receptor gene polymorphisms and receptor expression levels, patient responses to GLP-1RAs can vary significantly.[12] Specifically, some patients may experience altered expression or function of GLP-1 receptors due to genetic polymorphisms, which could weaken or completely block the metabolic effects of GLP-1RAs. For these patients, continuing the use of GLP-1RAs, despite a lack of efficacy, may not only fail to achieve treatment goals but also increase the risk of drug-related adverse effects, including common gastrointestinal issues and more severe side effects such as pancreatitis or gallbladder disease. Therefore, promptly identifying the ineffectiveness of GLP-1RAs in certain patients and discontinuing their use can offer multiple benefits. First, it can reduce patient exposure to potential unnecessary side effects, improving the overall risk-benefit ratio of the treatment. Second, avoiding ineffective treatment can significantly reduce unnecessary healthcare costs, making it more economically sustainable. There is growing evidence that genetic polymorphisms in GLP-1 receptor genes can significantly impact an individual's response to GLP-1RAs. These genetic variations may lead to differences in receptor expression levels or functional activity, affecting the drug's efficacy in treating both obesity and OSA. For example, some patients with certain GLP-1 receptor gene polymorphisms might exhibit reduced receptor expression or altered receptor signaling, resulting in a diminished response to GLP-1RAs. Personalized medicine approaches can help identify patients who are more likely to benefit from GLP-1RA therapy by evaluating their genetic profiles. By conducting genetic testing before treatment initiation, clinicians can tailor therapy to those with the highest likelihood of responding, thereby improving treatment outcomes and minimizing unnecessary exposure to medication for non-responders. This approach not only enhances treatment efficacy but also reduces the risk of adverse effects, such as gastrointestinal discomfort or more severe complications like pancreatitis or gallbladder disease. Furthermore, personalized medicine can optimize dosing regimens, as patients with certain genetic variants might require higher or lower doses to achieve the desired therapeutic effect. It can also help monitor potential resistance to GLP-1RAs over time, allowing for therapy adjustments as needed. Personalized adjustments based on patient genetic information and drug response are key to ensuring optimal resource utilization and maximizing patient benefit. Additionally, many patients may experience weight regain after discontinuing GLP-1RAs, sometimes returning to pre-treatment levels. Evidence suggests that this weight rebound after stopping GLP-1RA treatment may reverse its cardiometabolic benefits,[13] a problem that warrants further investigation in future research.

SGLT2 inhibitors have recently demonstrated significant efficacy in treating cardiovascular diseases, diabetes, and related metabolic disorders. Although research on their use in patients with OSA is still in its early stages, SGLT2 inhibitors have shown potential benefits. In the VERTIS CV study, ertugliflozin reduced the incidence of OSA by 48% compared to placebo.[14] Additionally, a meta-analysis of data from nine large randomized controlled trials evaluated the potential association between SGLT2 inhibitors and several respiratory diseases.[15] The results showed that SGLT2 inhibitors reduced the incidence of OSA by 65% compared to placebo.

The potential benefits of SGLT2 inhibitors for OSA patients can be explained through several mechanisms[16]: (1) SGLT2 inhibitors promote urinary glucose excretion, leading to weight loss. Since obesity is a major risk factor for OSA, weight loss can reduce upper airway resistance, thereby decreasing the frequency and severity of apneas; (2) During supine sleep, bodily fluids may shift from the lower limbs to the thoracic and neck regions, increasing upper airway resistance and exacerbating OSA symptoms. SGLT2 inhibitors reduce overall fluid load, lowering the occurrence of this fluid shift, thus helping to improve respiratory function during sleep; (3) Nocturia can disrupt sleep, interrupting the rapid eye movement (REM) cycle and shortening REM periods, which may reduce the incidence of REM-related OSA events.

In terms of long-term management, GLP-1RAs, such as liraglutide and semaglutide, have shown significant improvements in reducing the AHI, weight, and cardiometabolic risk factors in OSA patients. The benefits of GLP-1RAs appear to be twofold: they not only support weight loss, which is critical for reducing OSA severity, but they also have independent mechanisms that may improve sleep quality by acting on hypothalamic GLP-1 receptors that regulate sleep-wake cycles. These effects suggest that GLP-1RAs could provide sustainable management of OSA, especially in patients with concurrent obesity or metabolic syndrome. For SGLT2 inhibitors, studies have shown a notable reduction in the incidence of OSA, with mechanisms involving weight loss and fluid management during sleep. By reducing bodily fluid retention and promoting weight loss, these medications help decrease upper airway resistance, a key factor in OSA pathophysiology.

Despite these positive outcomes, concerns remain regarding the potential for rebound effects after discontinuing GLP-1RAs. Evidence suggests that many patients tend to regain weight once treatment is stopped, often returning to pre-treatment levels. This weight rebound could potentially reverse the improvements in OSA symptoms, as obesity is a major contributor to OSA severity. The rebound effect also implies a possible return of cardiometabolic risks, such as increased blood pressure, insulin resistance, and inflammation, which were initially mitigated during treatment. Therefore, discontinuing GLP-1RAs may necessitate alternative strategies to maintain weight loss and prevent OSA relapse. As for SGLT2 inhibitors, although data is less conclusive, discontinuation might similarly lead to a gradual return of weight and fluid retention, potentially diminishing the positive effects on OSA. Given that the mechanism of action for SGLT2 inhibitors involves maintaining fluid balance and reducing body weight, discontinuing treatment could lead to a recurrence of fluid shifts during sleep, exacerbating OSA symptoms.

Given these considerations, both GLP-1RAs and SGLT2 inhibitors may require long-term, if not lifelong, administration to maintain their benefits in OSA management. This approach aligns with the chronic nature of both obesity and OSA, where long-term treatment is often necessary to manage these conditions effectively. However, the need for ongoing treatment raises important ques-

tions about patient adherence, potential side effects, and economic sustainability, all of which should be carefully weighed in clinical decision-making.

Currently, the dual gastric inhibitory polypeptide (GIP)/GLP-1 receptor agonist tirzepatide has been shown to significantly reduce AHI in OSA patients.[4] Additionally, dual GLP-1/glucagon receptor agonists and triple GLP-1/GIP/glucagon receptor agonists are being explored for their potential effects in overweight or obese patients with OSA. Future studies need to be longer-term and more rigorously designed randomized controlled trials to thoroughly assess the safety and efficacy of GLP-1 and SGLT2 inhibitors in various patient populations and to better determine their effectiveness in reducing OSA severity and improving cardiovascular health. It is particularly important to clarify whether these treatments require long-term or intermittent use and to understand their long-term effects on OSA. Robust data on the risk-benefit ratio of these drugs will be crucial for clinical decision-making. In the future, physicians in the field of sleep medicine should enhance their skills by integrating obesity management with GLP-1 therapy as a treatment option for patients, which will require a deeper understanding of the potential side effects and challenges that patients might face, such as resistance to injection therapies.

Acknowledgments

None.

Funding

The authors declare that no financial support was received for the research, authorship, or publication of this article.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Writing the original draft (LH), reviewing and editing the manuscript (TF). All authors have approved the final version and publication of the manuscript.

References

[1] Malhotra A, Grunstein RR, Fietze I, Weaver TE, Redline S, Azarbarzin A, *et al*. Tirzepatide for the Treatment of Obstructive Sleep Apnea and Obesity. N Engl J Med 2024;391:1193–1205. doi:10.1056/NEJMoa2404881, PMID:38912654.

[2] Ng SSS, Tam WWS, Lee RWW, Chan TO, Yiu K, Yuen BTY, *et al*. Effect of Weight Loss and Continuous Positive Airway Pressure on Obstructive Sleep Apnea and Metabolic Profile Stratified by Craniofacial Phenotype: A Randomized Clinical Trial. Am J Respir Crit Care Med 2022;205(6):711–720. doi:10.1164/rccm.202106-1401OC, PMID:34936531.

[3] Gourdy P, Darmon P, Dievart F, Halimi JM, Guerci B. Combining glucagon-like peptide-1 receptor agonists (GLP-1RAs) and sodium-glucose cotransporter-2 inhibitors (SGLT2is) in patients with type 2 diabetes mellitus (T2DM). Cardiovasc Diabetol 2023;22(1):79. doi:10.1186/s12933-023-01798-4, PMID:37005640.

[4] Papaetis GS. GLP-1 receptor agonists, SGLT-2 inhibitors, and obstructive sleep apnoea: can new allies face an old enemy? Arch Med Sci Atheroscler Dis 2023;8:e19–e34. doi:10.5114/amsad/161170, PMID:37153372.

[5] Nolen-Doerr E, Stockman MC, Rizo I. Mechanism of Glucagon-Like Peptide 1 Improvements in Type 2 Diabetes Mellitus and Obesity. Curr Obes Rep 2019;8(3):284–291. doi:10.1007/s13679-019-00350-4, PMID:31124035.

[6] García de Lucas MD, Olalla Sierra J, Piña Fernández J. Liraglutide treatment in a patient with HIV, type 2 diabetes and sleep apnoea-hypopnoea syndrome. Diabetes Metab 2015;41(1):102–103. doi:10.1016/j.diabet.2014.10.005, PMID:25455831.

[7] Blackman A, Foster GD, Zammit G, Rosenberg R, Aronne L, Wadden T, *et al*. Effect of liraglutide 3.0 mg in individuals with obesity and moderate or severe obstructive sleep apnea: the SCALE Sleep Apnea randomized clinical trial. Int J Obes (Lond) 2016;40(8):1310–1319. doi:10.1038/ijo.2016.52, PMID:27005405.

[8] Jiang W, Li W, Cheng J, Li W, Cheng F. Efficacy and safety of liraglutide in patients with type 2 diabetes mellitus and severe obstructive sleep apnea. Sleep Breath 2023;27(5):1687–1694. doi:10.1007/s11325-022-02768-y, PMID:36542275.

[9] Gomez-Peralta F, Abreu C, Castro JC, Alcarria E, Cruz-Bravo M, Garcia-Llorente MJ, *et al*. An association between liraglutide treatment and reduction in excessive daytime sleepiness in obese subjects with type 2 diabetes. BMC Endocr Disord 2015;15:78. doi:10.1186/s12902-015-0074-6, PMID:26637348.

[10] Pauza AG, Thakkar P, Tasic T, Felippe I, Bishop P, Greenwood MP, *et al*. GLP1R Attenuates Sympathetic Response to High Glucose via Carotid Body Inhibition. Circ Res 2022;130(5):694–707. doi:10.1161/CIRCRESAHA.121.319874, PMID:35100822.

[11] Gault VA, Hölscher C. GLP-1 receptor agonists show neuroprotective effects in animal models of diabetes. Peptides 2018;100:101–107. doi:10.1016/j.peptides.2017.11.017, PMID:29412810.

[12] Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art. Mol Metab 2021;46:101102. doi:10.1016/j.molmet.2020.101102, PMID:33068776.

[13] Wilding JPH, Batterham RL, Davies M, Van Gaal LF, Kandler K, Konakli K, *et al*. Weight regain and cardiometabolic effects after withdrawal of semaglutide: The STEP 1 trial extension. Diabetes Obes Metab 2022;24(8):1553–1564. doi:10.1111/dom.14725, PMID:35441470.

[14] Wojeck BS, Inzucchi SE, Neeland IJ, Mancuso JP, Frederich R, Masiukiewicz U, *et al*. Ertugliflozin and incident obstructive sleep apnea: an analysis from the VERTIS CV trial. Sleep Breath 2023;27(2):669–672. doi:10.1007/s11325-022-02594-2, PMID:35596030.

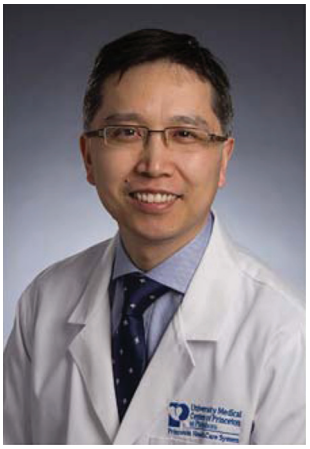
[15] Qiu M, Ding LL, Zhan ZL, Liu SY. Use of SGLT2 inhibitors and occurrence of noninfectious respiratory disorders: a meta-analysis of large randomized trials of SGLT2 inhibitors. Endocrine 2021;73(1):31–36. doi:10.1007/s12020-021-02644-x, PMID:33559806.

[16] Tanriover C, Ucku D, Akyol M, Cevik E, Kanbay A, Sridhar VS, *et al*. Potential Use of SGLT-2 Inhibitors in Obstructive Sleep Apnea: A new treatment on the horizon. Sleep Breath 2023;27(1):77–89. doi:10.1007/s11325-022-02606-1, PMID:35378662.

ERHM

Exploratory Research and Hypothesis in Medicine [Online ISSN: 2472-0712] publishes original exploratory research articles and state-of-the-art reviews that focus on novel findings and the most recent scientific advances that support new hypotheses in medicine.

Biography of the Editor-in-Chief



Prof. Lanjing Zhang (Editor-in-Chief)

Prof. Zhang is the Vice Chair of Pathology and Director of Gastrointestinal and Liver Pathology at Department of Pathology, Princeton Medical Center, Plainsboro, NJ. He is also a Visiting Professor in Biological Sciences at Rutgers University, Newark, NJ and holds visiting/adjunct professorship at several universities. His research is focused on clinical epidemiology, machine learning, computational biology, health services research and cancer biology. Dr Zhang has published more than 80 peer-reviewed articles, including those in Lancet, JAMA, JAMA Oncol, Nature and Cancer Cell. He is a co-investigator of an NIH R01 grant and co–principal investigator of 2 Rutgers University grants.

Updated on April 30, 2025

Published by Xia & He Publishing Inc.
14090 Southwest Freeway, Suite 300, Sugar Land, Texas, 77478, USA
Telephone: +1-409-420-2868
E-mail: service@xiahepublishing.com
Website: www.xiahepublishing.com